



TITLE:

Chromosome Behaviour in the Interkinesis (I) : Observation of Pollen Mother Cells in Tradescantia reflexa

AUTHOR(S):

Kato, Kazuo

CITATION:

Kato, Kazuo. Chromosome Behaviour in the Interkinesis (I) : Observation of Pollen Mother Cells in Tradescantia reflexa. Memoirs of the College of Science, Kyoto Imperial University. Ser. B 1935, 10(4): 251-262

ISSUE DATE:

1935-02-25

URL:

<http://hdl.handle.net/2433/257819>

RIGHT:

Chromosome Behaviour in the Interkinesis
I. Observation of Pollen Mother Cells in
Tradescantia reflexa

By

Kazuo KATO

(Botanical Institute, Kyoto Imperial University)

With Plates XIV—XVI and 2 Text-figures

(Received September 25, 1934)

While numerous observations and discussions in regard to the chromosome cycle have been made by previous investigators, the behaviour of chromosomes in the phase commonly called the resting stage, remain as yet largely obscure. In this phase accurate observation is most difficult, and the opinions hitherto put forward have been largely based on the results of observation of the two phases to which the phase in question is contiguous. While as a rule in the somatic mitosis chromosomes enter this phase, in the meiotic divisions they show manifold modifications. In one of the extreme cases, they directly pass to the second division without entering this mysterious phase (SCHRADER, 1923; WAKAYAMA, 1931; TUAN, 1931)¹⁾ and in the other they show every successive morphological change ordinarily taking place in the somatic mitosis. There are many intermediate cases of varying degrees which seem to afford evidences more substantial to the knowledge of the chromosome behaviour in question than those which can be obtained by direct observation of the somatic mitosis. With this in view, the present investigation was carried out. In this paper the terms interkinesis and interphase are used in the original senses of GRÉGOIRE (1905) and LUNDEGÅRDH (1912).

1) It is also known in animal oögenesis that the interkinesis is commonly omitted (cf. WILSON, 1928, p. 532).

Before going further the writer wishes to express his sincere gratitude to Prof. Y. KUWADA, at whose suggestion this study was undertaken, for his kind criticisms throughout the investigation.

Method

Pollen mother cells in *Tradescantia reflexa* were fixed with the Bonn modification of FLEMMING'S solution for about 24 hours, having been previously treated with CARNOY'S mixture for some minutes (1/2, 1, 2, 3 or 5). This method of fixation, especially in the case where CARNOY'S mixture was allowed to act on material for one minute was found to be the best of all the mixtures used singly or in combination. Sections were cut in varying thickness from 6-12 micra, and were stained exclusively with HEIDENHAIN'S iron alum hæmatoxylin. For comparison of the results some observations by the acetocarmine smear method were also made.

Observation

In obtaining the precise knowledge of the chromosome behaviour in the interkinesis it is necessary to follow successive stages of the chromosome transformations, the katachromasis and anachromasis. In the present investigation, therefore, observations were made from the heterotype metaphase down to the homotype metaphase.

Heterotype metaphase. In fixed material the structure of the metaphasic chromosomes is mostly obscure, but in some cases where the differentiation is satisfactory the spiral structure can be observed with more or less clearness. In these cases the chromatic thread which forms the spiral is quite thick as shown in Figs. 1-9, and appears to be of homogeneous structure. In the chromosomes shown in Fig. 1 and those in Fig. 9 in which they are in tension being pulled towards the poles, the spiral course of the threads can be more or less clearly traced. The number of coils seems to be constant in a given chromosome. In most of the chromosomes it is five or six (Figs. 1, 2, 3 and 4; cf. NEBEL 1932 a, b). In some cases every gyre or turn of the spiral appears to be disconnected from the adjacent ones, so that the whole chromosome appears as if it is composed of a pile of discs embedded in the ground substance or matrix (Figs. 2 and 4; cf. SANDS, 1923). In acetocarmine preparations, as first announced by FUJII, (FUJII, 1926; cf. ISHII, 1931; KUWADA, 1932; KUWADA and NAKAMURA, 1933, 1934), the threads form-

ing the spirals in question are not of a homogeneous solid structure as in the fixed material, but are again spirals of the lower order with gyres much shorter in diameter and far more numerous in occurrence than those of the spirals of the higher order which they form (Fig. 37). The spirals observed in the fixed preparations must, therefore, correspond to those of the higher order.

