Phase-contrast Cinematographic Studies on Meiosis in Orthopteran Spermatocytes

II. Chromosomal movement in the first meiotic anaphase

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The mitotic spindle plays its proper role in separating the daughter chromosomes to the poles in anaphase. Of all the mitotic processes, this anaphasic movement of chromosomes has been studied with the most zeal and a considerable number of the reports have been published. It was the principal purpose for this study to measure precisely this movement and to analyse quantitatively the data by correlating it with the structural changes of the spindle and cytoplasm occurring simultaneously. Besides the chromosome separation and the changes in diameters of the spindle and cell in the course of the separation, the present author's attention was drawn as well to the change of the chromosomal shape and the lines traced by the moving kinetochores through anaphase. Both data are indispensable to kinematic analysis of the anaphasic movement.

The author was able to observe and photograph with success the continuous spindle fibers by phase-contrast microscopy of the dividing spermatocytes of several species of grasshoppers. In the present report, he will deal with the observations and the results of measurements on the same materials and state a possible interpretation concerning the chromosomal movement in the anaphase I.

Materials and methods

The primary spermatocytes observed were those from the following kinds of grasshoppers: *Podisma mikado*, *Gastrimargus transversus*, *Dittoternis japonica*, *Oxya* sp., *Gampsocleis buergeri*, etc. Hanging drop preparations were made from the testis of these insects. The cells were photographed with an oil immersion phase-contrast objective (Tiyoda, dark-low contrast 90 $\times$) beginning with metaphase I ending with telophase I at intervals of 8 or 9 seconds. SAKURA-Konimicro 35 mm films were used and the exposure time was about 2 seconds. Details of the experimental methods were identical with those in the previous paper (Izutsu 1958). All procedure were carried out at room temperatures 25°–31°C.

To measure the chromosome separation and the changes in diameters of

the spindle and the cell, the negative films were projected with the aid of an enlarger on a table top and, on every 10th frame as a rule, outlines of the chromosomes, the spindle and the cell presented in the same optical cross section were traced on paper. The spindle was particularly well-outlined by mitochondrial bundles at the equator. The spindle poles could be identified as the points towards which the mitochondria or the spindle fibers converged. In some cases, it was possible to locate them directly as the points of centrioles when they were clearly photographed. The transverse diameter of the spindle was measured as the inner equatorial distance between the mitochondrial bundles covering the spindle surface. Since, however, the mitochondria are very fine and the border between the spindle and the cytoplasm was often invisible in *Gampsocleis buergeri*, no attempt was made to measure the diameters of the spindle (Fig. 4). For studies of the chromosome separation, each of the distances between the partner kinetochores, i.e. the leading ends, of both the separating chromosomes lying far from and near the spindle axis were measured. In addition, the longitudinal and transverse diameters of the cell along the spindle axis and the equatorial plate were measured respectively and simultaneously. The distances were then plotted against time.

Errors in measurements of the chromosome separation and the cell diameters were less than 0.2μ and those in measurements of the spindle diameters were less than 0.5μ , though they may have mounted up to 2μ in that of the spindle length.

In most cases, it was impossible to obtain real kinetochore paths of the chromosomes by tracing the successive positions of the kinetochores marked through film projection, because the cell and spindle continuously changed in their shapes and positions in the course of division. Accordingly, the kinetochore paths were studied on the graphs made by the following procedure.

Fig. 1. Side view of a primary spermatocyte metaphase (schema). P_1 and P_2 : spindle poles; B_1 and B_2 : bivalents lying at both ends of the metaphase chromosome configuration; $(d_1 + d_2)/2$: distance between the bivalents lying at both ends of the metaphase chromosome configuration.

