MONOCENTRIC MITOSIS WITH SEGREGATION OF CHROMOSOMES IN SCIARA AND ITS BEARING ON THE MECHANISM OF MITOSIS[1]

I. The Normal Monocentric Mitosis. II. Experimental Modification of the Monocentric Mitosis

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I. The Normal Monocentric Mitosis

It has become a generally accepted principle in cytology that a prime essential for complete mitosis is the presence of a bipolar or multipolar spindle (cf., *e.g.*, Wilson, 1925, p. 168; Gray, 1931, p. 172) and that in a unipolar field, although the chromosomes may divide, they cannot segregate into two groups. In flies of the genus *Sciara,* however, the first spermatocyte regularly undergoes as a normal process a monocentric mitosis which effects a regular and precise segregation of chromosomes (Metz, 1926*a*; Metz, Moses, and Hoppe, 1926). Since this process deviates widely from that observed in ordinary mitosis it has invited careful analysis, and has led to a series of studies involving several species.

Occurring as it does in the reduction division, the mitosis in question segregates homologous chromosomes. Consequently genetic as well as cytological methods have been used to trace the behavior of individual chromosomes (Metz, 1926*b*, 1927, 1928, 1929), and to determine the principle underlying the segregation process (see below). The present brief account is concerned primarily with evidence bearing on the mechanism of chromosome movement.

In the prevailing theories of mitosis[2] there has been a strong tendency to attribute the movement of the chromosomes to the poles in anaphase to forces of attraction or repulsion, or both, emanating from outside the chromosomes, and to consider the chromosomes themselves

[1] The second part of this investigation has been aided by a grant from the National Research Council, Committee for Research on Problems of Sex. The writer is indebted to Mrs. Philip B. Armstrong (M. Louise Schmuck) for assistance in that part of the investigation.

[2] See, *e.g.*, Wilson, 1932; Sharp, 1926; Bělař, 1929*a*, *b*, *c*; Gray, 1931.

as playing a relatively passive rôle, or at most as exerting a mutual repulsion.

So far as I am aware, no significant indications have been observed in ordinary mitoses that autonomous activity on the part of the chromosomes themselves plays an important part in their anaphase movements,[3] although various lines of evidence suggest that they may exhibit such activity at other stages.[4]

In the monocentric mitosis in *Sciara* the anaphase movement of certain chromosomes exhibits characteristics which strongly suggest that it is due in large part to the activity of the chromosomes themselves. These characteristics will be considered briefly below.

As noted in the papers referred to above, the monocentric mitosis in *Sciara* also presents evidence bearing on the much-debated question of the rôle played by the so-called " spindle fibers " in mitosis. It indicates that, whatever their precise nature may be, they represent a functional reality and reflect an activity operating on the chromosomes in the direction of the pole. (The active agent here may be the " insertion region " of the chromosome, or may be external to the chromosome.)

In the present studies observations have been made on both sectioned material and smears. In all cases the testes were dissected out and freed from surrounding material before fixation. On account of their small size, this permitted almost direct contact of the cells with the fixing fluid even in the case of the sectioned material. In the smears the cells were, of course, brought immediately into direct contact with the fixative. Excellent fixation was secured in both ways. Various fixatives have been used, but most of the observations have been made on material fixed in Gilson's mercuric-nitric mixture and stained in Heidenhain's iron haematoxylin. Flemming's fluid has been used, but with unsatisfactory results due to the presence of large chondriosomes which obscured the mitotic figure in most cells (see Metz, Moses, and Hoppe, Fig. 34). At the stage in question these chondriosomes usually form a saucer-shaped or cup-shaped mass lying peripherally and often extending about halfway around the cell. They have received special study by Mr. W. L. Doyle (paper in press).

Unfortunately details of the achromatic figure are not as satisfactorily brought out after fixation with Gilson as by other methods, and for this reason certain of the finer aspects of the figure have not yet been analyzed. The features essential for present purposes, however, are re-

[3] Since this was written, papers by Bleier (1931) and by Wilson (1932) have come to hand, both emphasizing the possibility of such autonomous activity. The literature on the subject is reviewed by both authors.

