

MITOSIS IN *TILIA AMERICANA* ENDOSPERM

JOHN W. FUSELER

From the Marine Biological Laboratory, Woods Hole, Massachusetts 02543, and the University of Pennsylvania Program in Biophysical Cytology, Biology Department, Philadelphia, Pennsylvania 19174

ABSTRACT

The endosperm cells of the American basswood *Tilia americana* are favorable experimental material for investigating the birefringence of living plant spindles and anaphase movement of chromosomes. The behavior of the chromosomes in anaphase and the formation of the phragmoplast are unique. The numerous ($3n = 123$), small chromosomes move in precise, parallel rows until midanaphase when they bow away from the poles. Such a pattern of anaphase chromosome distribution has been described once before, but was ascribed to fusion of the chromosomes. The bowing of chromosome rows in *Tilia* is explainable quantitatively by the constant poleward velocity of the chromosomes during anaphase. Peripheral chromosomes are moving both relative to the spindle axis and laterally closer to the axis, whereas chromosomes lying on the spindle axis possess no lateral component in their motion, and thus at uniform velocity progress more rapidly than peripheral chromosomes relative to the spindle axis. The chromosomes are moved poleward initially by pole-to-pole elongation of the spindle, then moved farther apart by shortening of the kinetochore fibers. In contrast to other plant cells where the phragmoplast forms in telophase, the phragmoplast in *Tilia* endosperm is formed before midanaphase and the cell plate during midanaphase, while the chromosomes are still in poleward transit.

Direct observation of meiosis and mitosis in living plant cells and tissues has been made on numerous species (see Pekarek, 1932, and Becker, 1938, for reviews). Notably, earlier studies did not utilize endosperm mitosis or meiosis in pollen mother cells, because it was thought that cutting of the tissue produced irreversible dissociation in the cytoplasm and nuclei which hindered division (Becker, 1938). This notion was proved invalid by Inoué (1953) who found pollen mother cells of *Lilium longiflorum* to be excellent material for the study of cell division (meiosis) in plants. Likewise, Bajer and Molé-Bajer (1954) showed that endosperm of various plants, especially the blood lily *Haemanthus katherinae*, is highly suited for the study of plant mitosis.

Detailed observation of dividing plant cells by polarized light microscopy has been limited to *Lilium* pollen mother cells (Inoué, 1953) and *Haemanthus* endosperm (Inoué and Bajer, 1961). The number of plant species in use for polarizing microscope investigation is limited because many plant cells have thick, birefringent or refractile cell walls, and highly refractile starch granules of plant cell cytoplasm which severely limit direct observation of the spindle. It is rare to find botanical material in which these cell constituents are at a minimum or absent.

Quantitative analysis of chromosome movement in dividing plant cells has centered on the petal and stamen hair cells of *Tradescantia* (Belar, 1929; Barber, 1939; Wada, 1950) and *Haemanthus* endo-

sperm (Bajer, 1957; 1958 *a* and 1958 *b*; 1968 *a* and 1968 *b*; Bajer and Allen, 1966; for review see Bajer and Molé-Bajer, 1972).

The endosperm of the American basswood tree *Tilia americana* has been found to be a highly suitable material for the in vivo study of plant mitosis. The *Tilia* endosperm cells are comparable to those of *Haemanthus* in terms of clarity of the cytoplasm and absence of a cell wall.

Here for the first time, quantitative analysis of anaphase chromosome movement and the accompanying changes in spindle fiber birefringence are described for an individual species. The anaphase mitotic events of *Tilia* endosperm, which have not been previously described, are unique in comparison to cell division in other plants and animals.

MATERIALS AND METHODS

The mature *Tilia americana* possesses a compact, symmetrical form, is 70–90 ft in height and 2–4 ft in trunk diameter. The natural distribution of the species includes much of the eastern half of the United States (Harlow, 1957; Collingwood and Brush, 1964). The tree commences flowering about the 1st wk in July at Woods Hole, Mass., and about 1 mo earlier in the Philadelphia, Pa., area. The perfect, five-petaled, white- or cream-colored flowers are borne in clusters from a stalk which is attached midway to a leafy bract. The period of flowering lasts for about 10–12 days during which pollination occurs. After this period the corollas wither and the seed begins to form. The ovule, after about 3 wk of development, measures 6–7 mm in length and contains large, clear endosperm cells. The mitosis of these cells is exceptionally suitable for polarized light microscope studies, although this condition exists for only 3–4 days. After this short period the endosperm cells become much smaller and contain an abundance of refractile, cytoplasmic, starch granules, which obscure the mitotic spindle birefringence and chromosomes.

Fresh material was collected for each experimental run. Only the leaflike bract, bearing the developing seed of the proper size, was removed from the tree.

