MICROMANIPULATION STUDIES OF CHROMOSOME MOVEMENT

I. Chromosome-Spindle Attachment and the Mechanical Properties of Chromosomal Spindle Fibers

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ABSTRACT

We have used micromanipulation to study the attachment of chromosomes to the spindle and the mechanical properties of the chromosomal spindle fibers. Individual chromosomes can be displaced about the periphery of the spindle, in the plane of the metaphase plate, without altering the structure of the spindle or the positions of the nonmanipulated chromosomes. From mid-prometaphase through the onset of anaphase, chromosomes resist displacement toward either spindle pole, or beyond the spindle periphery. In anaphase a chromosome can be displaced either toward its spindle pole or laterally, beyond the periphery of the spindle; however, the chromosome resists displacement away from the spindle pole. When an anaphase half-bivalent is displaced toward its spindle pole, it stops migrating until the nonmanipulated half-bivalents reach a similar distance from the pole. The manipulated half-bivalent then resumes its poleward migration at the normal anaphase rate. No evidence was found for mechanical attachments between separating half-bivalents in anaphase. Our observations demonstrate that chromosomes are individually anchored to the spindle by fibers which connect the kinetochores of the chromosomes to the spindle poles. These fibers are flexible, much less extensible than the chromosomes, and are able to pivot about their attachment points. While the fibers are able to support a tensile force sufficient to stretch a chromosome, they buckle when subjected to a compressive force. Preliminary evidence suggests that the mechanical attachment fibers detected with micromanipulation correspond to the birefringent chromosomal spindle fibers observed with polarization microscopy.

KEY WORDS birefringence · chromosome movement · micromanipulation · microtubules · mitosis · spindle fibers

The morphological events which comprise mitosis have been described in great detail for a wide variety of cell types. However, the actual mechanism of mitotic chromosome movement has not been determined. Attempts to explain chromosome movement by such diverse mechanisms as the depolymerization of microtubules (9, 20, 21), the sliding of interdigitating microtubules (24, 26,

28), the lateral association ("zippering") of non-parallel microtubules (1), or the participation of actin and myosin (7, 13, 14, 16, 30) clearly demonstrate our lack of understanding of the production and regulation of mitotic forces. Each of these theories contains the implicit assumption that the spindle fibers both generate and transmit the force for chromosome movement. The different molecular mechanisms of force production proposed by these various theories are based primarily upon the chemical and mechanical properties which they postulate for the spindle fibers. Thus, to differentiate between these or any other models of mitosis, a detailed knowledge of both the chemical and mechanical properties of the spindle fibers is required.

Direct experimental evidence for the physical attachment of chromosomes to the spindle was provided by early centrifugation (3, 31, 32, 34) and micromanipulation studies (8). However, these experiments do not permit deductions about the properties of the spindle fibers themselves. More recently, in a series of elegant micromanipulation experiments, Nicklas and his co-workers have described the attachment of chromosomes to the spindles of grasshopper spermatocytes and have characterized the mechanical properties of the chromosomal spindle fibers (26, 27, 29). They have demonstrated that chromosomes are individually attached to the spindle by fibers which anchor at the kinetochore of the chromosome and at the spindle pole. These "attachment fibers" are more rigid than the chromosomes, and may be modeled mechanically as a string or a thin wire (29). Although the chromosomes are normally firmly attached to the spindle, individual chromosomes can be experimentally detached by severing their chromosomal fibers with the tip of a microneedle. Detached chromosomes cease moving until mechanical attachment to the spindle has been re-established (25).

Nicklas and Staehly did not directly observe the mechanical attachment fibers which they described. Instead they inferred their existence from the response of individual chromosomes to manipulation. Thus, the relationship of the mechanical attachment fibers to the birefringent chromosomal fibers seen with polarization microscopy remains unclear.

We have investigated the general features of chromosome-spindle attachment and the mechanical properties of spindle fibers in the primary spermatocytes of two insect species: the crane fly,

Nephrotoma ferruginea and the grasshopper, *Trimeratropis maritima*. The results of the micromanipulation experiments reported here confirm the earlier observations of Nicklas and Staehly (29), and provide new information about the mechanical properties of chromosomal spindle fibers during anaphase. In addition they provide preliminary evidence that the birefringent chromosomal fibers are the structural elements which are responsibile for the observed anchorage of chromosomes to the spindle. The relationship between birefringent fibers and the mechanical attachment fibers observed with micromanipulation will be the subject of a companion paper to this article (5).

MATERIALS AND METHODS

Investigations were carried out on primary spermatocytes of the crane fly, *Nephrotoma ferruginea* Fabricius and the grasshopper, *Trimeratropis maritima* Harris. Crane flies were reared in the laboratory according to the method described by Begg (4) and Forer (11). Grasshoppers were periodically collected from a wild population in Brigantine, N. J. They were maintained in the laboratory at a temperature of 25°–30°C on a diet of fresh dandelion leaves, lettuce, and commercial rabbit food (Wayne Rabbit Ration, Allied Mills, Inc., Chicago, Ill.).

Cell Culture Chamber for Micromanipulation

The culture chamber used in these studies consists of a rectangular aluminum plate with a ¾ inch square cut out of one side (Fig. 1). 22-mm square glass cover slips
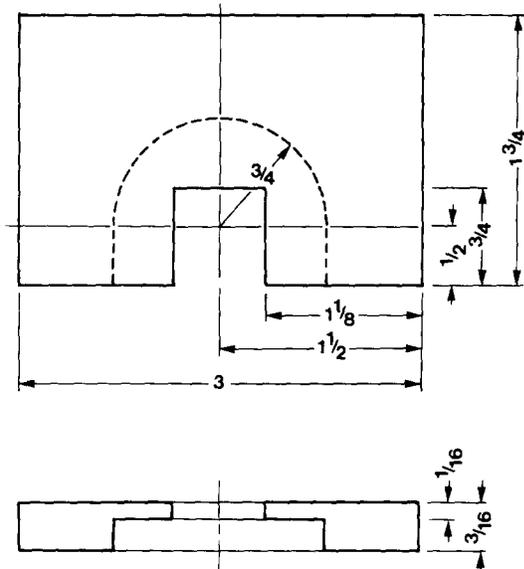

FIGURE 1   Micromanipulation chamber, top and front views. Dimensions given in inches.

were cemented to the upper and lower surfaces with polyvinyl alcohol, forming a culture chamber which is open at its front face. Spermatocyte smears were made on the upper cover slip before attaching it to the chamber. The chamber was prepared for use by attaching the bottom cover slip and adding 0.4 ml of Halocarbon 10–25 fluorocarbon oil (Halocarbon Products Corp., Hackensack, N. J.) to the well thus formed. The oil served both to flatten the cells and to protect them from dehydration. The volume of oil used was sufficient to cover the cells completely when the upper cover slip was attached. The micromanipulation needle was inserted through the open front face of the chamber and approached the cells from beneath (Fig. 2). Cell cultures were maintained at a temperature of 22°–25°C.