In the fixed material the spiral seems generally to be single, but in some cases where the so-called chromosome bridge is formed the bridge is frequently double (Figs. 2, 5, 6 and 7; cf. KUWADA, 1927, Fig. 5). In Fig. 8 which represents a more advanced stage than Fig. 7, the connecting bridge that appears to be single-stranded shows a somewhat winding aspect in the middle. With the ammonia-acetocarmine method, KUWADA, (1932) has recently demonstrated with his collaborator that the bridge consists of two spirals of the lower order.

In Figs. 2 and 3 it is shown that the spindle fiber is directly attached to the spiral thread. From Figs. 11 and 12 too, some idea about this relation of the spiral to the insertion point of the spindle fiber may be acquired. This observation is directly opposed to the view of McCLINTOCK (1930) and NEBEL (1932 a), who have pointed out that the spiral does not pass through the point to which the spindle fiber attaches.

Anaphase-telophase. In anaphase, the chromosomes present not infrequently a rugged appearance on the surface, suggesting their internal spiral structure. The point of spindle fiber insertion was not directly determined, but from the shape of a double V which the chromosomes assume in this stage, it seems likely that it is approximately median except for some which appear to be sub-terminal.

In the course of the anaphasic migration, the chromosomes are drawn out to a certain extent, and become more or less thinner. In the late anaphase, the drawn-out state is often very conspicuous in the proximal portion of the chromosomes. In Figs. 11 and 12 in each of which a chromosome of the shape of a double V is shown, comparatively thin chromatic threads are found joined together at the point of spindle fiber insertion, i. e. at the apex of the V, and each thread is connected with the spiral of each chromosome arm. It seems likely that these thin threads connecting the spirals and the insertion point are the parts of the spirals drawn out. In his Fig. 26 SAX (1930) has shown a similar case in *Lilium regale*.

According to his verbal information, IWATA has also observed such a case in *Lilium longiflorum*. These cases seem to show that the spiral thread passes through the point of fiber insertion.

At the end of the anaphase all the chromosomes become somewhat shorter, and come in contact laterally with one another, beginning in the part near the pole and advancing towards the distal end at which they remain free from one another for a pretty long time. After having been kept together at the pole for a while, they begin to separate again from one another. The nuclear membrane is formed meanwhile. When the nucleus is generally increased in volume, it is noticed that the chromatic spirals tend to loosen out. The daughter nuclei thus formed generally assume a lenticular shape which is flattened on the side of the equator of the cell. This peculiar shape of the nuclei and their orientation to the equator of the cell are regularly observed phenomena in this stage in acetocarmine preparations, whereas such is not always the case in fixed material owing to the fact that the original shape is often subjected to a distortion to a greater or less extent by the action of the fixative employed.

In the stage some time after the nuclear membrane has been formed, the chromatic threads are thinner than those found in the chromosomes in the metaphase and anaphase, and they no longer form the regular spiral, but assume an irregular zig-zag shape. In this stage the ground substance in which the chromatic threads are embedded loses its staining capacity to hæmatoxylin, so that the threads stand out more and more clearly against the ground substance. The extent to which the chromosomes are thus transformed in the telophase is different in different chromosomes even in the same nucleus. In the upper right-hand corner of the nucleus reproduced in Fig. 14 the chromatic threads are quite free from the ground substance, while most of the chromosomes in the same nucleus are found still furnished with an amount of matrical substance. In one of the chromosomes in Fig. 15 which is in a transition state to the complete disappearance of the matrical substance, the chromatic thread is found rather regularly coiled around the matrix. When the chromatic threads are free from the matrix, the spirals of the threads are generally deformed into irregular shapes, though the individual spirals are still clearly distinguishable from one another.

It may be added here that a small nucleus-like body is often found in *Tradescantia reflexa* (Fig. 13) as has been reported by

NAWASCHIN (1911)¹⁾, DARLINGTON (1929) and NEBEL (1932 a) in some *Tradescantia*. It is highly probable that this body is the supernumeral dwarf nucleus derived from a fragment of a chromosome or a whole chromosome which lagged behind the others in division (NEBEL, 1932 a). In one case it was observed that the structure of such a dwarf nucleus was quite the same as that of the ordinary nucleus lying by it (cf. NEBEL, 1932 a).

