MEMOIRS OF THE COLLEGE OF SCIENCE, KYOTO IMPERIAL UNIVERSITY, SERIES B,
VOL. IX, NO. 2, ART. 4, 1933

Behaviour of Chromonemata in Mitosis

I. Observation of Pollen Mother Cells in *Tradescantia reflexa*

By

Yoshinari KUWADA and **Takeshi NAKAMURA**

(Botanical Institute, Kyoto Imperial University)

With Plate VIII and 3 Text-figures

(Received Oct. 20, 1933)

In this paper the results[1] obtained from observation of pollen mother cells in *Tradescantia reflexa* are reported. The observations were made in stages from the metaphase of the heterotype division to the prophase of the homotype division.

In his observation of iron acetocarmine preparations of pollen mother cells in a species of *Tradescantia*, probably *T. reflexa*[2] FUJII reached the conclusion that in the heterotype division the chromonemata are in the doubly coiled state in the chromosomes (FUJII, 1926). While in the metaphase these double-coiled chromonemata appear to be single, the chromosomes containing them are divided in the anaphase into two longitudinal halves, in each of which a chromonema similarly double-coiled is contained. This couple of facts compelled us to a further observation, but a clear demonstration of the double-coiled nature was not easily reached. While working with fresh material with a new method for another purpose, however, we unexpectedly came across beautiful figures of chromonemata showing a clear double

1. The main result was published by one of us in Japanese with an English résumé (KUWADA, 1932). In that paper the material plant was described as *Tradescantia virginica*. This plant was formerly described as *T. virginica*. Recently, however, it was pointed out by MAKINO (1931) that it is not *T. virginica*, but *T. reflexa*, RAF.

2. In the original paper, only the Japanese name of the plant, which has hitherto been identified as *Tradescantia virginica*, was given.

coiling. A parallel investigation with fixed material which was necessary to complement the results we obtained in the homotype prophase was carried out by Mr. KATO[1]. In the present paper only the results obtained so far with fresh material are given.

The observations were made in summer, 1931, 1932, and 1933. When in September, 1931, we discontinued the observation for that year, we received ISHII's paper (1931a) in which the double coiling of the chromonemata is described and figures more or less schematically drawn are given.

Method

Besides the ordinary acetocarmine method, we employed our new method, in which pollen mother cells, smeared on a slide glass with a very small drop of a 3 % cane sugar solution, are exposed to ammonia vapour for a few seconds before they are stained with acetocarmine. As starch grains in the pollen mother cells often preclude accurate observation, observations were commonly made after the preparations had been gently heated with the flame of a match in order to cause the starch grains to swell. Another method in which pollen sacs in toto were exposed to ammonia vapour was also tried, but this method was found inferior to the method mentioned first, it being very apt to bring about variable results.

While the ammonia method can meet the purpose of demonstrating the coiling nature of the chromosomes in the dividing stages, it hardly enables us to follow the stages one after another in the period from interkinesis to the ensuing prophase, owing to the fact that the normal appearance of the spiremes is more or less disguised in the swelling caused by the method, and accordingly the ordinary acetocarmine method was used for the study in those stages.

Observation

In the ordinary smear preparations prepared by staining material with neutral violet extra (N. V. E.), the chromatic spirals in metaphase chromosomes appear to be simple spirals, the threads forming them presenting themselves as a relatively homogeneous structure, but in those stained with acetocarmine, a certain structure is visible which strongly suggests that the threads are also spirals of the lower order