Preparation of Spermatocyte Smears

CRANE FLY: The technique for making crane fly spermatocyte smears is described in detail elsewhere (4) and may be summarized as follows: Larvae at the proper stage of development were surface sterilized by brief immersion in 70% ethanol, and the testes were dissected out under Halocarbon 10–25 oil. After the removal of adhering fat body, the testes were transferred to a biologically clean (17) cover slip containing a drop of Halocarbon oil and torn open with a pair of fine watchmaker's forceps. A number of slender processes were drawn out from the mass of cells with the tip of the forceps. The cells at the edges of these processes were usually well flattened and suitable for micromanipulation.

GRASSHOPPER: The testis was dissected out into a watchglass containing grasshopper Ringer's solution

(29) at a pH of 7.4. Spermatocyte smears were made by transferring 6–10 testicular lobes to a clean cover slip, cutting open the thick distal ends with a pair of iris scissors, and drawing the bundle of lobes across the cover slip in a spiral-shaped smear.

Micromanipulation

All experiments were carried out using the Ellis piezoelectric micromanipulator (10). Microneedles were constructed from 1-mm-diameter glass capillary tubes. Using a De Fonbrune microforge, the end of the needle was bent at an angle of ~45° to the shaft. The needle tip was then drawn out to a diameter of <0.2 μm with the micromanipulator and a miniature heater which was mounted on the microscope stage.

Chromosome micromanipulation was carried out using phase-contrast optics. Pre- and postmanipulation observations were made with either phase-contrast optics to determine chromosome position, or polarization optics to visualize spindle fiber morphology. The tip of the microneedle was inserted into the cell and either placed along side the chromosome to be manipulated or inserted into the body of the chromosome itself. Chromosome attachment to the spindle was tested by pushing the chromosome with the microneedle as diagrammed in Fig. 3.
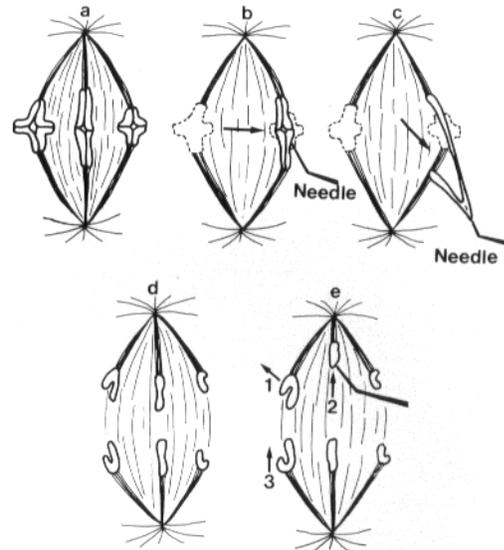
FIGURE 3 Diagrammatic representation of the various micromanipulations. (a–c) Lateral displacement of a chromosome in prometaphase and metaphase. (a) Before insertion of microneedle. (b) Needle inserted and chromosome displaced to edge of spindle. (c) Bivalent stretched by application of additional force. (d and e) Anaphase manipulations. 1, Half-bivalent pushed laterally. 2, Half-bivalent pushed toward its spindle pole. 3, Half-bivalent pulled away from its spindle pole.
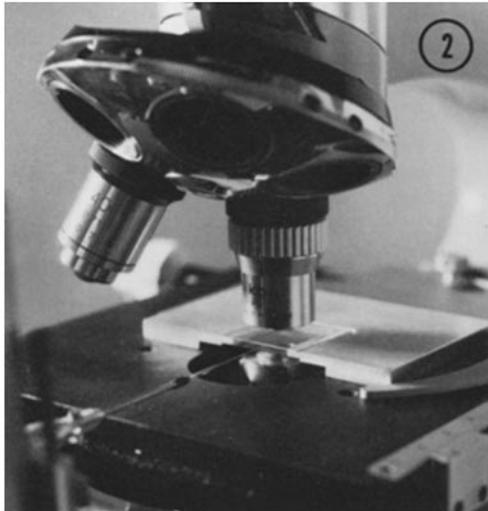
FIGURE 2 Micromanipulation chamber mounted on the microscope stage. The needle is inserted through the open front face of the chamber. The head of the micromanipulator is in the left front of the frame.

Microscopy

Illumination was from a 100-W mercury arc lamp (Illumination Industries, Sunnyvale, Calif.) filtered with a 1-cm path length CuSO₄ heat absorbing filter followed by a high transmission type B-2 Interference Filter (Baird Atomic, Inc., System Components Division, Bedford, Mass.) to provide 546-nm light. The illuminator was equipped with a Schneider f1.8, 8- to 64-mm zoom lens and a removable, centerable annular aperture for use in phase-contrast microscopy.

For polarization microscopy, HN-22 unlaminated sheet polaroid was used for both the analyzer and polarizer. A strain-free Olympus 20 × objective (NA 0.40) was used as the condenser with either a strain-free Olympus 40 × objective (NA 0.65) (Olympus Corp. of America, New Hyde Park, N. Y.) or a Nikon rectified 40 × objective (NA 0.65). Birefringence was measured visually with a Brace-Köhler rotating mica compensator (Γ = 21.5 nm).

For phase-contrast microscopy a 40 × Tiyoda phase-contrast objective (NA 0.71) was used in conjunction with the condenser described above. The condenser was converted to phase contrast by projecting into its front focal plane an image of an annular aperture which matched the phase annulus of the objective. By replacing the normal compensator with a ¼ λ plate, the light intensity of the system was increased to a useable level without removing the analyzer or polarizer. With practice the microscope could be converted from one optical system to the other in <30 s.

Photogrpahy

Photographic records were made with a Nikon Microflex camera system using either Kodak Panatomic-X or Kodak Plus-X 35-mm film. Magnification was calibrated by photographing a stage micrometer scale.

Data Analysis

Chromosome positions were measured from photographs printed at a final magnification of 1,500 for crane fly spermatocytes and 1,700 for grasshopper cells. Measurements were made using a Hewlett-Packard Model 9864A Digitizer in conjunction with the Hewlett-Packard Model 9830A programmable calculator (Hewlett Packard Co., Palo Alto, Calif.). Data were analyzed with the calculator, and graphs were drawn with its associated Model 9862A Plotter.

RESULTS

Tolerance to Micromanipulation

Trimeratropis and *Nephrotoma* spermatocytes differ significantly in their tolerance to micromanipulation. *Trimeratropis* spermatocytes withstand prolonged and even violent operations, while *Ne-*

phrotoma cells are easily killed by apparently gentle manipulations. Approx. 80% of the *Trimeratropis* and 30% of the *Nephrotoma* spermatocytes survive manipulation. Cells which are injured during manipulation usually undergo a sudden and rapid lysis. Their cytoplasm disperses and their chromosomes become highly refractile.