The author chose the two bivalents lying at both ends of the metaphase chromosome configuration, whose two spindle poles were clearly photographed from metaphase I to the end of anaphase I. Four straight lines, each running through the kinetochores of the bivalents and parallel to the longitudinal axis of the spindle, were drawn. Then, each of the distances between the lines through kinetochores oriented towards the same spindle pole was measured (d_1 and d_2 in Fig. 1). The values of the arithmetic mean of these two distances ($(d_1 + d_2)/2$,

for brevity it may be referred to as the distance between the bivalents lying at both ends of the metaphase chromosome configuration) were plotted as

ordinate and the values of a half of the distance between the separating homologous kinetochores as abscissa. Since each bivalent has its own kinetochore distance, two curves were obtained from each motion picture sequence of anaphase separation. By this procedure it was possible to draw curves in every case where a true lateral view of the cell was provided and the direction of the division axis could be identified, even if the positions of the spindle poles could not be located accurately. When the resulting curves are parallel to abscissa at a given point of the axis, it is suggested that the kinetochore regions of a chromosome group passing towards the same spindle pole are moving parallel to each other. Further we might conclude that the kinetochore regions are closing in toward one another, if a curve is decreasing, and conversely that, they are diverging from one another, if a curve is increasing.

Results and discussion

In the spermatocytes observed, the metaphasic spindle is surrounded with thread-like mitochondria and the bivalent chromosomes are distributed through the spindle body. In the case of a univalent X-chromosome, however, it sometimes happens that its larger portion lies in the cytoplasm and only the kinetochore region exists in the periphery of the spindle. Though individual bivalents oscillate in this stage as if pulled a little towards one pole and then towards the other, the amplitude undergoes gradual decrease. They are some distance from and not adherent to one another, suggesting that there is a slight repulsion among them due to an electric charge. However, there are few exceptions, for example, in the case of *Gampsocleis buergeri*, in which the chromosomes adhere to one another in the normal metaphasic process of the second division.

1) Chromosome separation and changes in spindle and cell diameters in anaphase I

Graphs showing the chromosome separation as well as the changes in spindle and cell diameters were made from four motion picture sequences of the primary spermatocytes of *Gastrimargus transversus*, four of *Dittopternis japonica*, two of *Podisma mikado*, four of *Oxya* sp. and five of *Gampsocleis buergeri*. Figs. 2, 3 and 4 represent the graphs obtained in *Dittopternis japonica*, *Podisma mikado* and *Gampsocleis buergeri*, respectively. As shown in these figures, the shape of the chromosome-separation curve was almost sigmoid in every case: the separation velocity of the kinetochores increases gradually till it reaches a fairly constant rate, and decreases gradually at the end of anaphase. While the spindle, whose length attains a minimum at the end of metaphase or the beginning of anaphase, elongates definitely in anaphase, though the length often fluctuates in the first half of this period. At the end of anaphase, when its length reaches a maximum, the distance between the separating chromosome groups also reaches a maximum. It may be generally admitted that the chromosome separation begins almost simultaneously with an increase in transverse diameters of the cell and the spindle

and with a decrease in longitudinal diameters. A similar fact has been reported in the primary spermatocyte division of *Psophus stridulus* L. (Jacquez and Biesele 1954). This fact seems to suggest a tractile mechanism of the chromosome separation. For a decrease in the spindle length is what might be expected, if the pulling of the chromosomes by the chromosomal spindle fibers were causing a slight compression in the rest of the spindle. Therefore, these

Fig. 2. Graphs showing the chromosomal separation and the changes in spindle and cell diameters in the first meiotic anaphase of *Dittopternis japonica* at 28.5°C. The same cell as in Fig. 9.

somal fibers, and (2) the elongation of the spindle.