The endosperm cells are flattened in a separate hanging drop chamber before they are mounted on the temperature control slide. The temperature control slide allows the temperature of the cells to be maintained at a given temperature by passing thermostatically controlled cooling solution under a window upon which the cells are mounted. The base of the hanging drop chamber consists of a biologically clean (see glassware) slide, coated on one side with glucose agar gel (3% glucose, 0.5% agar in distilled water). A spacer made of a hollowed square of filter paper (no. 3 Whatman) is mounted on the coated side of the slide, and is saturated with melted glucose agar solution. The top of the chamber consists of a biologically clean 18 mm × 18 mm cover slip coated on

one side with the glucose agar gel. These coated slides and cover slips are kept in a moist chamber until ready to be used. A small ridge of Vaseline is placed around the perimeter of the cover slip just before use.

The ovule is prepared by slicing off its apex with a scalpel (no. 11 blade) and removing the excess endosperm fluid with the tip of a wedge of filter paper (no. 541 Whatman). Stainless steel dissection instruments which are cleaned and sterilized in 70% ethanol solution before use are employed throughout the preparation of the slides and ovules. The remaining contents of the ovule are squeezed on to the coated side of the cover slip, which is immediately inverted and mounted on the previously prepared slide. This hanging drop preparation is sealed with valap (by volume, 40% Vaseline, 40% anhydrous lanolin, and 20% paraffin, melting point 55–57°C; cooked for 24–48 h at 60°C until thoroughly blended) to prevent desiccation of the cells. If sufficient humidity is not maintained between the slide and the cover slip, the cells rapidly lyse. The sealed preparation is inclined at a 45° angle for 20–30 min to flatten the cells (Bajer, 1954).

When the cells have obtained the desired degree of flattening as seen by phase-contrast microscopy, the cover slip is removed from the slide with sharp-pointed forceps. The cells are immediately covered with a drop of FC-47 fluorocarbon oil (3M Co., St. Paul, Minn.). The cover slip bearing the cells is inverted and immediately placed on a pool of FC-47 oil, which is contained in a 14-mm square well constructed of four strips of Teflon tape each 4 mm wide, on the window of the temperature control slide (Inoué et al., 1970). Extreme care is required in bringing the two drops of oil together to prevent any air bubbles from being trapped under the cover slip. A trapped air bubble can move freely in the low viscosity oil against the cover slip and can lyse all the cells it passes over, rendering the preparation unusable. Excess FC-47 oil is removed with filter paper and the preparation is sealed with valap. *Tilia* endosperm cells remain healthy for 10–14 h at 20–22°C by this method of preparation.

Glassware

All slides and cover slips, except the temperature control slide, are cleaned by sonication for 5 min in hot Alconox solution, then rinsed 10 times in tap water, 10 times in distilled water, and 10 times in pure distilled (Ballentine, 1954) water. After the final rinse the slides and cover slips are stored in biologically clean screw-cap specimen jars filled with 85% ethanol-Ballentine distilled water solution. Such exacting cleaning of the glassware is necessary to maintain healthy endosperm cells for long periods of time. As needed, small quantities of slides and cover slips are dried with Kimwipes (Kimberly-Clark Corp., Neenah, Wis.) and stored in small glass culture dishes with lens paper to protect them from dust and other foreign materials. This is important since such material is usually highly birefringent and/or refractile

and interferes with observations in the polarizing microscope.

The window of the temperature control slide is cleaned with mild white soap, rinsed with tap water, distilled water, Ballantine distilled water, and finally with pure 85% ethanol. After the final rinse the temperature control slide is dried with a Kimwipe.

Microscopy

Observations were made with a modified Leitz Ortholux polarizing microscope, using either a specially selected N-22 Polaroid sheet or a Glan-Thomson prism as a polarizer. The condenser consists of a Leitz UMK 32X (NA 0.4) long working distance objective mounted on a rectifier from an American Optics condenser. The objective lenses used were either Leitz UMK 32X (NA 0.4), or the Nikon rectified 20X (NA 0.4) and 40X (NA 0.65). The illumination for the polarizing microscope is provided by an HBO-200 mercury vapor lamp. Light from this source is filtered with a 546-nm narrow band pass, high transmission, interference filter in combination with a glass heat cut filter. This wavelength of green light has no detrimental effect on the dividing cell of *Haemanthus* endosperm (Inoué and Bajer, 1961). Spindle birefringence was measured visually using a Brace-Kohler compensator modified for making rapid measurements (Inoué et al., 1970). Photographic records were made with the Leitz Ortholux Orthomat system using 35-nm Kodak Plus-X film.

Outputs from the Brace-Kohler compensator, the Ortholux Orthomat automatic camera, and the thermistors in the temperature control slide plumbing, and a digital clock are recorded automatically on a strip chart recorder. Thus, for any given time during the experiment the spindle retardation can be obtained from the series of spikes representing the compensator setting; also, the time and the duration of exposure of the photograph and the temperature of the specimen are known.