Those spermatocytes which survive manipulation generally undergo a normal anaphase and show no difference in viability compared to unmanipulated control cells in the same preparation. All cells which are considered in this report completed a normal anaphase subsequent to manipulation.

Prometaphase and Metaphase Manipulations

GENERAL FEATURES OF CHROMOSOME-SPINDLE ATTACHMENT: In late prometaphase and metaphase each chromosome exhibits birefringent fibers extending from its kinetochores to both spindle poles. The edge of the spindle demarcates the normal maximum length of the chromosomal spindle fibers. Thus, the displacement of the kinetochores beyond the spindle boundary[1] would require an increase in the length of these fibers. The displacement of a chromosome about the periphery of the spindle, however, would not necessitate a change in fiber length.

Fig. 4 shows the results of an experiment in which a chromosome is manipulated about the circumference of the spindle. The bivalent designated by the arrow is displaced three-quarters of the way across the spindle without disturbing the positions of the nonmanipulated chromosomes or altering the structure of the spindle (0.0 and 3.9 min). As the bivalent is pulled further across the spindle, it shows no resistance to displacement until it reaches the spindle boundary, whereupon the kinetochores resist further displacement (8.4 min). These results demonstrate that the chromosomes are individually anchored to the spindle by "fibers" which attach to the kinetochores of the chromosomes and to the spindle poles. In addition, these observations suggest that the "attachment fibers" are able to pivot about their site of anchorage at the poles.

If instead of engaging a chromosome at the periphery of the spindle the tip of the microneedle

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[1] We use the term spindle boundary to denote the interface between the spindle body and the rest of the cytoplasm and not to imply any physical structure.
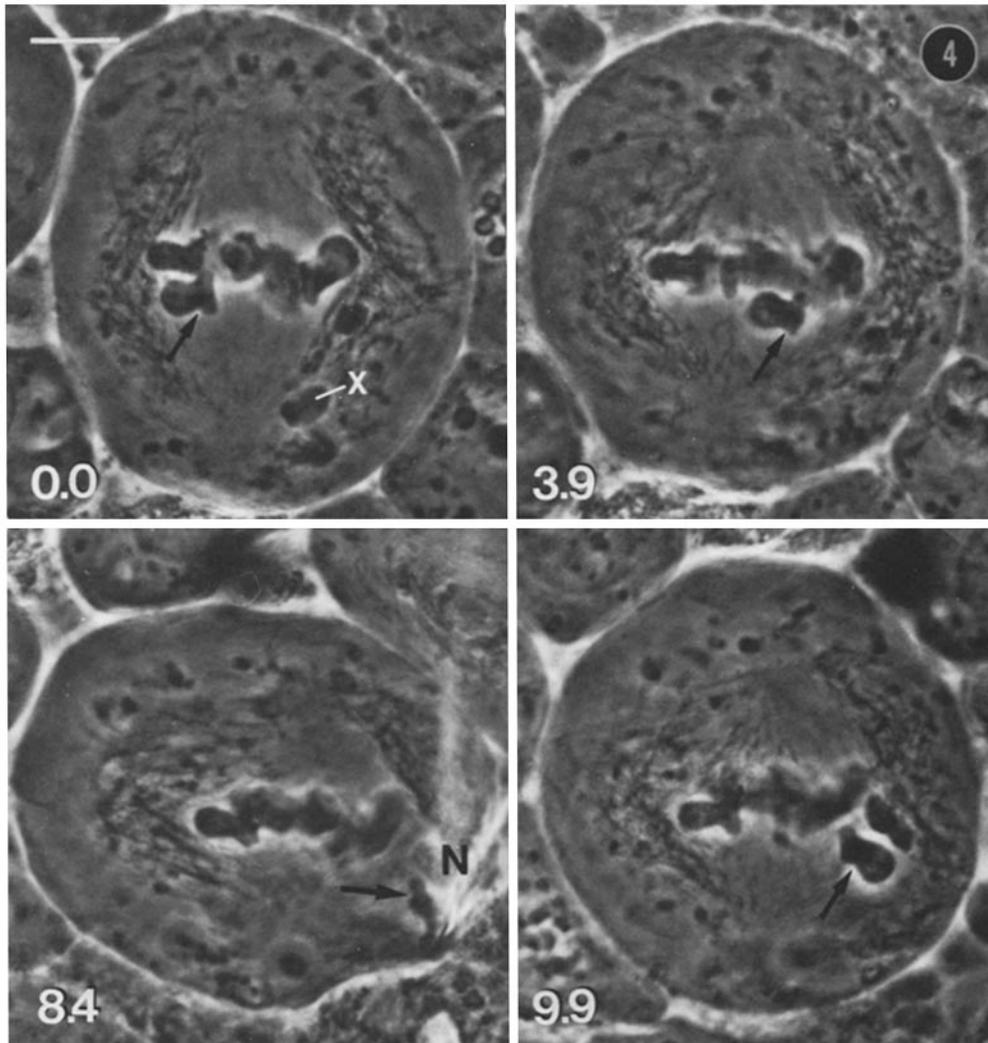
FIGURE 4 Translation of an individual chromosome about the spindle periphery in a *Trimeratropis* spermatocyte. The cell is in late prometaphase. The arrow indicates the manipulated chromosome. X, Unpaired univalent sex chromosome. N, Microneedle. In this and in all subsequent plates, the time with respect to the manipulation is given in minutes in the lower left of each frame: 0.0 min, before insertion of the microneedle; 3.9 min, chromosome displaced three quarters of the distance across the spindle; 8.4 min, chromosome pulled to edge of cell; 9.9 min, chromosome remains within the spindle boundary. Note that it has been turned over by the operation. Bar, 10 μm.

is inserted into the body of the spindle, it cannot be moved laterally without distorting the structure of the entire spindle. However, the needle can be moved parallel to the interpolar spindle axis without significantly altering the spindle's structure. These observations are consistent with the known ultrastructural properties of the spindle.