The curve of chromosome separation changes in shape with the temporal relations of the two processes. When they act simultaneously as in the spermatocytes of the grasshoppers, *Chorthophaga viridifasciata* (Ris 1949) and *Psophus stridulus* L. (Jacquez and Biesele 1954), and in the cultured chick and mouse cells (Hughes and Swann 1948), it is S-shaped. In cells in

these curves on anaphase movement seem to be accounted for by the view held by many investigators (Ris 1943, 1949; Hughes and Swann 1948; Inoué 1952; Jacquez and Biesele 1954; Makino and Nakanishi 1955; Amano 1957 and others), who consider that chromosome separation in anaphase consists of two separate processes, (1) the movement of the chromosomes to the poles due to the shortening of the chromo-

which both the processes are separated in time, i. e. at first the chromosome are drawn to the spindle poles and then, after an interval, the spindle stretches, the chromosome separation gives rise to an inflected curve, as shown in the newt cells (Boss 1954) and in the spermatocytes of the grasshoppers, *Podisma sapporensis* and *Acrydium japonicum* (Makino and Nakanishi 1955). According to the the above-mentioned classification the primary spermatocytes in every species of the grasshoppers studied by the present author belong to the first category.

In early telophase there is a period of pause, in which both the spindle elongation and the chromosome separation cease. In telophase, when the approach of the chromosomes to the nearest points to the poles is completed, the curve of the spindle length

Fig. 3. Graphs showing the chromosomal separation and the changes in spindle and cell diameters in the first meiotic anaphase of *Podisma mikado* at 29.5°C. The same cell as in Fig. 10.

is almost parallel to that of the chromosome separation. As the cleavage furrow deepens, the spindle is constricted by it and again elongates separating the chromosome groups further. These changes of the spindle in the cleavage phase may be just a reflection of cytoplasmic action, as suggested by Jacquez and Biesele (1954).

2) Continuous spindle fibers

As reported by the author (1958), the continuous spindle fibers were

possible to be clearly observed and photographed in the living anaphasic spermatocytes of *Dittopternis japonica*, *Oxya* sp., *Gestrimargus transversus*, *Patanga japonica*, etc. The image of these fibers is especially clear in anaphase of the cells whose metaphasic spindle has shown a distinct fibrous structure. In metaphase of such cells, however, it is difficult to discriminate these

Fig. 4. Graphs showing the chromosomal separation and the changes in cell diameters in the first meiotic anaphase of *Gamposcleis buergeri* at 29.0°C. The same cell as in Fig. 11.

fibers in vitro are in a pathological division, because in these cells the chromosome separation and the cleavage furrow progress quite normally, without any occurrence of pathologic phenomena such as chromosome stickiness or chromosome bridge. In addition, the shape of the chromosome-separation curve is almost identical with that in other normal anaphase. That of the spindle elongation and the time required from the beginning of anaphase to the appearance of cleavage furrow are also equal to those of normal control. This fact has been ascertained in the first spermatocytes of *Oxya* sp., *Gastrimargus transversus*, *Dittopternis japonica*, etc.

Ris (1949) attributes the spindle elongation in anaphase to an increase in its volume which manifests itself in a pronounced elongation. He maintains further that the growth is nowhere localized in the spindle. Recently, Amano (1957), however, has assumed in his new-extension-fiber theory that the spindle elongation results from an increase in length of the extension fibers, a new name of the continuous fibers termed by him. According to his theory, a total number of the extension fibers is nine, corresponding to that of canalicules included in a centriole, and they are skeletal structures of the

fibers from the chromosomal spindle fibers, owing to a visual obstruction of the halo around the chromosomes. But when the meiosis enters anaphase the continuous fibers are easily identified in the interzonal region. These are thin fibers (about 0.2μ in thickness), which are connecting the two spindle poles in the central spindle region, as observed in ordinary fixed preparations. In Figs. 5-8, the fibers photographed in the first division of *Gastrimargus transversus* are clearly demonstrated.

Now, it might not be admitted that such cells showing clearly the continuous

spindle which are produced and extended by the action of the centrioles, the spindle poles (Amano 1954. 1957). This theory, further explaining the fact that the centrioles, in some protozoa, produce the spindle fibers in the dividing stage and form the flagella in the resting stage, is interesting because the component fibrils of the spindle comparable in diameter to those in

Figs. 5-8. Course of division from early anaphase to telophase in a first spermatocyte of *Gastrimargus transversus*. In this cell the metaphase spindle showed distinct fibrillar structure. Arrows indicate continuous spindle fibers. Time interval from the commencement of photographing is indicated on each microphoto. Scale on Fig. 5. Room temperature: 29.0°C. Phase-contrast.

the flagella have been known to exist in several kinds of mitotic cells (Cleveland 1953 ; Inoué 1953 ; Takeda 1956, 1957 ; Tanaka *et al.* 1956 ; Amano 1957 ; de Harven and Bernhard 1956).

