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The Cytology of Trisomic Mutations in a Wild Species of Oenothera

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VII—The Cytology of Trisomic Mutations in a Wild Species of Oenothera

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[Plates 22–24.]

Genetical

In September, 1932, seeds were collected of a wild *Oenothera* on the peninsula known as Penzance, near Wood's Hole, Massachusetts, from plants growing by the roadside. The following year a culture of fifty plants was grown in the experimental grounds at Regent's Park. They were quite uniform in character except two plants to be mentioned later. This species in cultivation has foliage remarkably like that of the *Oenothera Lamarckiana* of de Vries' experiments, but with leaves somewhat narrower, more pointed, and less crinkled than in that species. The flowers were, however, small like those of *O. biennis*. It is proposed to call this species *O. paralamarckiana*, not only on account of its resemblance to *O. Lamarckiana*, but also because it produces numerous trisomic mutations, in fact, in a much higher proportion than they have ever appeared in *O. Lamarckiana* itself. A full technical description of this new species will be published elsewhere. Figs. 1 and 2 are from photographs of typical plants of the 1933 culture in the rosette and flowering stages respectively.

The original culture from wild seeds contained one dwarf mutation, unbranched, with smaller flowers, fig. 3, and another plant with narrower, smooth leaves. There was also some variation in the amount of red pigment on the midribs of the leaves. In the light of subsequent knowledge, it is necessary to conclude that the smooth leaved plant was probably a trisomic mutation. The dwarf might also be expected to have 15 chromosomes, if not more, since its offspring nearly all had 15. While dwarfs in *Oenothera*, such as *nanella*, have been found to have 14 chromosomes, other dwarfs such as *O. nutans* mut. *nana* are known to be trisomic (Catcheside, 1933). Unfortunately, the material collected from the present dwarf mutant did not yield stages for determining the chromosome number, which must therefore remain uncertain although it was probably 15. A typical plant of the original culture of *paralamarckiana* was examined cytologically by Mr. C. E. Ford and found to have a ring of 14 chromosomes at diakinesis, *i.e.*, the catenation is the same as in most other small-flowered species of *Oenothera*.

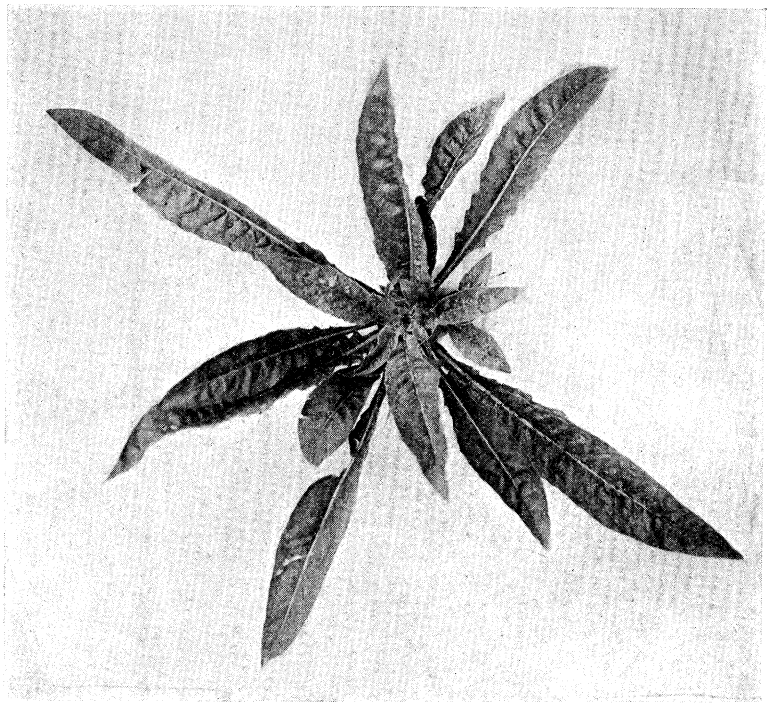

FIG. 1—*O. paralamarckiana* rosette 1933

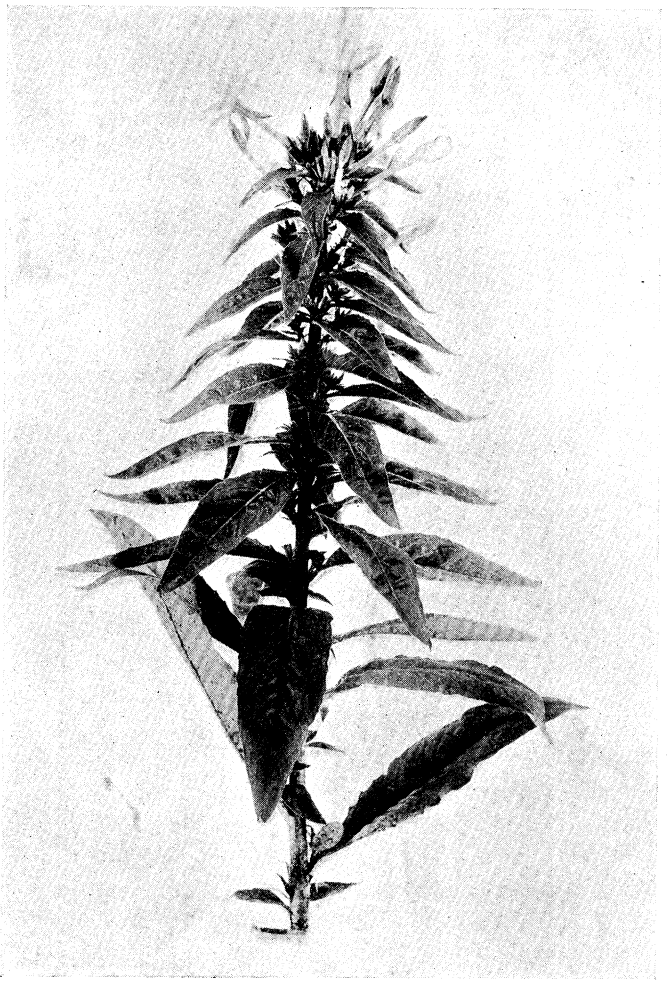

FIG. 2—*O. paralamarckiana* in flower 1933

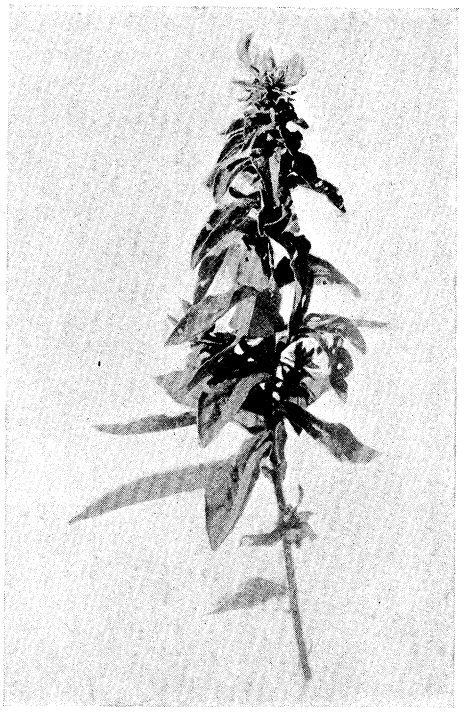

FIG. 3—Dwarf mutant in flower 1933

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In 1934, three F_2 cultures of these plants were grown. (1) Culture 124.34 was from the dwarf pollinated by a normal tall. Twenty seedlings germinated in April and were transferred to a box in May. In July there were 18 surviving rosettes and 17 of them afterwards flowered. An extraordinary feature is that every one of them was trisomic. Fifteen of these belonged to a single uniform type with sterile pollen, leaves more markedly crinkled than in the parent type but less broad, less blunt, and less bullate than in the trisomic mutant *lata*. This type will be called *semilata* (*cf.* GATES and THOMAS, 1914). It is represented as a rosette in fig. 4 and

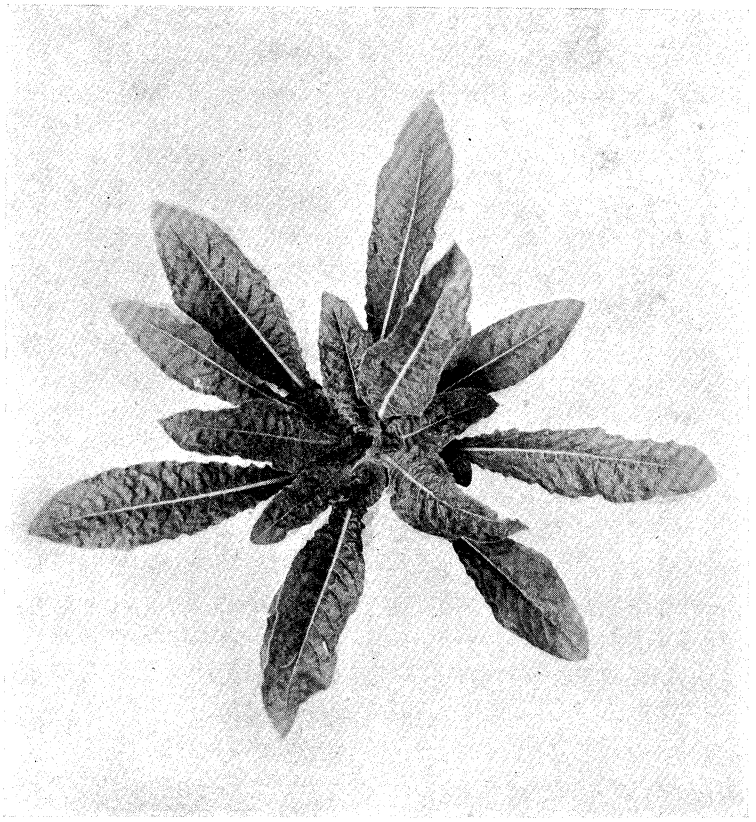

FIG. 4—*O. paralamarckiana* mut. *semilata* rosette 1934

flowering in fig. 5. The differences from the parent (diploid) type can be made out by comparing with figs. 1 and 2. The extra chromosome here produces similar effects to those in *lata* but less marked. It may be provisionally regarded as a chromosome homologous with the extra in *lata* but modified, perhaps through segmental interchange. Although only one of these 15 plants has been investigated cytologically, yet all their characters are so uniform that they were certainly all trisomic with the same extra chromosome present. One other plant (II. 4) differed in having much larger leaves, nearly smooth, hanging down the stem ; and pink midribs. A side branch is represented in fig. 6. It was thought that this plant might be triploid or tetraploid, but it proved to be trisomic. The pollen grains

appeared empty ; they were mostly triangular, but some were four-lobed. Finally, the remaining plant, I. 3, of this culture had leaves somewhat larger than the *semilata* type, but nearly smooth, and it also had 15 chromosomes. Fig. 7 shows a side branch. Much of the pollen in this plant appeared good, but the grains were mostly quadrangular, the number of germination pores ranging from four to eight or even more, with many of the grains irregularly shaped. The pollen was

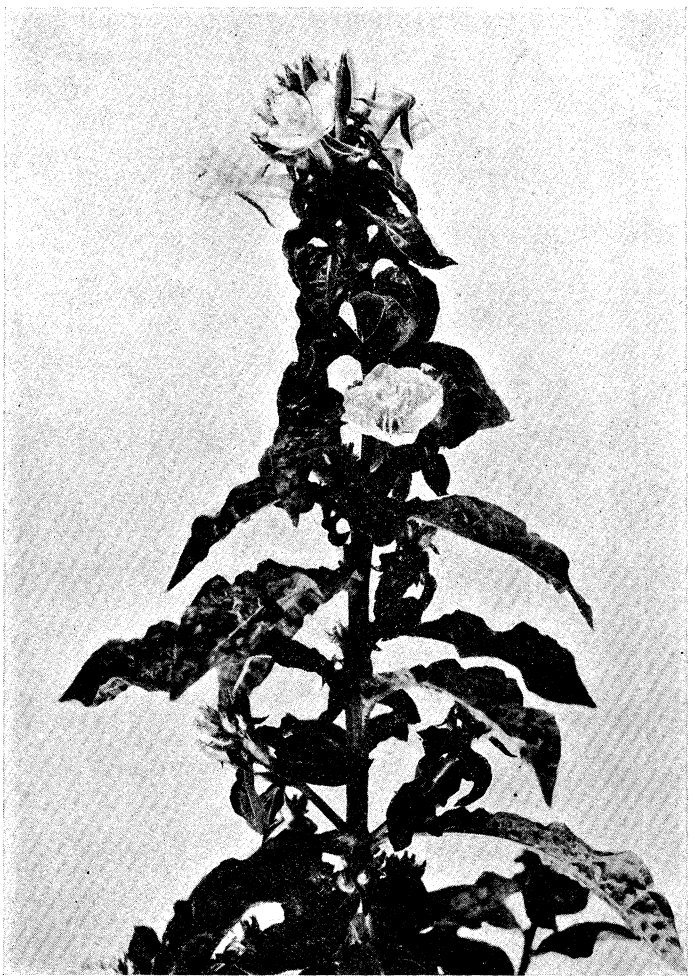

FIG. 5—*O. paralamarckiana* mut. *semilata* in flower 1934

recognizably different from that of the tetraploid *O. gigas*, which has quadrangular grains. Every plant in this culture was thus trisomic.* From this it is concluded that while the tall (normal) parent had a ring of 14 chromosomes the dwarf probably had 15. If this were so, then only megaspores with 8 chromosomes functioned in

* No record was kept of germination in the seed pan after the first month, and it is therefore possible that the diploid seeds showed delayed germination in comparison with the trisomics, but there is no direct evidence of this.

fertilization or only trisomic seeds germinated promptly. On the other hand, if both parents of this culture had 14 chromosomes, the non-disjunction (presumably in the female parent, *i.e.*, the dwarf) must have been extraordinarily high even if differential germination rates be brought in to account for the absence of diploid offspring. It is much more likely that the dwarf was trisomic. Possibly it might even have had 16 chromosomes.

