THE ORIGIN OF ISO-CHROMOSOMES

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(With Plates V and VI and Fifteen Text-figures)

Most chromosomes have an intercalary centromere. They consist, as we say, of two arms which are joined by the centromere, or more properly a gene-string which passes through it. Regular division of the chromosome depends on a lengthwise splitting of the gene-string followed by a lengthwise splitting of the centromere itself, and the explosive separation of the two halves.

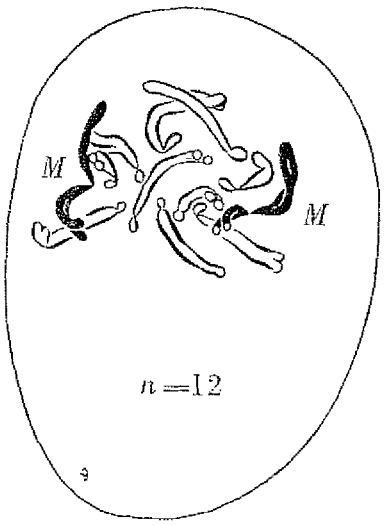
Now just as the gene-string can be broken crosswise by short wave irradiation, so the centromere itself can be broken to give a new fragmented centromere which is terminal (Rhoades, 1938, in *Zea Mays*). The new telocentric chromosome produced in this way is subefficient, that is to say it reproduces and divides with less regularity than its complete companions. It can apparently restore the intercalary position of its centromere by secondary change, the two sister chromatids becoming concurrent arms. The broken ends within the centromere can join up like the broken ends of other parts of the chromosomes. The new chromosome with two identical arms may then be described as an iso-chromosome.

Telocentric chromosomes may also arise under natural conditions, and the circumstances of their origin have been made clear in a triploid *Tulipa* (Upcott, 1937) and a diploid *Fritillaria* (Darlington, 1939*a*). In the two plants the condition of the crosswise splitting or misdivision of the centromere is the same, namely, the presence on the spindle of unco-oriented chromosomes at the first anaphase of meiosis, or of unsplit chromosomes at the second anaphase. In the triploid tulip this is a consequence of indifferent co-orientation of trivalents and non-co-orientation of univalents. In the diploid fritillary it is a consequence of non-co-orientation of bivalents as well as of univalents. In both cases, however, there must be a specific and exceptional capacity for misdivision, or incapacity for correct division, inherent either in the centromeres or in the organization of meiosis.

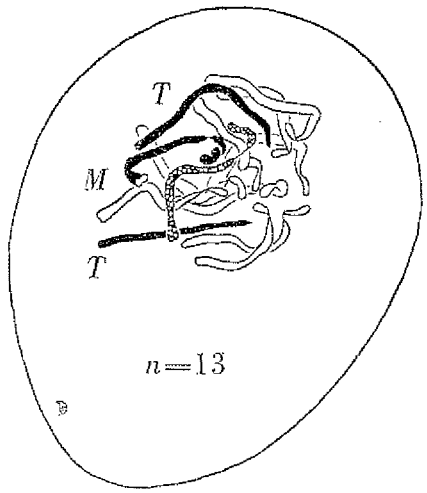
In my previous account I have discussed both the structural and genetic implications of this behaviour. It now remains to discover its genetic consequences by cytological study. The misdivided chromosomes,

so far as they are stable, will give the functional telocentrics that I have already described at second anaphase. It is necessary to see what happens at the next mitosis in the pollen grain.

In the diploid *Fritillaria karadaghensis* (as it is now identified) I found misdivision in about 5 % of the pollen mother cells. This process could give complete and balanced haploid pollen by a complementary orientation of two homologous misdividing chromosomes. Such an orientation I illustrated in Figs. 4 *a*, *b*, and in an imperfect form in Figs. 5 *b*, *e* and *d* of my paper. This special requirement must however reduce the frequency of the expected telocentrics in the pollen grains.