3) Paths of kinetochores from the end of metaphase I to the late anaphase I
In Figs. 9, 10 and 11 representative graphs are demonstrated, from which

we can obtain the informations of the lines along which the kinetochores move from the end of metaphase to late anaphase. Arrows in the graphs indicate the time of the completion of the separation of the homologous chromosomes

Fig. 9. Graph indicating kinetochore path from metaphase to late anaphase of a bivalent lying at one end of the metaphase chromosome configuration in a primary spermatocyte of *Dittopternis japonica*. Abscissa: a half of the distance between partner kinetochores of the bivalent. Ordinate: distance between bivalents lying at both ends of the metaphase chromosome configuration. The arrow shows the time when distal ends of the partner chromosomes in each of the two bivalents were just separated. For explanation see text. The curve on the other bivalent, which was similar in shape, was omitted.

lying at both ends of the metaphase chromosome configuration. The curves indicate that in Figs. 9 and 10 the kinetochore regions approach one another at the start of anaphase, and that in Fig. 11 they approach one another after diverging a little at the start of anaphase. After the distal ends of the chromosomes are completely separated, the paths diverge generally more or less from one another. There is then a period at which the kinetochores move parallel to each other, followed by their mutual approach in late anaphase. Assuming an error of two micra in the measurement of the spindle length, the calculated error in the value plotted as ordinate was about 0.6μ , and the error in abscissa was probably less than 0.2μ . Though the value of the above-mentioned changes in kinetochore paths did not exceed the

Fig. 10. Graph indicating kinetochore path from metaphase to late anaphase of a bivalent lying at one end of the metaphase chromosome configuration in a primary spermatocyte of *Podisma mikado*. Abscissa, ordinate and arrows are as in Fig. 9. For explanation see text. The curve on the other bivalent, which was similar in shape, was omitted.

given estimated errors in some cases, similar changes were found in almost every case and also, the curves which were made by plotting at as short intervals as possible gave rise to similar results. So it may be safely said

that such paths are taken in reality by the moving kinetochores in anaphase. On the other hand, transverse diameter of the spindle is unchanged (Makino and Nakanishi 1955) or increases a little (Ris 1949, Jacquez and Bieseke 1954) in anaphase. Concerning the width of the spindle the author has found no report maintaining its decrease in anaphase. Also in the spermatocytes measured in the present study the transverse diameter of the spindle increases at least in early anaphase (Figs. 2 and 3). It is clear, therefore, that the kinetochore paths obtained in the present study cannot be explained from the changes in the spindle width. A qualitative explanation by the author is as follows:

The mutual approach of the kinetochore regions at the start of anaphase in Figs. 9 and 10 appears to be explained from the poleward attraction onto the kinetochores through the chromosomal fibers: the component force acting parallel to the longitudinal axis of the spindle results in a stretch of the proximal chromosomal arms till the separation of the distal ends is completed, while the transverse component force shifts the kinetochores towards

the spindle axis. Thus, the kinetochores approach the spindle axis so long as the partner chromosomes do not get free from each other. In the mechanism at early meiotic anaphase, an autonomous terminalization movement of the chiasmata is also involved in addition to the pulling by the chromosomal fibers. Therefore, if the rate of terminalization movement is smaller than that of shortening of the chromosomal fibers, the kinetochore regions will approach one another at early anaphase as in the cases of Figs. 9 and 10. If the rate of terminalization-movement exceeds that of shortening of the chromosomal fibers, the kinetochores will tend to diverge from one another as shown in Fig. 11.