[4] See, *e.g.*, McClung, 1927; Lewis, 1932; Lewis and Lewis, 1932.

vealed clearly enough in the sections studied. In the smears the achromatic figure is so exceedingly delicate in structure as to be almost invisible, due presumably to the delicacy of the fixation and the similarity of the refractive indices of the materials in the figure. The general nature of the structure, however, is clearly indicated by the configuration and behavior of the chromosomes.

All the material is from pedigreed cultures which have been kept in the laboratory, in some cases for many generations.

The principal characteristics of the monocentric mitosis in *Sciara coprophila,* Lint. may be summarized as follows: (For details see Metz, Moses, and Hoppe, 1926.)

1. The spermatogonial chromosome group as shown in Text Fig. 1 typically consists of ten members: three pairs of rod-like chromosomes, one pair of small V-shaped chromosomes, and one pair of much larger V-shaped chromosomes (the " limited " chromosomes). The " limited " chromosomes are limited to the germ-line, due to elimination from somatic nuclei in early cleavage.[5]

2. No synapsis occurs. In prophase of the first spermatocyte division (Text Fig. 1) the univalent chromosomes appear to be distributed at random, but about equidistant from one another, about the periphery of the nucleus when the nuclear membrane breaks down.

3. After the nuclear wall vanishes a half spindle appears and the chromosomes, apparently without changing their locations, all become oriented toward the single pole, each with a " spindle fiber " extending from the normal insertion point on the chromosome toward the pole. No centrioles have been identified.

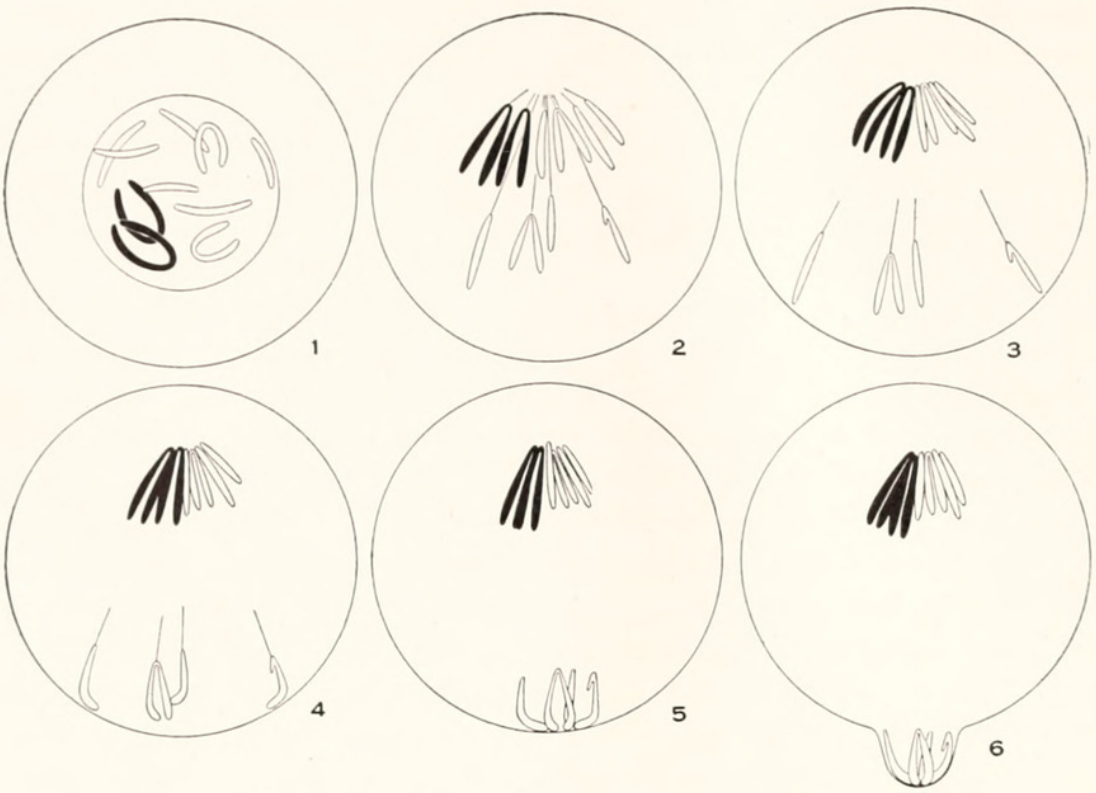
4. The chromosomes do not become arranged in any one plane and nothing comparable to an equatorial plate or metaphase stage is found. On the contrary, they move from their prophase positions either directly toward, or directly away from, the pole (Text Fig. 2).