Text-fig. 1.



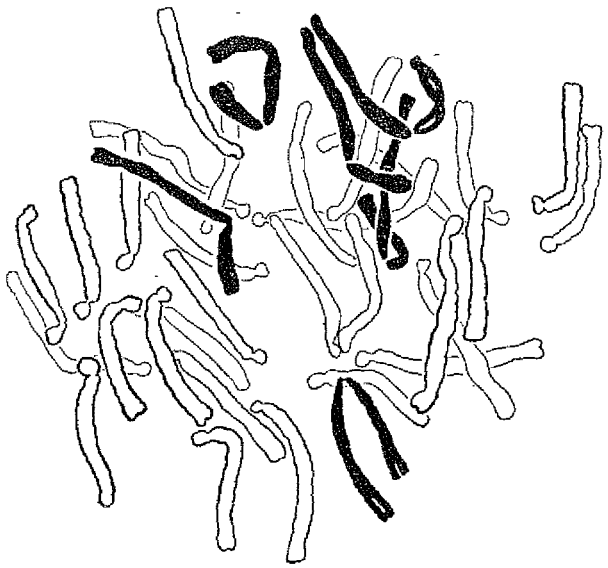
Text-fig. 2.

Text-figs. 1, 2. Pollen grains of *Fritillaria karadaghensis* fixed in La Cour's 2 BE and stained with gentian violet. Text-fig. 1, normal complement: Text-fig. 2, abnormal with one *M* replaced by two telocentrics. The stippled chromosomes show a broad centric constriction. × 1100.

Examining thirty-five pollen grain mitoses in metaphase and anaphase I found that thirty-three had normal complements (Text-fig. 1) and two abnormal. One of these had an extra chromosome, probably the telocentric arm of an *S*-chromosome. One had two long telocentrics, apparently replacing one of the *M*-chromosomes (Text-fig. 2). This could have arisen from the complementary misdivision of two homologues already suggested. It is interesting as showing how a "fragmentation" of an *M*-chromosome such as is often to be inferred in experiment and from natural comparison could have taken place. In *Fritillaria pudica* for example, whose 13 chromosomes suggest this process, several *S*'s

are difficult to distinguish from telocentrics (Darlington, 1936). The same kind of change has been assumed in the Orthoptera to account for the differences between the *Stenobothrus* and *Mecostethus* types of complement as well as between those of races and species in *Hesperotettix*.

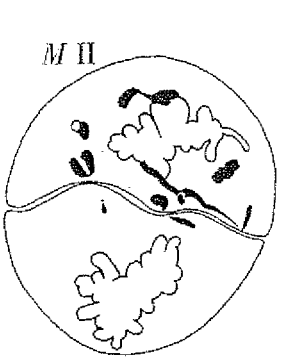
These observations show nothing of the origin of iso-chromosomes. Better evidence may be obtained, however, in two ways. First, a more satisfactory technique than the Flemming-gentian violet smears used for *Fritillaria karadaghensis* is the maceration and removal of pollen grain walls followed by Feulgen staining (cf. legend to Pl. VI). Secondly,



Text-fig. 3. *F. latifolia major*, $3x=36$. Copy of microphotograph in Plate V showing that the six *M*-chromosomes are none of them equal-armed, and that the 30 *S*-chromosomes all have a considerable short arm. ×1200.

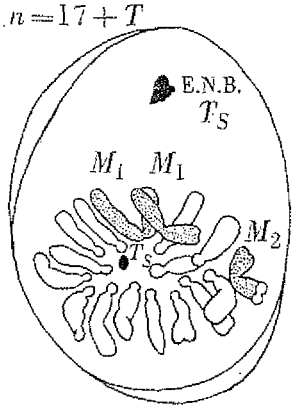
misdivision is to be expected at either first or second division in the obligatory univalents of triploid forms. And in the pollen grains there should be no differential elimination of those carrying the products of misdivision, since all pollen grains but one in 2048 (where $x=12$) should be unbalanced from normal binomial segregation of the extra set.

