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On the Structure of the Anaphasic Chromosomes in the Somatic Mitosis in *Vicia Faba*, with Special Reference to the So-called Longitudinal Split of Chromosomes in the Telophase.

By

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With Plate I.

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In my previous paper (1921) on the so-called longitudinal split of chromosomes in the telophase I expressed the view that "there is some probability that certain mechanisms exist, probably during the process of shortening, which give such a basis to the telophasic chromosomes as to present a feature of zigzag or coiled arrangement of the essential parts". At that time it seemed to me that a spiral or zigzag aspect of the chromatic threads in anaphasic chromosomes shown in Fig. D in SHARP'S paper (1913) on the somatic chromosomes in *Vicia*, an aspect which was rightly held by him to be an artifact, might suggest what the structure of the anaphasic chromosomes was, the structure which may account for the mechanism, because I thought that there must be a certain condition in the chromosome which gave rise to such. But I had myself no such evidence to support my view, and I hesitated to discuss the problem further. The year after my paper had been published (1922), MARTENS' paper on the cycle of the somatic chromosomes in *Paris quadrifolia* L. appeared, in which a spiral or zigzag structure of chromosomes in various stages of mitosis was schematically illustrated, and I came to hope that more knowledge of the structure of the anaphasic chromosomes might be reached than we have at present. In this hope, I studied chromosomes in root-tips of *Vicia Faba* fixed with FLEMMING'S strong solution which had proved to be the best in my previous work, but I was unable to demonstrate anything new. On examining, however, a material fixed with BENDA'S modification of FLEMMING'S solution I got a preparation, in which a beautiful spiral or a like structure was found in chromosomes in every mitosis. I doubted at first if it was a normal occurrence in this fixation, but on repeating the same procedure in making preparations I came to the conclusion that it was a normal occurrence, if a material was fixed with BENDA'S solution, though this fixative was inferior to FLEMMING'S solution for other stages. This spiral structure of the anaphasic chromosomes in the somatic mitosis in *Vicia* is seen also in Fig. 53 of ROBYNS' paper, which was drawn from a material fixed with BENDA'S solution. BARANETZKY described and illustrated in his Fig. 41 this spiral structure of the chromosomes in *Tradescantia* in the fresh state as early as 1880. His view was contested by BALBIANI, STRASBURGER and others, who regarded this structure as simply a series of chromomeres closely arranged one after another, and has been rather ignored by his contemporaries and succeeding investigators[1]. VEJDOVSKY (1911–1912) has observed the structure in the chromosomes of *Ascaris* and other animals and has designated the spiral threads " chromonema ".

Now that it is very important to get more precise knowledge as to the structure of the anaphasic chromosomes, which may enable us to judge which of the two opinions in regard to the stage at which the longitudinal splitting of chromosomes takes place is right, I shall give the results I have so far obtained briefly in the following lines.

I used as material root-tips of *Vicia Faba* fixed with BENDA'S solution and imbedded in paraffin in the usual way, the alcohol used for dehydration being in a series of percentages beginning from $2\frac{1}{2}$ %.

1) See VEJDOVSKY, *p.* 6 *ff.*

For staining, HEIDENHAIN'S haematoxylin has been exclusively used.

The spiral aspect of the chromatic thread in the chromosomes can be most clearly seen in the anaphasic chromosomes. It can be seen only in well differentiated preparations, in which the matrix of the chromosome is faintly stained or entirely uncoloured, while the spiral chromatic thread remains deeply stained. One can hardly hope to get an ideal differentiation in all the chromosomes in a mitotic figure and also in all the parts in a chromosome. In some parts of the chromosome, the structure is in fact very clear, while in others it may be obscure, the variation being probably due to two conditions, fixation and differentiation. It may be objected that this spirality seen in the chromsomes is only an artifact such as SHARP has drawn in his Fig. D. It may, indeed, in a strict sense, not be natural. All those observed in fixed material must be at least more or less different from their natural state. Nevertheless they enable us to get a glimpse of their morphological characters or structures in the natural state or at least the general principle of the structure. For instance, the fibre structure of the achromatic spindle, in which the points of attachment of the fibre to the chromosomes are very important in the study of the behavior of chromosomes in mitosis, can only clearly be recognized in fixed material, and yet we have every reason to believe that it should not be overlooked as merely an artifact. The spiral structure of the chromosomes makes its appearance as constantly as other structures observed in the fixed materials do, structures, the existence of which is generally accepted as actual. Moreover, as will be seen later, we have reason to recognize it as showing the natural state of of the chromosome structure.