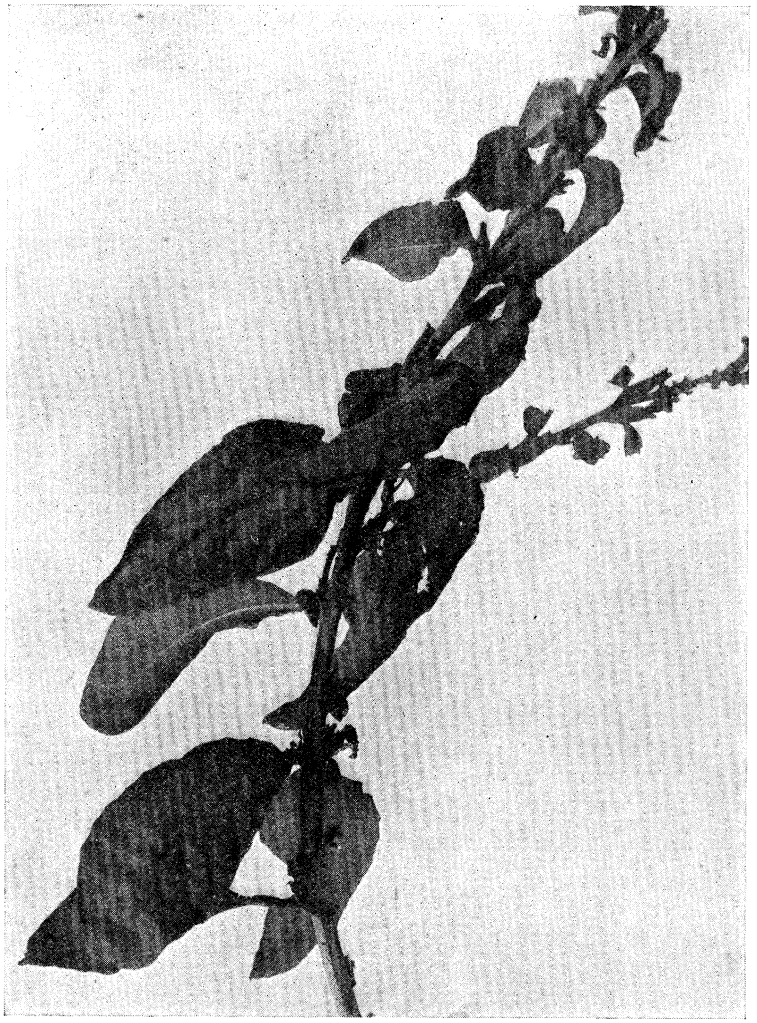

FIG. 6—Large-leaved trisomic, No. 124.34, II. 4

The second F_2 culture, 18.34, of these plants was from open-pollinated seeds of the dwarf mutation. About 40 germinated, and 33 were pricked off a month later. Twenty-five of these survived to be planted out, one of which died in the rosette stage. Only one aberrant plant appears to have been a hybrid with another culture. The remaining 23 plants were classified as follows: (*a*) *semilata* type like that of culture (124); 12 plants, two of which remained rosettes while the remainder,

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which flowered, all had dry and empty anthers. (*b*) "Rubrinervis" type,* with red midribs and more or less good pollen; 8 plants, two of which remained rosettes. Four of these plants, with nearly all bad pollen, were (probably) trisomics. (*c*) Two extraordinarily hairy plants of a new type which may be called *hirsuta*, fig. 8. The leaves were small, smooth, subentire, waved, and very heavily pubescent. The buds were rounded and stout and the anthers empty, so this was also clearly a trisomic

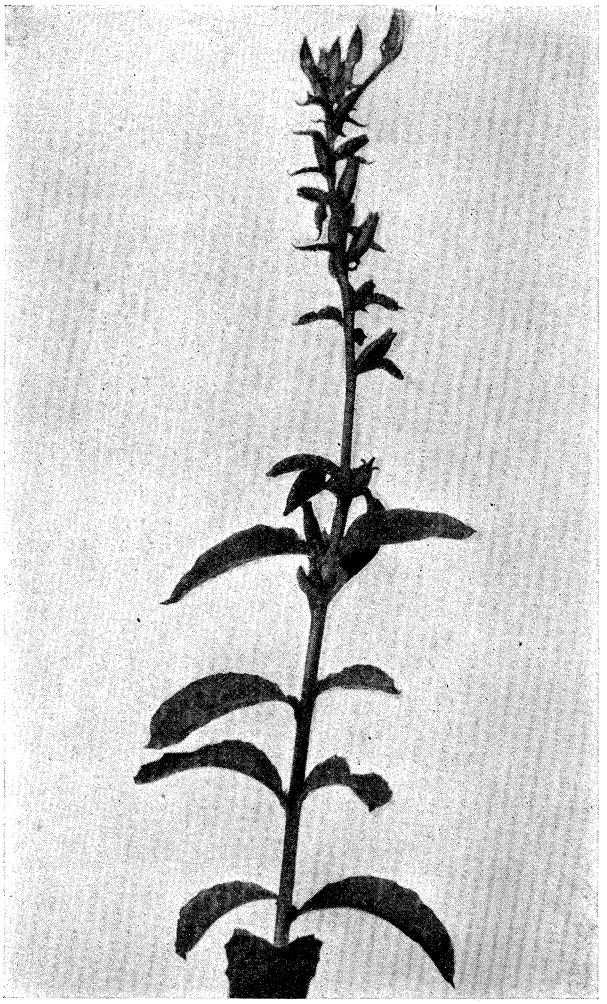

FIG. 7—Smooth-leaved trisomic, 124.34, I. 3

type. The buds, leaves, and capsules were also densely hairy. (*d*) One plant was a dwarf like the parent, but branched and bushy. It had some good pollen.

The third F_2 culture, 19.34, was from selfed seeds of the same (typical) plant which had been used to cross with the dwarf. Unfortunately only one or two seeds germinated and but one reached maturity. This strengthens the evidence that

* This was probably the same as the larger leaved mutant, II. 4, in Culture 124, fig. 6.

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trisomic seeds of this species germinate better than diploids. The surviving plant was typical *paralamarckiana* with good pollen and doubtless diploid.

From the foregoing it will appear that a dwarf (probably trisomic) mutation from wild seeds of this species when crossed with the tall type produced 17 plants, all trisomic, belonging to three different types, but 15 of them *semilata*; while unguarded seeds of the same dwarf produced 23 plants, 12 of them *semilata*,

FIG. 8—*O. paralamarckiana* mut. *hirsuta*

8 "*rubrinervis*," but with varying amounts of bad pollen, one dwarf like the parent, and two identical plants of a new (*hirsuta*) type, which was probably trisomic. Of the 23 plants at least 19 were probably trisomic. This wild species thus produces an astonishingly high percentage of trisomic mutations, much higher even than *Lamarckiana*; and the offspring of the dwarf were practically all trisomic—a situation which has never occurred before. In this connection it may be pointed out that the senior author and others grew cultures of *O. Lamarckiana* and its relatives at Wood's

Hole Laboratory 25 years ago, at a distance of a mile or two from where the seeds of *O. paralamarckiana* were collected. There is thus the possibility that the latter might be descended from natural crosses between the pollen of *O. Lamarckiana* and the small-flowered wild local species. This, however, appears improbable.

Whether *O. paralamarckiana* is a hybrid of *O. Lamarckiana* or not, the fact remains that it has survived under natural conditions at Penzance for a period of some 25 years, and therefore appears to be entitled to recognition as a wild species unless it can be shown to be of hybrid origin. *O. Lamarckiana* has never been found wild in America, except a small clump of plants found by the senior author in company with Professor MARIE-VICTORIN, at Lotbinière in Quebec Province, October, 1932. These plants were on the roadside by a cultivated field near a château with an English garden, and had very probably escaped from there.

Although many attempts have been made, notably by DAVIS, to show that *O. Lamarckiana* originated as a garden hybrid, yet there is no evidence that its status as a species is different from that of other early species such as *O. biennis* and *O. grandiflora*. Neither *O. biennis* nor *O. Lamarckiana* have hitherto been found truly wild in America. The status of *O. Lamarckiana* will not be further discussed here, but it may be pointed out that DAVIS, who has been the chief protagonist of the view that *O. Lamarckiana* is of hybrid origin, probably in English gardens, is constrained to admit that the specimen collected in Eastern North America by MICHAUX, in the eighteenth century (see DAVIS, 1927, p. 338, plate V) and which has been identified by various botanists with *O. Lamarckiana*, "has no affinities with *O. grandiflora* Solander." He further admits (DAVIS, 1926, p. 367) that this specimen agrees with *Lamarckiana* in bud and flower characters and in pubescence. That being so, the difference in foliage which he points out by no means "removes it entirely from this species." On the contrary, the very important characters of agreement indicate that it is at least closely related to that species, and makes it certain that a large-flowered *Oenothera* with the bud, flower, and pubescence characters of *O. Lamarckiana* grew wild somewhere in Eastern North America in the eighteenth century.

Since all the types of mutational behaviour discovered in *O. Lamarckiana* have subsequently been found in such species as *O. biennis* and *O. grandiflora*, there is no longer any genetical reason for assuming that *Lamarckiana* differs in origin from other species. It is generally recognized that the wild species of *Oenothera* are nearly all crypthybrids, as shown by the catenation of their chromosomes. Hybridization must, therefore, have played a considerable but by no means predominating part in the production of the present species as we know them. The evolution of the genus has been discussed elsewhere (GATES, 1933).

CYTOLOGICAL

The bulk of these observations were made on material collected from one trisomic plant, II. 3, belonging to the type *semilata* in the culture 124.34. The culture contained 15 plants of this identical type. Material was also collected and studied

from the single smooth-leaved (trisomic) plant, I. 3, and from the large-leaved trisomic mutant, II. 4, but no essential differences in their cytological behaviour were discovered. The main account will therefore be based upon the *semilata* material. The results agree in many features with an earlier investigation (GATES and THOMAS, 1914) of trisomic *Oenotheras* belonging to *lata* and *semilata*, but subsequent improvements in technique have made possible a much more detailed history of the chromatin threads, and light can now be thrown upon many points then obscure. We are indebted to Mr. C. E. FORD for making certain of the preparations.

Methods

The flower buds in various stages of development were collected in the field between 1 and 3 p.m. on very bright sunny days of August, 1934. The fluids used for fixing were Bouin, Allen's modification of Bouin and Navashin's fluid, with, in the last case, a previous treatment of the material for a few seconds in Carnoy's mixture. The formulae of Carnoy's and Navashin's fluids were those given by MAEDA (1930). The sepals were removed from the buds before fixing and an exhaust pump was always used. The materials were kept in the fixative for 5 to 6 hours. Of the three fixatives used, Navashin's following treatment with Carnoy gave the most satisfactory results.

Paraffin sections were cut from 10–16μ in thickness, thus enabling a study of complete cells, which is necessary for a critical understanding of nuclear structure and chromosome configuration. The gentian violet-iodine stain, which was mainly used, is superior to Heidenhain's haematoxylin in giving a clearer structure of the chromosomes at all stages, but *Oenothera* material is sometimes difficult to stain. Before staining, the slides were mordanted in 1% chromic acid for 15 minutes and rinsed in water. The slides could afterwards be differentiated to any extent according to the requirements.

Description

The pollen mother cell nucleus in the resting condition consists of threads which are so extraordinarily fine that their relationships cannot be followed, fig. 9, Plate 22, but small stained granules of varying size and number are found apparently attached to these threads. The nucleolus is sometimes vacuolate. The heterotypic prophase begins with a transformation of this almost submicroscopic threadwork into threads which are still extremely delicate but which can be followed for considerable distances, although their relationships to each other can still not be clearly traced, figs. 10, 11, Plate 22. Numerous chromatic granules are still present as thickenings of these threads. Probably one of the main changes has been a shortening and thickening of the individual threads composing the resting nucleus. A further feature of interest is that in some nuclei, see figs. 10 and 11, two definite deep-staining granules are visible, attached to the nucleolus and connected with chromatic threads. These represent the nucleolar body first described by LATTER

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(1926) in *Lathyrus* and since recognized in the pollen mother cells of a number of other plants. From present knowledge it is evident that these two bodies and their attached threads represent the particular pair of chromosomes which produces the nucleolus in telophase at a particular locus of their length. At these and later stages the nucleolus frequently retains a more deeply staining outer rim or shell, figs. 10–13, Plate 22.

Fig. 12, Plate 22, represents a further stage in the shortening and thickening of the chromatic threads in the nucleus. They now assume a superficially beaded appearance, and the larger chromatic aggregations previously present, fig. 11, have disappeared. A small protein crystalloid is sometimes present in the nucleolus, as in fig. 12. Neither now nor at earlier stages can free ends of threads be seen in uncut nuclei. For reasons which will appear later, the beaded character of the leptotene thread at this time is believed to be due to the fact that it is composed of two threads twisted about each other and so forming nodes and internodes. The threads are presumably double also in the earlier stages represented by figs. 10 and 11, but they may be too near the limits of resolution to show any indications of this. There is no indication as to what happens to the numerous granules which disappear between figs. 11 and 12.

Figs. 13–19, Plate 22, represent more or less successive stages of synizesis. In these stages free ends are never observed in uncut nuclei. It therefore appears probable that all the chromosomes are already arranged end-to-end at this time. Frequently a single nucleolar body, formed by the fusion of two, is visible in the nucleolus, figs. 13–15, 18, 19, and in some cases the thread passing through this body can be clearly traced over the surface of the nucleolus, figs. 14, 18, 19. The same conditions were described in three species of *Oenothera* by Sheffield (1927).

In all stages of synizesis there is a region, usually adjacent to the nucleolus, where the threads are too densely compacted to be followed, and from this area loops arise. Throughout this period, portions of the thread are no doubt constantly moving about and rearranging themselves. In the meantime, the thread is shortening and thickening throughout its length. Synizesis itself probably represents a sensitive condition of the nucleus, when the delicate threads are easily compacted together by the entrance of the fixing agent.

As the rearrangement of the threads progresses, the loops thrown out from the mass become more conspicuous, and as the thread shortens greatly during this period the loops also diminish in number. A stage is reached in which the split character of the leptotene thread is evident in places, figs. 19, 20, Plate 22, where the threads are lying parallel and not twisted about each other. This untwisting of the halves of the split leptonema to form parallel threads is therefore concomitant with the shortening of the leptonema as a whole. Only certain portions of the thread undergo this untwisting, and a certain amount of twisting remains in all the definitive chromosomes. The progressive shortening of the threads finally leads to the pachynema or second contraction stage, figs. 21, 22, Plate 22, in which seven loops in a continuous very heavy thread are clearly present. This stage evidently

corresponds with the brochonema described (LATTER, 1926) in Lathyrus. In some cases the extra chromosome can also be seen attached between two of the loops. It is clear that each of the seven loops in the pachytene nucleus consists of a pair of chromosomes arranged end-to-end. No evidence of parasynaptic pairing of the ends of chromosomes has been obtained in this material, nor has anything been observed which would suggest the formation or terminalization of chiasmata. One can only conclude that, although, apparently, it does sometimes occur, this hypothesis has been over-emphasized as an explanation of the meiotic phenomena in *Oenothera*.

The characteristic stage represented by figs. 21 and 22 is followed by a spreading out of the pachynema, the beginning of which is shown in fig. 23, Plate 22. Here it is clear that the chromosomes are arranged end-to-end, and the double connections between chromosomes can sometimes be seen, each chromosome being a double structure composed of two laterally approximated threads from the leptonema stage onwards. Further spreading out of the chain leads to such conditions as fig. 24, Plate 22, which shows probably a chain of 15 chromosomes in various degrees of condensation. Fig. 25, Plate 22, is a more typical diakinesis stage showing the 15 uncompacted chromosomes with uneven margin in a chain. The connections between them are double in some and appear single in others. Up to this stage, it appears that the whole of the chromatin is arranged either in a closed ring of 14 chromosomes with the extra attached to it by one end, or, more often, in a chain of 15 chromosomes the extra being at one end of the chain. In fig. 25 there is also a fine connection between the nucleolus and one chromosome, which may be significant.

First meiotic division

The nuclear membrane has persisted until now, and the nucleolus is still present, although diminished in size. In fig. 26, Plate 22, the nuclear membrane and the nucleolus have disappeared, the ring or chain of 15 chromosomes have become much more compact and are being pulled into V shapes by the forces that finally align them in a zigzag arrangement on the spindle. Each arm of a chromosome remains attached to a different chromosome. It seems clear that the body of the spindle and the forces which align the chromosomes arise within the nucleus itself. The karyolymph of the nucleus has become " anisotropic " through the stresses set up in it, which orient the apices of the now V-shaped chromosomes, the " spindle-fibre attachment " being at the middle. It appears that these movements of orientation in prometaphase and the consequent strains set up, frequently lead to breaking of the now delicate connections between adjacent chromosomes in the ring or chain. Fig. 27, Plate 22, represents a later prometaphase stage in which the chromosomes are much more compacted, the connections very delicate, forming a chain of 15, but the chromosomes have not yet become V-shaped nor taken up a zigzag arrangement. The tensions normally set up by the spindle have not yet developed, and the 15 chromosomes are left oriented mostly in one plane on the equator of the spindle.

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Figs. 28 and 29, Plate 22, are first metaphase groups slightly different from fig. 27. The connections between chromosomes have disappeared and the chromosomes are all aligned in a single plane, as in a somatic metaphase. The spindle forces which normally produce a zigzag arrangement of the chromosomes in two planes have failed to develop. It may be expected that all these chromosomes will split and undergo a pseudohomotypic mitosis similar to that described in the embryo sac mother cells of *Taraxacum* by GUSTAFSSON (1934). Such a condition would yield a dyad of diploid pollen grains, but no later stages of this process (which must be rare) have been found. In the pseudohomotypic metaphase three things must happen; (1) breakdown of the connections between chromosomes; (2) loss of the tensions which would normally pull alternate chromosomes to the same pole; (3) the development of fresh tensions which separate the halves of each chromosome, presumably by the splitting of the spindle fibre attachment and the repulsion of its two halves. Fig. 30, Plate 22, a neighbouring cell to fig. 27, Plate 22, shows an intermediate condition in which the connections between chromosomes have disappeared, but the chromosomes are still in two planes and some of them still partly retain their V-shape.