Satisfactory material is provided by *F. latifolia major* ($3x=36$). The processes of misdivision at meiosis are less clear than in the diploid owing to the crowding of the cell and the high number of ordinary laggards. Its results can, however, be seen at first telophase and second metaphase (Text-fig. 4). No inversion bridges were found in this form by Frankel (1934) and I have confirmed his observation on the new material.



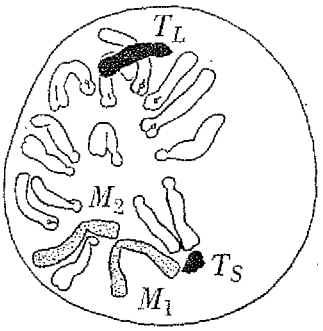
Text-fig. 4.

$n=17+T$



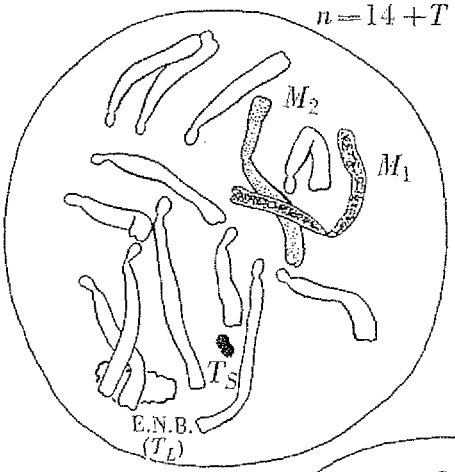
Text-fig. 5.

$n=17+2T$



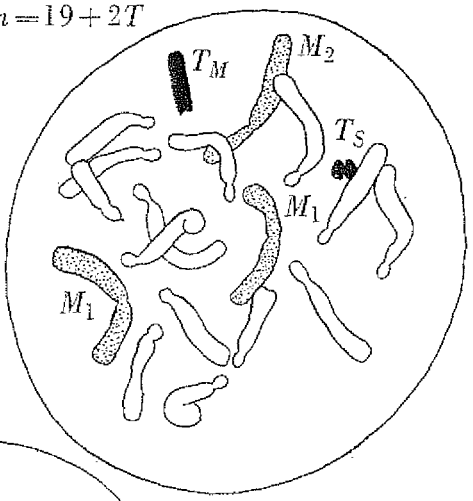
Text-fig. 6.

$n=14+T$

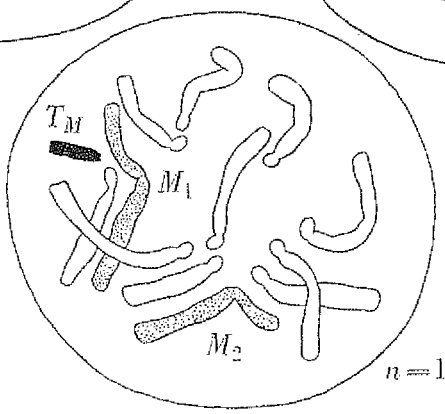


Text-fig. 7.

$n=19+2T$

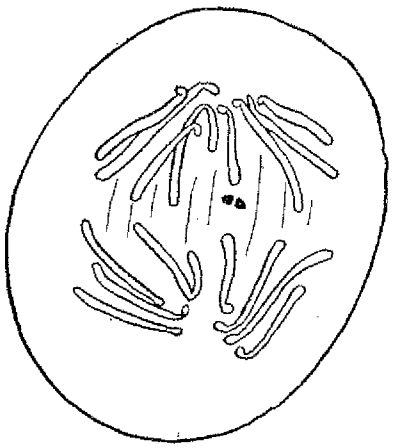


Text-fig. 8.