I use the term "spiral", but it is of course not a regular spiral. It may appear, in fact, more or less regular in a certain chromosome or in a certain part of a chromosome. Generally speaking, however, it is by no means regular. The pitch of the spiral may be different at any level of the spiral, and the irregularity may go even so far that one can hardly recognize the spiral character. Even in the cases where the spirality is rather regular, the diameter of a cross section of the spiral at one level may be different from that at another (see Figs. D, E, F, G, H, I, J, and K for these descriptions). These irregularities seem to suggest that the spirality is an outcome from what we may suppose to occur when a more rigid thread stretched out in a straight line and imbedded in a less rigid matrix is put under a stress along its long axis as a consequence of contraction of the matrix. The spirality seems to tend to grow more regular, as the stages go on.

In his very careful investigations on the chromosomes of *Vicia Faba* SAKAMURA has presented excellent illustrations of the M-chromosome in stages of metaphase and anaphase. I tried to take measurements of the length of these chromosomes in his illustrations, and got the following results[1] :—

In early metaphase	(Pl. XIII,	Fig. 1) ········	52 mm
,, ,, ,,	(,,	Fig. 2) ········	54
,, later ,,	(,,	Fig. 4) ········	50
In anaphase	(,,	Fig. 8) ········	44

These are the M-chromosomes in the somatic mitosis and all are magnified to the same degree. I tried again to measure the M-chromosomes in the heterotype division which are reproduced in his Figs. 24 and 25, both magnified to the same degree, but on a smaller scale than those in the somatic mitosis. The results are :—

In early anaphase	(Pl. XIII,	Fig. 24) ·····	30–32 mm
,, later ,,	(,,	Fig. 25) ······	29

These results show that the chromosomes in mitosis get more shortened as the stages go further. It may be said without any exact measurement that the chromosomes in the telophase are much more shortened than those in the earlier stages, and those in the prophase are longer than those in the metaphase and anaphase (SHARP, 1921.).

1) I did not try to measure the chromosomes drawn in his Fig. 3, as they are folded in the middle, so that an exact measurement can hardly be obtained.

LUNDEGÅRDH says: "Wie sich die Chromosomen in der Metaphase fortwährend verkürzen und verdicken, machen sie es auch in der Anaphase, etwa, was schon FLEMMING (1882, S. 236) beobachtet hat" (1912, p. 449). FLEMMING has a statement to the effect that a short and thick chromosome in the telophase is much shorter than that in the anaphase and is in volume nearly as large as one of the split halves of the metaphasic chromosomes, which is much longer than this.

The data just given are in keeping with the view that the spirality of the chromatic thread seen in the anaphasic chromosomes may have a causal relation to the contraction of the chromosomes. What, then, is the structure of the chromosomes in metaphase and prophase, where the chromosomes are much longer than those in the anaphase? We never find the spiral structure in these chromosomes such as is seen in the anaphase, but a string-of-pearls structure. This latter structure is especially prominent in a late prophase or an early metaphase where the longitudinal halves of the chromosomes are still lying side by side. A paired arrangement of the "pearls" or chromomeres is seen here (Fig. A). Not seldom a part of a string made up of a series of these chromomeres, where the pearl structure is very obscure, as is usually the case with the anaphasic chromosomes, shows a zigzag aspect (Figs. B, C), which may be looked upon as the beginning of the spirality. Somewhat the same aspect is drawn by LUNDEGÅRDH (1912) in his Fig. 44 (a double chromosome lying horizontally at the right-centre of the figure). As to the origin of the spirality VEJDOVSKY (1911-1912) expresses the same view that a chromomere structure of chromosomes in an earlier stage gives rise to a spiral structure as a result of the contraction of the chromosomes (p. 62). He observes, however, this spirality in the chromosomes already in a late prophase in the spermatogenesis of *Decticus*. DEBSKI (1897) describes a distortion of the prophasic nuclear threads in *Chara*, but it seems to me that this has nothing to do with the present problem.