1. To be published later.

with a shorter diameter. This doubly coiled or spiral-within-spiral structure is more clearly demonstrated by our ammonia method described above, the chromosomes being enormously swollen under the influence of the ammonia vapour without the chromonemata being much affected (See Fig. 1, KUWADA 1932, especially 4th turn from the top of the chromosome on the right hand side). Optical cross sections of the turns of the larger spirals, or the spirals of the higher order, are of ring form, indicating this complicated structure; in a longitudinal median optical section, a chromosome appears as if it is composed of rings longitudinally arranged in two rows. These rings appear to be single, and this singleness leads us to the conclusion that a single small spiral is coiled into a large secondary spiral. If the small (primary) spiral is actually single, the longitudinal split of the chromonemata to which the arisal of the chromonemata in the anaphasic chromosome halves is due, must take place after the metaphase when the formation of the secondary spiral is complete. This would, however, make separation of the daughter spirals in the anaphase very difficult (cf. KUWADA, 1927). For the separation to take place easily, the split must have been initiated before the coiling, and consequently the spirals must be double already in the metaphase.

In order to settle the question whether the spiral is single or double, the following observations were made:

1) On the supposition that observation of anaphase chromosomes which are dividing longitudinally may disclose the doubleness of the spirals, they were observed with special attention, but the complication of the figures due to overlapping of the two separating spirals did not allow us to get any clear idea as to how they are brought about from a mother spiral. In the metaphase we have been able so far to confirm in a number of cases that two spirals of the second order come by their separation from each other out of a single-appearing spiral of the same order. In Figs. 1—3 a sequence of the separating process is shown. In Fig. 1 every turn of the one spiral is found at the level between two consecutive turns of the other; in Fig. 2 some corresponding turns of both spirals are found nearly on the same level, while others still keep their alternate positions; in Fig. 3 all the corresponding turns of both spirals are nearly on the same level. In Fig. 4 the spirals in the upper part of the chromosome are quite separated and free from each other, while in the lower half a certain complication probably caused by a chiasma obscures the condition.

2) If the duality exists, it should be clearly observable in the

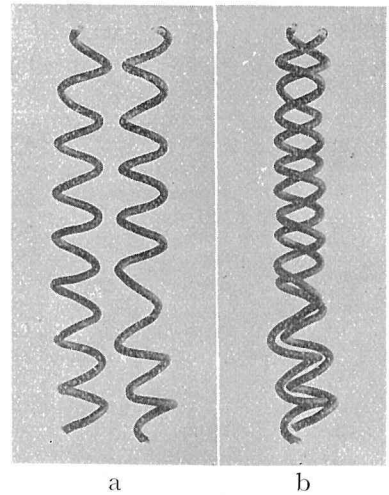
region where the spiral is uncoiled. From this point of view the region where a chromosome was connected with another was observed. So far as the results we obtained are concerned, the threads (more properly speaking, the primary spirals) forming the secondary spirals contained in these chromosomes are always in contact with each other at their ends (Figs. 5, 6). When these chromosomes are pulled towards the opposite poles, this conjunction of the threads is broken with more or less difficulty. When the breaking is difficult, the end parts of the threads on the side of the point of conjunction are drawn out from the coil. It was not infrequently observed that in these parts the threads are double (Figs. 7, 8). In N. V. E. preparations the spiral structure of these double threads is obscure, but in acetocarmine preparations prepared by the ammonia method it is often clear (Fig. 3, KUWADA, 1932). In the ordinary acetocarmine preparations too, cases where these parts showed this structure were often met with. In Fig. 9 the spiral structure is clearly shown, but the spiral is single. In Fig. 10 the structure is not so clearly presented as in Fig. 9, but the thread is recognisably double. It seems likely that in the former case (Fig. 9) one of the pair of junctions has been broken and the parts of the threads thus released from tension have been drawn back into the main body of their respective chromosomes, while in the latter (Fig. 10) both remain unbroken.

3) The singleness mentioned above of the chromatic rings, or the cross sections of the primary spirals, seems to show that every turn of the secondary spiral is single. This apparent contradiction to the result mentioned above (2) led us to ask ourselves whether the secondary spiral is not in reality composed of two spirals so loosely disposed to each other, that cross-sections of turns of them are found quite detached from each other. If this is the case, the rings may be simple in the presence of two primary spirals in a chromosome. With this question in view, turns of the secondary spirals were followed one to another in chromosomes lying on the plane of optical section. So far as cases where critical observation was possible are concerned, however, no such duality as might be expected from the question presented above could be revealed, whenever the chromosomes appeared to contain a single regular spiral.