The zigzag arrangement corresponding to a normal first metaphase is represented in figs. 31 to 40, Plates 22 and 23. At this stage the catenation varies considerably, probably owing to breakages of some of the delicate connections, for the evidence is that at earlier stages there is always complete catenation of the chromosome threads. Occasionally the whole ring of 14 chromosomes may now be traced, with the extra always attached by one end only to the connection between two other chromosomes, fig. 39. It is then a rod oriented horizontally in the middle plane of the spindle and between the two rows of V-shaped chromosomes making up the zigzag ring. More usually this ring is broken up at metaphase, so that chains of varying lengths are formed. Thus, fig. 34 shows the same condition, except that instead of a ring of 15 chromosomes there is a chain of 11 (including the extra oriented at the side of the spindle as the 4th chromosome from one end of the chain and the 8th from the other end) and a separate chain of 4 which has been displaced in the figure. Fig. 40 shows a chain of 9 and a chain of 5, with the extra lying unattached in the middle plane of the spindle. In fig. 33, in which the chromosomes are not yet fully oriented, there is a chain of 6 and a chain of 9, the extra being presumably at one end of the latter chain. In other cases the chains are still more broken up, usually into pairs. Thus in fig. 35 there is a chain of 9 with three (displaced) rod pairs. Fig. 38, from plant I. 3, shows a chain of 11 not yet fully oriented, and a (displaced) chain of 4. Fig. 37 shows two chains of 4, three rod pairs and the extra unattached. In fig. 36 is represented the rare condition of a ring pair—in addition to two rod pairs, a chain of 5 and a chain of 4. In this case it is equally probable (1) that the extra has formed the ring pair with its homologue (the corresponding chromosome of the same complex), or (2) that it has attached itself to a homologous chromosome to form the chain of 5. Fig. 31 shows another rare condition, in which a trivalent is formed, presumably by the extra and its two homologues. This group together with the chain of 5 and the chain

of 7 are only partly oriented (prometaphase). The same is true of fig. 32, which shows a chain of 5, chain of 9 and a single chromosome. The catenations in some of these cells were confirmed by Mr. D. G. Catcheside.

The various configurations found in 60 pollen mother cells are tabulated in Table I. They show 26 different configurations. A chain is represented by parentheses, and a ring by a circle, the minus sign meaning an attachment to the ring and the plus sign a separate group of chromosomes. The configuration (7) — 1 — (3) represents a chain of 10 chromosomes with the extra attached by

Table I—Configurations in 60 p.m.c. of *semilata* Trisomic

	Catenation	Number of nuclei
1	(15)	6
2	⑭ — 1	1
3	(13) + ②	1
4	(13) + (2)	3
5	(12) + (3)	1
6	(11) + (4)	5
7	(9) + (6)	4
8	(9) + (5) + 1	4
9	(9) + (4) + (2)	2
10	(9) + (3) + (3)	1
11	(8) + (7)	4
12	(8) + (5) + (2)	2
13	(8) + (4) + (2) + 1	2
14	(8) + (4) + (3)	2
15	(7) + (4) + (2) + ②	1
16	(7) + (5) + (3)	4
17	(7) + (4) + (2) + (2)	2
18	(7) + (6) + (2)	2
19	(7) — ② + (6)	1
20	(7) — 1 — (3) + (4)	1
21	(5) + (4) + 3 (2)	2
22	(5) + 2 (4) + (2)	3
23	(5) + (3) + 3 (2) + 1	1
24	(5) + (3) + 2 (2) + (3)	2
25	(5) + 2 (4) + ②	1
26	3 (4) + (3)	2
		60

one end between the seventh and eighth, fig. 34. The most frequent catenation was a chain of 15, found in six cells. Next most frequent was a chain of 11 and a chain of 4. Owing to the presence of the extra chromosome it is impossible to determine whether the breaks are more frequent between homologues or between

pairs, but they probably occur with equal frequency at any point. It will be seen that in the 60 cells a ring pair only occurs four times, once attached to a chain of 7, while a chain of 3 occurs eleven times and three cells were found to have two chains of 3.

It appears that up to diakinesis all the chromosomes are attached end-to-end. This chain is afterwards frequently broken at various places when the strains are set up which orient the chromosomes in a zigzag arrangement at metaphase.

When a chain contains an odd number of chromosomes, *e.g.*, Nos. 4 and 7, it is usually regarded as including the extra attached to one end; but in such figures as 31 and 32 this cannot be so. Here the break in the ring must have come between homologous chromosomes. Of course, as has long been recognized (GATES, 1923), the homology of the chromosomes in a pair cannot be so strict in the catenated *Oenothera* as in other genera, and it is probable that the homology of the chromosomes in some pairs is closer than in others. In these forms, where there is no evidence of laterally paired ends, it is probable that the homologies of the various pairs are not very close, *i.e.*, the homologous chromosome ends may be very short, too short for side-by-side pairing. These conclusions appear to be supported by the fact that breaks in the chain not infrequently occur between chromosomes which are regarded as homologues.

There is a period in first metaphase when the connections between chromosomes are stretched to the utmost. Then the forces applied at the spindle fibre attachment to the middle of each V-shaped chromosome increase to the point of breaking these connections. This is the beginning of anaphase, as represented in figs. 41–43, Plate 23. Fig. 41 is unusual. Seven chromosomes are passing to one pole, 6 to the other, and two chromosomes left in the median plane of the spindle are being stretched out by forces apparently pulling in opposite directions. This condition is evidently similar to that described in another trisomic *lata*-like mutation (GATES and THOMAS, 1914, fig. 49). Other cases of like character have been interpreted as resulting from the failure of chiasmata to terminalize, but it is obviously impossible to apply such an interpretation here because these are evidently whole chromosomes lying with both ends free. Fig. 42 is a later anaphase, which shows 8 chromosomes passing to one pole and 7 to the other. Some of the chromosomes are also undergoing fragmentation. In fig. 43 one chromosome, presumably the extra, is left in the equatorial region and appears to have attached to it on opposite sides, spindle fibres pulling towards the two poles. This will probably result in the two halves unwinding from each other and separating, if the pulling forces are great enough to bring this about.

Interkinesis and second division—In the great majority of cases the separation of chromosomes is 8 + 7, as in fig. 44, Plate 23, which represents interkinesis. The chromosomes clearly show their split condition, these halves corresponding to the split halves of the leptotene thread. The chromosomes repel each other at this time, no doubt because they bear a surface charge of electricity. The ends of the four arms also characteristically repel each other, although the split halves remain

closely attached in the middle. Fig. 45, Plate 23, shows a not very infrequent condition in which 9 chromosomes enter one daughter nucleus. The extra chromosome is also sometimes left out of the cytoplasm, but only rarely in this material.

The second division is represented in figs. 46–53, Plates 23 and 24. Fig. 46 is a homotypic prophase in which $7\frac{1}{2}$ chromosomes are found on each spindle. Evidently the two halves of the extra have separated in the first division. In fig. 47 the numbers are respectively 8 and 7 split chromosomes. Figs. 48 and 49 are from two sections of the same pollen mother cell, the left-hand spindle with 9 chromosomes being in early anaphase, while the right-hand spindle has 6 chromosomes which have not yet begun to separate. It is significant that the spindle with 9 chromosomes is already in advance of the one with 6. The latter may complete its mitosis but will not form viable pollen grains. Fig. 50 shows nearly the same condition, but with 8 and 7 chromosomes. In fig. 51 the spindles are in mid-anaphase with 8 divided chromosomes on one spindle and 7 on the other. Fig. 53 shows the same condition at a somewhat later stage. The last three cases will all result in a tetrad of pollen grains, two of which have 8 chromosomes and two 7. In fig. 53 is shown a later stage, in which the four telophase nuclei all have 7 chromosomes, the two halves of the extra being left behind on the spindles. This is probably what commonly happens when the extra splits in the first division.

Fig. 54, Plate 24, represents the 15 chromosomes in a somatic cell of the petal epidermis.

Meiosis in another trisomic

The following six figures are from the trisomic plant II. 4, a side branch of which is represented in fig. 6. One of the slides prepared from this plant was very strongly destained to bring out the internal structure of the chromosomes. Fig. 55, Plate 24, represents a first metaphase in which the chromosomes are not yet fully oriented. There is present a chain of 4, chain of 7, a ring pair, as in fig. 36, Plate 22, and a rod pair. In fig. 56, Plate 24, we have two chains of 4, a rod pair and a chain of 4 with the extra attached by one end to the end of the second chromosome. In fig. 57, Plate 24, there are two chains of 4, three rod pairs and the extra, while in fig. 58, Plate 23, there are three chains of 4, a rod pair and the extra. A chromosome in the chain on the left is constricted into an hour-glass shape by being twisted on its axis. In fig. 59 the same thing has happened to several of the chromosomes. We have here a chain of 4, chain of 3, two rod pairs, and four single chromosomes, all of which show a median twist. The preparation from which figs. 58–60, Plate 23, were drawn was specially destained to a point at which the outlines of the chromosomes are difficult to follow. The appearance thus gained is that of a series of peripheral dots which are presumably optical sections of a compact double spiral chromonema in each chromosome, although it was not possible to follow the thread.

The late anaphase in fig. 60 shows 7 chromosomes at one pole and 8 approaching the other, three of them lagging. Several of the chromosomes in these figures have a constriction, which is generally but not always median. This condition has

been described before (GATES and SHEFFIELD, 1929) in the homotypic anaphase chromosomes of both megaspores and microspores in *O. rubricalyx*, where it appeared as a transverse segmentation of the chromosomes. From these destained preparations it is clear, however, that this appearance is simply due to a twist of the chromosome on its axis. It probably means that the matrix of the chromosome is so thin and pliable that it can undergo a twist and constriction at a point where the chromonemata cross. The false appearance of a transverse split in the anaphase chromosomes thus finds an explanation.

DISCUSSION

The extraordinary genetical feature of *O. paralamarckiana* is the very high proportion of trisomic mutations which it produces. Very probably the dwarf mutation from wild seeds had 15 chromosomes, since its offspring nearly all had 15, but the approach to 100% rather than 50% of trisomics among its offspring may have been due to delayed germination of the diploid seeds. In this connection it may be pointed out that wild seeds of an *Oenothera* from the Canadian prairies at Saskatoon, kindly sent by Professor W. P. THOMPSON, produced in 1934 a rosette with leaves of the typical *lata*-form but otherwise showing the characters of the species. There can therefore be no doubt that trisomic mutations occur in the wild, but whether they survive is another question. The occurrence of trisomic *lata* mutations in *O. biennis* is well known (GATES and THOMAS, 1914), and they have since been described from various other sources. A considerable frequency of non-disjunction, which leads to the formation of trisomic mutations, is known to occur in wild species of *Oenothera*. Thus, SHEFFIELD (1927) found in the pollen mother cells 4% in *O. novae-scotiae*, 7% in *O. eriensis*, and 6% in *O. ammophila*, and still higher frequencies have been observed. While pollen grains with an extra chromosome are known to be at a disadvantage in competition, so that they seldom function in fertilization, the same apparently does not apply to megaspores, and as the mechanism of meiosis is the same in both, non-disjunction must also occur in the production of megaspores. It follows inevitably that any wild *Oenothera* showing catentation may be expected to produce trisomic seeds. That they never lead in this genus to the production of species with 16 chromosomes is indicated by the fact that all the wild species hitherto studied agree in having 14.*

It is worth noting here that six trisomic types with 17 chromosomes have been found (STUBBE, 1934) among a large number of other aberrants in the offspring of X-rayed plants of *Antirrhinum majus*. They are more or less highly male sterile and differ from the type in such characters as leaf size, shape and colour, size and shape of flower, colour of lip and density of the raceme.

* One tetraploid species, *O. glauca*, has, however, been found (SCHWEMMLE, 1924); and the same author (1928) found in the F_2 from *O. Berteriana* × *odorata* a diploid plant with certain tetraploid (4*b*) branches. The latter when selfed, yielded two *gigas* offspring with 28 chromosomes.

The catenation in trisomics

Cytological studies of a considerable number of trisomic mutations have now been made. Håkansson (1926) found the mutations *stricta*, *curta*, *obscura*, *dentata*, and *dependens* from the Swedish *O. Lamarckiana* to have 15 chromosomes. Some of these forms were observed to have a free pair and a ring of 13, often broken. In a later paper (1928) he examined the chromosome linkage in the megaspore mother cells of various forms, including three trisomic mutants, *lata*, *stricta*, and *pulla*; *lata* was found to have a chain of 13 and a free pair, *stricta* was probably the same, while in the *pulla* mutant was found mostly a ring of 6 with three pairs and a trivalent, thus combining the trisomic and the half-mutant condition. In a later paper (Håkansson, 1930) the chromosome arrangements in the pollen mother cells of a number of primary trisomics were examined, including *lata*, *dependens*, *stricta*, *longepetiolata*, *pallescens*, *pulla*, and *liquida*, in all of which the most frequent arrangement was a chain of 13 and a free pair. In *nitens* was found a chain of 9 and 3 pairs, while a form called *curta* had a chain of 11 and 2 pairs. The latter does not segregate *Lamarckiana*. A secondary form from the trisomic *cana* had $14\frac{1}{2}$ chromosomes. The maximum catenation was a chain of 11 and two pairs, the half-chromosome being at the end of the chain.

In a trisomic derivative of *cana*, Goodwin (1933) found a chain of 13 and a ring bivalent, the chain breaking into smaller chains and branched chains (with the extra attached); also a chain of 10 with a ring-and-rod trivalent and a ring pair. Catcheside (1933) made a more detailed study of two trisomics. In *O. nutans* mut. *nana* the maximum linkage found was a ring of 14 with the extra attached by one end, the maximum possible being a chain of 13 closed by a ring pair. In a *lata* mutant from *rubrinervis* × *blandina* the maximum was a ring of 4 joined to a ring pair by a seventh chromosome, together with 4 ring pairs as in *rubrinervis*. He cites a trisomic mutant from *O. deserens* × *nutans* F_2 examined by Mr. E. D. Sweet in this Laboratory, in which the catenation is a ring of 10, a ring pair and a triple arc. Finally, Verbrugge (1934) investigated the catenation in a trisomic mutant from *O. rubricalyx* × *blandina* in which the maximum was a chain of 9 chromosomes and three pairs. These results are compiled in Table II, from which it will be seen that in all the primary trisomics from *O. Lamarckiana* examined the extra chromosome belonged to the ring of 12, whereas in the half-mutant and in the trisomic from *deserens* × *nutans* it belonged to a free pair.

All these results are summarized in Table II (p. 244).

The method of synapsis

In the earlier literature of the subject *Oenothera* was regarded as the outstanding example of telosynapsis in plants. This was based upon the fact that in the forms which we now speak of as showing catenation, more or fewer of the chromosomes are arranged end-to-end in a ring in diakinesis and on the heterotypic spindle until the connections between them are broken in anaphase. It is now known that in

most of the wild North American species the 14 chromosomes are thus normally arranged in a closed ring. It has also been recognized from the first that the chromosomes in the chains and rings are essentially whole somatic chromosomes, and nearly all workers on the subject are agreed that for an understanding of the

TABLE II—MAXIMUM CATENATION IN VARIOUS TRISOMICS

Parent	Trisomic mutant	Maximum catenation observed	Author
Swedish *O. Lamarckiana*	*stricta*	undetermined	HÅKANSSON, 1926
	obscura	? (13) + (2)	,, ,,
	dentata	(13) + (2)	,, ,,
	dependens	(13) + (2)	,, ,,
O. Lamarckiana	*lata*	(13) + (2)	,, 1928
	stricta	(13) + (2)	,, ,,
	half mut. from *pulla*	(6) + 3 (2) + 3	,, ,,
	dependens	(13) + (2)	,, 1930
	longepetiolata	(13) + (2)	,, ,,
	pallescens	(13) + (2)	,, ,,
	pulla	(13) + (2)	,, ,,
	liquida	(13) + (2)	,, ,,
	nitens	(9) + 3 (2)	,, ,,
	curta	(11) + 2 (2)	,, ,,
	secondary from *cana*	(11) + 2 (2)	,, ,,
O. Lamarckiana cana	*cana* derivative	(13) + (2)	GOODWIN, 1933
O. nutans	*nana*	(14) − 1	CATCHESIDE, 1933
O. rubrinervis × *blandina*	*lata*	(4) − 1 − (2) + 4 (2)	,, ,,
O. deserens × *nutans*	trisomic	(10) + (2) + 3	SWEET, 1933
O. rubricalyx × *blandina*	,,	(9) + 3 (2)	VERBRUGGE, 1934
O. paralamarckiana	*semilata*	(15)	present paper
	trisomic	(9) + (6)	,, plant II. 4
	,,	(11) + (4)	,, ,, I. 3

complicated genetical results the chromosomes must normally have fixed places in the ring, homologous chromosomes alternating and alternate chromosomes generally passing later to the same pole. And since the species and their complexes for the most part remain constant, crossing-over in the middle portions of the chromosomes is not to be expected.