The chromomere structure of the chromosomes has been observed by many investigators. FLEMMING (1882) described and somewhat schematically illustrated this structure in metaphase as well as in prophase in 1882. STRASBURGER (1884) illustrated this in his Fig. 32 more in detail, in which "Microsomenscheiben" are shown to be connected with each other by "Hyalopasma". The chromomeres are arranged close to each other in metaphase or a late prophase, while they are distributed at certain distances from each other in an early prophase, each chromomere being connected with the matrix of the chromosome. This latter structure is demonstrated by MÜLLER (1912) and others. It has been shown by WENRICH that two given chromomeres have a definite distance between them, so that in the parasyndesis, homologous chromomeres are arranged in pairs (McCLUNG, 1924, p. 618). This paired arrangement in the prophase of the heterotype division has been clearly observed also by ALLEN (1905) in *Lilium canadense* and by myself in *Lathyrus*.

In a very early prophase in which chromatic anastomoses are just beginning to be broken down, we find a "new" chromosome running in an "old" chromosome in a form of zigzag or somewhat spiral (KUWADA, 1920). In these new chromosomes fixed with FLEMMING'S solution, I was unable to find any chromomere structure. The material fixed with BENDA'S modification was inferior to the latter for the study of prophase and telophase. When the prophase stage proceeds, and a true longitudinal splitting comes to take place, the chromomere structure is, however, often reported to occur. MÜLLER (1912) is of the opinion with which OVERTON (1922) agrees, that the chromomere structure is to be seen before the longitudinal splitting of chromosomes takes place (p. 9) which he maintains occurs in the prophase and first at thin portions of the chromosomes (p. 12), viz. portions between chromomeres. He seems to be inclined to think that this phenomenon shows a material difference between a series of portions of chromosomes (p. 12). The existence of the chromomeres is accepted by such a distinguished cytologist as STRASBURGER, while it is denied by some others. In the present paper I want to leave the problem as to the existence of the chromomere structure before the longitudinal splitting of the chromosomes open. At present, however, I am inclined to think, from the stand point of the chromosome theory of heredity, that the chromomeres appearing after the splitting of the chromosomes can not be looked upon as morphological units of the same order as those chromatic bodies appearing long before the splitting, even if these are actually proved to be present.