According to Freundlich (1927), Bernal (1940) and Östergren (1945), the spindle is most probably of a tactoid-like structure and, consequently, the chromosomes are influenced by a force which strives to remove them out of the spindle in a transverse direction (Östergren 1945). As the chromosomes are drawn towards the spindle axis against this force, it seems quite reasonable that the kinetochore regions should recede from the spindle axis just after the distal ends of the chromosomes are completely separated. As an additional force or the alternative to this force may be considered a repulsion among the chromosome bodies due to electric charge. This force, if present,

Fig. 11. Graph indicating kinetochore path from metaphase to late anaphase of a bivalent lying at one end of the metaphase chromosome configuration in a primary spermatocyte of *Gampsocleis buergeri*. Abscissa, ordinate and arrows are as in Fig. 9. For explanation see text. The curve on the other bivalent, which was similar in shape, was omitted.

will tend to separate the chromosomes from each other, thus acting in the spindle in the same manner as the force arising in the spindle tactoid.

The reason why the kinetochores then move along parallel lines would be that the spindle elongation takes place at this period and the force arising in the spindle tactoid and/or the body repulsion among the chromosomes keeps them at an almost constant distance from one another. At late anaphase, however, the kinetochores would be pulled to the spindle axis as a result of extreme shortening of the chromosomal fibers.

At mitotic anaphase of endosperm cells of *Haemanthus katharinae* Bak. reported by Bajer (1954), the kinetochore paths are somewhat different from those obtained in this study. In *Haemanthus katharinae*, the kinetochores move along parallel lines at the beginning of anaphase, and then deviate towards the spindle poles, and at late anaphase, chromosome paths again become parallel. According to him, the chromosome movement in this late anaphase is caused by the action of the "pushing body". In meiosis of the grasshopper spermatocytes the arms of the homologous chromosomes are associated in the chiasmata even at the beginning of anaphase, while the two chromatids in the mitotic cells of *Haemanthus katharinae* are attached to one another only by the kinetochore region (Molè-Bajer 1958). Therefore, the difference in the kinetochore paths between the two kinds of cells appears to be dependent upon the resistance against the poleward pulling force in the chiasmata and the kinetochore regions: the chromosomes of the spermatocytes need to be pulled polewards by a force stronger than that in mitosis, strong enough to make the kinetochores approach to the spindle axis against the opposing force arising in the spindle tactoid and/or the body repulsion among the chromosomes.

Summary

Phase-contrast cinematography was used for the study on the meiotic processes from metaphase I to telophase I in the spermatocytes of several species of grasshoppers, obtaining the following results.

- 1) The chromosome separation and the changes in spindle and cell diameters in anaphase I were measured on the cine-films. The graph of chromosome separation plotted against time was S-shaped in each spermatocyte examined. In the anaphase the chromosomes approach the spindle poles, while the spindle body elongates definitely, the two processes occurring almost simultaneously.

- 2) The continuous spindle fibers were clearly observable in the living, anaphasic cells. So these component fibers of the spindle exist in vivo as a morphological reality and are not an artificial product. Furthermore, it was conjectured that the elongation of the continuous fibers results in stretching the spindle in anaphase.

- 3) The kinetochore paths of the bivalents were followed from metaphase

I to the end of anaphase I on the cine-films. They are grouped into two types: i) in which the kinetochores approach to the spindle axis progressively from the start of anaphase to the complete separation of the chromosomal tips, and ii) in which they approach to the spindle axis after moving parallel to or receding a little from the axis at the start of anaphase. In both the types, the kinetochores recede somewhat from the axis just after the separation of the chromosomal tips and then, they move more or less parallel to the spindle axis, till they deviate towards the spindle pole at the end of anaphase.

4) All the results mentioned above seem qualitatively well explained on the following assumption: an autonomous terminalization of the chiasmata is active at the early anaphase I, the chromosomes are carried to the spindle poles by the traction of the chromosomal fibers and the elongation of the spindle, and the chromosomes are affected in the spindle by a force which tends to push them out of the spindle and/or by a body repulsion among them.

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