4) The results mentioned above drove us to another question—whether the singleness of the chromatic rings is merely a seeming phenomenon. With this question in view, the cover glass was pressed. A case of the results obtained was shown in Fig. 4 in the previous

paper (KUWADA, 1932). In that figure, the duality of the turns is clearly shown though the primary spirals are a good deal broken. A good many figures in which the doubleness was suggested were obtained, but in many cases the primary spiral was broken to a greater or less extent. In Fig. 11 a, b, c, the doubleness is indicated by arrows.

5) In preparations where the cover glass is not intentionally pressed, some turns of the secondary spiral are often found to be markedly thicker than those in which the chromatic rings appear to be single. In these broad turns the rings (cross-sections) are two in number, being arranged side by side in the shape of the figure 8. This couple of rings is so oriented as to have the longitudinal axis of the 8 nearly perpendicular to the length of the turns, indicating that the broad turns consist of two independent primary spirals running closely together side by side (Figs. 12, 13, cf. Text-fig. 2).



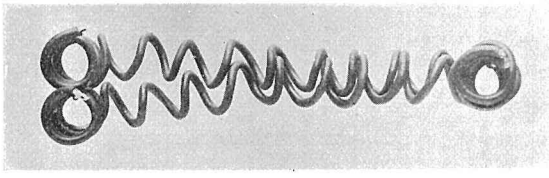
a b

Text-fig. 1. A wire model showing what appearances are brought about by combining two spirals coiled in different directions. Note that the difference in level on which the corresponding turns of the spirals are found results in different appearances as shown in the upper (front view) and the lower half (side view) of the model. a. The two spirals separated, b. combined.

6) Even in the case where the secondary turns are as thick as those of which the cross-sections appear to be single rings, a pair of the primary spirals can be clearly recognized, if the directions of the coiling of the spirals are opposite to each other. An example is shown in Fig. 11 a (cf. Text-fig. 1).

7) The effects of ammonia vapour are of two main types. In the one, only the matrix of the chromosomes, and in the other, both matrix and chromonemata are affected. In the former there are several subtypes. In one of these the secondary spirals are drawn out to a considerable extent without showing practically any considerable change in the primary coiling (Fig. 14). Fig. 16 shows an extraordinary case where only a few traces of the turns of the secondary spiral are found, most of them having been drawn out. The primary spirals thus drawn out from their secondary coiling appear just like the spiremes in the late prophase of the second division. Even in this drawn-out or un-

coiled state from the secondary coiling, the doubleness of the primary spirals is for the most part not clearly observed, it being obscured by the component spirals being disposed so closely to each other that they appear as a single spiral, but in places it can be observed with more or less distinctness (See also Fig. 14). In Fig. 15 chromosomes are shown which still keep their double-coiled state. In an end turn of the secondary spiral indicated by an arrow the component primary spirals are clearly separated from each other through a half turn length, while they appear as one thicker spiral in the other half, corresponding turns of them being found so closely in contact with each other as to present such an appearance. The condition is practically the same as that found in the chromosome shown in Fig. 4, in which only the secondary coiling is visible.