5. Certain chromosomes regularly move toward the pole in the customary fashion. These include both of the large " limited " chromosomes and one member of each of the other four pairs of chromosomes. Genetic evidence indicates that among the latter four pairs it is regularly the maternal member which passes to the pole. This evidence appears to be complete for at least three of the four pairs in one species.[6]

6. The remaining four chromosomes—homologs of four which go to the pole—regularly move " backward " directly away from the pole, following radial, and hence diverging, paths as far as possible from the common center. When the chromosomes reach the periphery of the cell their courses are deflected and made to converge, ultimately bringing

[5] See Metz, 1931; DuBois, 1932; Schmuck and Metz, 1932.

[6] Metz, 1927 and unpublished evidence of Dr. Helen B. Smith and the writer.



TEXT FIGS. 1–6. Schematic representation of the movements of the chromosomes in the monocentric first spermatocyte division in *Sciara coprophila* Lint.

Diagram 1. Prophase just before dissolution of the nuclear membrane. The chromosomes are scattered, apparently at random, about the periphery of the nucleus.

Diagram 2. Beginning of migration of the chromosomes—six toward the pole and four directly away from it.

Diagram 3. Slightly later stage, after the six chromosomes have reached the pole and the other four have progressed to the periphery of the cell. The two median "retreating" chromosomes are presumed to be at high and low levels, respectively, and at the periphery.

Diagrams 4–6. Successively later stages showing the migration of the chromosomes along the periphery of the cell away from the pole, and the beginning of the extrusion process at the point farthest from the pole.

Actually the paths of the retreating chromosomes are only rarely equidistant from one another as shown in these diagrams (see text), and the chromosomes which pass to the pole do not ordinarily retain their polar orientation later than the stage shown in Diagram 3.

The two "limited" chromosomes are represented in black. These regularly pass to the pole and are retained. Among the other chromosomes precise segregation of homologs is effected. The hook at the proximal end of the retreating chromosome at the right, due to sub-terminal fiber attachment, is presumably characteristic of its homolog also, but is not evident in the latter, probably because of the lack of stretching. As will be seen from the camera drawings (Plate I), the retreating chromosomes are comparatively more elongate than the diagrams indicate. (See also Figs. 14–18 in Metz, Moses, and Hoppe, loc. cit.)

them together at a peripheral point opposite the single pole. Subsequently they are extruded in a finger-like process, where they degenerate (Text Figs. 3–6).

7. During their "backward" movement the four chromosomes retain their reversed orientation, moving with the spindle fiber insertion point hindmost, and the free end, or ends (in the case of the V-shaped member) foremost.

8. The posterior portion in these chromosomes is commonly taut and slender and often almost detached from the remainder, giving convincing evidence of a force, represented by the "spindle fiber," operating in the direction of the pole and in opposition to the movement of the chromosome (see Figs. 7 and 8).

9. In contrast, the apical ends of these chromosomes are thick, usually curved or twisted, and lie free, with no indication of any traction operating on them (see photographs, Plate I). It is clear that they are not being "pulled" in the direction of movement.

It is thus possible to distinguish the operation of two forces on the four retreating chromosomes. One is a retarding force, represented by the so-called "spindle-fibers" operating in the direction of the pole and only on one region of the chromosome, the insertion point. The other, and superior, activity operates in the opposite direction, and involves the chromosome as a whole, serving to cause the movement described above. It is the latter activity which seems to be performed mainly by the chromosome itself rather than by an outside agent.