The phase-contrast system consists of a Nikon microscope body and condenser with Zeiss phase objectives. Illumination for the phase-contrast microscope was provided by a tungsten filament lamp, filtered for 546 nm green light as described for the polarizing light microscope.

All exposed film was developed in 0.25 strength Kodak Microdol-X for 13 min at 68°F with 30-s intervals of agitation. Fixation, clearing, and washing of the film were carried out at the same temperature.

Magnification was calibrated by photographing a standard stage micrometer through the same optical system as the cells.

OBSERVATIONS

The description of mitotic events and analysis of chromosome movement in *Tilia* endosperm is based on observations on about 50 cells over three flowering seasons (1970–1972).

Since the poleward velocity of chromosomes (Barber, 1939; Ris, 1949) and spindle retardation (Inoué, 1959; 1964; Inoué and Sato, 1967) are affected by temperature, all observations on *Tilia* endosperm reported in this paper were made at a given constant temperature. At 24°C, which is approximately optimum for *Tilia*, the time from nuclear membrane breakdown to the end of telophase averages about 90 min. The typical stages of *Tilia* mitosis are illustrated by representative photographs in Figs. 1 and 4.

Prometaphase to Metaphase Events

After nuclear membrane breakdown, in early prometaphase, the numerous small chromosomes, ($3n = 123$, Dermen, 1932), are scattered in the central region of a weakly birefringent spindle. No distinct birefringent fibers can be resolved at this early stage. During prometaphase (Figs. 1 A, 4 A), which lasts about 0.5 the total time of mitosis, the chromosomes oscillate back and forth for short distances about the metaphase plate and parallel to the spindle axis. The total spindle birefringence and the spindle length increase as prometaphase progresses. About midway through prometaphase, tiny points of birefringence, in contact with the individual chromosomes, become visible. These points of strong birefringence are the first clear manifestations of the kinetochore fibers. This pattern of kinetochore fiber formation seen in *Tilia* appears to be similar to that described for *Lilium* pollen mother cells just before onset of anaphase (Inoué, 1953), and at early metaphase in *Haemanthus* endosperm mitosis (Inoué and Bajer, 1961).

The transition from prometaphase to metaphase is striking. The chromosomes cease to oscillate and rapidly become aligned in a straight row on the metaphase plate. The birefringence of the entire spindle increases during this transition until it becomes stable at metaphase. Surprisingly enough, despite the large number of chromosomes, the kinetochore fibers become distinct (Fig. 1 B) and their birefringence is observed to increase until it is stronger than that of the general spindle.

The distribution of birefringence along the length of the metaphase kinetochore fiber in *Tilia* is similar to that seen in other dividing plant cells (Inoué, 1953; 1964; Inoué and Bajer, 1961). Immediately adjacent to the kinetochore the fiber possesses a tiny point of strong birefringence. The birefringence of the kinetochore fiber gradually decreases until it drops more rapidly where the fiber begins to taper poleward. The numerous