Interkinesis. In the interkinesis the nucleus is generally increased in volume, and its lenticular shape in the late telophase is transformed to the ovoidal. By taking this change in shape of the nucleus and also the grade in development of the cell wall formed between the daughter nuclei into consideration one can easily determine the successive stages in the interkinesis.

In the early interkinesis the spirals are much loosened and partly drawn out into zig-zag threads. The threads are not smooth, but present a wavy or corrugated or sometimes a knotted appearance in which the knots represent the turns of the spiral (Figs. 17 a, b; cf. Fig. 13 in MAEDA'S paper, 1928). This knotted appearance can also be observed in the acetocarmine preparations (Fig. 38). These threads are found confined within separate territories which are arranged radially leaving a clear space at the centre (RABLE'S polar field; Fig. 16).

As the stage advances, the process of drawing-out of the spirals proceeds further, and it becomes finally very hard to trace the threads through their whole length; they run through irregular courses and come nearer or are superimposed upon one another. In this stage the matrix seems almost lost, and the threads are thinner than the spiral threads in the telophase. They no longer present the wavy or knotted appearance, but are found loosely coiled into spirals of small gyres. The so-called katachromatic transformation of the chromosomes seems to reach its climax at this stage. A typical nucleus in this stage is reproduced in Figs. 18 and 39, the latter of which was taken from an acetocarmine preparation. From these figures it seems highly probable that the interkinetic spirals of small gyres correspond to those of the lower order (chromonemata) which are visible in fixed material only when they are loosened out from the coiling of the higher order. Thus it may be said that the spirals found in the interkinesis in both fixed and

1) This body has been called by him chromatin-nucleolus.

acetocarmine preparations must be the primary spirals (spirals of the lower order) as pointed out by FUJII (1926). KUWADA (1932) states that only the spirals of the lower order are evident in the interkinesis the coiling of which is irregular in the advanced interkinesis, unravelling taking place to a certain extent.

Though in this stage it is hardly possible to trace each individual spiral along its whole length, it seems beyond doubt that the ends of the spiral threads are not fused into a continuous thread, because free ends can be observed here and there.

The morphological relation between the spirals in sister chromosomes is not clearly observable in this stage, but there are some reasons to infer that these two are attached at the point of spindle fiber insertion. In the first place, as will be seen later, the two spirals in a comparatively early prophase of the homotype division are found attached to each other at the insertion point of spindle fiber, and in the second place, it has been found in *Rhoeo discolor* that sister chromatids are linked in pairs at the insertion point throughout the whole interkinesis (KATO, 1930; SAX, 1931). NEBEL (1932 a) who has observed this linkage in pairs in *Tradescantia reflexa*, states to the effect that the chromonemata in the interkinesis are four in number and are attached at the point of spindle fiber insertion, with the matrix practically non-evident.

In the late interkinesis, the loosely coiled spirals of small gyres in the mid-interkinesis are re-transformed to the threads of the wavily corrugated or knotted appearances that are presented in the early interkinesis. These threads now appear thicker than in the mid-interkinesis (Fig. 19).

Summing up the results obtained, we may say that the first change taking place during the interkinesis is loosening-out of the meiotic spirals which reaches its climax at the stage when the katachromatic transformation of the chromosomes is at the maximum point, and then a reverse change takes place. The morphological continuity of chromosomes is kept in the interkinesis in the form of chromonemata loosened or unravelled to a considerable extent from their compact form of spirals.

Homotype division. In the early prophase the nucleus is somewhat spherical in shape. In this stage the chromonemata which are of the corrugated or knotted appearance are distributed in the nucleus more uniformly than in the early interkinesis (Fig. 20). The chromonema of the corrugated appearance in the upper left-

hand corner of the nucleus in Fig. 20 runs through a zig-zag course which reminds us of the helix course of the spiral of the higher order. In Figs. 21 and 22 this aspect is more conspicuous than in Fig. 20, and the corrugated or knotted appearance of the chromonemata is almost lost. The chromonemata are now seen as regular spirals of large gyres similar to those found in the late telophase in the heterotype division or those found in the early interkinesis (comp. Fig. 23 with Figs. 15, 16 and 17 *a, b*) and are more easily distinguishable from one another throughout their whole length than in the earlier stage (Figs. 23, 24 and 40). This similarity in form between the spirals in the heterotype telophase and those in the early homotype prophase has been also mentioned by TAYLOR (1931) in *Gasteria*, and the coiled aspect of the nuclear threads which NEWTON (1926) has described in the homotype prophase in *Fritillaria Meleagris* corresponds to that shown in Fig. 24. The process through which these large spirals of chromonemata are formed is, briefly speaking, reverse to the process which takes place in the katechromatic transformation of the chromosomes from the heterotype telophase to the interkinesis.