As noted previously (Metz, 1926*a*), the chromosomes, during the movement in question, give the appearance of being carried by currents in the protoplasm. But careful study of a large amount of material prepared in various ways has convinced me that no true currents are involved and that the only flowing motion concerned is that of the material immediately around the individual chromosome. The details of the process will be presented in another paper. They may be summarized, however, by saying that each of the retreating chromosomes moves off independently, away from the pole, either in a small island of material (gel?) from the original half-spindle, or at the apex of a pseudopod-like outgrowth of this material.

Some idea of the independent movement of these chromosomes, and of the general aspect of the figures, may be gained from the drawings shown in Figs. 7, 8, and 10 and from the photographs in Figs. 15–17. For descriptions of these see explanation of figures.

That the movement is due to an activity of the chromosome itself is suggested mainly by two features. The first of these is the independent action of the individual chromosomes, each of which not only follows its

own course, but follows a course apparently determined only by the original position of the chromosome in relation to the pole. This original position appears to be determined at random. If two or more of the four chromosomes happen to be adjacent at the beginning they move away together and follow essentially the same path. If they are well separated at first their paths diverge widely (see, *e.g.*, Fig. 17). Furthermore, the path of any individual away from the pole may follow any radial line within the theoretical cone delimited by imaginary extension of the half spindle. In other words, it seems impossible to explain the phenomenon on the basis of predetermined currents or lines of flow in which the chromosomes are caught and transported.

The second feature concerns the position of the chromosome in the island or process of " spindle " substance in which it moves. This position is not easily determined with accuracy, for only occasionally is the outline of the mass revealed. However, numerous large chondriosomes and other stainable constituents present in the cytoplasm sometimes delimit the clear mass distinctly. Careful study of such cases indicates that typically the position of the chromosome is apical. Since, as already noted, there is clear indication that the movement of the chromosome is retarded by an activity represented by the " spindle fiber," it seems improbable that the chromosome would remain at the apex of the moving mass unless it were itself responsible for the movement. In other words, were the chromosome simply being transported by an outward flow of the material from the half spindle, the retarding " spindle fiber " force would presumably cause it to move less rapidly than the surrounding material and hence not remain at the apex of the outflowing mass.

As a result of the above considerations and others of a more detailed nature, the hypothesis is advanced that the retreating chromosomes move because of their own activity. It may further be postulated that this activity operates by bringing about a progressive alteration in the physical state (*i.e.*, by solation or gelation) of the protoplasm adjacent to the chromosome.[7] Presumably the simplest form of such activity would be one producing a change in front of or behind the chromosome—the former serving to pull the chromosome along, so to speak, and the latter acting more as a pushing agent. Without attempting a detailed discussion of such possibilities, it may be noted in this connection that the

[7] This hypothesis has been under consideration for more than two years, but has been withheld pending the completion of experiments which show that the chromosomes in question are alive and functional (see below). The possibility of bodies moving through the protoplasm by such means has been suggested to me by several physiologists, particularly Dr. Selig Hecht and Dr. S. O. Mast. To them and to Dr. Ralph S. Lillie I am greatly indebted for advice on numerous questions which have arisen in connection with the present study.

chromosomes do not give the appearance which would be expected if the activity were operating solely on the anterior (distal) ends. This is particularly true in the case of the V-shaped member. Often the two arms of this V are deflected near the ends in opposite directions, yet they do not continue to move in opposite directions and pull the chromosome out into a straight line. Rather, the chromosome as a whole continues to move, even with the apices deflected. This would indicate that the activity is graded along the length of the chromosome—or, perhaps more probably, that it is an activity not only of the stainable portion of the chromosome proper, but also of the chromosome sheath, and hence that it involves a relatively large mass of material whose shape may be modified without seriously affecting the movement.

Any antero-posterior differentiation of the chromosome required by such hypothesis is readily conceivable, since the "insertion point" of the spindle fiber distinguishes the posterior end of the chromosome and may well influence the general activity of this region.