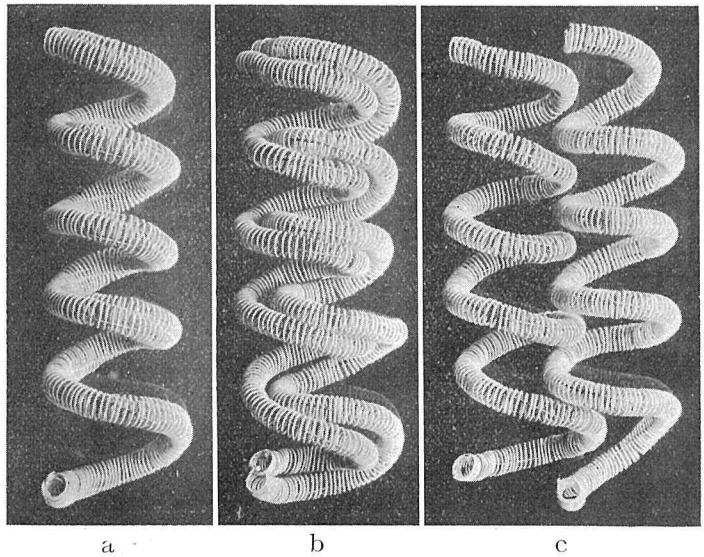


Text-fig. 2. A wire model representing a half turn of the coil of the higher order of a double-coiled, double spiral. On the left hand side the combined spirals are separating (cf. Fig. 15). At both ends, end views of the spirals are shown (cf. Figs. 12, 13). Note that the two spirals are coiled in the same direction.

From the facts mentioned above we may conclude that the secondary spiral turns must consist of two primary spirals, which combine into a seemingly double-stranded spiral, the one shoving into the other (Text-fig. 2). The direction of coiling of both these spirals is generally the same, but they may be opposite to each other. In the latter case the "double-stranded" nature of the spiral is easily perceptible, but in the former it is obscured, and is first revealed only when the two spirals are separated to a considerable extent. In preparations stained with N. V. E. too, this doubleness of the secondary turns is often observed (KUWADA, 1927, Figs, 3 and 4), but no such spiral structure of the (secondary) turns as that found in the case of acetocarmine preparations is observed, they appearing quite homogeneous.

No case was found which is favourable to the view of NEBEL (1932) that the heterotype chromosomes consist of four parallel cords of the ordinary single-coiled spiral chromonemata shoved into one another. As to the problem of the process by which the "double-stranded", double-coiled spiral (double-coiled, double spiral) which is separable into its component spirals is formed, we shall discuss that in a separate paper, but here we shall give briefly a possible method of formation, one which seems to us most probable at the present stage of our knowledge.

As will be seen later we are of the opinion that in the early prophase the chromonemata are thrown into a new coiling, as a result of which the old spirals which reappear in the "spiral stage" of the prophase are drawn out. If the longitudinal halves of a chromonema are each thrown into the new coiling, the two spirals thus formed will be quite independent from each other. The two halves of the chromonema are, however, associated very closely with each other, so that the growing spirals will be shoved into each other to form a seemingly double-stranded spiral, as they gradually grow into the difinite spiral form. If these chromonema halves are thrown again into another new coiling, a seemingly double-stranded, doubly coiled spiral which is quite separable into its component double coiled spirals will be formed (cf. Text-figs. 2, 3). In this case it is to be supposed that the drawing-out of the first produced spiral which should result from the second coiling, is prevented by the contracting force of the matrix substance, which grows richer in amount as the stage proceeds. From the stand point of the genesis of the spiral thus interpreted, the spiral denoted above the primary should really be called the secondary and *vice versa*. The question of which denotation is the more appropriate will be discussed in another paper.



a b c

Text-fig. 3 Wire models showing how the double-coiled, double spiral is separated into two double-coiled single spirals.

a. Double-coiled, double spiral.

b. The same with coils of the lower order separated (cf. Figs. 12, 13).

c. The same completely separated (cf. Figs. 1–4).

We have a second question—how can the separation from each other of these complicated spirals be brought about? The question is difficult to answer, but the phenomenon which we studied more closely in living staminate hair cells[1] than in pollen mother cells, that the chromonemata undergo a considerable swelling in the period from the end of the prophase to the biginning of the telophase, during which the separation of the component spirals is visible, seems to throw some light on the mechanism of the sepration in question. This seems plausible when we consider that the distance between the longitudinal axes of two chromonemata set closely together will grow greater as they swell; the greater the swelling is, the greater will be the distance, i. e. the separation.