In a study of the related genus *Eucharidium*, SCHWEMMLE (1926) made a number of interesting observations. He adhered to the older view of a "continuous spireme" which later segmented, whereas we now know this is only true when a ring of 14 chromosomes is present. He observed the leptotene thread to be clearly split, and he noted in the second contraction (pachytene) stage the presence commonly of seven loops corresponding to the seven pairs, as we have done in the present paper. Yet he arrived at the conclusion that this represented a parasynaptic method of reduction. He also found frequently in diakinesis a chain of 4 or of 6 chromosomes, the rest being in ring pairs.

The situation was further altered by the papers of DARLINGTON (1929, 1931), in which he applied to the meiotic chromosomes of *Oenothera* his conception of terminalization of chiasmata and thus brought some evidence in favour of parasynapsis in *Oenothera*. That evidence is cogent, however, only if terminalization on a large scale actually takes place. It can now be recognized that for a satisfactory interpretation of meiosis in *Oenothera* a comparative knowledge of the phenomena both in catenated and uncatenated species is necessary. Such information is now available, and it is clear that the uncatenated condition is the primitive one, from which catenation has arisen, since most plants, including some genera related to *Oenothera*, show the usual condition of free pairs. Catenation has probably arisen in the genus *Oenothera* itself, since such large-flowered species as *O. Hookeri* and *O. missouriensis* have 7 free pairs while most of the more northern (derived) species with small flowers have a ring of 14, intermediate conditions being found in a few species such as *O. biennis* and *O. ammophila*, as well as in various mutations.

A study was made (GATES and GOODWIN, 1931) of meiotic stages in *O. blandina* and *O. purpurata*, two derivative forms of *Oenothera* having 7 free pairs of chromosomes, and a rather meagre amount of evidence was obtained which was interpreted in favour of parasynaptic pairing. The prophase thread was recognized as double, with the two portions twisted about each other in the loop stage. The nature of this doubleness will be considered later. From a large number of nuclei studied, the chromosome pairs all formed complete rings at diakinesis. The only direct evidence which could be interpreted as terminalization of chiasmata was one metaphase plate with 7 pairs of rod chromosomes (fig. 35, Plate 18), a single one of which showed a pair of granules on the connecting strands. It is important to remember that in *Oenothera* free or uncatenated pairs of chromosomes normally form rings, *i.e.*, they are connected only at the ends. There is, moreover, a marked tendency for the ring pairs to be interlinked with each other, which evidently results from the fact that the chromosomes pair mainly at or near their ends. These conditions were clearly illustrated in the paper mentioned and it was pointed out that, so far as the middle portions of the chromosomes are concerned, the term asynapsis applied to them, *i.e.*, these portions were, for the most part, not paired at any stage of their development. CATCHESIDE (1931) was successful in obtaining more evidence of chiasma formation in a study of a derivative of *O. pallescens* having a ring of 6 chromosomes and 4 ring bivalents. At that time it seemed not unreasonable to

conclude that the ring bivalents had all been produced by an original side-by-side pairing of threads with formation of chiasmata, followed by their terminalization.

A different aspect has been placed on this matter, however, by later work. An investigation of HEDAYETULLAH (1933) on meiosis in *O. missouriensis*, showed that in this species with 7 ring bivalents the free ends of the 14 unpaired threads can be seen in the leptotene stage, which is followed by pairing of threads at or near the ends only. The middle portions of the chromosomes did not pair, but usually repelled each other from an early stage, and so by condensation the ring pairs were formed. Acrosyndesis is obviously a suitable term to describe this process. Evidence of chiasmata or their terminalization was not found, the indications being that the bulk of the ring pairs were formed without any such process. Thus in this wild and relatively homozygous species the homologous chromosomes pair only at their ends and repel each other in the medial portions, so forming ring bivalents. There is no evidence at all of chiasma formation in the usual sense in *O. missouriensis*. Such a history is suitably described as acrosyndesis of the chromosome ends with asynapsis of the middle portions. This confirms the suggestion of CATCHESIDE (1931) that synapsis might begin at the ends of the chromosomes, but shows that it does not proceed to the middle as he, perhaps naturally, assumed. In *Allium*, on the contrary, KOSHY (1934) finds that the zygotene pairing commences simultaneously at both ends and proceeds to the spindle fibre attachment in the middle.

EMERSON (1931), in a short study of an undescribed species of *Oenothera*, with seven free pairs of chromosomes, found conditions which are evidently closely similar to those in *O. missouriensis*. In six cells he found thread conditions of the chromosomes which he is inclined to interpret as side-by-side pairing. But his figures show the threads actually attached at their ends only to form a ring, and many of these rings show a medial repulsion as in *O. missouriensis*. Such figures-of-eight as occur do not in any way suggest true chiasmata, but simply a twist in the ring. EMERSON says, "Appearances of chiasmata were evident, but it seems impossible in this material to be sure that they actually are true chiasmata (*i.e.*, a cross between two of four strands)." He cites the fig. 2D of HÅKANSSON (1928) as showing "strong side-by-side pairing with appearance of chiasmata." This is similar to, though a little earlier than, our figs. 21 and 22. It shows several main loops, each composed apparently of two chromosomes connected at the end of the loop, and nothing that could be considered seriously as evidence of chiasmata. EMERSON's results are thus most reasonably interpreted as evidence of acrosyndesis with a superficial appearance resembling chiasmata.

It might be argued that in *O. missouriensis* and other species with free ring pairs of chromosomes the terminal portions only of the chromosomes are homologous, the central portions being non-homologous, and that this would account for the acrosyndesis. That argument is excluded, however, by a cogent bit of evidence obtained incidentally in a paper by VERBRUGGE (1934). In a study of the chromosome configurations in a trisomic mutant from *O. rubricalyx* × *blandina* she found a high degree of catenation of the 15 chromosomes, the maximum being

a chain of 9 with 3 free pairs. A single gigantic tetraploid pollen mother cell was found, however, with 15 separate ring pairs of chromosomes in heterotypic metaphase. This cell can only have arisen from a duplication (*i.e.*, autotetraploidy), probably in the last premeiotic telophase, of the 15 chromosomes normal to the plant. These chromosomes must be completely homologous throughout their length, yet they pair in the usual manner characteristic of *Oenothera*, *i.e.*, by acrosyndesis. The pairing of ends only in metaphase can therefore not be regarded as evidence that only the ends are homologous. There is no reason to believe that the ring bivalents of *O. missouriensis* are other than homologous throughout their length, like the laterally paired chromosomes in various other genera of plants. Manifestly, then, some other explanation of acrosyndesis in *Oenothera* will have to be found.

Kihara and Lilienfeld (1934) have recently made a study of cytomyxis and nuclear migration in the pollen mother cells of certain hybrids between *Triticum* and *Aegilops*. Complete nuclear migration was relatively rare, but they figure two cases in which a pollen mother cell has become binucleate in this way. The cells are, however, of normal size. If the two nuclei fuse they would produce a cell with a tetraploid nucleus. But the gigantic tetraploid cell found by Miss Verbrugge cannot have originated in this way. Its chromosomes must have arisen through previous duplication and therefore be homologous in the fullest possible sense. Yet in the 15 bivalents the chromosomes are paired only by their ends, and all the pairs are free, *i.e.*, the condition is exactly the same as in *O. missouriensis*. Håkansson (1926) similarly found in an anther of *Lamarckiana* × *biennis* a group of eight gigantic tetraploid pollen mother cells, doubtless all descended from an archesporial division in which the chromosomes were doubled. Unfortunately, the catenation of the chromosomes in these cells was not definitely determined. In the trisomic mutant *nana*, from *O. nutans*, Catcheside (1933) also found a loculus with a group of 14 tetraploid cells. Non-disjunctions were observed in them, but the catenation was not determined. As regards the *gigas* mutations, the indications are that catenation of the chromosomes is present. Thus in *O. Lamarckiana gigas* (Gates, 1911, figs. 20–22) the chromosomes are scattered in three or more planes on the heterotypic spindle in metaphase in the manner characteristic of catenated chromosomes, and in *O. gigantea* Håkansson (1926) found that there were usually two free pairs, seldom a tetravalent, the rest of the chromosomes being connected together.

The elaborate attempt of Darlington (1931) to force *Oenothera* meiosis into the same mould with plants having markedly different meiotic phenomena has clearly broken down. Several of Darlington's general assumptions and hypotheses regarding meiosis are involved in this breakdown, and a re-orientation of ideas has become necessary. In the first place it may be pointed out that it has been clear for some time that the old conception of the continuous spireme, either in meiotic or somatic prophase, is a thing of the past. It was natural that the early studies of *O. rubrinervis* and *O. Lamarckiana*, forms in which a high degree of catenation

present, should have resulted in the adoption of the conception of a continuous spireme, but we now know from the observations of many investigators that in organisms with free pairs of chromosomes their ends are separate from the earliest prophase stages, and all critical workers are apparently agreed on this point. In those *Oenotheras* with complete catenation the spireme is, on the contrary, not only continuous but also devoid of free ends. The catenated chromosomes of *Oenothera* are linked up end-to-end from the earliest stages of meiosis, whereas free pairs are free from the earliest stages. This applies equally when both types of arrangement are found, as they are in many biotypes, in the same cell.

Darlington's idea that chiasmata are necessary to produce meiotic pairing is disproved not only by the facts already cited in *Oenothera* but by various other lines of evidence, only one of which need be mentioned here. A number of cases of secondary pairing of chromosomes, both in heterotypic and homotypic mitosis, shows that this must be due to a residual attraction between similar elements and is entirely independent of chiasma formation. Generally, this secondary pairing does not express itself at all in diakinesis, at a time when the bivalents all repel each other owing to a superficial charge. Later, in both first and second metaphase, when these widely sundered bivalents lose their repulsion the mutual specific attractions express themselves. There is also evidence of attraction between the halves of the bivalent chromosomes, quite apart from any chiasmata which may occur. Absence of pairing cannot, therefore, be taken as proof of failure of chiasmata or of their complete terminalization either in *Oenothera*, where this argument has frequently been used, or in any other genus.

Another important matter is the interpretation of the prophase threads. In the early literature of *Oenothera* it was supposed that the spireme was a single thread. When evidence of its double nature was obtained (*e.g.*, Schwemmle, 1926; Darlington, 1929, 1931; Gates and Goodwin, 1931; Catcheside, 1931), it was somewhat hastily assumed that this represented a zygotene stage. Clear evidence has since been obtained (*e.g.*, Koshy, 1934, on *Allium*; Hoare, 1934, on *Scilla*) that the leptotene thread is split, just as the threads in any somatic prophase are split. Incidentally, this disposes of Darlington's hypothesis that the difference between the meiotic and somatic prophases consists primarily in a postponement of this split. No such postponement takes place.

The doubleness of the delicate prophase thread in *Oenothera*, observed in this and other papers, is not then a zygotene condition but is due to a split in the leptotene thread. This split reappears in heterotypic anaphase and telophase as the split in the homotypic chromosomes. The chromosomes of the heterotypic anaphase are therefore double, just as they are in an ordinary somatic anaphase, as shown by the work of Hedayetullah (1931) on *Narcissus*, Perry (1932) on *Galanthus*, Koshy (1933) on *Allium*, Hoare (1934) on *Scilla*, and others.

Thus we return by another route to a view resembling in many respects that expressed in the earliest paper on this subject (Gates, 1908), for we conclude that the first division separates chromosomes which have for the most part been connected

from the beginning by their ends only, while the second separates the halves of these chromosomes, which have been split from the leptotene stage onwards. In chromosome pairs the corresponding ends of two chromosomes have been connected with each other, while in the case of rings each chromosome of the ring is attached by each end to a different chromosome. The connections between chromosomes can sometimes be seen to be double, and this is to be expected since they represent portions of the split leptotene thread. There is normally no zygotene stage in *Oenothera*, although this stage is characteristic of many other plant genera. The reason for its absence in *Oenothera* is clear, namely, the repulsion which develops between the medial portions of the bivalent chromosomes. This repulsion is probably purely physical (electrical) in character. It develops, after pairing of the ends has already taken place, and is great enough to prevent the middle portions of the bivalent from pairing but not great enough to pull the ends apart. The ends are paired and remain paired, not because of chiasmata but because of the specific attraction between ends, which causes them to pair and which holds them together until they are separated in anaphase. When a spore mother cell in any *Oenothera* shows less than the maximum catenation, it is not therefore due to " failure of chiasma formation " but because of failure of two ends to pair, or subsequent breakage of the connections. Such failure to pair is to be expected, since it depends upon the chromosome ends coming within each other's sphere of influence so that their mutual attraction can take effect.

It need not be held that the chromosomes in a ring are attached only by their extreme tips. There is evidence that short terminal portions, probably varying in length in different cases, are paired side by side. The shorter these are, the more likely they are to be pulled apart in the jostling movements which clearly take place during prophase and metaphase. Chiasmata may also occur between these short paired ends ; but their numbers will be very few compared with the numbers which have been assumed to form and terminalize along the whole length of the chromosomes.

It may be pointed out here that the high frequency of non-disjunction in all the catenated *Oenotheras*, leading to a high frequency of trisomic mutations, appears to be a direct result of the fact that the bivalents are not paired throughout their length but that each catenated chromosome is attached at its ends to two others.

The nature of catenation

Linkage of chromosomes in diakinesis and first metaphase is now known to occur not only in *Oenothera* and *Datura* but in a number of other genera. It can be induced in *Datura* by crossing " B " races with a standard line (Belling and Blakeslee, 1926). In *Oenothera*, the crossing of two uncatenated forms can give rise to an F_1 with a ring of 4 or a ring of 6 (Gates and Catcheside, 1931). In *Pisum*, the crossing of certain races which have long been separated will also give rise to chromosome rings (Pellew and Sansome, 1931, E. R. Sansome, 1933). The rings of 4 and 6 discovered in maize (McClintock, 1930, Brink and Cooper, 1932)

are also being genetically investigated, but unlike the *Oenothera* rings they are only maintained in the stock by controlled breeding. Semi-sterile forms with rings of 4 have been produced from the homozygous *Oenothera blandina* by X-raying the pollen (CATCHESIDE, unpublished). Thus catenation appears (*a*) when certain forms are crossed; (*b*) after X-ray treatment. It is pretty evident that in the latter case segmental interchange of chromosomes has been induced and in the former it has previously occurred in one or both of the parents.

A comparison of the rings in *Oenothera* and maize brings out a fundamental difference in addition to that already mentioned. In maize the rings of 4 and 6 are apparently oriented on the heterotypic spindle according to chance, so that if the consecutive chromosomes are called A, B, C, D, they are equally likely to segregate AB, CD, or AD, BC. In *Oenothera*, on the other hand, the orientation is normally always the same, in a zigzag arrangement giving the separation AC, BD. It is known from various observations (*e.g.*, SHEFFIELD, 1929) that this orientation is at any rate sometimes taken up in the prophase nucleus before the spindle as such is visible, and it seems clear that this behaviour of the spindle fibre attachments is the fundamental cause of the difference in orientation between the rings of *Oenothera* and *Zea*. They take up a fixed orientation in one but not in the other. BRINK and COOPER (1932) have recognized that segmental interchange in maize has not led to the establishment of fixed complexes like those of *Oenothera*, and they point out that the hypothesis of segmental interchange as applied to *Oenothera* by DARLINGTON (1931) is inadequate to account for the persistence of the complexes and the failure of crossing-over to take place in the middle portions of the chromosomes. They attempt to account for this condition by the hypothesis that the medial portions of the chromosomes are homologous but with reversed orientation. It has already been pointed out, however, that in *Oenothera* meiotic chromosomes which are known to be completely homologous throughout their length still pair only by their ends. Some other explanation of this condition is therefore required. All are agreed that the complex-differences in *Oenothera* are determined by the middle portions of the chromosomes. That being so, the fact that the complexes and the phenotype normally remain constant from generation to generation shows that crossing-over does not normally occur in this region of the chromosomes. Obviously, therefore, true chiasmata do not occur in this region of the chromosomes, except as occasional occurrences leading to a change of catenation and a mutation.