The view that an anaphasic chromosome has a spiral structure is in accord with results obtained by CHAMBERS and SANDS (1923) in the microdissection of fresh material, that the chromosome in the pollen mother cells of *Tradescantia* is an elastic, jelly-like, nodulated cylinder, and possesses a cortex which differs markedly in refractive index from its central core. It seems to me that the more detailed study of SANDS of the *Tradescantia* chromosomes gives to this view a valuable support, although he has not laid stress on the spiral structure which he has observed (SANDS, 1923). The ring structure of a cross section, or of an end view, of a chromosome, and a central dark line in the anaphasic chromosomes seen in fresh material or a light line seen in fixed preparations, structures which are reported by some investigators such as BONNEVIE (1908) and LUNDEGÅRDH (1910), are also in accord with this spiral structure view. When the constriction of a chromosome goes so far that it seems as if the chromosome were divided into two merochromosomes, there appears sometimes a small chromatic body in the region of constriction (Figs. L, M). The morphological nature of this chromatic body can easily be understood, if we admit the spiral structure of the chromosomes, by assuming that in this region of the constriction the contraction of the chromosome is not complete, so that there may sometimes appear small chromatic bodies or chromomeres, and this assumption would afford us a suggestion as to the reason why the position of the constriction in the chromosome is definite in a definite chromosome. It may be considered, that such constriction of a chromosome is intensified in the anaphase by a force pulling the chromosomes apart, but this is not the only cause of the constriction, because the so-called "satellites" ("Trabanten") which may be looked upon as a case of chromosome constriction (TISCHLER, 1922, p. 628) may be found in the nuclear plate, where no such force operates. These chromatic bodies can hardly be understood by explanations such as those put forward by some investigators, that the chromosomes are of a tubular structure or made up of chromomeres which are cylindrically arranged in the matrix of the chromosomes. To compare these chromatic bodies with the chromomeres appearing in the metaphase I have reproduced them in Figs. N and O in the same magnification as the latter, which are reproduced in Fig. I. We find in these figures that both are nearly of the same size. In this connection I should like to mention that in the reducing division we often encounter the fact that there is a fine stained thread connecting the ends of the constituent chromosomes of a geminus which are being pulled apart towards the poles. The origin of the thread is probably due to the viscous nature of the chromosomes. If the chromosomes have a spiral structure, we can then consider the possibility that the chromatic spiral may be straightened out at the fusion region of the chromosome ends by the force pulling the chromosomes apart. If it actually occurs, there may be a case where a certain number of chromomeres are found in the thread. In such a case it would be natural to suppose that the chromomeres are no longer lying in contact with each other, but that on account of the force setting in they are brought back to the earlier condition, where they were arranged with certain distances in between. This condition is found in Fig. 15, b, Pl. 3 of McCLUNG's paper (1919) on the multiple chromosomes in *Hesperotettix viridis*, and it would have a bearing upon the phenomenon of the "Verlustmutation", if we assume the chromomeres as the seat of the inheritance units.

In the anaphase the chromomere structure is normally obscure, this probably being due to the fact that the chromomeres are brought into so close contact with each other by the stress produced as a result of contraction of the matrix of the chromosomes. But sometimes this structure can be seen with certain accuracy[1] (Fig. J, the lowest chromosome in the figure, at lower end). In this state of affairs the chromosomes would appear as if they were longitudinally split along their long axis as observed by Miss MERRIMAN (1904) and others. If the chromomeres in the spiral are longitudinally so arranged as to make four rows of them along the long axis of a chromosome, so that the chromosome may appear in the side view as if they consisted of two rows of chromomeres, another two lying behind them, the clear space between the chromomeres on a given level of the spiral will be continuous with that on the next level, so that the whole feature would suggest the longitudinal split of the chromosome. This situation of the chromomere arrangement is clearly demonstrated in *Tradescantia* by SANDS (1923). A certain chromosome was found "to have a rather regular four-rowed construction".

Now I understand the life history of the chromosomes provisionally as follows :— The chromomeres are obscure when the chromosomes are very young, but at about the time of, or a little after the longitudinal splitting of the latter they appear to be present. In the earlier stage the chromomeres stand at certain distances from each other, but in the later prophase when the chromosomes are contracting in their length, the distances between them become shorter until at last each chromomere comes in contact with another (cf. MÜLLER'S Figs. 10, 12). Thus the chromosome presents a pearl-necklace structure. When the stage comes to the metaphase and the chromosomes become much shorter, the string of pearls begins to take a zigzag form. I do not think, however, that the stage in which the spirality begins to appear is the metaphase also in other organisms as in the present case with *Vicia*, but it may be different in different organisms. In *Decticus* (VEJDOVSKY, 1911–1912) it must have occurred earlier than in *Vicia*. It is not, however, to be expected that the spiral structure can

1) It seems to have a causal relation to fixation.