In the interkinesis the larger coil is lost to sight, and even the smaller coil is extensively loosened, so that the nucleus appears to be filled with long, slender chromonemata running sinuously or coiled in part in a more or less irregular manner. This appearance is quite the same as that presented by the interphasic nucleus in young pollen grains.

The characteristic feature which is first recognisable in the very beginning of the prophase is the reappearance of the old spirals, that is, the spirals that are found before the interphase—the "spiral stage" (SHARP, 1929). In the present material the chromonemata are doubly coiled in the stages before the interkinesis, and accordingly the old spirals that reappear are not single-coiled spirals as in the case of somatic mitosis, but double-coiled ones. Both these spirals are drawn out gradually as the prophasic spiremes grow thicker. Mr. KATO has traced a series of these transformations successively in preparations from fixed material. An attempt to trace it stage after stage in the (ordinary) acetocarmine preparations ended in failure, but we wish here to mention the result we obtained which seems to give an important hint as to the mechanism of the uncoiling or drawing-out of the old spirals.

In the early stage of the drawing-out no clear internal structure is visible in the spiremes, but in a later stage a rugged appearance which seems to suggest that an internal change resulting in a new coiling of the chromonemata is taking place, becomes perceptible at the contour of the spiremes along the whole length. When the old spirals have been drawn out and the spiremes attain their full thickness, that is, when they are nearly as thick as the chromosomes in the

1. To be published later.

metaphase, they clearly show their internal, ordinary simple spiral structure. To put it in other words, the old spirals are drawn out as the new spirals grow more and more definite, the change which results in the new coiling of the chromonemata not beginning to occur at a certain definite point or points, but taking place evenly throughout their whole length. For comparison the prophase in pollen grains was observed. The result obtained was quite the same as that described above for the homotype division. The formation of a new coil in the prophase has been expressly stated by some authors (VEJDOVSKÝ, 1911–1912; ISHII, 1931b; NEBEL, 1932) and has been described by BONNEVIE (1908) as an internal differentiation of the spireme into the core and the cortical part.

The result of observation just mentioned above points to the conclusion that the increase in thickness of the spiremes does not necessarily mean the growth of the chromonemata in thickness, but shows the degree of their coiling. The condensation or the shortening of the spiremes into chromosomes may, therefore, be looked upon as a result of coiling of the chromonemata contained in them. "No change in the length of the chromonemata proper is necessary to account for the shortening and thickening of the chromosomes" (NEBEL, 1932, p. 277). This will be more fully discussed in another paper in connection with the results obtained from living staminate hair cells.

Summing up the results we obtained together with those obtained by our collaborator Mr. KATO, we may say that the chromonemata are visible in acetocarmine as well as in fixed preparations throughout all the stages from the heterotype division to the homotype division. As was announced by FUJII, the chromonemata are doubly coiled in the heterotype division, and singly in the homotype division. In the interkinesis both the primary and the secondary coil are set loose, so that it becomes hardly possible to distinguish them from one another throughout the length of individual chromonemata. At the out-set of the prophase the doubly coiled form taken up by chromonemata before interkinesis reappears. The coils, primary and secondary, that have thus reappeared, soon begin to be drawn out, as the new coiling of the chromonemata sets in. When this new coiling is fully accomplished, the chromosomes of the homotype division containing the ordinary simple spirals of chromonemata are formed.

Summary

1) The announcement by FUJII that in the heterotype division in pollen mother cells in *Tradescantia reflexa* the chromosomes are of double-coiled structure was confirmed by a new method in which pollen mother cells were exposed to ammonia vapour before being stained with acetocarmine. The double-coiled spirals in the metaphase are not single, but double (diad). According to the intensity of the influence of the ammonia vapour upon the cell, the matrix substance of the chromosome suffers a certain change in its colloidal state; in some case the larger spirals or the spirals of the higher order are drawn out, so that the double-coiled form of the chromonemata is reduced to the ordinary simple spiral.