In the above considerations two possibilities have been omitted which may immediately be raised as objections or alternatives to the hypothesis presented. One is the possibility that the movements under consideration result from an electromagnetic activity and simply reflect a repulsion between the four retreating chromosomes and the pole. Perhaps such a possibility cannot be ruled out at present; but the many difficulties it encounters when applied to mitosis in general [8] make its validity seem very doubtful. For this reason it is being considered only as a last resort.

The other possibility is that the chromosome movements under consideration do not represent true mitotic activity at all. Since the four retreating chromosomes are ultimately cast off and degenerate, it may be suggested that they are already inert and degenerating during anaphase and hence that they are to be considered essentially as foreign bodies whose elimination has no connection with the process of mitosis. This possibility seems to be definitely ruled out by the experimental evidence considered below.

If the hypothesis of chromosome movement presented above is correct it should be applicable to ordinary bipolar mitosis. In such ordinary mitosis the two forces or activities here identified because of their antagonistic action would have to act in the same direction and supplement, instead of oppose, one another. This feature will be considered in a subsequent, more detailed, account.

[8] See, *e.g.*, Wilson, 1925, p. 186; Gray, 1931, p. 167.

II. Experimental Modification of the Monocentric Mitosis

As intimated above, when one studies the normal processes of monocentric mitosis in *Sciara* spermatocytes, he is at once confronted by the question as to whether the chromosomes which retreat from the pole are in a normal state of activity or are in a degenerating condition. The answer to this question bears not only upon the immediate problems involved in the present paper, but also upon other aspects of our studies—particularly those concerned with sex determination, the influence of sex on chromosome behavior, and the interrelation between cytoplasm and chromosomes. For this reason especial attention has been devoted to the subject and numerous experiments have been performed in an effort to modify the normal procedure in such a way as to give critical evidence. Such evidence has now been secured. It is considered below, together with other features bearing on the question at issue.

It is evident, as already indicated, that normally the chromosomes which are cast off in the polar-body-like process or "bud" eventually degenerate. This is true not only in the case of the monocentric first spermatocyte division, but also in that of the dicentric second spermatocyte division which, of course, immediately succeeds the first. The ultimate degeneration in both cases is readily understood, because the "bud" contains almost nothing but the chromosomes, and in the absence of cytoplasm these obviously could not long continue their normal development.

The immediate problem is to determine whether, in the monocentric division, this degeneration takes place after the "bud" is extruded or is already well under way during the division itself. Before considering the experimental evidence attention may be called to two features in the normal processes which strongly suggest that the former alternative is correct. The first of these is the fact that after the chromosomes are extruded in the "bud" they appear to undergo changes in form and structure similar to those exhibited by the chromosomes retained in the cell, although it is difficult to trace these activities in detail in the narrow compass of the "bud," and they apparently do not continue through more than the interkinesis period. They do seem to indicate, however, that the discarded chromosomes are not inert at the time they are discarded.

The second line of evidence comes from the second division. It seems clear that, although the chromosomes which are discarded at this division eventually degenerate, they are not degenerating during the mitotic process, for the mitotic figure here is bipolar, the chromosomes show essentially typical behavior, and those which are discarded are sister halves of those which remain in the functional cell (spermatid).

Similar evidence is, of course, provided by the well-known phenomena of polar body formation in eggs, which show that the ultimate fate of chromosomes is not necessarily an indication of their condition during the preceding mitosis.

The final, and apparently conclusive, evidence, however, comes from experiments which show that when retained in the cell, instead of discarded, the chromosomes under consideration (in the monocentric division) remain alive and active, and hence that they must be alive and able to function during the mitosis itself.

The evidence is briefly as follows: Under suitable conditions, such as exposure to low temperatures,[9] the formation of the polar-body-like protuberance may be inhibited at either the first or second divisions. In such cases the chromosomes which would otherwise be eliminated are retained in the cell and are thereby enabled to continue functioning and to give evidence of their condition.

In the monocentric mitosis it is apparently possible to stop the " backward " movement of the retreating chromosomes at any stage of what would correspond to anaphase in typical mitosis. The subsequent positions of these chromosomes, and to some extent their behavior, appears to be determined by the time at which their migration is stopped. This is inferred from the fact that in the preparations examined the chromosomes are in the various positions corresponding to those through which they normally pass during the monocentric mitosis.