From the considerable literature on catenation in *Oenothera* and its important genetical results, only two papers need be cited here, CLELAND and BLAKESLEE (1931) and GATES and CATCHESIDE (1932). In these papers the chromosome configurations in quite a large number of species and their hybrids have been considered. The results are all consonant with a theory of segmental interchange, and it appears probable (GATES, 1933) that in nature both hybridization and segmental interchange have been concerned in producing the condition in *Oenothera* in which most of the wild species show complete catenation. But this was only made possible by a condition of the spindle which allowed of fixed orientation. The fifteen

possible configurations have now all been observed in different species, mutants and hybrids.

DARLINGTON (1931) has combined his theory of parasynapsis in *Oenothera* with BELLING's (1927) theory of segmental interchange, but as we have seen, parasynapsis in the current sense is not the general method of meiosis in *Oenothera*, and CLELAND and BLAKESLEE (1931) point out that the two are not necessarily associated. Nevertheless an explanation of how segmental interchange takes place in *Oenothera* must be found, and we adopt the view, based partly on unpublished observations in this laboratory, that it occurs through chiasma formation between chromosomes belonging in different complexes, following a figure-of-eight arrangement as DARLINGTON and others have suggested. It has long been evident that *O. rubrinervis*, with 4 free pairs of chromosomes and a ring of 6, has been derived from *O. Lamarckiana* having one free pair and a ring of 12 by a rearrangement of chromosome portions in which three new free pairs of homologous chromosomes are formed by segmental interchanges, involving certain chromosomes in the *Lamarckiana* ring.

Without entering into further details here, we thus reach the view that while the normal method of pairing in *Oenothera* is acrosyndesis, but with the extreme ends laterally paired in some cases, yet as a mutational event leading to a change of catenation an arrangement like a figure-of-eight may take place between the threads of chromosomes belonging to different complexes. This would no doubt occur when two non-homologous ends are thrown near each other in the leptotene stage or earlier. As the specific attractions between various parts are probably relative and not absolute, and as they may be expected to vary in intensity with the inverse square of the distance, it is not surprising that portions which exceptionally come to lie very near each other may become attached even although non-homologous, and may afterwards be pulled apart by the attached chromosomes, an interchange resulting owing to their attached ends. On the other hand, it is necessary to conclude that apart from mutations, crossing-over does not take place in the middle portions of the chromosomes either in homozygous forms with free pairs or in forms with complexes and chromosome rings. That being the case, true chiasmata are not to be expected in the middle portion of the chromosomes, except as rare occurrences leading to a mutation.

The genetical results with *Oenothera* indicate that while the complexes of each form generally maintain themselves without change, yet crossing-over occurs at times both in single pairs and in the catenated chromosomes. Hence chiasmata may be expected to occur in the end segments of the chromosomes in both cases, but not in the middle portions. The sequence of cytological events by which a mutation with a change of catenation arises, as in the origin of *rubrinervis* from *Lamarckiana* or *deserens* from *rubrinervis*, will, no doubt, be more fully elucidated by further cytological investigations.

The expense of growing the plants described in this paper was defrayed in part by a grant from the Royal Society.

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Summary

Wild seeds of an *Oenothera* from near Wood's Hole, Massachusetts, produced a dwarf mutation whose offspring (42 plants) from open-pollination and crossing with the tall were practically all trisomic, belonging to four—perhaps five—distinct types. This wild species has a ring of 14 chromosomes and shows some resemblances to *O. Lamarckiana* but has small flowers. The trisomics were all more or less pollen-sterile. The absence of diploids may have been due to the delayed germination of diploid seeds.

From a detailed cytological investigation, especially of the trisomic type known as *semilata*, combined with recent studies of *Oenothera* without catenation, various conclusions regarding *Oenothera* cytology are reached.

Two nucleolar bodies were frequently found, each attached to the thread in the early leptotene stage of meiosis. They were probably concerned in producing the nucleolus in the previous telophase.

The leptonema is split, like the threads in a somatic prophase, with the halves twisted about each other.

Shortening and thickening of these threads leads to the pachytene stage, in which the 15 chromosomes are still arranged end-to-end, usually in seven loops with the extra attached by one end.

The maximum catenation is a chain of 15 chromosomes, the extra being attached nearly always by one end only, but the chain is frequently broken in diakinesis.

There is no strictly zygotene stage in *Oenothera*, and such chiasma formation as takes place is normally confined to the ends of the chromosomes, *i.e.*, acrosyndesis. In forms with ring pairs, the medial portions of the chromosomes show repulsion or asynapsis. Terminalization of chiasmata, when it occurs, is confined to the terminal portions of the chromosomes. This conclusion is strongly supported by the genetical evidence, which indicates that the differences between the two complexes of any species reside in the medial portions of the catenated chromosomes. If crossing-over occurred in these portions the species would not breed true, as they normally do.

In forms with catenated chromosomes the corresponding split leptotene threads are arranged end-to-end from the earliest stages of meiosis. In forms with free pairs they are separate from the earliest stages. Less than the maximum catenation in diakinesis is not due to terminalization of chiasmata but to failure of corresponding ends to come close enough together to pair, or to breakage of the delicate connections. Chiasmata are not necessary to produce pairing.

Evidence is produced to show that the chromosomes pair only at or near their ends, even when they are very closely homologous throughout their length.

The chromosome rings in *Oenothera* persist, unlike those of *Zea*, because they have a fixed orientation on the spindle. The behaviour of the spindle fibre attachments which determine the zigzag arrangement of the chromosomes in the ring is fundamental to the maintenance of the condition of catenation.

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The occasional chiasmata with crossing-over which occur between non-homologous chromosomes in a ring result in a change of catenation and lead to a mutation.

Several pseudohomotypic mitoses were observed, in which the connections between the 15 chromosomes had broken down, they were all oriented in one plane and would probably all split to form a dyad of diploid pollen grains. In such cases there must be a complete change in the behaviour of the spindle fibre attachments.

By exceptional destaining it was demonstrated that the chromosomes in heterotypic anaphase are frequently constricted and twisted on their axis, and that they show indications of spiral structure at this time.

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Explanation of Plates

All figures were sketched at table level with the aid of a camera lucida. A 2 mm Zeiss Apochromatic, 1·3 aperture, and Zeiss oc. × 15 were employed for all drawings. Magnification × 2800. They are from pollen mother cells of *Oenothera paralamarckiana* mut. *semilata*, 124.34. II. 3.

Fig. 38, from pollen mother cell of plant I. 3. Figs. 55–60, from pollen mother cells of plant II. 4. In figs. 31–38, 40A, and 55–59, the various chromosome groups have been separated to make the drawing clear.

Plate 22

Fig. 9—Resting nucleus showing deeply staining granules attached to the reticulum, and the vacuolate nucleolus.

Figs. 10–11—Nucleus in early prophase, showing densely staining chromatin granules as thickenings of the threads. Two deep-staining bodies are attached to the nucleolus and connected with chromatic threads.

Fig. 12—Leptotene stage, showing the beaded appearance of the threads. A small protein crystalloid is present in the nucleolus.

Figs. 13–18—Successive stages of synizesis. A nucleolar body is present in figs. 13–15, 18, 19, and a chromatin thread is seen passing through this body.

Figs. 19–20—Showing the split character of the leptotene threads, which have untwisted and become parallel.

Fig. 21—Pachytene stage, showing thickened thread arranged in 7 loops with uneven margin. The extra chromosome is represented by X.

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Fig. 23—Spreading of the pachynema ; double connections between the chromosomes are evident.

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Fig. 25—Typical diakinesis nucleus, uncompacted chromosomes with uneven margins. Fifteen chromosomes in a chain, showing double connections in some. One chromosome is attached by a thread to the nucleolus.

Fig. 26—Nucleolus and nuclear membrane have disappeared. The chromosomes are in a ring or chain of 15 and are becoming V-shaped.

Fig. 27—Prometaphase stage with compact chromosomes.

Figs. 28–29—Exceptional first metaphase nuclei in which the connections between the chromosomes have disappeared and all the chromosomes are arranged in one plane.

Fig. 30—A metaphase in which the chromosomes are in two planes but the connections have broken down.

Fig. 31—Prometaphase, showing a trivalent, a chain of five chromosomes, and a chain of 7.

Fig. 32—Prometaphase, a chain of 5 chromosomes, a chain of 9 and a single chromosome.

Fig. 33—Metaphase, showing a chain of 9, and a chain of 6 chromosomes.

Figs. 34–40 are from the heterotypic metaphase.

Fig. 34—A chain of 10 chromosomes (the extra being attached between the 7th and 8th chromosomes from one end) and a chain of 4.

Fig. 35—A chain of 9 chromosomes and 3 rod pairs.

Fig. 36—A chain of 4, a chain of 5, a ring pair and 2 rod pairs.

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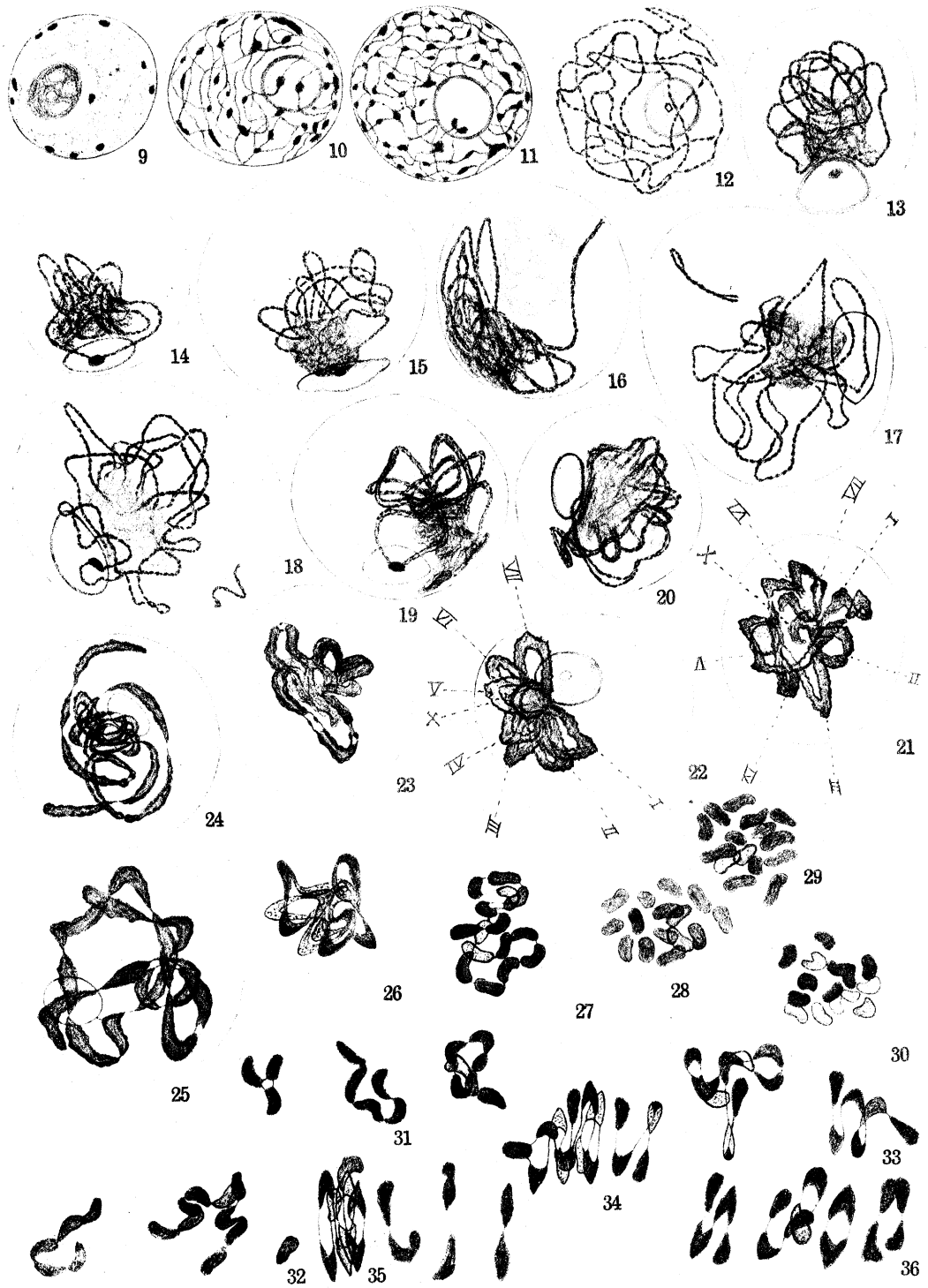

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Plate 23

Fig. 37—Three rod pairs, two chains of 4, and the extra chromosome unattached.
Fig. 38—A chain of 4 and a chain of 11.
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Trisomic II. 4

Fig. 58—Three chains of 4, one rod pair and a single chromosome. One chromosome in the chain on the left shows a constriction. The peripheral dot-like appearance of all the (destained) chromosomes probably represents a spiral structure.
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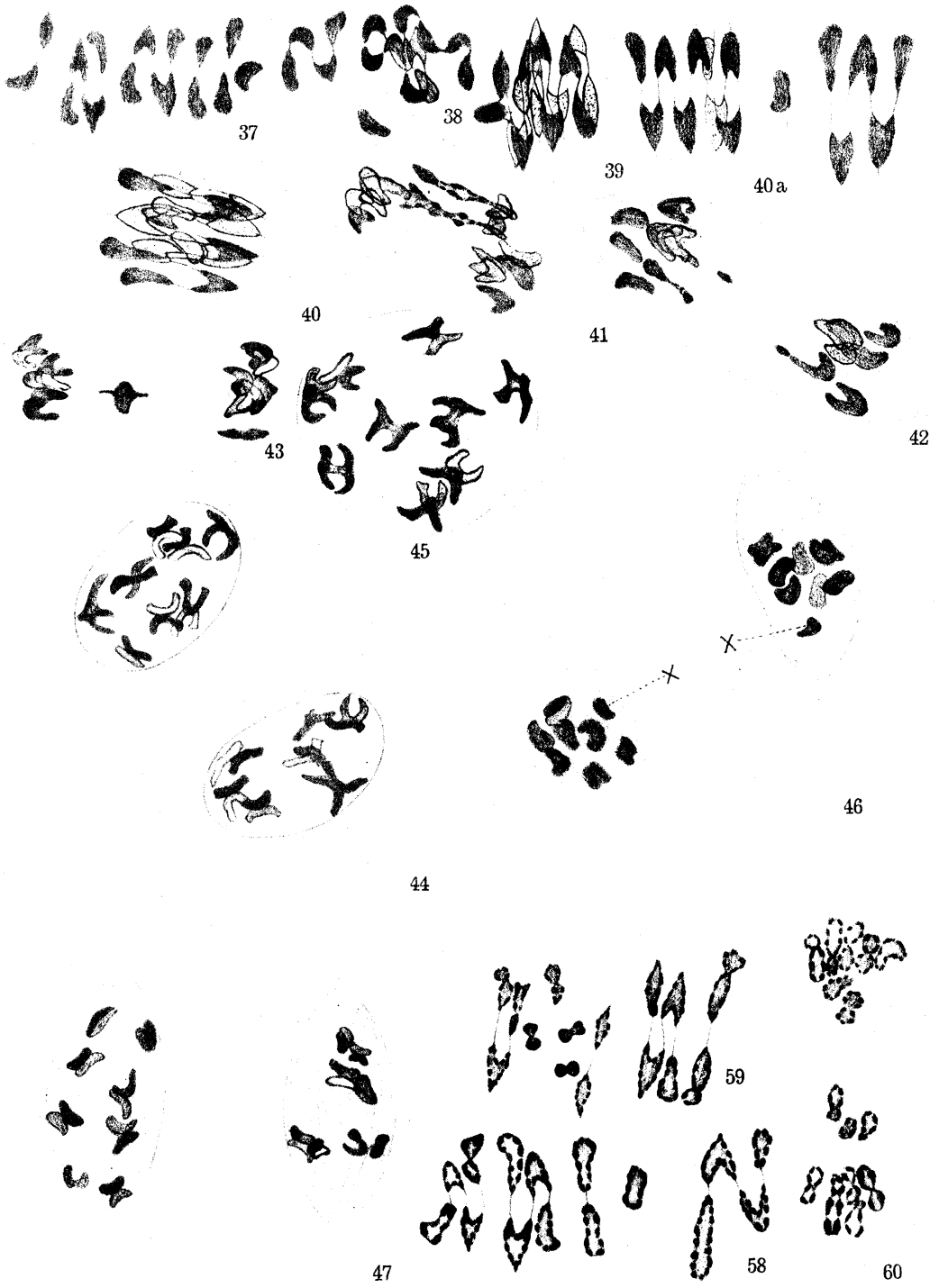

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Plate 24

Figs. 48–49—Two sections of the same pollen mother cell, the left-hand spindle with 9 chromosomes in early anaphase and the right-hand spindle with 6 in metaphase.