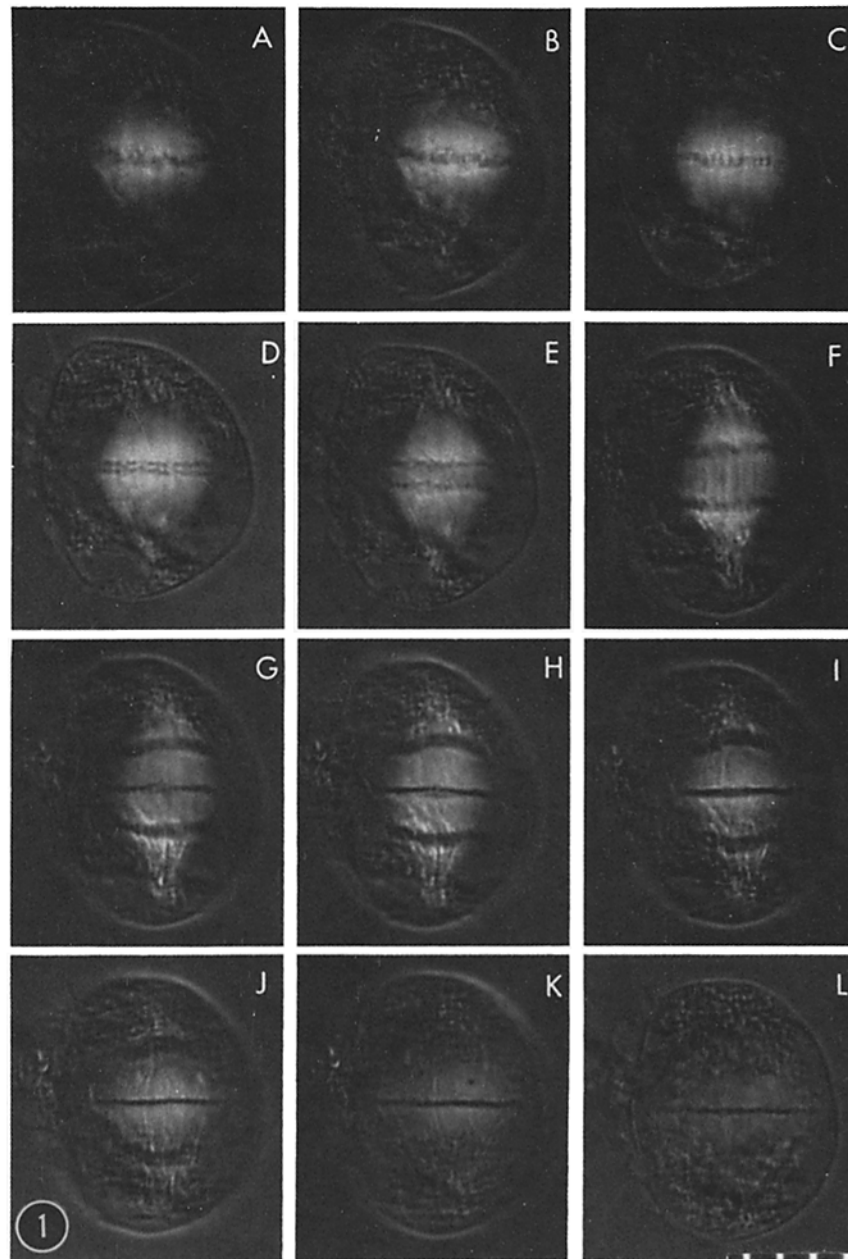


FIGURE 1 The sequence of mitosis in the *Tilia americana* endosperm cell (70g3;1) seen by polarized light microscopy. (Scale interval is 10 μm). **A** 18:53 h, prometaphase. The chromosomes not yet aligned on the metaphase plate. **B** 18:57 h, metaphase. The chromosomes aligned on the metaphase plate. **C** 19:08.8 h, full metaphase. The spindle fibers reach maximum retardation. **D** 19:09.4 h, onset of anaphase. The rows of chromosomes abruptly separate. **E** 19:11.1 h, early anaphase. The rows of chromosomes move synchronously poleward maintaining parallel alignment. **F** 19:14.3 h, mid-anaphase. The phragmoplast is complete and the rows of chromosomes begin to bow. **G** 19:17.5 h, mid-anaphase. Vesicles which form the cell plate begin to condense. The rows of chromosomes show distinct bowing. **H** 19:18.9 h, late anaphase. Cell plate becomes smooth and begins widening. **I** 19:20.5 h, telophase. The chromosomes begin to condense. **J** 19:25.8 h, mid-telophase. **K** 19:30.6 h, late telophase. Daughter nuclei reformed. **L** 19:34.9 h, end of telophase. Cell plate is almost complete. These photographs were selected from a series as being representative of the stages of mitosis described in the text.

kinetochore fibers and part of the continuous spindle fiber produce a region of strong birefringence which extends from the metaphase plate to about 50% of the length of the half-spindle. The birefringence in the polar region of the half-spindle is weaker. There are occasional local fluctuations of birefringence in both regions of the half spindle, the "northern lights effect" described by Inoué (1964).

The gentle taper of the metaphase spindle produces a broad spindle pole, which is reasonably distinct provided the cell is not overly flattened (Fig. 1 C). The distribution and amount of spindle birefringence and spindle morphology remain quite stable during metaphase, which lasts for 5-6 min. The parameters measured on the spindle for analysis of chromosome movement are shown in Fig. 2.

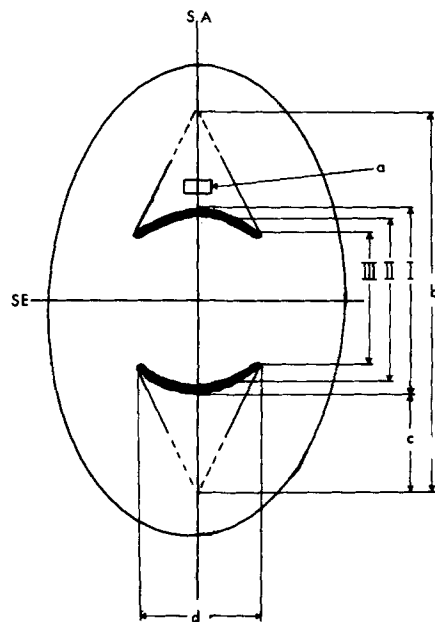


FIGURE 2 Diagrammatic sketch of a *Tilia* endosperm cell in mid-anaphase showing mitotic parameters which were measured. (a) Area in which spindle retardation was measured (see Fig. 3 A). (b) Apparent pole-to-pole separation. (c) Kinetochore to apparent pole distance. (d) Width of the spindle measured as width of the chromosome row. I Kinetochore-to-kinetochore separation on the spindle axis. II Kinetochore-to-kinetochore separation halfway between the spindle axis and the margin of the chromosome row. III Kinetochore-to-kinetochore separation at the margin of the chromosome row. S.A., spindle axis. S.E., spindle equator.