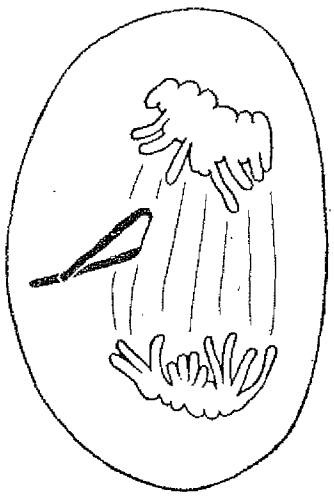


$n=12+T$

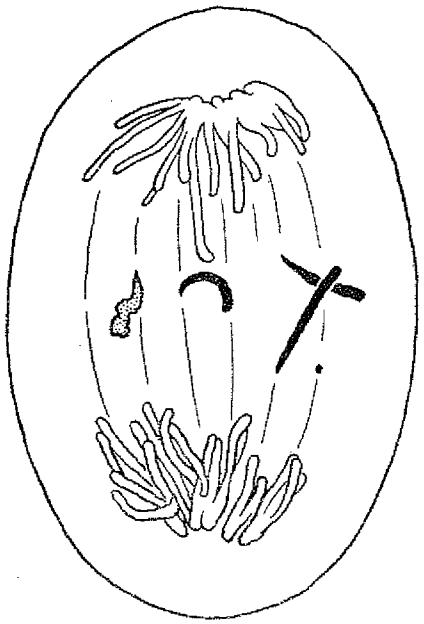
Text-fig. 9.



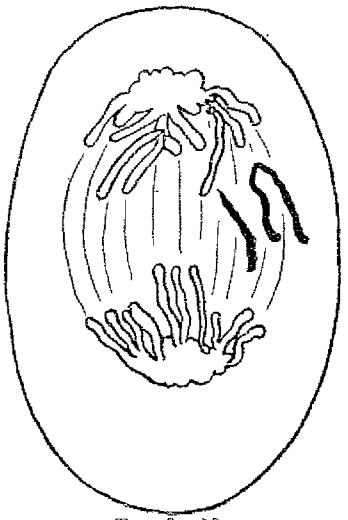
Text-fig. 10.



Text-fig. 11.



Text-fig. 12.



Text-fig. 13.

Text-figs. 4–13. *F. latifolia major*. Text-fig. 4, pollen mother cell at second metaphase showing the consequences of misdivision and other irregularities. Text-figs. 5–9. First pollen grain mitoses at metaphase extracted by maceration from pollen grain wall. *M*-chromosomes are stippled and new telocentrics black (T_S, T_M and T_L). *E.N.B.* extranuclear body, either telocentric or laggard chromosome. Note the non-orientation of most telocentrics and the variable spiralization in the different pollen grains characteristic of triploids (cf. Upcott & Philp, 1939). Text-figs. 10–13. Anaphase lagging of short and long telocentrics in the pollen grain. In Text-fig. 12 one has been outside the nucleus. In Text-figs. 12 and 13 one has divided and one chromatid has reached a pole successfully, the other is late in Text-fig. 12; it will not divide at all but will become an iso-chromosome in Text-fig. 13. × 900.

The chromosomes were counted in 111 pollen grains in seven smears. They show the usual slight differences in distribution which are to be attributed to the different rates of development of differently balanced individuals.

Of these 111 complements a proportion show the first abnormality at metaphase: the presence of telocentric chromosomes. These are of two clear types: short fragments derived from the short arms of *S*-chromosomes and longer fragments derived from the long arms of *S*- or perhaps from *M*-chromosomes. It is significant that the short fragments are disproportionately frequent, since the long telocentrics lag most frequently at meiosis. They are evidently most often lost.

A high proportion of grains have extra-nuclear bodies. Some of these may be lagging whole chromosomes from meiosis. Others seem to be the products of misdivision. Some remain outside the spindle (Text-figs. 5, 6). Others come on to the spindle, but continue to show signs of their extraneous origin in their corrugated outline (Text-fig. 12).