The resistance of kinetochores to displacement beyond the spindle periphery is observed from approximately mid-prometaphase to the onset of anaphase. Fig. 5 shows a metaphase cell with well-developed birefringent chromosomal fibers (−8.3 min, arrows). A bivalent lying at the edge of the spindle is stretched to more than three times its original length without displacing the kinetochores beyond the spindle boundary (0.0 min). In exper-
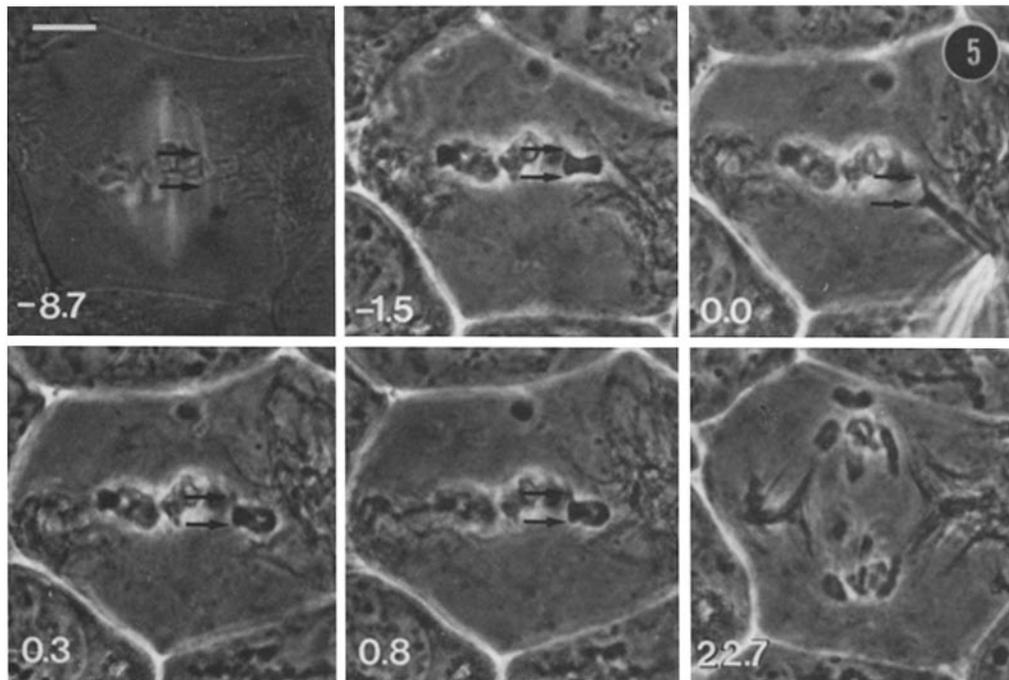
FIGURE 5 Lateral stretching of a *Trimeratropis* bivalent in metaphase. Arrows indicate the kinetochore positions. −8.3 min, Polarization optics. Birefringent chromosomal fibers are well developed. The chromosome is stretched (0.0 min), and when released returns to its original length within 0.8 min. The manipulation results in an increase of 1 μm in the kinetochore to pole distance. Bar, 10 μm.

iments on 14 *Trimeratropis* cells in which individual chromosomes were extensively and repeatedly stretched, the maximum displacement of a kinetochore away from a spindle pole was 2 μm. These results demonstrate that relative to the force required to stretch a chromosome, the chromosomal spindle fibers are comparatively inextensible structures.

The general features of chromosome-spindle attachment which were observed in *Trimeratropis* spermatocytes are also found in *Nephrotoma* cells. However, photographic documentation is less clear in the case of *Nephrotoma* due to two technical problems: (a) the smaller *Nephrotoma* cell is more easily displaced during manipulation and is usually moved across the cover slip by the microneedle before the force exerted becomes sufficient to stretch the chromosome; (b) the bright needle tip frequently obscures the small *Nephrotoma* chromosomes during the manipulation. However, as in the experiments on *Trimeratropis* spermatocytes, the lateral displacement and stretching of a chromosome results in only a slight increase in the

kinetochore to pole distance (a maximum of 1.5 μm in experiments on 12 cells). A representative example of this manipulation is presented for a prometaphase cell in Fig. 6.

CHROMOSOME ELASTICITY: These experiments also demonstrate the striking resiliency of *Trimeratropis* chromosomes. Manipulated bivalents return to within 1 μm of their original length within 0.3 min after being released from the microneedle (Fig. 6). Nicklas and Staehly (29) report similar findings for the chromosomes of the grasshopper, *Melanoplus differentialis*.

In marked contrast to these results, little stretching of *Nephrotoma* chromosomes was observed (Fig. 6). It is not clear whether this is because the chromosomes are less extensible or, as discussed previously, because the cell is pulled across the cover slip by a force which is less than that required to stretch a *Trimeratropis* chromosome.

MANIPULATION OF BIREFRINGENT CHROMOSOMAL FIBERS: The micromanipulation experiments reported here demonstrate the existence of fibers which attach the kinetochores
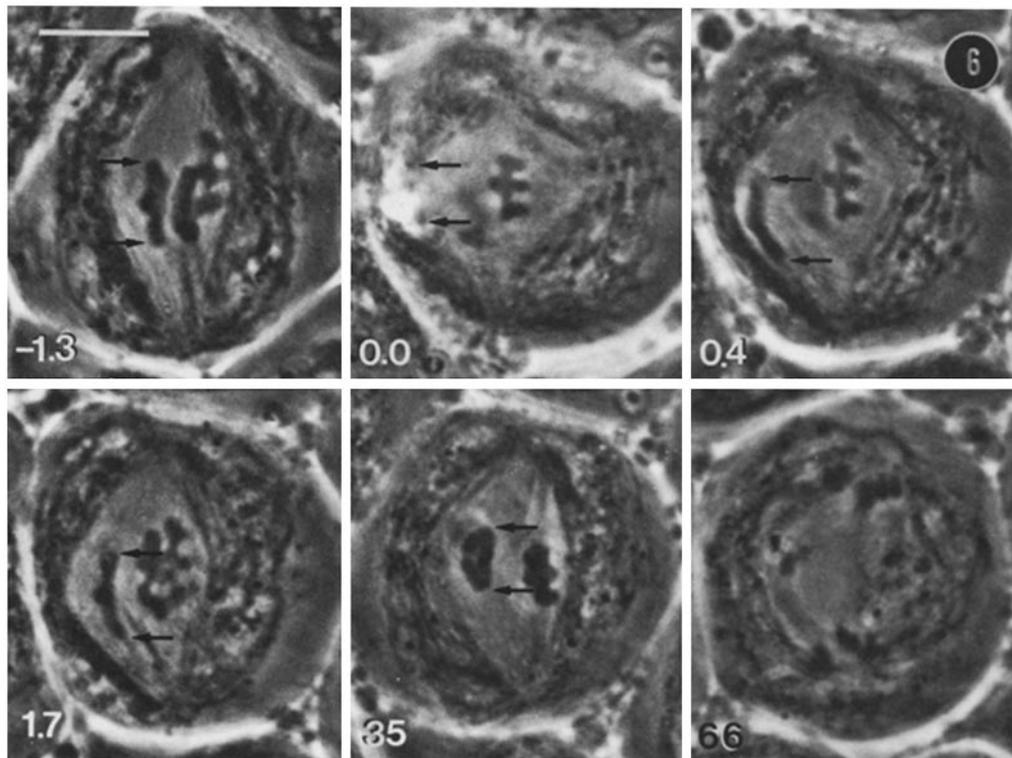
FIGURE 6 Lateral displacement of a *Nephrotoma* bivalent in prometaphase. Arrows indicate the kinetochore positions. When a bivalent is pushed laterally, it resists displacement beyond the spindle boundary (0.0 min). Upon removal of the microneedle (0.4 min), the bivalent can be seen to lie at the edge of the spindle, against the mitochondrial sheath. It gradually moves back toward the interpolar axis as the cell approaches metaphase. The kinetochore to pole distance is increased by 1.3 μm a a result of the manipulation. Bar, 10 μm.

of the chromosome to the spindle poles. If the birefringent chromosomal fibers serve this function, it should be possible to move a chromosome laterally by manipulating its associated fiber. To test this hypothesis, the microneedle was inserted into the cell and placed against the side of a birefringent chromosomal fiber while observing the cell with polarization optics. The needle was then pushed laterally against the fiber, and the behavior of the chromosome was observed with phase-contrast optics.