Description of Anaphase

The onset of anaphase is marked by an abrupt separation of all chromosomes (Figs. 1 D, 4 C) and a concurrent increase of the pole-to-pole distance of the spindle (Fig. 3 B, curve *b*). For the initial 4-6 min of anaphase, depending on the individual cell, the chromosomes move very slowly poleward. After this period all the chromosomes in the daughter rows accelerate rapidly, while maintaining precise alignment, and reach maximum poleward velocity in about 1-2 min. The chromosomes move synchronously poleward as two poleward parallel rows until they bow away from the poles in mid-anaphase (Figs. 1 F, 4 E).

The anaphase movement of chromosomes in

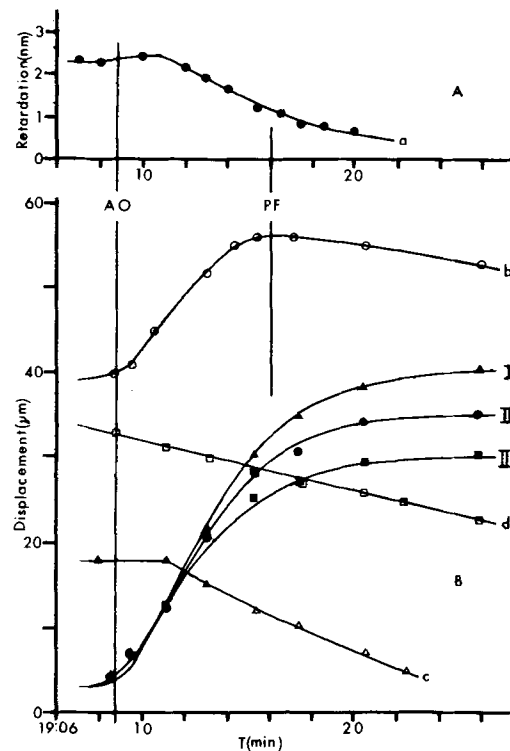


FIGURE 3 A Curve (a) Retardation of the spindle fibers in area (a) (Fig. 2) vs. time. B Typical example of the change with time of the spindle parameters defined in Fig. 2 for a single cell (cell 70g3;1; see Fig. 1). The small letters and numerals in this figure correspond directly to the distances shown in Fig. 2. The solid lines for curves I, II, and III were fitted by the method of least squares and are of the form $y = r^t$, where y is displacement (micrometers) and t is time, i and r are constants. Curves (b), (c), (d) were fitted by eye. AO, anaphase onset. PF, phragmoplast formation complete.