The details of these features will be left for a more complete account. Present interest centers in the fact that the retreat of the chromosomes has been stopped at various stages and that preparations have then been made at various subsequent time intervals. In consequence it is possible to reconstruct the sequence of stages showing the behavior of these retained chromosomes during the ordinary transformations of the second spermatocyte.

Development of the second spermatocyte in this treated material apparently progresses in the normal fashion. The chromosomes of the regular nucleus go through the usual interkinesis, prophase, metaphase, and anaphase stages. Coincidently the four artificially retained chromosomes go through a corresponding series of stages up to late prophase or metaphase. They become diffuse or reticular like the others during the telophase and interkinesis (see Fig. 12) ; they divide, and they condense into split prophase chromosomes during the prophase of the second spermatocyte. They appear to undergo these transformations

[9] The precise nature of the treatment required has not yet been determined. The conditions described here were first found in material purposely subjected to cold. Similar conditions have been observed in a few specimens taken from laboratory stocks which had probably been exposed to low temperature through cooling of the room.

regardless of their position in the cell or the stage at which they were interrupted in the monocentric division. They also appear to act independently of one another, in the sense that they exhibit essentially the same characteristics when widely separated from one another as when close together (see Figs. 13 and 14). When separated each is surrounded by a relatively transparent layer of protoplasm, which I interpret as an integral, although perhaps transitory, part of the chromosome, representing the chromosome "sheath" described by numerous observers in ordinary mitoses.[10] When close together, the chromosomes give the appearance of lying in a small nucleus; but I have been unable to detect a nuclear membrane and am inclined to attribute the appearance to the coalescence or approximation of the transparent layers or sheaths around the chromosomes.

In most cases these artificially retained chromosomes do not exhibit an increase in size corresponding to that of the chromosomes in the normal nucleus. They vary considerably in this respect and the evidence as a whole suggests that the growth is dependent on the position of the chromosome with respect to the normal nucleus—being greatest when there is a close proximity (see Fig. 14). This in turn suggests that the nature of the protoplasm immediately surrounding the chromosome is an essential factor in respect to growth, and that only the environment provided by the nucleus itself is suitable for promoting normal growth.

In no case has it thus far been possible to inhibit the monocentric mitosis in such a way as to retain all the chromosomes in the nucleus and thus get a second spermatocyte nucleus containing the total diploid group; but it seems almost certain from the above evidence that if this could be done the growth and activity of the chromosomes which are ordinarily eliminated (paternal members) would be equivalent to that of their (maternal) homologs.

As would be expected, the correspondence between the transformations of the retained chromosomes and the others ceases at the metaphase of the second spermatocyte division. In the absence of any mitotic apparatus (asters or spindle) the daughter halves of the artificially retained chromosomes cannot separate, hence they remain together during the anaphase of the second division. Whether or not they would subsequently exhibit further activity during the development of the spermatid has not been determined.

From the evidence presented above it seems clear that in the monocentric mitosis under consideration the retreating chromosomes are alive and are capable of taking an active part in the mitotic movements as postulated in the first part of the paper. This does not, of course, mean that they are necessarily entirely unaffected by the monocentric mitotic

[10] A good example is described by Metz and Nonidez, 1924.

phenomena under normal conditions, or that their activity is precisely like that of their homologs which go toward the pole. Indeed, it seems evident that a difference in activity does exist, that it is due to the sex of the parent from which the chromosomes were derived, and that it is in some way responsible for the opposite responses of the two sets of chromosomes.