The results of a typical experiment are presented in Fig. 7. The needle is placed alongside the upper chromosomal fiber of a single bivalent at the edge of the spindle. When the needle is moved laterally, the upper kinetochore rotates away from the spindle axis and the entire chromosome is displaced slightly in the direction of the push (0.0 min).

Sufficient force is exerted on the chromosome to increase the interkinetochore distance by 2 μm during the operation. However, the chromosome returns to its original length when the needle is removed (1.1 min). The manipulation does not disturb the positions of the other chromosomes, nor does it disrupt the manipulated birefringent fiber (6.5 min).

These results suggest that the birefringent chromosomal fiber is the mechanical attachment fiber; however, they do not rule out the possible participation of a nonbirefringent spindle fiber component in the attachment of chromosomes to the spindle. In addition, this experiment also demonstrates the flexibility of the chromosomal fiber. It is evident from the 0.0-min print that the chromosomal fiber is bent at nearly a 90° angle during the manipulation without disrupting the fiber.
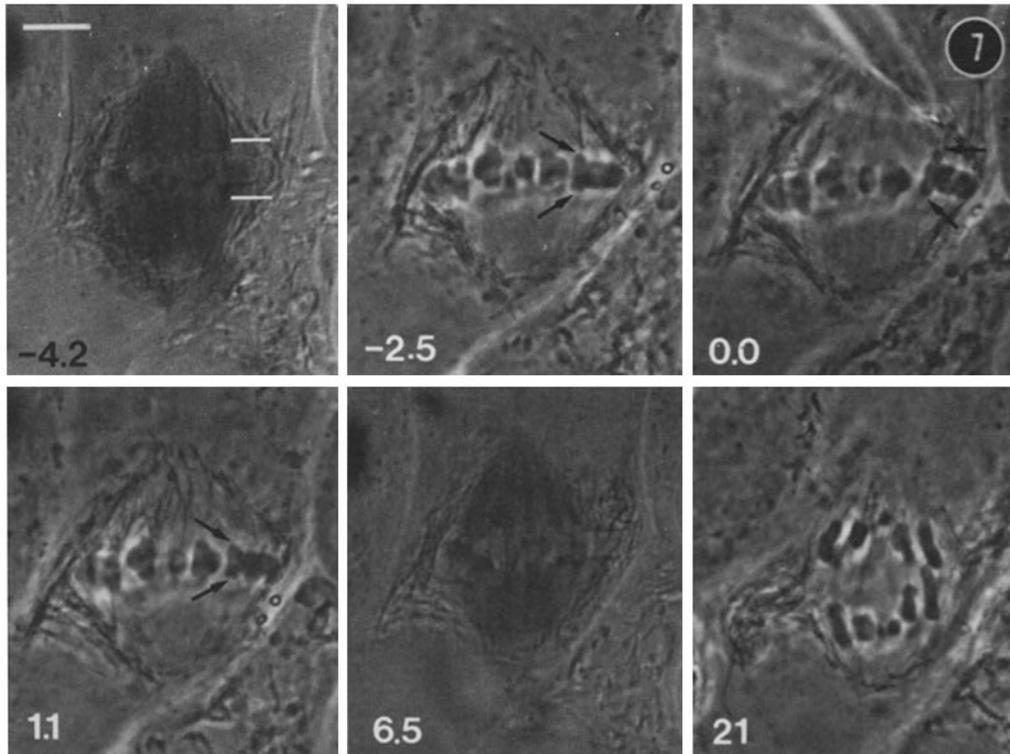
FIGURE 7 *Trimeratropis* bivalent displaced laterally by pulling on its chromosomal fiber. A single chromosome at the right-hand margin of the spindle is manipulated. The position of the kinetochores is indicated by arrows. The body of the chromosome extends toward the right-hand edge of the spindle. −4.2 min, polarization optics. Birefringent chromosomal fibers are well developed. Fibers extending from the kinetochores of the chromosome to be manipulated are indicated by the white bars. Note that the chromosomal fiber is not disrupted by the manipulations (6.5 min). Bar, 10 μm.

Anaphase Manipulations

GENERAL FEATURES OF CHROMOSOME-SPINDLE ATTACHMENT: During prometaphase and metaphase a bivalent is attached to both spindle poles and therefore cannot be displaced significantly from the equator: a pull toward either pole is resisted by the fiber attached to the opposite pole. In anaphase each half-bivalent should be attached to only that spindle pole to which it is migrating; thus, the resistance of a half-bivalent to both lateral and poleward displacement could be examined.

The half-bivalent indicated by the arrow in Fig. 8 a is displaced laterally, beyond the edge of the spindle (3.2 min). Despite the manipulation it continues to migrate toward the spindle pole at the same rate as the other chromosomes (7.9 min). The operation has no effect upon the position or

subsequent movement of its sister half-bivalent.

The cell shown in Fig. 8 b is in early anaphase. One half-bivalent is pushed toward the upper spindle pole (0.0 min) where it remains (10.1 min). The sister half-bivalent is unaffected by the manipulation and continues its poleward migration normally. When the half-bivalent in Fig. 8 c is pulled away from its spindle pole (0.0 min), the chromosome arm stretches into the opposite half-spindle, but the kinetochore is not displaced by the operation (0.6 min). The results of these three experiments taken together demonstrate that a bivalent is attached to only one spindle pole in anaphase; however, the other aspects of chromosome-spindle attachment which were described previously remain unchanged.