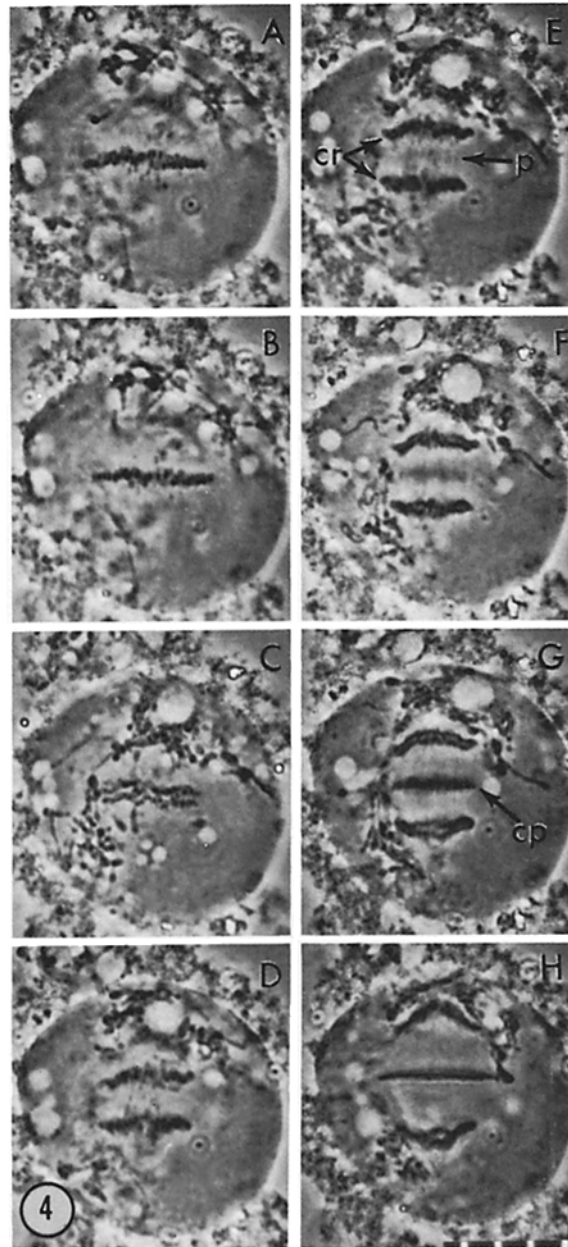


FIGURE 4 The sequence of mitosis in *Tilia* endosperm cell (71g6:1) seen by phase-contrast microscopy. (Scale is 10 μ m.) A 02:18 h, prometaphase. B 02:21 h, metaphase. C 02:39 h, onset of anaphase. D 02:48 h, early anaphase. E 02:52 h, mid-anaphase. Phragmoplast formation complete. F 02:55 h, mid-anaphase. Cell plate formation. G 03:02 h, telophase. H 03:26 h, late telophase. *p*, phragmoplast; *cr*, chromosome rows; *cp*, cell plate. These photographs were selected from a series as being representative of the stages described in the text.

Tilia is accomplished by two mechanisms. Initially, from the onset of anaphase to early anaphase, the chromosomes are displaced by pole-to-pole elongation of the spindle (Fig. 3 B, compare

curve I with curves *b* and *c*). Between early and mid-anaphase there is a combination of pole-to-pole elongation of the spindle and shortening of the kinetochore fibers. The pole-to-pole elongation of

the spindle ceases in midanaphase and the chromosomes are moved to their final position by shortening of the kinetochore fiber (Fig. 3 B, curve *c*). The occurrence of these two mechanisms of anaphase chromosome movement overlap sufficiently in time, resulting in a continuous displacement of chromosomes, which can be described by a single function.

The time course of poleward movement of the daughter chromosomes lying at different distances from the spindle axis, measured as the increase in distance between separating kinetochores, describes a series of S-shaped curves (Fig. 3 B, curves I, II, III). The observed data were fitted to the exponential function (Gompertz, 1825, quoted in Peters and Van Voorhis, 1940):

$$y = r^t, \quad (1)$$

by the method of least squares (solid lines in curves I, II, III in Fig. 3 B). In the equation, $y_t = y_m r^t$, y_t is the observed kinetochore separation at any time (t) and y_m is the maximum separation of the kinetochores in telophase; t is the time in minutes after anaphase onset, and i and r are constants. These constants i and r were determined for chromosomes on the spindle axis (position I), halfway between the spindle axis and the margin of the chromosome row (position II), and the margin of the chromosome row (position III), by the equation:

$$\log \log y = (\log r)t + \log \log i.$$

This equation linearizes the S-curves and yields values of r and i for chromosomes at positions I, II, and III which are shown in Table I. These values of i and r for each position were found to be the same for cells observed in Philadelphia in 1970 and cells observed in Woods Hole in 1972 which

TABLE I

Computed Values of i and r Obtained from Single Cells for Chromosomes at Positions I, II, and III

	I	II	III
Cell I. 70g3 Philadel- phia ($T, 24.8^\circ\text{C}$)			
i	0.063	0.075	0.127
r	0.70	0.69	0.71
Cell I'. 72h1 Woods Hole ($T, 25.0^\circ\text{C}$)			
i	0.064	0.079	0.125
r	0.69	0.71	0.70

were maintained at 24.8°C and 25°C , respectively. The term r is about the same value for each chromosome in the spindle at this high temperature and probably reflects a general characteristic of anaphase movement possessed by chromosomes and/or their respective kinetochore fibers. The value of i increases for chromosomes lying at increasing distances from the spindle. Each individual chromosome in the row would possess a unique value of i , which may simply be a function of the geometry of the half-spindle.

Analysis of Chromosomes Row Bowing

I conceive that bowing of the rows of chromosomes away from the spindle poles arises in the following manner. When the kinetochore-to-pole distance remains constant, only minimal or no bowing of the rows of chromosomes is observed. The entire half-spindle, including the chromosomes, is moved poleward as a unit. When the pole-to-pole elongation of the spindle ceases and the chromosomes are moved poleward by shortening of their kinetochore fibers, bowing of the chromosome rows occurs. It appears that all of the chromosomes maintain equal velocity toward the spindle pole at any given time during anaphase, and that the chromosomes toward the margin of the spindle have a greater distance to travel with respect to the chromosomes on the spindle axis; those chromosomes farther removed from the spindle axis will be progressively retarded in displacement parallel to the spindle axis, resulting in the bowed configuration.