In the middle of Fig. 25 sister chromatids which run otherwise free from each other are attached at the point of spindle fiber insertion. The pair of these sister chromatids assumes the form of an X, or less frequently a double V (Fig. 27). These forms presented by the pair of chromatids are generally not observable in the stage earlier than that shown in Fig. 25.

In Figs. 25 and 26 some of the spirals are extensively drawn out. The change in the form of the spiral by unravelling becomes more and more evident as the stage proceeds (Figs. 27 and 28), though there still remains some trace of the spiral winding which is represented by the sinuosity of the spireme. In acetocarmine preparations, the spiremes in this stage show a spiral structure in which the gyres are much smaller than those of the meiotic spirals or the spirals of the higher order observed in the heterotype division. When the spiremes become thickened the sinuosity disappears and the individual chromosomes are now clearly distinguishable from one another (Fig. 29). When they approach the stage at which the nuclear membrane disappears, the corresponding arms of the sister chromatids come nearer to each other (Fig. 30). In Figs. 31-36 the series of change shown in Figs. 25-28 is illustrated again by a set

of chromosomes picked up from the nuclei corresponding in stage to those shown in these figures.



Text-figs. 1 and 2. Showing 12 chromosomal elements in the heterotype and the homotype metaphase respectively.

In the metaphase the 12 chromosomes¹⁾ which are much thinner and longer than those in the heterotype division are so arranged as to have their points of spindle fiber insertion lie on the equatorial plane and their arms directed towards the poles (Fig. 42). The contraction in length of the metaphase chromosomes from the prophase spiremes is less conspicuous in the homotype division than in the heterotype division. In Fig. 42, a metaphase group of chromosomes taken from an acetocarmine preparation is shown. In these chromosomes the spiral structure is very pronounced. The turns of these spirals are more numerous than those of the meiotic spirals in the heterotype division. The same result has been obtained by SHINKE (1930) in *Lilium*, *Rhoeo*, *Allium*, *Tricyrtis*, *Najas* and *Hosta*.

Summing up the results obtained we may say that the meiotic form of the spiral in the heterotype telophase is repeated in the early prophase of the homotype division. The spirals found in these two stages are closely similar in shape. The threads forming the spirals grow, then, thicker and shorter, and the spirals are gradually drawn out. When they are considerably drawn out, the threads (spiremes) come to show their internal spiral structure.

1) According to NEBEL (1932 a, b) the haploid number of chromosomes is 6 in *Tradescantia reflexa*, RAF. In our material which was identified by MAKINO (1931) as *Tradescantia reflexa*, RAF. it is 12 as shown in Text-figs. 1 and 2 (cf. ANDERSON and DIEHL, 1932).

Summary

1) In fixed material the chromatic spirals of the chromosomes in the heterotype metaphase appear to be single-coiled and usually to be single-stranded. In acetocarmine preparations the threads forming these spirals are found not to be solid threads but spirals of small gyres; the spirals are, therefore, doubly coiled.

The threads connecting two associated chromosomes are observed occasionally to be clearly double, a fact which may show that the single appearing spirals in the metaphase are in reality double.

2) In the late telophase the spirals of large gyres begin to unravel. The unravelling reaches its maximum at the point at which the katachromatic transformation of the chromosomes is ended. Thus in this stage only the spirals of small gyres which are now visible also in fixed material are evident, most of the matrix substance being lost in this stage. No anastomoses are found in the nucleus.

3) In the beginning of the homotype prophase the spirals of small gyres which appear now again to be solid threads in fixed material come to run through a helix course, thus the whole figure closely resembles the spirals of large gyres in the heterotype division. In *Tradescantia reflexa* the chromosomes, thus, pass through the interkinesis in the form of chromonemata.

4) Successive stages in the unravelling of the larger spirals in the homotype prophase to form metaphase chromosomes are observed in fixed material. In acetocarmine preparation the chromosomes thus formed are found to be spirals of small gyres, in which the turns are smaller in diameter and larger in number than those of the spirals of large gyres in the heterotype division.