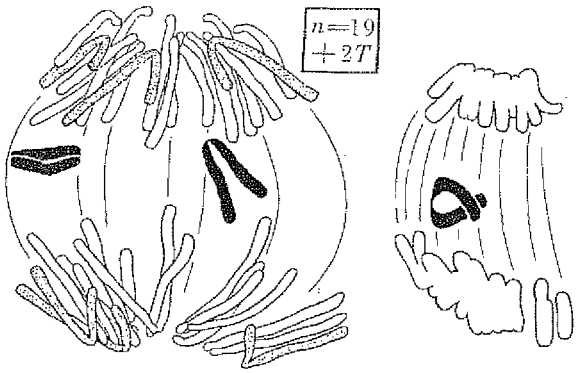
Table II indicates in fact that the extranuclear bodies correspond in frequency to the deficiency of long fragments. Some long telocentrics may in addition be overlooked at metaphase since they are less dissimilar from the normal *S*-chromosomes than are the short-arm fragments.

The most striking consequence of misdivision is seen in a pollen grain containing the two complementary products of breakage at the centromere of an *S*-chromosome. Lagging on the anaphase spindle side by side are the long telocentric and the telocentric (Plate VI, fig. 5). This combination I have found only twice (Table III), but the lagging is characteristic.

Both types of telocentric are frequently distinguished by their lying a little off the plate as though their congression were slower than usual (Text-fig. 8 and Pl. VI). At anaphase this delay becomes even more obvious. Short telocentrics may be seen lagging and lying crosswise in the spindle (Text-fig. 10). Long ones may divide after the normal chromosomes and their halves may nevertheless reach the poles successfully (Text-fig. 12), but their delay may be more serious. Both halves still attached to the centromere may then be carried to one pole (Text-figs. 14, 15, and Pl. VI).

In considering these figures it is important to recognize the means of distinction between a secondarily misdividing telocentric and a normal daughter *M*-chromosome. The six *M*-chromosomes of *F. latifolia major* are illustrated in Text-fig. 3 and in Pl. V, as well as in the pollen grains.

It will be seen that their arms are unequal. The arms of the telocentric, on the other hand, being sister chromatids, are equal and at an early stage can be seen to lie parallel. The second distinction consists in the two anaphase groups being equal and symmetrical apart from the laggard (Text-fig. 14).



Text-fig. 14. Text-fig. 15.

Text-figs. 14, 15. *F. latifolia major*. Delayed division of telocentrics leading to asymmetry on the spindle and the inclusion of an undivided iso-chromosome in one daughter nucleus. ×1100.

There is no doubt therefore that delayed telocentric chromosomes can pass whole to one daughter nucleus at mitosis and in doing so provide the necessary condition for the origin of iso-chromosomes.

TABLE I

Chromosome numbers (12–22) *of* 111 *pollen grains of* Fritillaria latifolia 3*x*, *omitting telocentric fragments* (*seven slides*)

Class	12	13	14	15	16	17
Observed	1	0	15	18	9	20
Percentage	0·96	—	14·3	17·1	8·5	19·0
Binomial	0·02	0·33	1·6	5·4	12·1	19·3
Class	18	19	20	21	22	
Observed	13	16	9	3	1	
Percentage	12·4	15·2	8·5	2·8	0·96	
Binomial	22·5	19·3	12·1	5·4	1·6	

CONCLUSION

In the original misdivision we saw how centromeres would split or explode before they had divided or reproduced. Here the process is reversed, for they divide but do not split. And the result is also a reversal of form, for the one-armed telocentric returns to the two-armed structure

from which it arose with the genetically important difference that the two arms are identical.