POLEWARD DISPLACEMENT OF HALF-BIVALENTS: The poleward displacement of a half-bivalent in anaphase has been analyzed in a
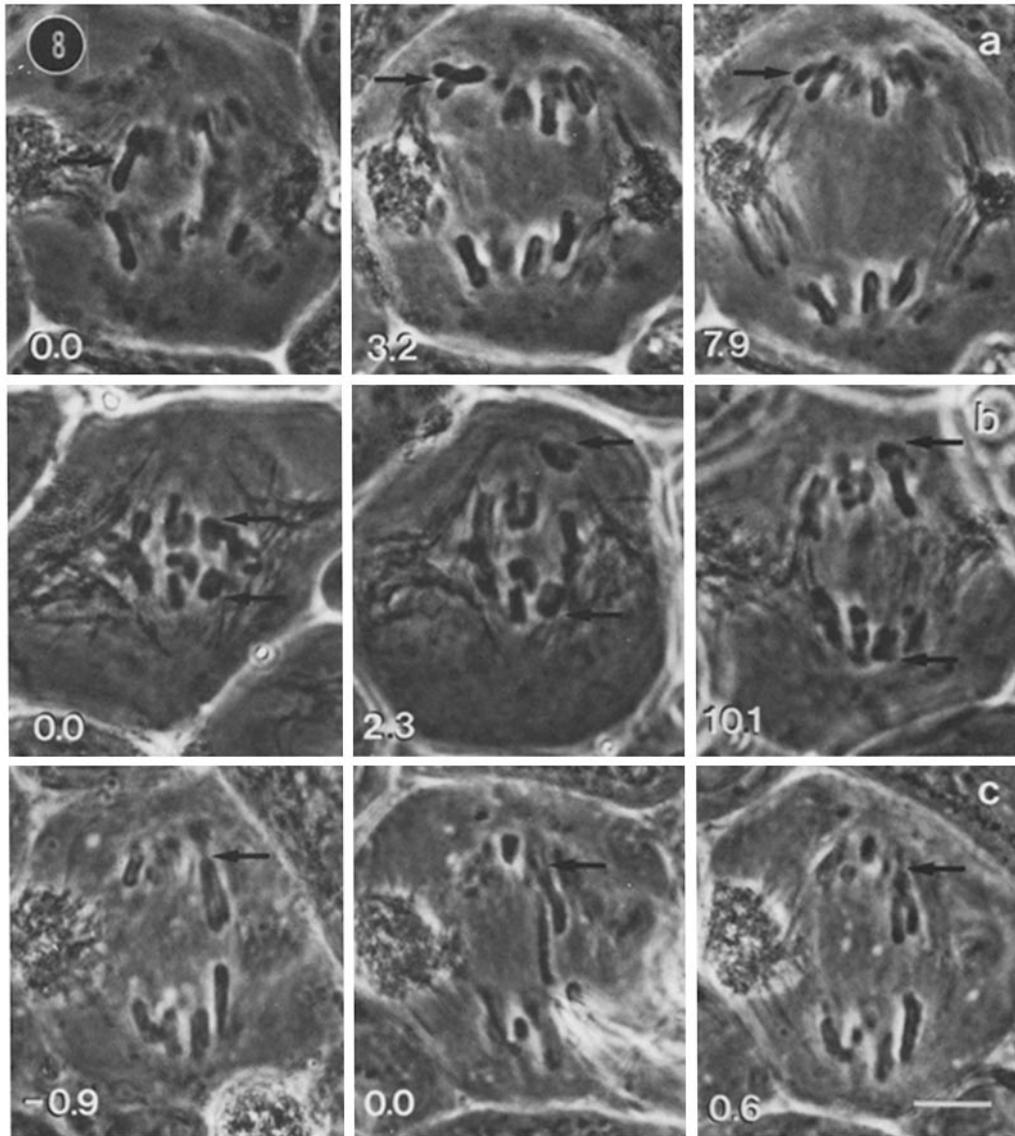
FIGURE 8 Summary of anaphase manipulations in *Trimeratropis*. The three sequences are from different cells. *(a)* Lateral displacement of half-bivalent away from spindle axis. Arrow indicates manipulated half-bivalent. *(b)* Displacement of half-bivalent toward its spindle pole. Arrows indicate manipulate half-bivalent and its sister. *(c)* Stretching of half-bivalent away from its spindle pole. Arrow indicates position of the kinetochore. Bar, 10 μm.

total of five *Nephrotoma* and three *Trimeratropis* spermatocytes. Identical results were obtained for all eight cells. A half-bivalent which is displaced toward its spindle pole in anaphase stops moving until the other chromosomes in the same half-spindle reach a similar distance from the pole. The manipulated half-bivalent then resumes its pole-

ward migration together with the nonmanipulated ones.

The effects of the poleward displacement of a half-bivalent are more clearly seen in *Nephrotoma* spermatocytes since these cells have fewer chromosomes. Such an experiment is presented photographically in Fig. 9 and graphically in Fig. 10.
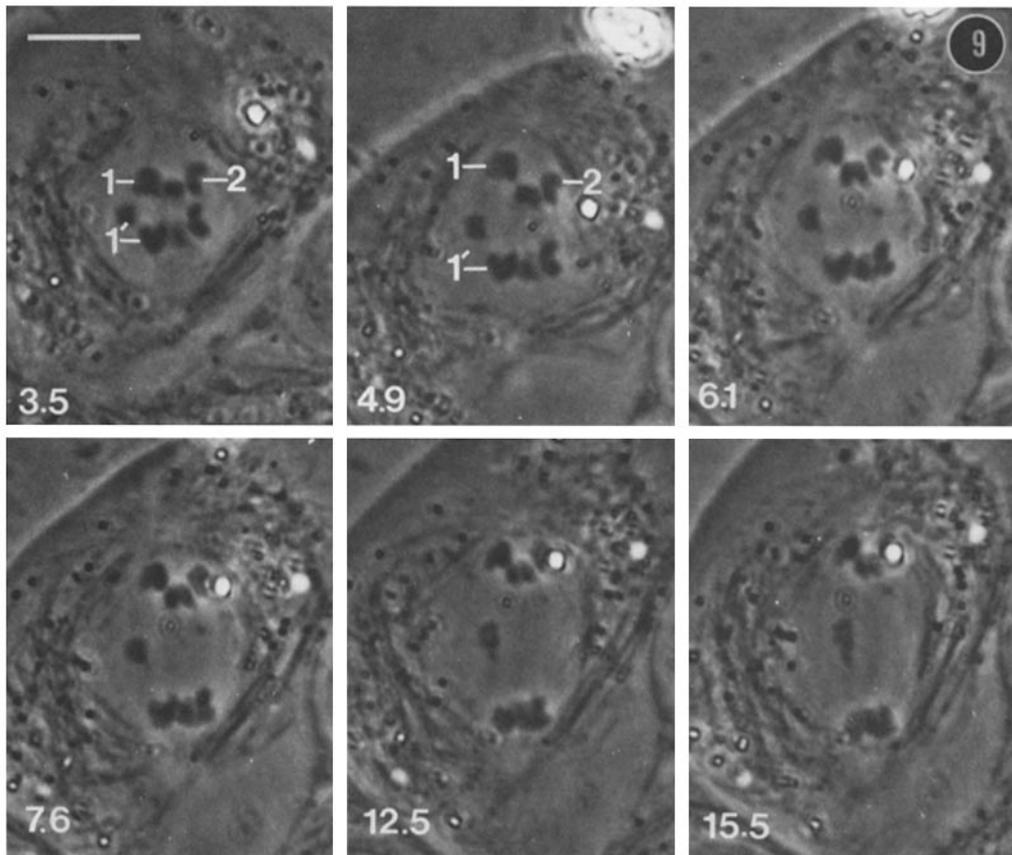
FIGURE 9 Poleward displacement of a *Nephrotoma* half-bivalent in anaphase. *1*, Manipulated half-bivalent; *1'*, sister half-bivalent; *2*, a nonmanipulated half-bivalent used as reference. Details in text. Bar, 10 μm.