Literature Cited

- ANDERSON, E., and DIEHL, D. G., (1932) Contributions to the *Tradescantia* problem. Jour. Arnold Arboretum, Vol. 13.
- DARLINGTON, C. D., (1929) Chromosome behaviour and structural hybridity in the *Tradescantiae*. Jour. Gen., Vol. 21.
- FUJII, K., (1926) The recent progress in cytology and its methods of investigation. (Japanese) Proc. Japan. Assoc. Adv. Sci., Vol. 2.
- GRÉGOIRE, V., (1905) Les résultats acquis sur les cinèses de maturation dans les deux règnes I. La Cellule, T. 22. (cited from WILSON'S "the cell in development and heredity").
- ISHII, T., (1931) On the structure of chromosomes. (Preliminary note) Japan. Jour. Gen., Vol. 7.

- KATO, K., (1930) Cytological studies of pollen mother cells of *Rhoeo discolor*, HANCE with special reference to the question of the mode of syndesis. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, Vol. 5.
- KUWADA, Y., (1927) On the spiral structure of chromosomes. Bot. Mag. Tokyo, Vol. 41.
- (1932) The double coiled spiral structure of chromosomes. Bot. Mag. Tokyo Vol. 46.
- KUWADA, Y., and NAKAMURA, T., (1933) Behaviour of chromonemata in mitosis I. Observation of pollen mother cells in *Tradescantia reflexa*. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, Vol. 9.
- (1934) Behaviour of chromonemata in mitosis II. Artificial unravelling of coiled chromonemata. Cytologia, Vol. 5.
- LUNDEGÅRDH, H., (1912) Das Caryotin in Ruhekern und sein Verhalten bei der Bildung und Auslösung der Chromosomen. Arch. f. Zellforsch., Bd. 9.
- MAEDA, T., (1928) The spiral structure of chromosomes in the sweat-pea (*Lathyrus odoratus*, L.). Bot. Mag. Tokyo, Vol. 42.
- MAKINO, T., (1931) Miscellaneous note on plants. XLVIII. Jour. Japan. Bot., Vol. 7.
- McCLINTOCK, B., (1930) A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea mays*. Proc. Nat. Acad. Sci., Vol. 16.
- NAWASCHIN, S., (1911) Über eine Art der Chromatindiminution bei *Tradescantia virginica*. Ber. d. Deutsch. Bot. Ges., Bd. 29.
- NEBEL, B. R., (1932 a) Chromosome structure in Tradescantiae I. Methods and morphology. Zeitschr. f. Zellforsch. u. mikr. Anat., Bd. 16.
- (1932 b) Chromosome structure in Tradescantiae II. The direction of coiling of the chromonema in *Tradescantia reflexa* Raf., *T. virginiana* L., *Zebrina pendula* Schnizl. and *Rhoeo discolor* Hance, Ibid., Bd. 16.
- NEWTON, W. C., (1926) Chromosome studies in *Tulipa* and some related genera. Jour. Linn. Soc., Vol. 47.
- SANDS, H. C., (1923) The structure of the chromosomes in *Tradescantia virginica*, L. Amer. Jour. Bot., Vol. 10.
- SAX, K., (1930) Chromosome structure and the mechanism of crossing over. Jour. Arnold Arboretum, Vol. 11.
- (1931) Chromosome ring formation in *Rhoeo discolor*. Cytologia, Vol. 3.
- SCHRADER, F., (1923) A study of chromosomes in three species of *Pseudococcus*. Arch. f. Zellforsch., Bd. 17.
- SHINKE, N., (1930) On the spiral structure of chromosomes in some higher plants. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, Vol. 5.
- TAYLOR, W. R., (1931) Chromosome studies on *Gasteria*. III. Chromosome structure during microsporogenesis and the postmeiotic mitosis. Amer. Jour. Bot., Vol. 18.
- TUAN, H. C., (1931) Unusual aspect of meiotic and postmeiotic chromosomes of *Gasteria*. Bot. Gaz., Vol. 8.
- WAKAYAMA, K., (1930) Contribution to the cytology of fungi I. Chromosome number in Agaricaceae. Cytologia, Vol. 1.
- WILSON, E. B., (1928) The cell in development and heredity. 3rd. ed. New York.

Explanation of Plates

Figs. 1-36 were drawn with the aid of *ABBE's* camera lucida using *ZEISS's* imm. 1/12 and comp. oc. 18. Figs. 37-42 are photomicrographs taken from acetocarmine preparations, using *ZEISS's* apoch. imm. 2 mm. and comp. oc. 12.