Several mechanical questions arise from these observations. There is a delay in the congression, orientation and splitting of the new terminal centromeres. Is this delay due to their terminal position, and characteristic of it, or is it due to their being only fractional centromeres lacking some of their centrogenes? Or, a third possibility, is it due to the broken ends within the centromere reuniting before metaphase? Has the terminal centromere the same property of reunion that the broken ends of chromosomes possess, or is it potentially stable? And is it possible for two broken centromeres to fuse to give new *M*-type chromosomes?

TABLE II

Frequencies of short and medium telocentrics and extranuclear bodies in different slides of Fritillaria latifolia *3x at metaphase*

Slide	Total nuclei	Short *ff*	Long *ff*	Extra-nuclear bodies	Mean chromo-some no.	Mean *ff* no.
2	53	25	5	2	16·5	0·5
6	18	3	3	8	17·1	0·3
3, 5, 7	14	5	1	9	17·4	0·4
4*	23	—	—	8	18·3	0·0
5	3	1	—	2	—	—
Total	111	34	9	29	16·9	0·4

* Telocentrics in anaphase cells only.

TABLE III

Frequencies of long and medium (L) and short (S) telocentrics showing delay in about 100 *pollen grain anaphases in* Fritillaria latifolia *3x (about an equal number with S only behaved normally)*

1S	*2SS*	*1L*	*2LL*	*L+S*	Total *ff*
5	1	8	4	2	27

The answers to these questions are indicated by Text-figs. 11 and 15. We see that when division, although delayed, is successful, a thread joins the products just as it does in the first misdivision. This suggests that the original breakage has been followed by fusion which will lead to repeated breakage. The structure of the iso-chromosome is already represented by the apparent telocentric at metaphase. In fact we seem to have the same situation as that described by McClintock (1939) for ordinary broken ends which can reunite (under certain conditions) to

give an endless repetition of breakage and reunion, a repetition from which the passage of the whole iso-chromosome to one pole will of course provide an escape.

The evidence that iso-chromosomes, arising as they do in *Fritillaria*, are, or can be, functional depends on the observation of such chromosomes in the regular life cycles of maize, *Datura* and other plants. It is not however necessary that all iso-chromosomes should be functional, for this reason: If a centromere contains x centrogenes it can be broken into fragments having any number of centrogenes less than x just as the nucleolar organizer can be broken into different-sized fragments, according to McClintock (1934). The iso-chromosome arising secondarily can then have any number from 2 to $2(x-1)$ or even $2x$ centrogenes. The larger fragments may fail to double; the smaller ones may fail to divide; but various results are likely to be produced from those of about half the proper size. It thus seems that misdivision is a means and, in fact, the only means we know, of adaptation in the size and functions of the centromere.

At first sight it might have seemed absurd that a rare and ruthless accident like misdivision should have any value in evolution. We now see that it may act in two ways. The first is in its effect on the genetic structure of chromosomes. The indications of fragmentation and fusion at the centromere already referred to in the Orthoptera, and in *Fritillaria* itself, have been difficult to understand on the basis of random structural changes. Centric fragmentation now becomes merely misdivision and centric fusion a not improbable consequence of it.

The second effect of misdivision is on the mechanical properties of the centromere. We may particularly look for signs of this effect in the sex chromosomes of animals whose special methods of co-orientation, segregation or elimination at meiosis may often require special properties in their centromeres (e.g. in *Sciara* or *Cimex*, Darlington, 1939*b*). Where the mechanical evidence is lacking we may turn to the structure of the chromosomes. For example, it now appears that the two arms of the *Y*-chromosome in *Drosophila melanogaster* contain homologous series of genes in the two segments next to the centromere (Neuhaus, 1937). Since these segments are of necessity the most conservative of all, their similarity is an indication that the *Y* is derived from an iso-chromosome. That such a chromosome should afterwards shed the greater part of one arm is further an indication that the effect of its origin on the centromere was the profitable one.