Fig. 50—Same stage but with 7 and 8 chromosomes.

Fig. 51—Homotypic mid-anaphase with 8 divided chromosomes on one spindle and 7 on the other.

Fig. 52—Homotypic late anaphase with two groups of 7 and two groups of 8 chromosomes.

Fig. 53—Four telpohase nuclei, each with 7 chromosomes. The two halves of the extra, which show a constriction, are left on the spindles.

Fig. 54—Somatic metaphase with 15 chromosomes.

Trisomic II. 4

Fig. 55—A rod pair, a ring pair, a chain of 7 and a chain of 4 in heterotypic metaphase.

Fig. 56—Two chains of 4, one rod pair and a chain of 4, the extra being attached by one end to the end of the second chromosome.

Fig. 57—Three rod pairs, two chains of 4, and a single chromosome.

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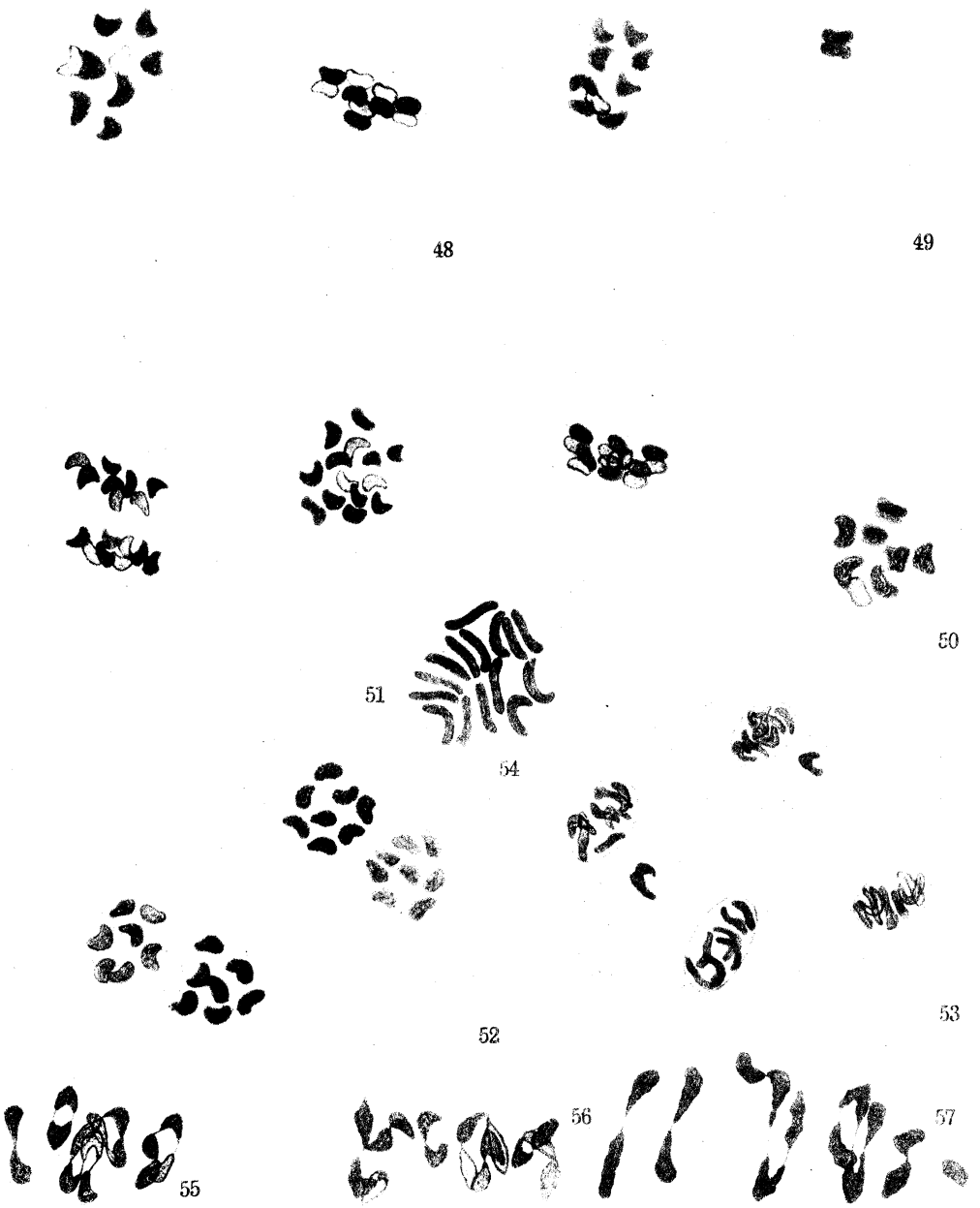

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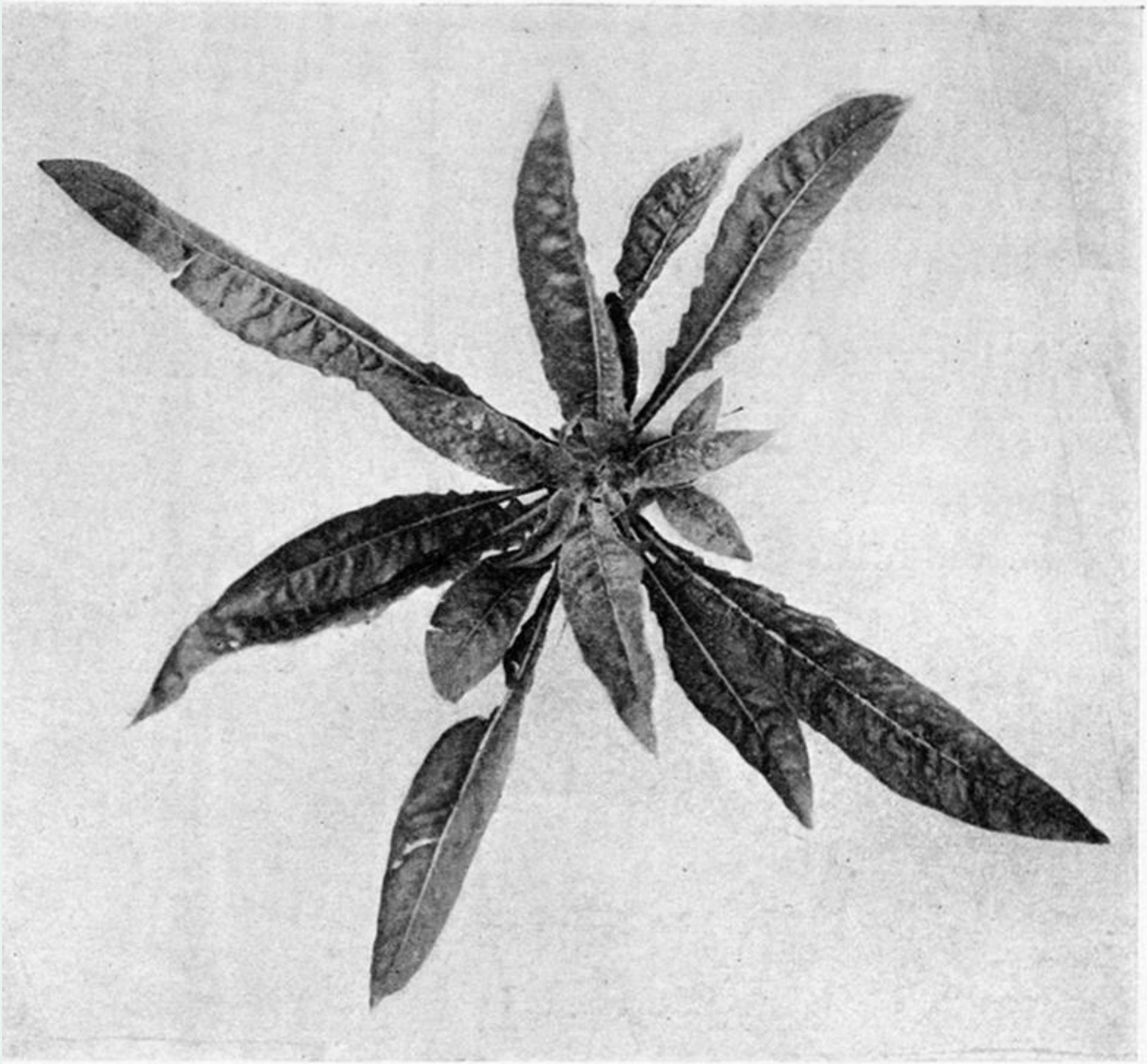

FIG. 1—*O. paralamarckiana* rosette 1933

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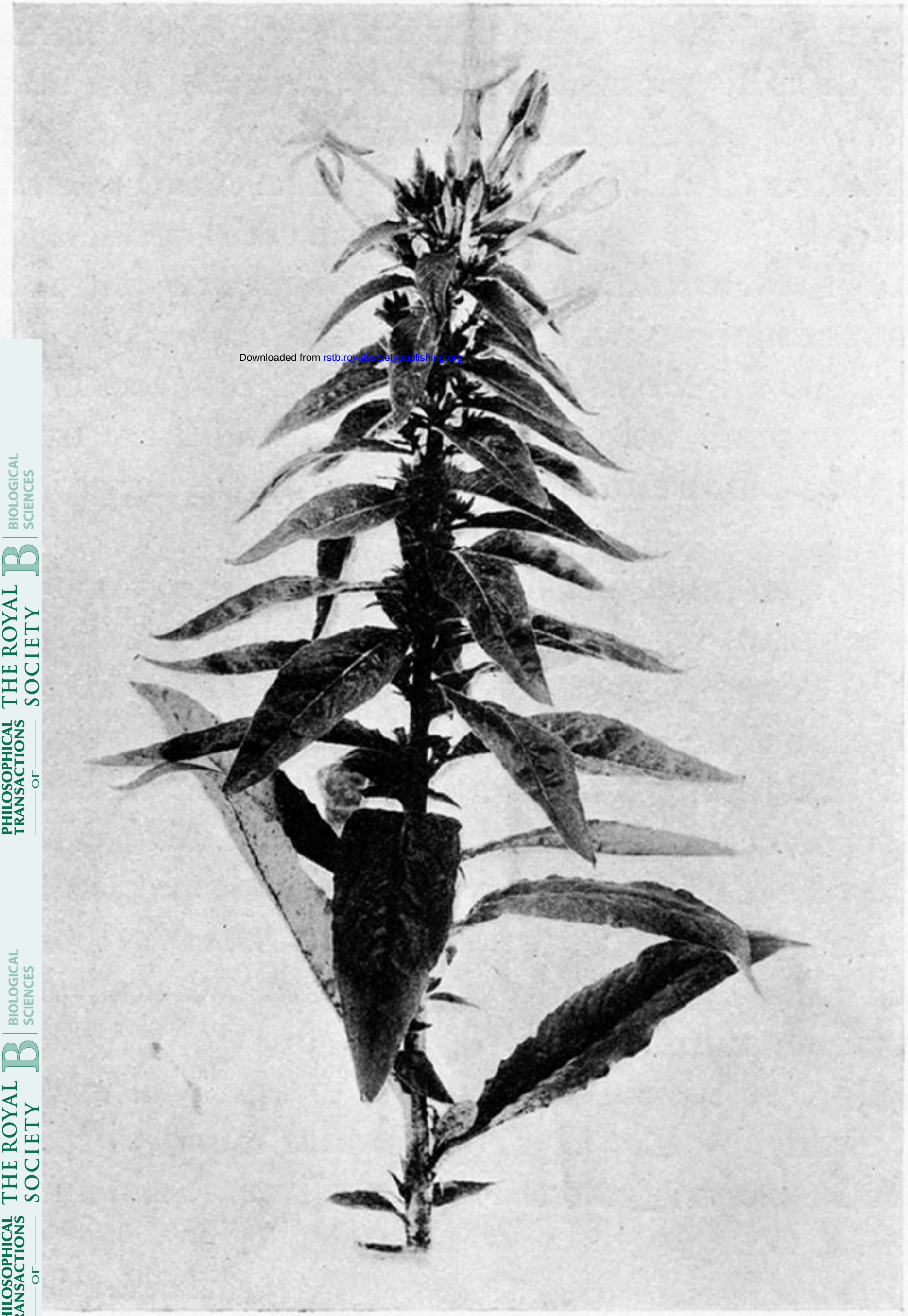

FIG. 2—*O. paralamarckiana* in flower 1933

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g. 3—Dwarf mutant in flower 1933

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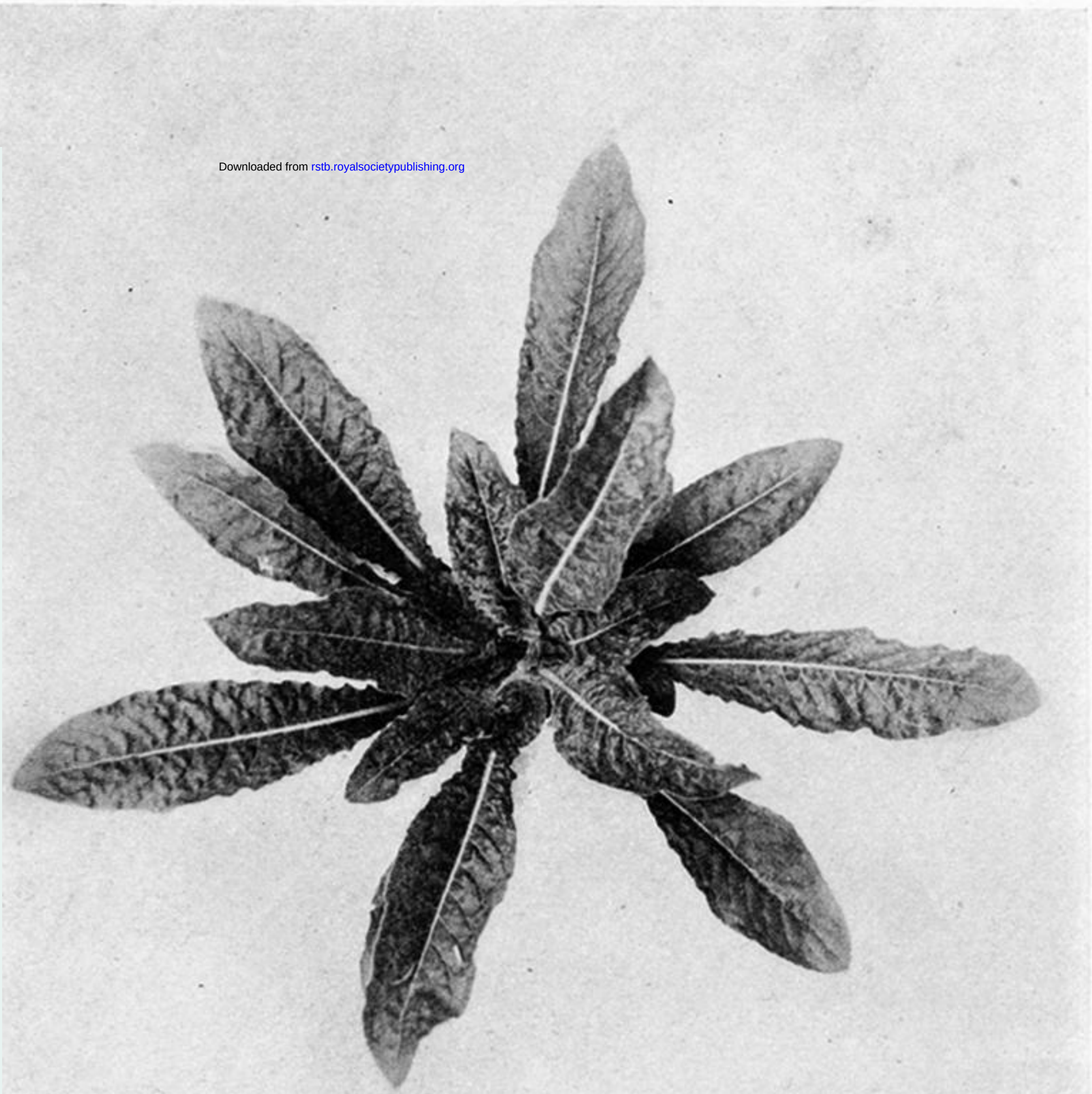