Figs. 1-9. I-metaphase.

Fig. 1. A bivalent.

Fig. 2. Three chromosomes linked in chain. The middle chromosome is linked with one of its neighbours by two threads.

Fig. 3. A univalent chromosome.

Fig. 4. A bivalent.

Figs. 5, 6 and 7. Bivalents in which the two chromosomes are linked with each other by two threads.

Fig. 8. The same in which the two chromosomes appear to be connected with a single crooked thread.

Fig. 9. A bivalent showing spirals clearly.

Figs. 10-12. Chromosomes in I-anaphase in which the longitudinal halves are clearly separated from each other.

Fig. 13. A dwarf nucleus found near by a nucleus of normal size.

Fig. 14. I-telophase. Some of the spirals are free from the matrix.

Fig. 15. Late telophase with matrix nearly lost. Unravelling of the spirals is noticeable here and there.

Fig. 16. Early interkinesis showing the corrugated appearance of the threads unravelling from the spirals. Matrix disappears completely, but the original position of the chromosomes is still recognizable.

Fig. 17 *a* and *b*. Showing knotted appearance of the threads running through the course of a loose helix.

Fig. 18. Mid-interkinesis. Only small coils are evident, the maximum point of unravelling of the meiotic spirals being reached.

Fig. 19. Late interkinesis showing thickening of nuclear threads.

Fig. 20. Early II-prophase showing loosely winding aspect of nuclear threads visible here and there.

Fig. 21. The same.

Fig. 22. The same showing coiled aspect of nuclear threads which is now conspicuous.

Fig. 23. The same showing thickened nuclear threads which are coiled into rather regular spirals of large gyres.

Fig. 24. Middle II-prophase. Nuclear threads are thicker than in the preceding stage.

Figs. 25 and 26. Later stage. In some of the chromosomes the spirals of the nuclear threads are considerably uncoiled.

Fig. 27. An advanced stage in which coiling aspect of the nuclear threads is markedly diminished. Sister chromatids attached to each other at the insertion point of spindle fiber are shown.

Figs. 28 and 29. More advanced stages. In Fig. 29 the coiled aspect of the nuclear threads entirely disappears.

Fig. 30. A stage near the II-metaphase.

Figs. 31-36. Showing successive stages of unravelling of chromosomes. Figs. 31 and 36 correspond in stage to Figs. 25 and 28 respectively.

Fig. 37. Chromosomes in the I-metaphase showing the spiral-within-spiral structure. Note that the thick spirals are made up of spirals of small gyres.