Misdivision, therefore, is likely to owe its importance in evolution

less to its more obvious effect in modifying the genetic structure of the chromosome, for which it provides an awkward alternative, than to its less obvious effect in modifying the mechanical properties of the centromere, for which it seems to be indispensable.

SUMMARY

Following misdivision of the centromere at meiosis in diploid and triploid *Fritillaria* new telocentric chromosomes are formed whose broken ends rejoin within the centromere. This type of chromosome is delayed at metaphase and anaphase in the pollen-grain mitosis. It may then, either break again at the centromere, or pass without separation to the pole as a new iso-chromosome.

The misdivision and the origin of the iso-chromosome are each likely to be important as affecting the genetic structure of the chromosome and the mechanical properties of the centromere.

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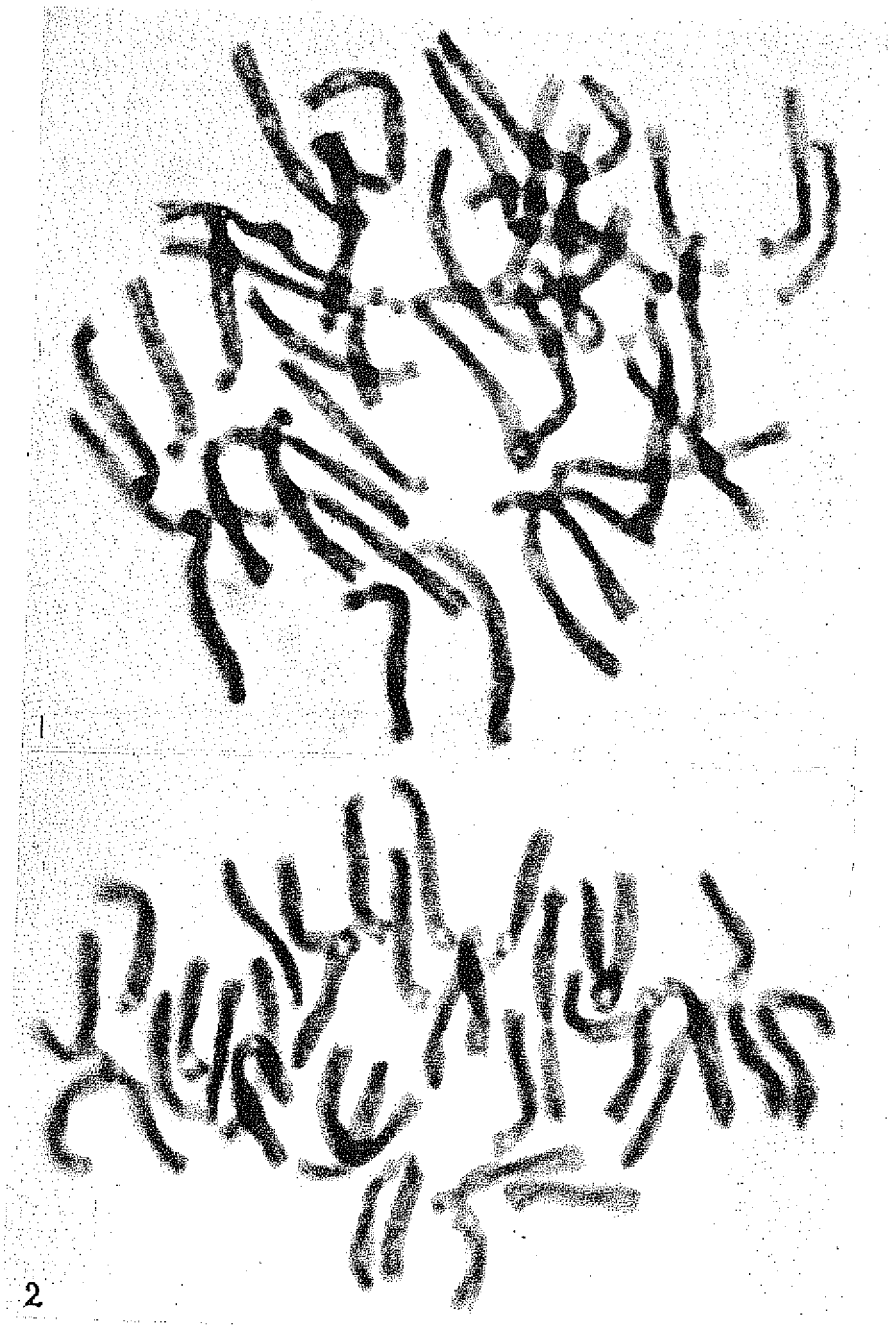