SUMMARY

I

The present paper supplements previous studies on the monocentric first spermatocyte division in *Sciara* and considers the findings in relation to the general problem of the mechanism of mitosis. Contrary to generally accepted principles of mitosis, an accurate segregation of chromosomes is effected in a unipolar field during the mitosis in question. The segregation is highly selective in that paternal chromosomes react in opposite fashion from their maternal homologs and pass away from the pole instead of toward it. These chromosomes which "retreat" from the poles exhibit characteristics which are believed (1) to demonstrate the functional reality of the so-called "spindle fibers," which in this case retard the movement of the chromosomes, and (2) to suggest that the opposing force or activity responsible for the movement of the chromosomes is due to an activity of the chromosomes themselves. The hypothesis of "autonomous" movement on the part of the chromosomes is suggested particularly by two lines of evidence:

(1). Each retreating chromosome in its movement directly away from the pole acts independently of the others and may move along any radial line within the theoretical cone delimited by imaginary extension of the half spindle. Its path appears to be determined entirely by its original position with respect to the pole.

(2). Each retreating chromosome appears to move in a small mass of protoplasm derived from the original half spindle, and to maintain its position at the *apex* of this mass during the movement. On account of the opposing activity represented by the so-called "spindle fiber," it seems improbable that the chromosome could retain this apical position if it were not itself responsible for the movement.

The suggestion is made that the anaphase movement of chromosomes may be primarily due to such activity of the chromosomes themselves and that this activity serves to bring about movement by producing localized alterations in the viscosity of the adjacent protoplasm.

II

Experimental evidence is reviewed which shows that the retreating chromosomes are alive and therefore theoretically capable of function-

ing as postulated during the movements in question. This evidence also bears on the interrelations between sex and chromosome behavior to be considered subsequently.

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PLATE I

All figures are from spermatocytes of *Sciara coprophila* Lintner, fixed in Gilson's mercuric-nitric fluid and stained in Heidenhain's iron haematoxylin. Figures 7–14 are from camera lucida drawings made at table level, using 1.5 mm. objective and No. 12 ocular. They are reduced approximately three-fifths in reproduction. The drawings were made by Dr. Esther Carpenter, with the exception of that for Fig. 11 which was made by Miss Louise H. Buck. Figures 7–10 are from un-

treated specimens showing certain features of the normal monocentric mitosis; Fig. 11 from a specimen treated with cold; Figs. 12–14 second spermatocytes from specimens presumably exposed to cold in the laboratory during the first division. Figures 7–10 are from sections; 11–14 from smears.

FIG. 7. Primary spermatocyte in approximately the stage represented in Text Fig. 4. The group of six chromosomes has reached the pole and lost its earlier orientation. The four retreating chromosomes have begun to converge after reaching the periphery. Note the two chondriosomes separating the V-shaped chromosome at the left from the others and showing that this chromosome has moved through part of the chondriosome mass (only part of which is shown).

FIG. 8. Slightly later stage, corresponding approximately to that shown in Text Fig. 5. The chromosome in black at the right has apparently passed through the mass of chondriosomes along the path indicated. The chondriosomes in the center lie at about the level of the chromosome at the extreme left, which may have passed through the opening between the two clusters.

FIG. 9. Slightly earlier stage than the preceding. In this cell the four retreating chromosomes are not widely separated. They have come into contact with the saucer-shaped mass of chondriosomes preparatory to passing through it. The contact has evidently served to bend or fold their distal ends, giving a clumped appearance. This is shown clearly by the V-shaped chromosome at the left. Although not very evident in the figure, the transparent sheath around each chromosome is distinct in the preparation; it serves to keep the chromosome proper from touching the chondriosomes.

FIG. 10. Incomplete figure at approximately the same stage as the preceding. Three retreating chromosomes are shown—two rods and the V. The one at the left is at a higher level than the others and is completely cut off from the periphery by chondriosomes, only a few of which are shown. The sheath surrounding this chromosome is in contact with the chondriosomes. This chromosome would subsequently have passed between the chondriosomes to the periphery. Such cases seem to demonstrate that the chromosomes are not transported by currents and that each is at the apex of the outflowing mass of protoplasm in which it lies, if there is any such mass in addition to the transparent " sheath."

FIG. 11. Primary spermatocyte from smear made from material treated with cold for 48 hours and dissected and fixed in cold. Cell slightly flattened. The four retreating chromosomes are long and slender and widely divergent—the V-shaped one lying at the upper left of the figure. Whether or not their elongation is due to the cold is uncertain, but it is probable that their movements were greatly slowed down, if not stopped, by the treatment. The chromosome at the lower right lies partly among the chondriosomes and is curved partially around one of them as shown in the figure. The achromatic figure is not visible in this cell.