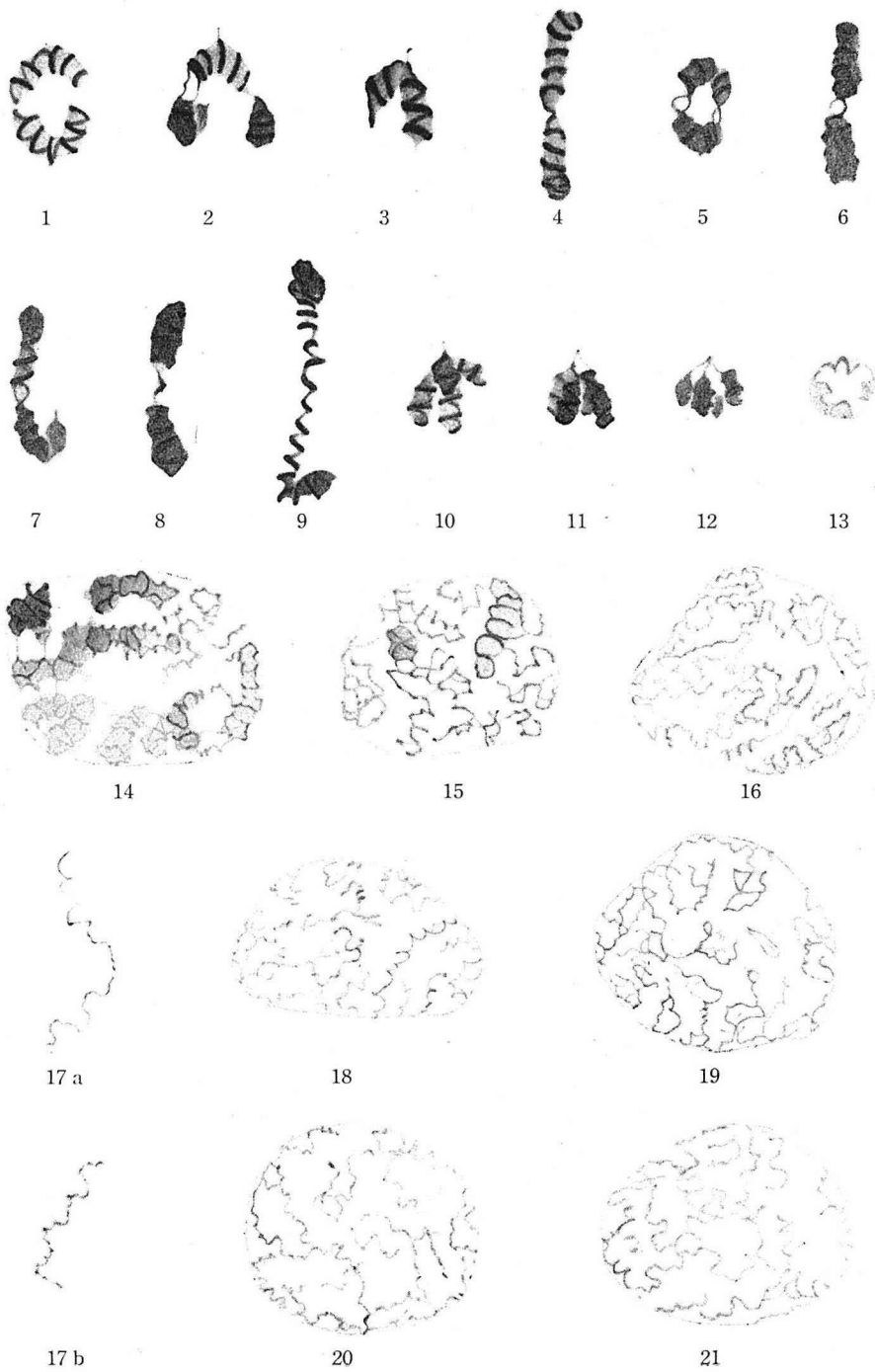
Fig. 38 Interkinesis just before mid-interkinesis. The nucleus is filled with spirals of small gyres. Even traces of thick spirals are barely visible.

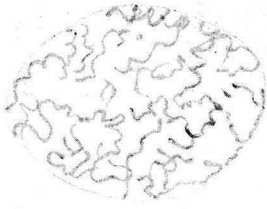
Fig. 39. Mid-interkinesis. Only spirals of small gyres are evident in the nucleus.

Fig. 40. Early II-prophase. Coiled threads are thicker.

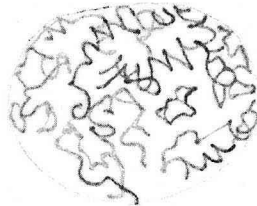
Fig. 41. Middle II-prophase. Coiled spiremes are made up of spirals of small gyres.

Fig. 42. Chromosomes in the II-metaphase.