This explanation of bowing of the chromosome rows can be quantitatively substantiated by the following treatment. When the displacement equation (equation [1]) is differentiated with respect to time, the velocity of a chromosome at any position in the spindle parallel to the spindle axis is obtained. This velocity equation is:

$$dy/dt = \bar{V}_{(t)n} = [(ln i)(ln r)]r^t \dot{r}^t, \quad (2)$$

since $y = r^t$, then:

$$\bar{V}_{(t)n} = [(ln i)(ln r)] r^t y_{(t)n}, \quad (3)$$

where n defines the chromosomes at position I, II, or III. The calculated velocities $\bar{V}_{(t)n}$ parallel to the spindle axis for chromosomes at positions I, II, and III on the chromosome row are given in Fig. 5.

From the geometry of the half-spindle, the velocity toward the pole (\bar{V}_p) of each chromosome is the resultant of its velocity parallel to the spindle axis ($\bar{V}_{(t)n}$) and its velocity toward the spindle axis

(\bar{V}_w). That is $\bar{V}_{p(t)n} = \bar{V}_{v(t)n} + \bar{V}_{w(t)n}$ in vector notation (see Fig. 6). For chromosomes on the spindle axis ($\bar{V}_w = 0$) and (\bar{V}_p) is equal to (\bar{V}_v). This is the velocity for chromosomes at position I, and is defined as $\bar{V}_{v(t)}$. The initial assumption for the explanation of bowing states that the velocity toward the pole at time (t) for all chromosomes is equal, namely $\bar{V}_{p(t)n} = \bar{V}_{v(t)}$. The velocity of the chromosome toward the spindle axis ($\bar{V}_{w(t)n}$) is determined from Fig. 3 B, curve *d*. $\bar{V}_{u(t)n}$ for chromosomes at any position may then be determined graphically (Fig. 6).

If the assumption that all chromosomes at time (t) maintain the same velocity toward the spindle pole is valid, then $\bar{V}_{u(t)n}$ should equal $\bar{V}_{v(t)n}$ calculated from equation (2). The displacement values $y_{(t)n}$ calculated by substitution of $\bar{V}_{u(t)n}$ into equation (3) must then equal the observed displacements (y , Fig. 3 B, curves I, II, and III). As shown in Fig. 7, the graphically determined values of $\bar{V}_{u(t)II}$ (circles) and $\bar{V}_{u(t)III}$ (triangles) are in close agreement with the values of $\bar{V}_{v(t)n}$ (solid lines) computed from equation (2). The displacement of $y_{(t)n}$ (triangles in Figs. 8 and 9 for chromosomes at positions II and III, respectively) calculated by substituting the graphically determined values of $\bar{V}_{u(t)n}$ in equation (3) shows close agreement with observed values of chromosome displacement, y (squares in Figs. 8 and 9) and the least squares

lines (solid curve) fitted to the y values. By close agreement, it is meant that the calculated values of displacement fall within the 95% confidence limits determined for the least squares curve fitted to the observed displacement values. These observations seen in Figs. 8 and 9 provide quantitative justification of the explanation presented for the bowing of chromosome rows in late anaphase.

Anaphase Spindle Birefringence Changes

The birefringence of the half-spindle measured the same distance, 3–4 μm , from a row of chromosomes on the spindle axis, remains fairly constant for a few minutes after the chromosomes have separated at anaphase onset, then begins to decay. The presence of this lag period before the spindle birefringence begins to decay varies in duration from cell to cell. In other cases, however, the birefringence of the half-spindle may begin to decay immediately after the chromosomes separate. In general, when the chromosomes approach their maximum poleward velocity in early anaphase, the half-spindle birefringence begins to decay exponentially. Analysis of the decay of anaphase birefringence and its significance is presented elsewhere (Fuseler, 1972). The time constant of the exponential decay of half-spindle retardation is not affected by the bowing of the chromosome rows or the transition of chromo-