be seen before the longitudinal splitting of the chromosome. If such is said to occur, further critical investigations will be needed, because we shall then encounter certain difficulties which may not be in accord with the chromosome theory of heredity. WILSON (1925) discusses this point and points out the weakness of the chromonema hypothesis from this point of view (p. 896 ff). In the anaphase, where the chromosomes are still shorter, the process of zigzag or spiral formation is complete in every part of the chromosome, thus presenting the spiral structure of the latter. In late anaphasic or early telophasic chromosomes this spiral structure was not clearly seen in my preparations. A chromomere-like structure is sometimes to be seen. It is not a real chromomere structure, but I think, turns of the spiral closely in contact with each other present such a feature. A spiral structure can clearly be seen in the telophase only when a certain structural change or "vacuolization" takes place in the chromosomes. In this stage the essential part of the chromosomes seems to be zigzag rather than spiral. This structure is maintained throughout the interphase or so-called resting stage, although it becomes obscure in the chromatic anastomoses. When the nucleus is ready for the next division, however, the anastomoses are broken down, and individual chromosomes become again clear. A zigzag new chromosome appears in the old chromosome as a result of breaking down of the chromatic anastomoses. They soon begin to straighten out. This straightening out seems to be connected with lengthening of the chromosomes (growth period), at full length of which the longitudinal splitting is supposed to occur. Thus the life cycle of the chromosomes is complete.

If we accept the theory of the linear arrangement of the genes in the chromosomes, the spiral structure of the anaphasic chromosome must stand utterly opposed to the opinion that the longitudinal splitting of the chromosomes takes place in the anaphase or telophase. If it does occur in these stages of mitosis, it will not result in qualitatively equal halves of a chromosome and as a consequence of this, the division is not equational, but a reduction in a certain sense, because the longitudinal splitting of the chromosomes in these stages does not mean a longitudinal splitting of the chromatic spiral or the essential part of the chromosome which is the only structure in the chromosome that retains its individuality throughout all the stages of the life cycle of the chromosome, but a transversal division of each turn of the spiral.

In conclusion I wish to express my sincere thanks to Prof. E. B. Wilson of Columbia University, New York, for the encouragement and suggestions conveyed in his letter.

Literature cited.

ALLEN, C. E. (1905), Das Verhalten der Kernsubstanzen während der Synapsis in den pollenmutterzellen von *Lilium canadense*. Jahrb. f. wiss. Bot. XLII.

BARANETZKY, J. (1880), Die Kerntheilung in den Pollenmutterzellen einiger *Tradescantien*. Bot. Zeit. Jahrg. XXXVIII.

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

CHAMBERS, R. and SANDS, H. C. (1923), A dissection of the chromosomes in the pollen mother cells of *Tradescantia virginica* L. Journ. Gen. Physiol. Vol. 5.

DEBSKI, B. (1897), Beobachtungen über Kerntheilung bei *Chara fragilis*. Jahrb. f. wiss. Bot. Bd. XXX.

FLEMMING, W. (1882), Zellsubstanz, Kern und Zelltheilung. Leipzig.

KUWADA, Y. (1921), On the so-called longitudinal split of chromosomes in the telophase. Bot. Mag. Tokyo. Vol. XXXV.

LUNDEGÅRDH, H. (1910), Ueber Kernteilung in den Wurzelspitzen von *Allium Cepa* und *Vicia Faba*. Svensk Bot. Tidsk. Bd. 4.

——— (1912), Chromosomen, Nukleolen und die Veränderungen im Protoplasma bei der Karyokinese. Beiträg. z. Biolog. d. Pflanz. Bd. XI.

MARTENS, P. (1922), Le cycle du chromosome somatique dans les Phanerogames. I. *Paris quadrifolia* L. La Cellule, t. XXXII.

McCLUNG, C. E. (1917), The multiple chromosomes of *Hesperotettix* and *Mermiria* (Orthoptera). Jonrn. Morphol. Vol. 29.

——— (1924), The chromosome theory of heredity. General Cytology edited by E. V. Cowdry. Chicago.

MERRIMAN, M. L. (1904), Vegetative cell division in *Allium*. Bot. Gaz. Vol. XXXVI.