FIG. 4—*O. paralamarckiana* mut. *semilata* rosette 1934

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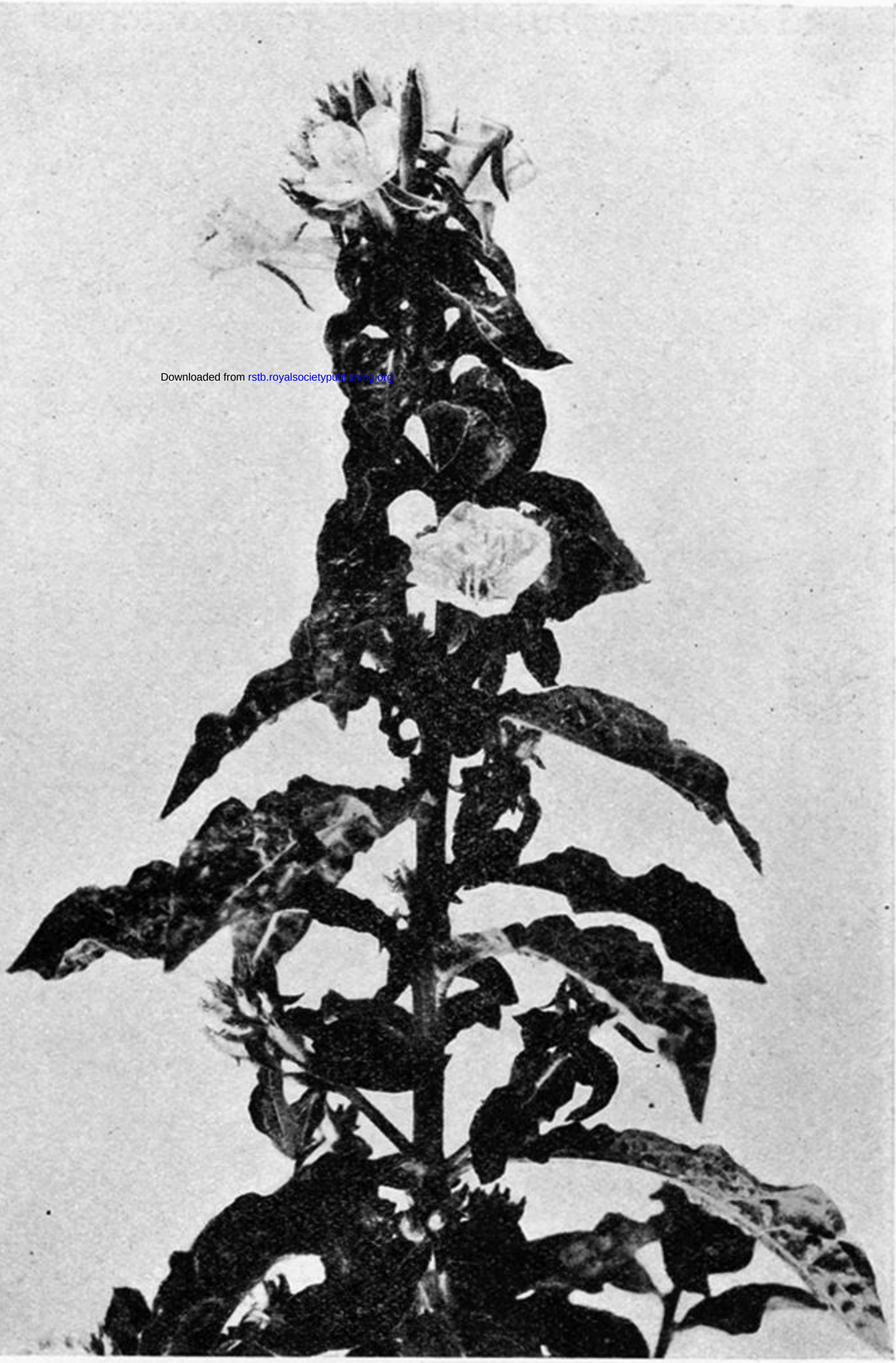

FIG. 5—*O. paralamarckiana* mut. *semilata* in flower 1934

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FIG. 6—Large-leaved trisomic, No. 124.34, II. 4

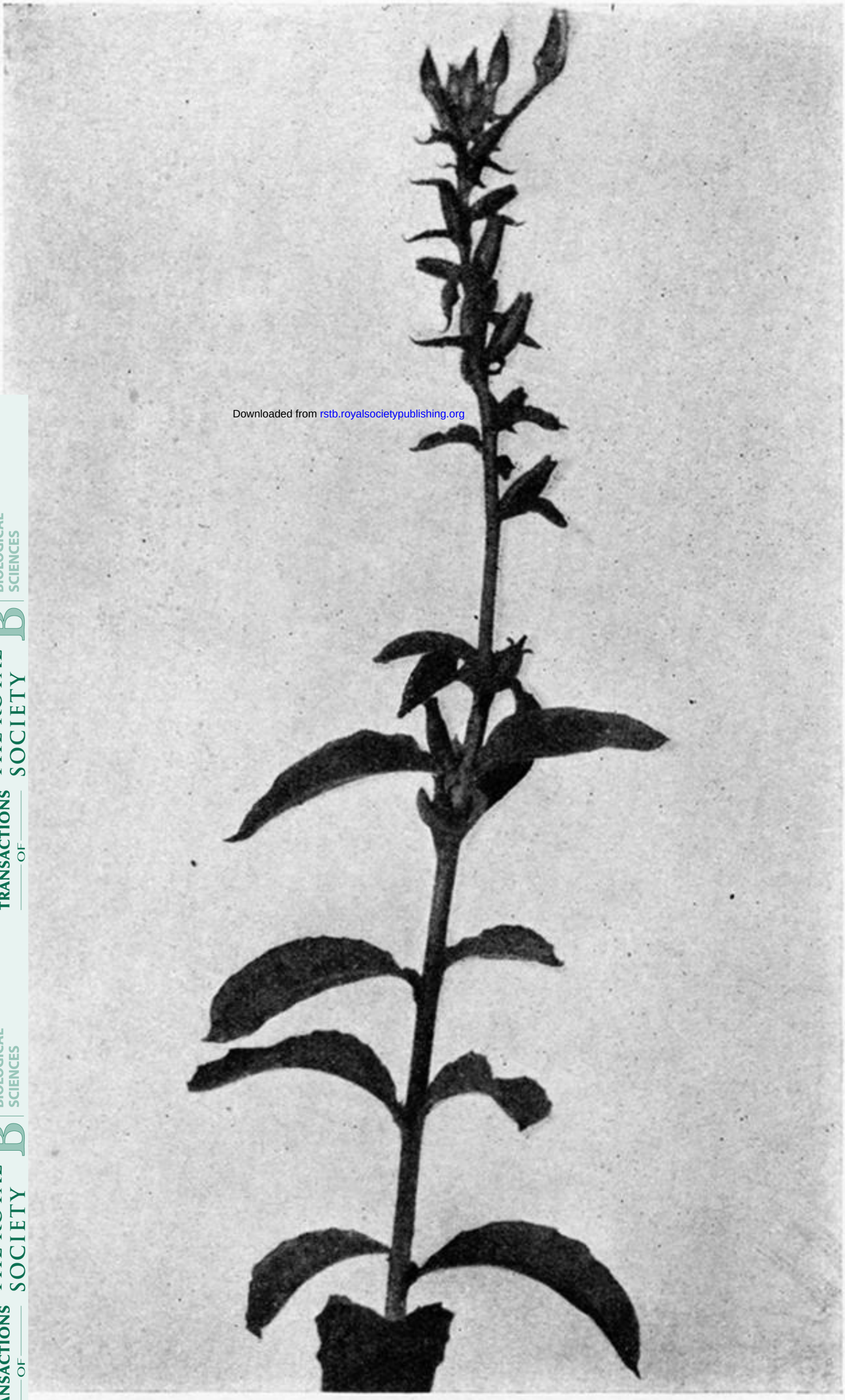

FIG. 7—Smooth-leaved trisomic, 124.34, I. 3

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FIG. 8—*O. paralamarckiana* mut. *hirsuta*

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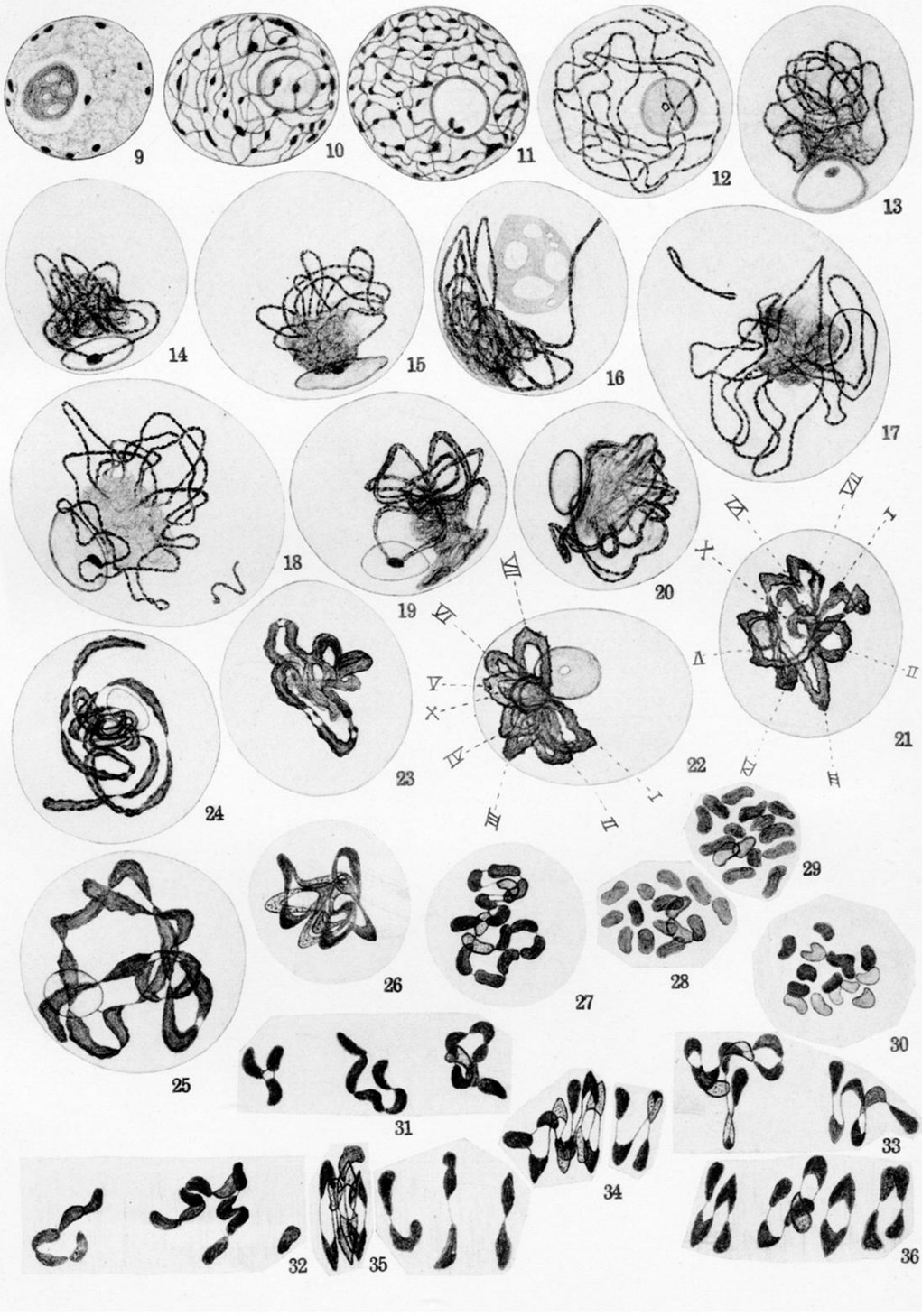

PLATE 22

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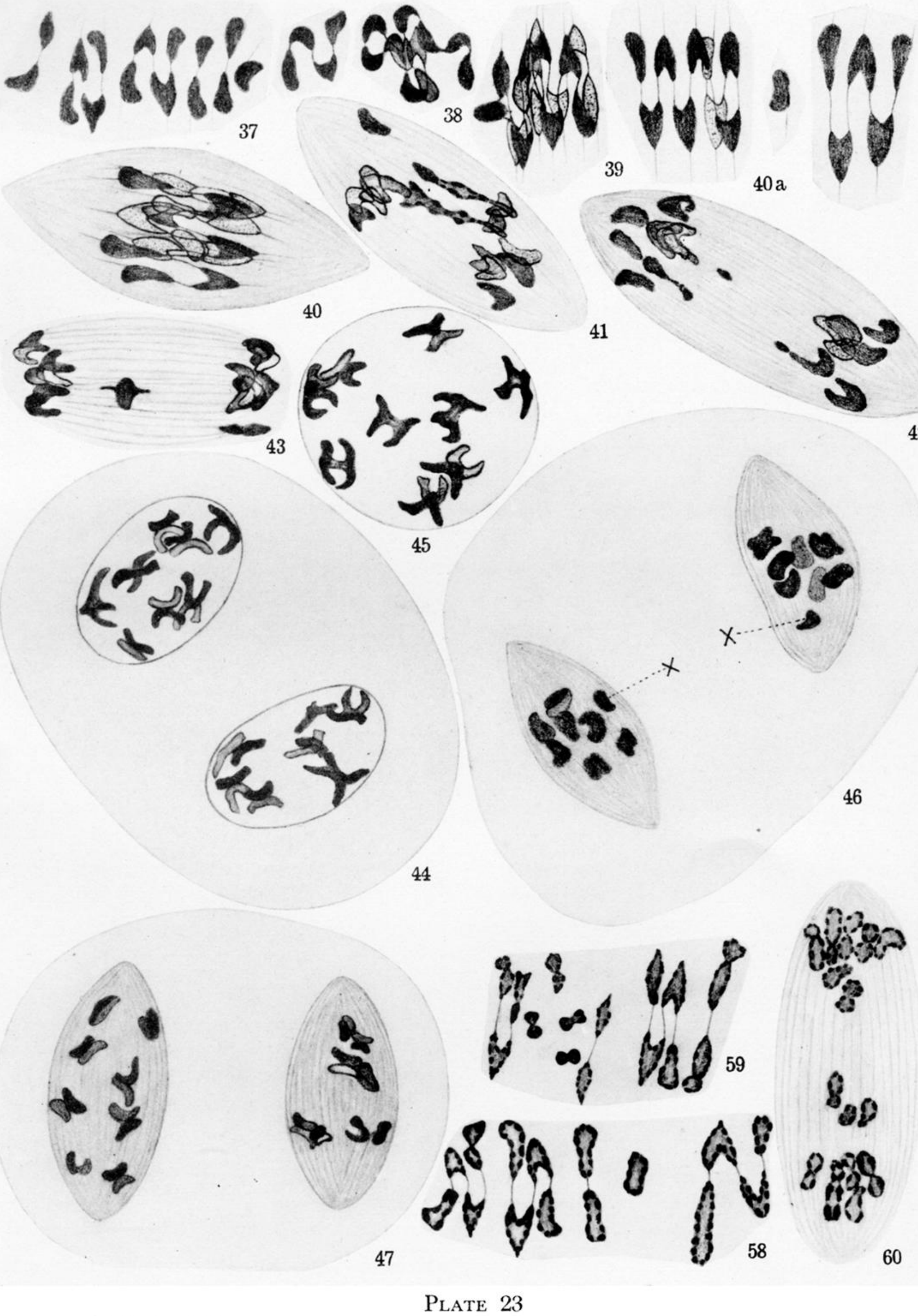