FIGS. 12–14. Second spermatocytes in which the chromosomes which should have been discarded at the first division have been retained, presumably due to exposure to cold.

FIG. 12. Interkinesis stage showing the chromosomes of the regular nucleus above and the artificially retained ones in a cluster resembling a nucleus below. The latter chromosomes are exhibiting the same interkinetic changes as those in the regular nucleus.

FIG. 13. Later stage from same testis as the preceding, showing the regular chromosomes, above, in metaphase of second division, seen in side view (compare with figures in Metz, Moses, and Hoppe, 1926) and the artificially retained chromosomes near lower right. The latter have divided and condensed and are in essentially the same stage as the others. Each is surrounded by its transparent " sheath," which in this case is clearly revealed by the relatively dark cytoplasm surrounding them.

FIG. 14. Later stage (middle anaphase) of second division from same testis. As in preceding three cases (all from smears) the achromatic part of the mitotic figure is practically invisible (see above under "methods"). The position of the spindle is clearly indicated, however, by the orientation of the chromosomes passing to the poles (for explanation of this mitosis see Metz, Moses, and Hoppe, 1926). The four lowermost chromosomes, all split, are the artificially retained ones—marked *r*. They lie near, but not in, one end of the spindle. The one shown in solid black lies at a high level directly above this pole of the spindle. Note that in this cell the retained chromosomes appear to be fully as large as those from the regular nucleus. In the cells of this testis only one large "limited" chromosome is present—a condition frequently found. The daughter halves of this chromosome in the present figure lie at the left of the spindle, going to opposite poles. The daughter halves of the small V are at the right of the spindle.

FIGS. 15–17. Photographs with explanatory drawings of three primary spermatocytes during the monocentric mitosis; from sections.

FIG. 15. A cell showing the retreating chromosomes shortly after they have reached the periphery and their paths have begun to converge. The chromosomes at the pole have lost their orientation and become irregularly clumped. *a*. Outline camera drawing; note that the V-shaped chromosome is at the right. *b*. Composite photograph taken at different levels to show relative horizontal positions of the retreating chromosomes. The two outer ones are at a high level and the two inner ones at a low level. *c* and *d*. Photographs at high focus and low focus, respectively, showing the features just mentioned.

FIG. 16. Similar illustrations of a cell at a slightly later stage. The V-shaped chromosome, near middle of the retreating group, and the rod at the right are at higher levels than the other two. *a*. Camera drawing (see Fig. 17, Metz, Moses, and Hoppe, 1926). *b*. Photograph at high focus showing the two upper chromosomes, with indications of the other two which are not entirely out of focus. *c*. Photograph at low focus, using higher magnification, showing the two lower chromosomes. As indicated by the figures, the paths of the retreating chromosomes are converging. The two lower members are moving upward and toward the right; the V is moving downward, and the rod at the right is moving downward and toward the left.

FIG. 17. Similar illustrations of an unusually small cell at an earlier stage than either of the two preceding. Three of the retreating chromosomes, two rods and the V, are readily seen at short distances from the other chromosomes, which are close together. *a*. Composite photograph taken at different levels. *b*. Outline camera drawing designed to supplement the composite photograph in showing the general composition of the figure, and the comparative horizontal positions of the chromosomes. Apparently the fourth retreating chromosome is the long one projecting from the compact group down toward the retreating V. This member is not completely separate from the group which has gone to the pole, although it lies below most of the members of this group. The identity of this chromosome is not certain, however, because the cell may be cut, and also the clustered chromosomes are too closely clumped to make individual identification possible. Note the wide divergence of the three conspicuous retreating chromosomes. Presumably none of them has quite reached the periphery. *c*. High focus showing the retreating hooked rod, at left. *d*. Lower focus showing the group near the pole and also indicating the vertical distance between the retreating rod just mentioned and the two other members shown in the next figure. *e*. Still lower level showing the retreating V and the rod seen at the right in *a* and *b*.

PLATE I