Half-bivalent No. 1 is pushed ~3 μm closer to its spindle pole (3.5–4.9 min, Fig. 9). The manipulated half-bivalent stops moving until half-bivalent No. 2 reaches it (7.6 min), whereupon it resumes its poleward migration at the normal anaphase velocity (12.5–15.5 min and Fig. 10). Thus, the manipulated chromosome re-initiates movement at the time it would normally have reached that distance from the pole. The manipulation does not affect the movement of the nonmanipulated sister half-bivalent (No. 1', Fig. 9).

A half-bivalent which is displaced completely to a spindle pole remains at the pole for the duration of anaphase (Fig. 8 b). Although the manipulated half-bivalent does not move with respect to the pole, it separates normally with the rest of the chromosomes during spindle elongation.

THE EFFECT OF POLEWARD DISPLACE-MENT OF A HALF-BIVALENT UPON ITS BIRE-FRINGENT CHROMOSOMAL FIBER: Half-bivalents which have been displaced poleward in anaphase do not show organized birefringent fibers. Instead, an area of diffuse birefringence is found between the manipulated half-bivalent and its spindle pole (Fig. 11). However, clear chromosomal fibers are associated with the nonmanipulated half-bivalents. In contrast to a normal chromosomal fiber whose birefringence can be extinguished by rotating the specimen through 45°, the region between the manipulated chromosome and its spindle pole retains some diffuse birefringence regardless of the angle it makes to the analyzer and polarizer. This observation suggests that the parallel alignment of microtubules normally found in the chromosomal fiber is disorganized by the manipulation.

MECHANICAL ATTACHMENT BETWEEN SISTER HALF-BIVALENTS: As the preceding re-

sults demonstrate, no evidence was found for mechanical connections between sister half-bivalents in anaphase in spermatocytes from either species.

These results were consistently observed in *Trimeratropis* spermatocytes and in *Nephrotoma* spermatocytes from freshly prepared smears. However, as the *Nephrotoma* spermatocyte preparations aged, mechanical connections began to develop between sister half-bivalents. In all cases where this mechanical connection was detected, chromosomal bridges could be seen between the separating sister half-bivalents. Both the frequency and
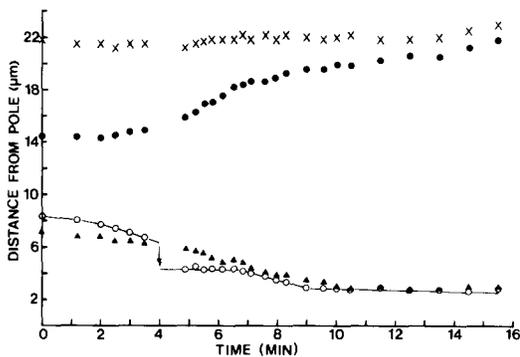

FIGURE 10 Graph of manipulation shown in Fig. 11. All distances are measured from the spindle pole toward which the manipulated half-bivalent was displaced. ×, Position of second spindle pole; ○, manipulated half-bivalent; ●, sister half-bivalent; ▲, nonmanipulated half-bivalent. The velocity of the manipulated half-bivalent after the resumption of movement is the same as before the operation (0.6 μm/min).

degree of bridging increased with the age of the preparation. Half-bivalents with distinct chromosomal bridges could not be displaced poleward. When a bridged half-bivalent was displaced laterally, its sister half-bivalent moved as well. Similar chromosomal bridging was also found in *Trimeratropis* spermatocytes when the pH of the Ringer's solution used was below 7.2. However, at a pH of 7.2 or above, chromosomal bridges were seldom observed.

DISCUSSION

Attachment of Chromosomes to the Spindle

The results of the micromanipulation studies reported here confirm and extend the previous observations of Nicklas and Staehly (29). Chromosomes are individually anchored to the spindle by "fibers" which attach the kinetochores of the chromosomes to the spindle poles. In prometaphase and metaphase each bivalent is attached to both spindle poles by a pair of these fibers, while in anaphase each half-bivalent is attached to only that pole to which it is moving.

The fibers which attach the chromosomes to the spindle are flexible, less extensible than the chromosomes, and are able to pivot about their attachment points. A comparison of prometaphase-metaphase and anaphase manipulations demonstrates that while these fibers are able to support a tensile force which is sufficient to stretch the chromosome, they buckle when subjected to a compressive force.
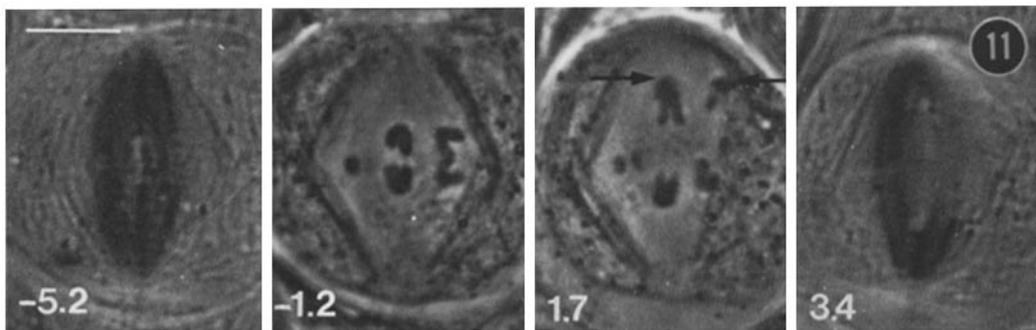

FIGURE 11 Response of birefringent chromosomal fiber to poleward displacement of a half-bivalent in anaphase. Arrows indicate kinetochores of manipulated chromosomes. −5.2 min, Polarization optics. Birefringent fibers extend from the kinetochores of the chromosomes to both spindle poles. −1.2 min, Anaphase onset. 1.7 min, Two half-bivalents displaced toward upper spindle pole. 3.4 min, Distinct birefringent chromosomal fibers extend between the unmanipulated half-bivalents and the lower spindle pole, while only diffuse birefringence is observed between the kinetochores of the manipulated half-bivalents and the upper spindle pole. Bar, 10 μm.