MÜLLER, H. A. C. (1912), Kernstudien an Pflanzen. I u. II. Arch. f. Zellforsch. Bd. VIII.

OVERTON, J .B. (1922), The organization of the nuclei in the root tips of *Podophyllum peltatum*. Trans. Wisconsin Acad. Sci., Art, Lit. Vol. XX.

ROBYNS, W. (1924), Le fuseau de caryocinese et le fuseau de cytocinese dans les divisions somatiques des Phanerogames. La Cellule. t. XXXIV.

SAKAMURA, T. (1915), Ueber die Einschnürung der Chromosomen bei *Vicia Faba* L. Bot. Mag. Tokyo. Vol. XXIX.

SANDS, H. C. (1923), The structure of the chromosomes in *Tradescantia virginica* L. Am. Journ. Bot. Vol. X.

SHARP, L. W. (1913), Somatic Chromosomes in *Vicia*. La Cellule. t. XXIX.

——— (1921), An introduction to cytology. New York.

STRASBURGER, E. (1884), Die Controversen der indirecten Kerntheilung. Bonn.

TISCHLER, G. (1922), Allgemeine Pflanzenkaryologie. Handb. d. Pflanzenanat. Bd. II. Berlin.

VEJDOVSKY, F. (1911–1912), Zum Problem der Vererbungsträger. Prag.

WILSON, E. B. (1925), The Cell. New York.

Explanation of Plate I.

All the figures except Fig. J are microphotographs taken with Zeiss' apochr. imm. 1.5mm and a comp. oc. 12 and are enlarged from the original negatives to: Figs A, B, C, D, E, F, N, O and P, $\times 1.5 \times 1.5$ and Figs. G, H, I, K, L and M, $\times 1.5$ and reproduced without retouching. Fig. G is reproduced from an enlargement from an original plate taken by myself, by Prof. Y. KAMADA of the Imperial College of Applied Art of Tokyo, to whom I wish to take this opportunity of expressing my thanks. Fig. J has been drawn by me with the aid of an Abbe's camera lucida using Zeiss' apochr. imm. 1.5 and comp. oc. 18 for outlines and comp. oc. 12 for studies in detail. All the figures are from root-tips of *Vicia Faba*.

Fig. A. Chromosomes in metaphase, a double chromosome at left showing chromomere structure.

Fig. B. The same. About the middle, there is a double M-chromosome showing e-constriction, in which a part of one of the daughter-chromosomes shows a beginning of zigzag winding.

Fig. C. The same. Note a double chromosome at right. The zigzag aspect is seen in upper part of one of the daughter chromosomes at left.

Fig. D. Chromosomes in anaphase. Somewhat irregular spiral aspect of a chromosome is seen at right hand side of the figure.

Fig. E. The same. The spiral aspect of a chromosome is seen in the middle of the figure.

Figs. F, G, H. All from one and the same original negative showing a spindle figure in anaphase. Fig. F. Whole figure, in upper group of chromosomes of which there are two chromosomes at focus, both showing the spiral structure. Fig. G. Only the upper group is shown in the smaller magnification. Fig. H. Negative figure of the same showing the spiral structure of the left hand side chromosome in Fig. F and Fig. G (right hand side in Fig. H) more clearly.