2) In the interkinesis the larger spirals are lost to sight, and even the smaller spirals (spirals of the lower order) are extensively deformed. The nucleus thus formed presents a close resemblance in structure to the nucleus in young pollen grains.

3) In the homotype prophase a new coiling of the chromonemata takes place, as a result of which the old spirals that have reappeared in the "spiral stage" are drawn out.

Literature Cited

BONNEVIE, K. (1908): Chromosomenstudien. Arch. f. Zellforsch. Bd. I.

FUJII, K. (1926): Recent Progress in Cytology and its Methods of Investigation. (Japanese). Japanese Assoc. Adv. Sci. Vol. II.

ISHII, T. (1931a): On the direction of coiling of the spiral in the chromosome. (Japanese). Bot. Mag. Tokyo. Vol. XLV.

—— (1931b): On the structure of chromosomes (Preliminary note). (Japanese). Japanese Journ. Genetics, Vol. VII.

KUWADA, Y. (1927): On the Spiral Structure of Chromosomes. Bot. Mag. Tokyo. Vol. XLI.

—— (1932): The Double Coiled Spiral Structure of Chromosomes. (Japanese). Bot. Mag. Tokyo. Vol. XLVI.

MAKINO, T. (1931): Miscellaneous Notes on Plants. XLVIII. Journ. Japanese Bot. Vol. VII.

NEBEL, B. R. (1932): Chromosome Structure in Tradescantiae. I. Ztschr. f. Zellforsch. u. mikrosk. Anat. Bd. 16.

SHARP, L. W. (1929): Structure of Large Somatic Chromosomes. Bot. Gaz. Vol. LXXXVIII.

VEJDOVSKÝ, F. (1911—1912): Zum Problem der Vererbungsträger. Prag.

Explanation of Plate VIII

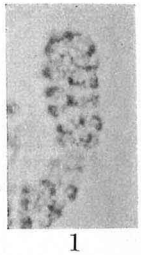
All the figures are microphotographs of I-metaphase chromosomes in pollen mother cells in *Tradescantia reflexa*. Figs. 1, 2, 4, 9, 10, 14, and 15 were taken with Zeiss' apochrom. imm. 1.5 mm. and comp. oc. 15×, and Figs. 6—8 with Zeiss' apochrom. imm. 2 mm. and comp. oc. 15×. Figs. 3 and 11—13 were taken with Zeiss' apochrom. imm. 1.5 mm. and Leitz's periplan. oc. 15×, and Fig. 16 with the same objective and periplan. oc. 25× with a Leitz Makam camera. Fig. 5 is an enlargement to ×1.5 from the original negative taken with Zeiss' homog. imm. 1/12 and oc. 4.

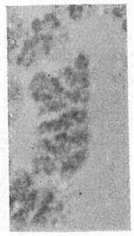
Figs. 1—4. Prepared by the ammonia method. In Figs. 1—3 the double coiled nature of the chromonemata is more or less clearly seen. In Fig. 4 it is concealed.

Fig. 5. Prepared by the ordinary method of smearing, stained with N. V. E.

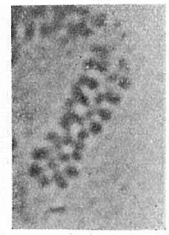
Figs. 6—10. The same stained with acetocarmine. In Figs. 6—8 the double coiled aspect is recognizable in places; in Figs. 9—10 this structure is largely destroyed by the intentional pressure on the cover glass.

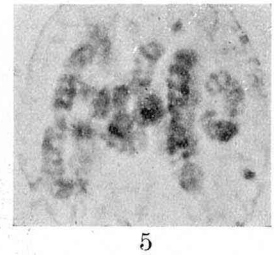
Figs. 11—16. Prepared by the ammonia method. Arrows indicate doubleness of the spiral. Fig. 11a, b, c, are three consecutive optical sections. In Figs. 14 and 16 the coils of the higher order are drawn out to a greater or less extent; in the latter figure it is carried out to the extreme, giving rise to the disappearance of the ordinary compact form of chromosomes in the I-metaphase.