The birefringent chromosomal fibers which extend from the kinetochores of the chromosomes to the spindle poles are the obvious candidates for the mechanical attachment fibers characterized in these micromanipulation studies. By pulling a birefringent chromosomal fiber laterally with the microneedle, the associated chromosome can be both displaced in the direction of the pull and stretched, demonstrating that the fiber is a substantial structure which is capable of transmitting force to the chromosomes. While these results suggest that it is the birefringent fiber which transmits the force to the chromosomes, they do not rule out the possibility that a nonbirefringent fiber may be the structural element detected by micromanipulation. The relationship of birefringent chromosomal fibers to the mechanical attachment fibers will be considered in detail in a subsequent paper (5).

Poleward Displacement of a Half-Bivalent in Anaphase

When a half-bivalent is displaced toward its spindle pole in anaphase, it stops moving until the nonmanipulated half-bivalents reach a similar distance from the pole. The manipulated half-bivalent then resumes its poleward migration at the normal anaphase rate. A half-bivalent which is displaced completely to the pole does not move further. Thus a chromosome which is prematurely displaced toward its spindle pole reinitiates movement only at the time it would normally have reached that same distance from the pole.

This dramatic behavior of the manipulated chromosome suggests that the chromosomal fiber, which is normally under tension in anaphase, bends and slackens as a result of the poleward displacement of a half-bivalent, but continues to shorten at a normal rate. Thus the fiber behaves as if it were a flexible cable which is being reeled in at the spindle pole. As the fiber shortens, it cannot exert a force on the manipulated half-bivalent until the slack is taken up and the fiber is again under tension (Fig. 12). If the chromosomal fiber continues to shorten at the normal anaphase rate, the manipulated half-bivalent should only begin to move again when the nonmanipulated half-bivalents reach it. This is exactly what is observed.

An area of diffuse birefringence is observed between the kinetochore of the manipulated half-bivalent and the spindle pole, rather than a distinct
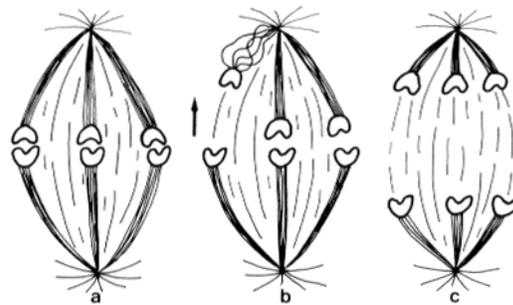

FIGURE 12 Interpretation of a birefringent chromosomal fiber's response to the poleward displacement of a half-bivalent in anaphase. (a) Anaphase onset. (b) Half-bivalent displaced toward upper spindle pole. The microtubules which comprise the fiber lose their cohesion, separate laterally, and bend. The manipulated half-bivalent stops moving. (c) The nonmanipulated half-bivalents reach the manipulated chromosome. Shortening of the manipulated chromosomal fiber has restored tension, and the manipulated chromosome resumes its motion toward the pole.

chromosomal fiber. This observation indicates that the microtubules which comprise the fiber lose their parallel alignment and buckle as a result of the manipulation (Fig. 12). Ultrastructural studies of manipulated cells are planned in order to investigate the response of kinetochore microtubules to the poleward displacement of a chromosome in anaphase.

It is highly unlikely that the observed cessation in movement of a manipulated half-bivalent results from a mechanical disruption of its chromosomal fiber. Nicklas and Staehly (29) have directly demonstrated the persistence of a mechanical connection to the spindle in half-bivalents which have been displaced toward the pole. A manipulated half-bivalent can be pulled back to its original position, but resists further displacement toward the equator, demonstrating that its chromosomal fiber remains physically intact.

Stability of a Chromosomal Fiber in Anaphase

Physical tension has been shown to be necessary for the stabilization of chromosomal spindle fibers (18, 19, 27). When both kinetochores of a bivalent are oriented to a single spindle pole, the chromosomal fibers are not under tension, and the bivalent reorients until it achieves a bipolar orientation (2, 27). In grasshopper spermatocytes the average time required for this reorientation is 16 min (27)

and in crane fly spermatocytes, ~10 min (2). The results of the micromanipulation studies presented in this paper demonstrate that a half-bivalent which has been displaced toward its spindle pole in anaphase does not reorient, even though the tension in its associated chromosomal fibers is relaxed as a result of the manipulation. However, the time elapsed before the half-bivalent resumes its poleward movement never exceeds 8 min in *Trimeratropis*, and 4 min in *Nephrotoma* spermatocytes. It is therefore unclear whether the lack of tension produced by these manipulations was too short-lived to permit reorientation, or whether physical tension is not required for fiber stability in anaphase.

Physical Attachment of Sister Half-Bivalents in Anaphase

In *Trimeratropis* spermatocytes no evidence was found for a mechanical connection between sister half-bivalents in anaphase. A half-bivalent could be manipulated poleward or laterally without affecting its sister half-bivalent or the other non-manipulated chromosomes. Nicklas and Staehly (29) report similar findings for *Melanoplus* spermatocytes.

Similar results are found for *Nephrotoma* spermatocytes in freshly prepared smears. However, as the cell preparations age, mechanical connections develop between sister half-bivalents in anaphase. The presence of mechanical connections between sister half-bivalents is always correlated with the formation of chromosomal bridges. When clear chromosomal bridges are visible, the lateral displacement of a half-bivalent results in the coupled movement of its sister half-bivalent. Bridged half-bivalents also resist poleward displacement. These observations suggest that the physical linkage of sister half-bivalents in anaphase is an artifact produced by the cell culture conditions. It is interesting to note that chromosomal bridges also develop in *Trimeratropis* spermatocytes when they are cultured at low pH.

The results presented here differ from those of Forer and Koch (15). They conclude from micromanipulation studies of crane fly spermatocytes that sister half-bivalents are normally mechanically linked in anaphase. However, chromosomal bridges are visible in some of their micrographs (see, for example, their Fig. 1, 0.0-min print). These authors suggest that the mechanical connections which they detect with micromanipulation

may be responsible for the striking coordinated behavior of sister half-bivalents which is observed in response to UV-microbeam irradiations (12). However, direct mechanical coupling is not necessarily a prerequisite for the coordinated movements of chromosomes. In spermatocytes of the mole cricket, *Gryllotalpa hexadactyla*, orientation of a heteromorphic bivalent determines the orientation of the X chromosome (6, 33), yet no mechanical connection between the two chromosomes is detectable (6). It would be interesting to know whether sister half-bivalents in *Trimeratropis* spermatocytes show a coordinated response to UV-microbeam irradiation. Sister half-bivalents in another species of grasshopper (*Oxya sp.*) do not show such a response (22, 23).

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