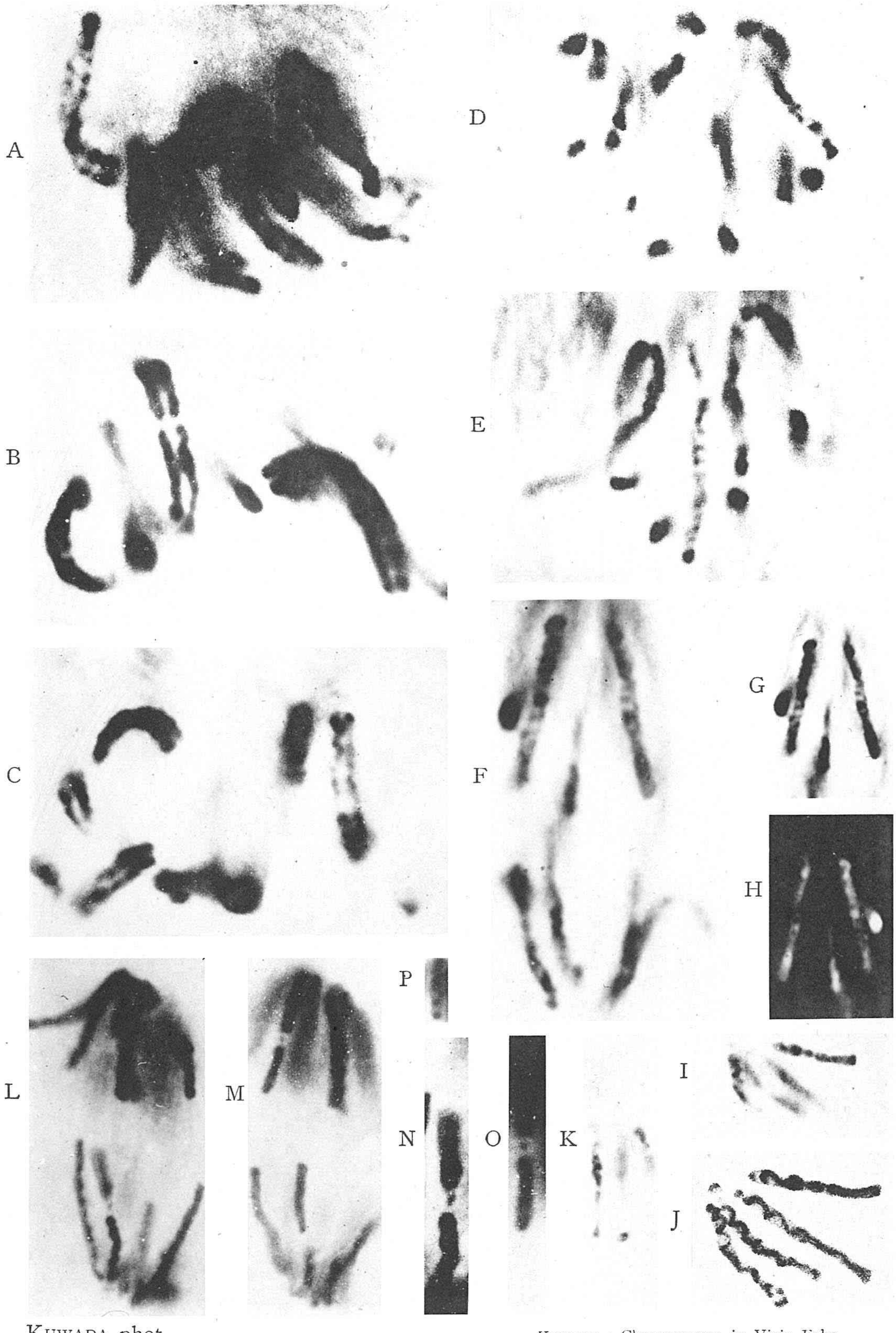
Figs. I, J. One of the chromosome groups in anaphase. In Fig. I the spiral aspect is seen at upper part of the lowest chromosome. Fig. J. Drawn from the same.

Fig. K. Anaphasic chromosomes, the left hand side one showing the spiral structure.

Figs. L, M. Two microphotographs at different focus from one and the same spindle figure, each showing a chromatic body in the region of e-constriction of the sister M-chromosomes.

Figs. N, O. M-chromosomes in Fig. L and Fig. M enlarged.

Fig. P. Upper portion of M-chromosome seen in Fig. M, showing its spiral structure. The chromatic body which is situated near the lower end of the portion is cut out from the figure. The portion is put in the same relative position as in Fig. M.



KUWADA phot.

KUWADA: Chromosomes in Vicia Faba.