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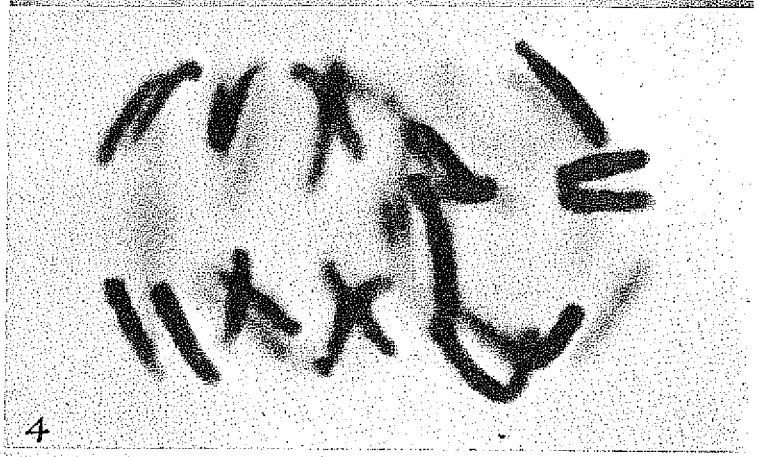
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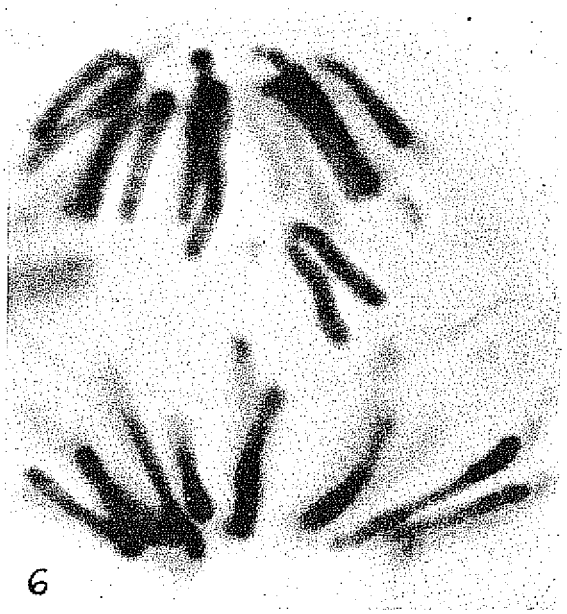
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EXPLANATION OF PLATES V AND VI

PLATE V

Ovarian mitoses in *F. latifolia major* ($3x=36$) at metaphase and beginning of anaphase, showing the positions of the centromeres in *M*- and *S*-chromosomes (cf. Text-fig. 3). Length range: 15–29μ. Average proportionate length of *M* to *S*: 1·0–0·71. Smear preparation after Feulgen hydrolysis. ×1800.

PLATE VI

Pollen grains of *F. latifolia major* in side view of metaphase (Fig. 3) and successive stages of anaphase showing delay in congression, orientation and division of long and short telocentrics. Fig. 5 shows both in the same cell. Preparation from anther macerated in equal parts of 95% alcohol and concentrated HCl for 10 min. before Feulgen hydrolysis. ×1600.

Preparations by L. La Cour, microphotographs by H. C. Osterstock.