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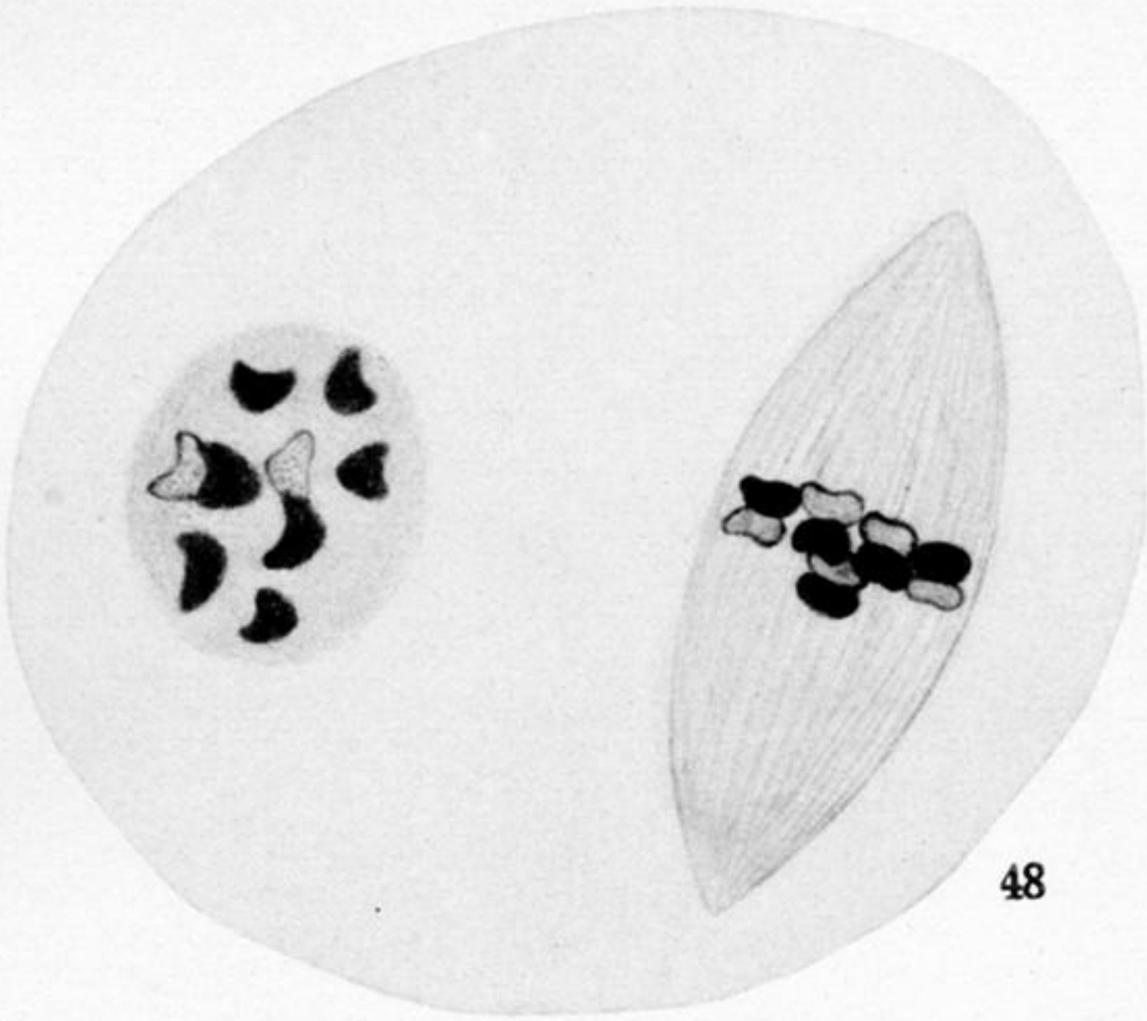

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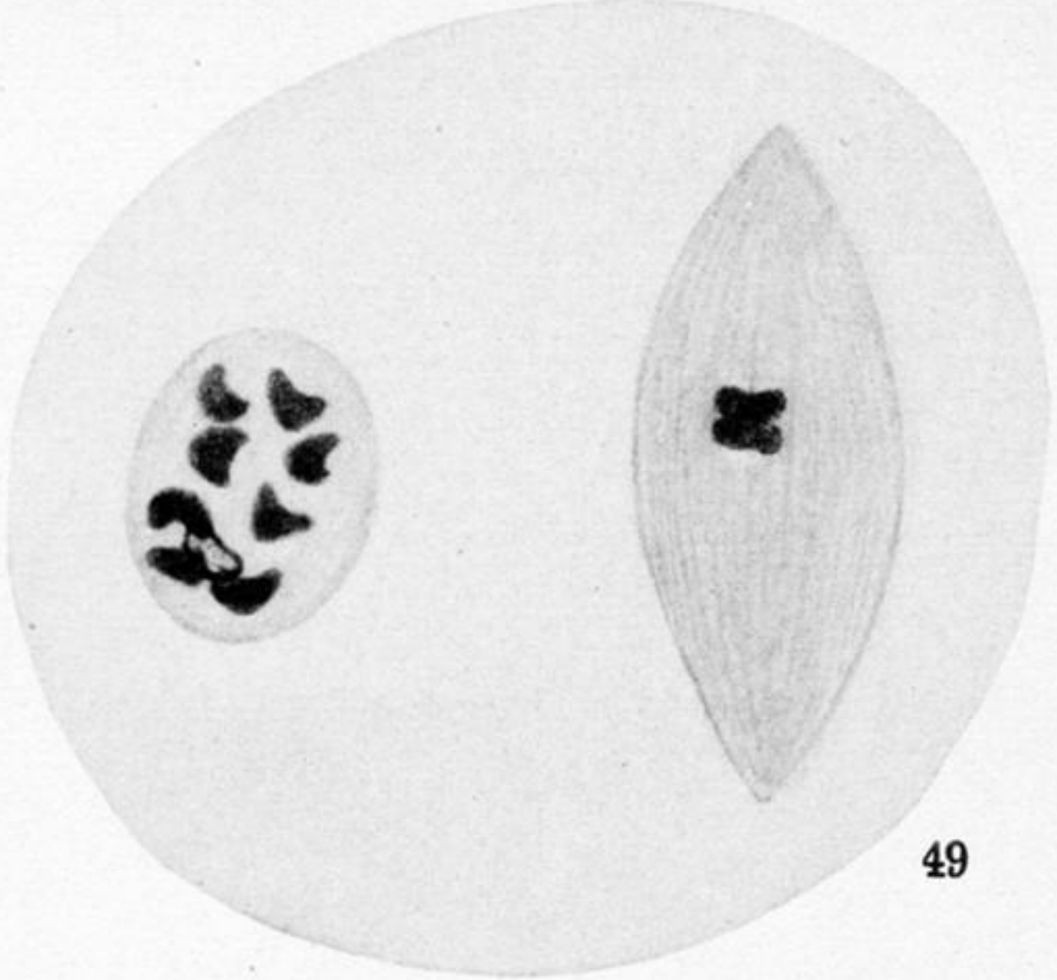

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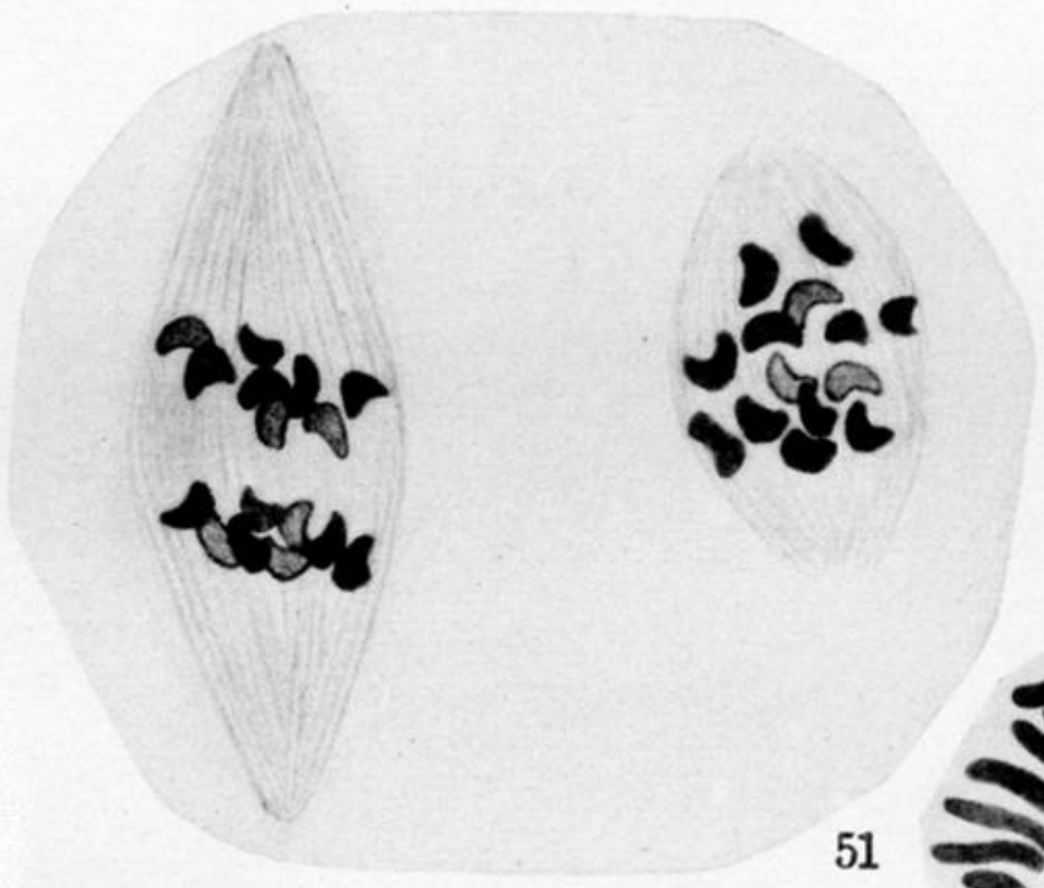

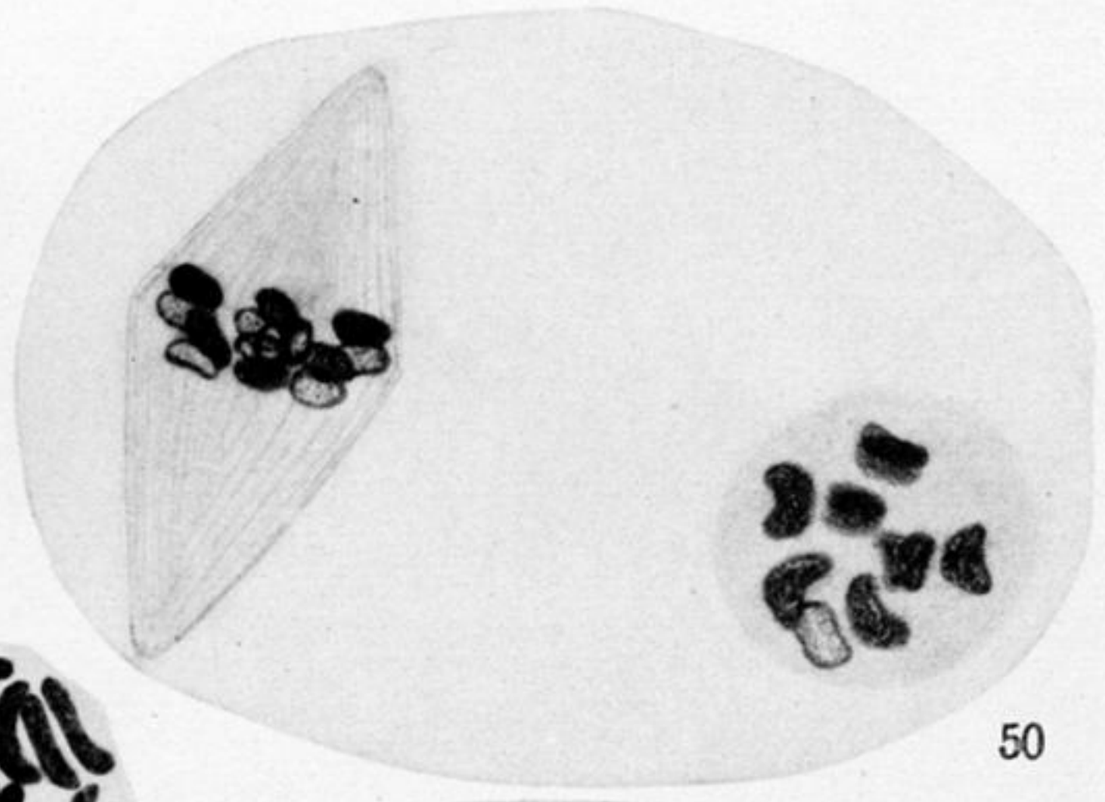

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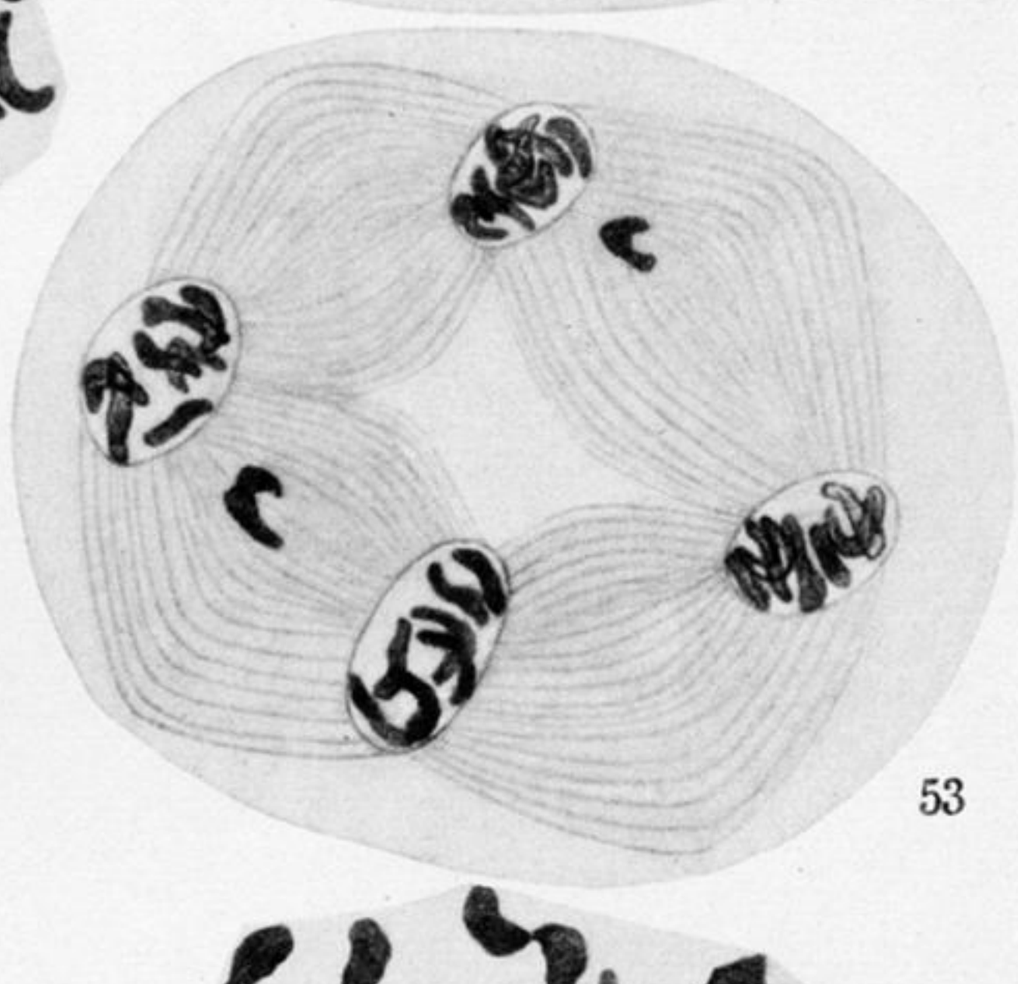

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FIG. 51—Homotypic mid-anaphase with 8 divided chromosomes on one spindle and 7 on the other.

FIG. 52—Homotypic late anaphase with two groups of 7 and two groups of 8 chromosomes.

FIG. 53—Four telpohase nuclei, each with 7 chromosomes. The two halves of the extra, which show a constriction, are left on the spindles.

FIG. 54—Somatic metaphase with 15 chromosomes.

TRISOMIC II. 4

FIG. 55—A rod pair, a ring pair, a chain of 7 and a chain of 4 in heterotypic metaphase.

FIG. 56—Two chains of 4, one rod pair and a chain of 4, the extra being attached by one end to the end of the second chromosome.

FIG. 57—Three rod pairs, two chains of 4, and a single chromosome.