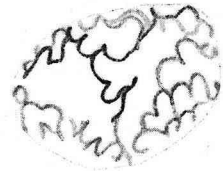




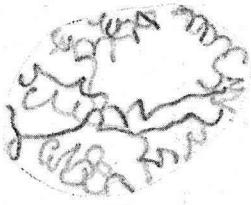
22



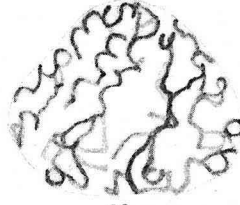
23



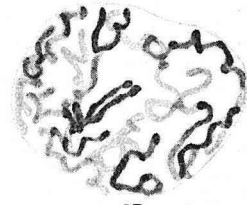
24



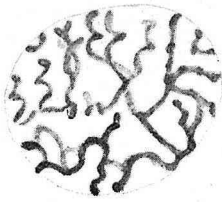
25



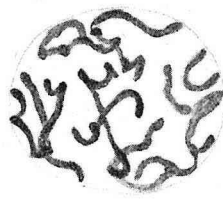
26



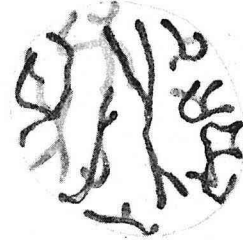
27



28



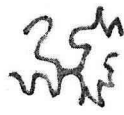
29



30



31



32



33



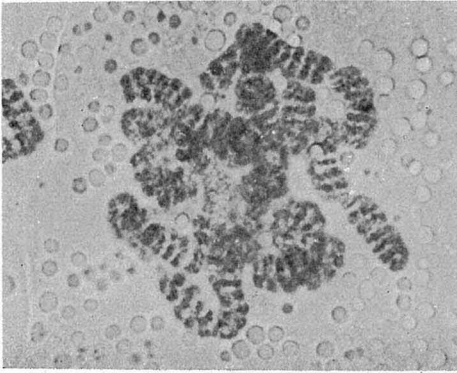
34



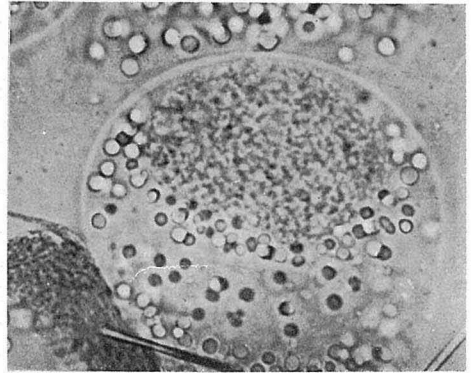
35



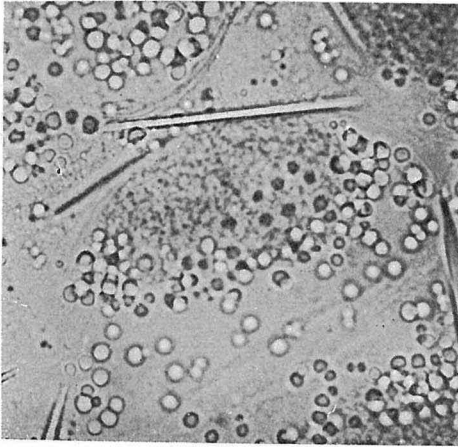
36



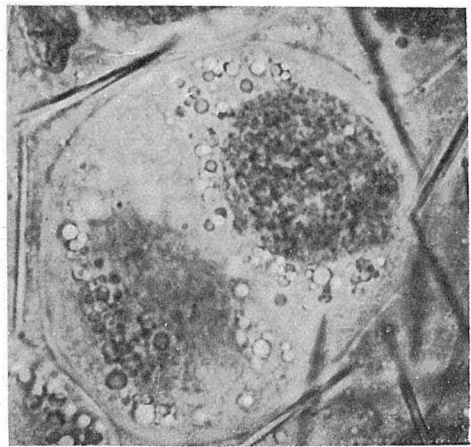
37



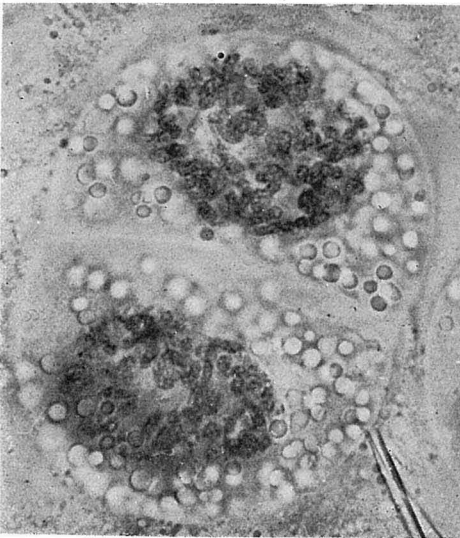
38



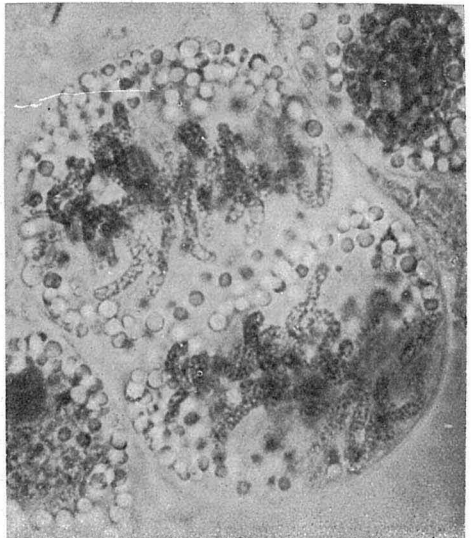
39



40



41



42