1


2


3


4

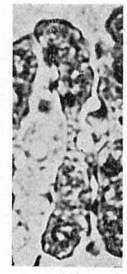

5

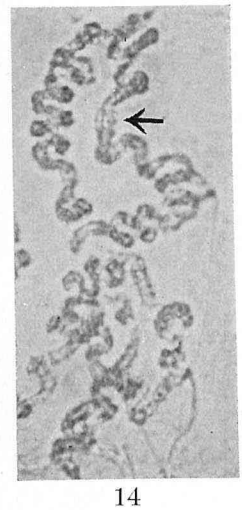

6

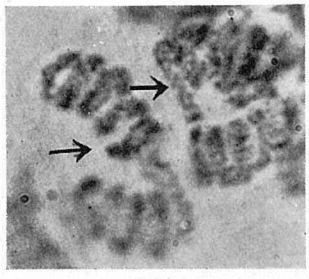

7

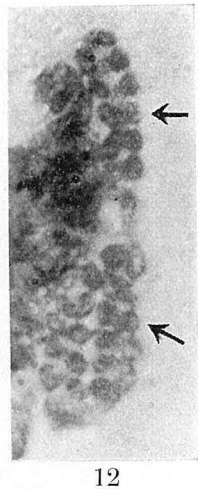

8

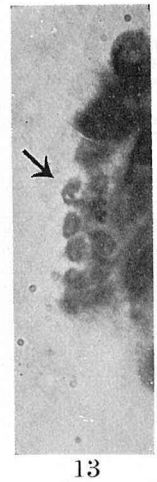

9

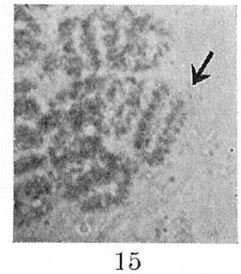

10

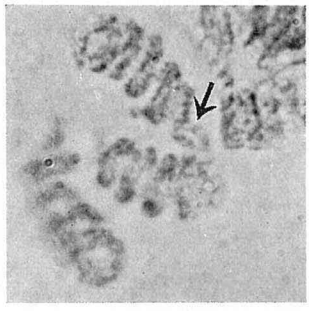

14

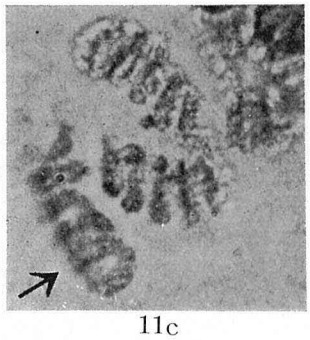

11a

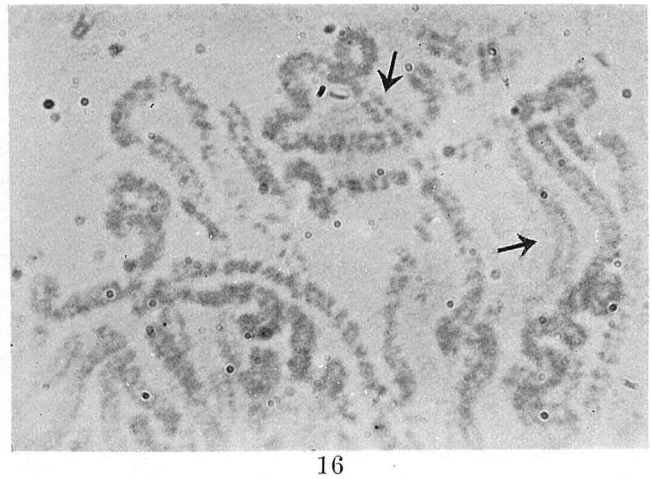

12


13


15


11b


11c


16