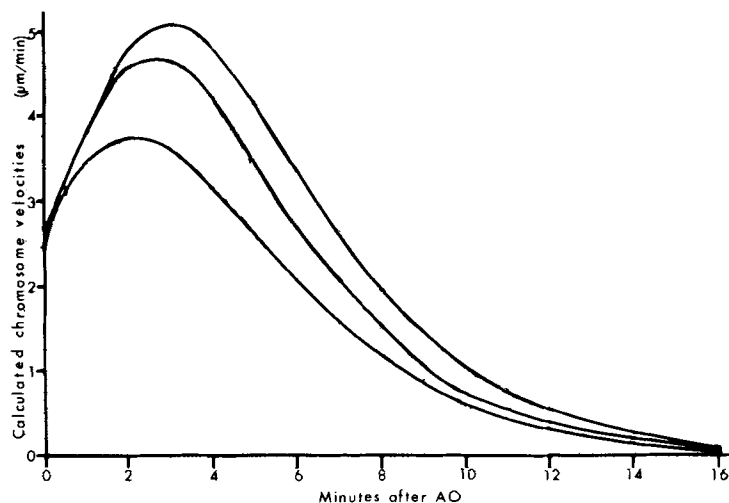


FIGURE 5 The calculated velocity of chromosomes parallel to the spindle axis at positions I, II, and III determined by equation (2); and the values of t and r as shown in Table I. The time taken for anaphase onset (AO) was 19:09.4 h as seen in Fig. 1 D. Since the chromosomes have already separated and are moving poleward this is not the precise time of anaphase onset, and displacement $y_{(t)n}$ is not zero, the velocity of the chromosomes calculated from equation (2) will not be zero at anaphase onset by this treatment.

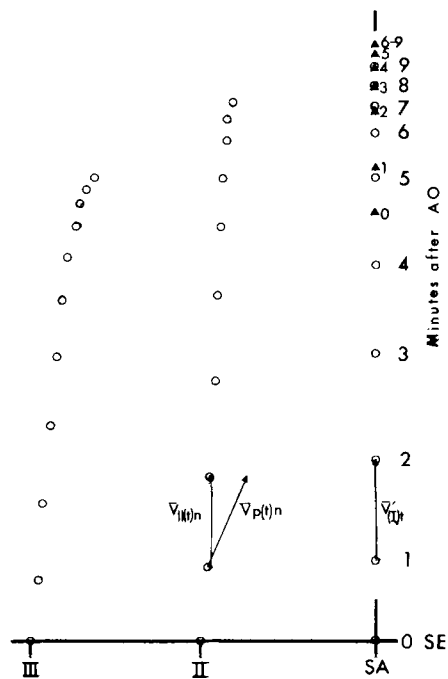


FIGURE 6 Accumulative velocity plot for the independent determination of $\bar{V}_{v(t)n}$ from geometry of the spindle assuming that bowing of the chromosome rows is the result of all the chromosomes maintaining equal velocity toward the poles at any time during anaphase. A quarter of the spindle is represented. $\bar{V}_{v(t)n}$ is equal to $\bar{V}_{p(t)n}$, the velocity of the chromosomes toward the pole at any time, t , at position n , N is II and III. $\bar{V}_{v(t)n}$ is the graphically determined velocity of the chromosomes parallel to spindle axis (SA) at time (t) and position n . The solid triangles and small numbers refer to the positions of the apparent spindle pole. 0 min is defined as anaphase onset (AO). Open circles and large numbers refer to the chromosome velocities after anaphase onset. SE is the spindle equator. Refer to text for details.

some movement from pole-to-pole elongation to movement by kinetochore fiber shortening, or by phragmoplast formation. In telophase, when the chromosomes have ceased moving, the half-spindle birefringence is extremely weak or not detectable.

A small portion of the kinetochore fiber, about $1\text{-}\mu\text{m}$ long, immediately adjacent to the kinetochore, maintains constant birefringence until late anaphase when it decreases. The remainder of the kinetochore fiber birefringence, which largely comprises the half-spindle, decays exponentially as described above (Fig. 3 A and 10 B). The kinetochore fibers undergo a slight increase in length from onset of anaphase to midanaphase. In mid-

anaphase, when the rows of chromosomes begin bowing, the kinetochore fibers undergo a linear decrease in length, which continues until telophase (Fig. 3 B, curve c). The measurements of the changes in length of the kinetochore fibers are approximate because the poleward ends of the fibers are diffuse, and the exact location of the poles is difficult to determine.

Phragmoplast Formation

The interzonal fiber birefringence remains weak for 3–5 min after the initial separation of the rows of chromosomes at onset of anaphase. From early to midanaphase there is a rapid increase in the interzonal fiber birefringence, which may reach a value of retardation equal to or greater than that of the metaphase spindle (Fig. 10). By midanaphase, the uniformly birefringent interzonal fibers have condensed into strongly birefringent, individual, parallel fibers of the phragmoplast (Fig. 1 F). Small vesicles and particles of the rapidly forming cell plate are seen to condense in the middle of the phragmoplast at this time (Figs. 1 G, 4 F). The surface of the cell plate, which is irregular in midanaphase, becomes smooth with the completion of condensation of the vesicles and particles in late anaphase. The cell plate begins widening perpendicular to the spindle axis by late anaphase, and makes contact with the cell membrane in telophase. During this period, late anaphase to telophase, the birefringence of the cell plate fibers undergoes a slow exponential decay. Weak, residual birefringent fibers may persist on the periphery of the cell plate for 1 h or more after the end of telophase.

DISCUSSION

Synchronous movement of numerous small chromosomes as seen in *Tilia* anaphase is fairly common in the dividing cells of both plants and animals. Endosperm mitosis in the dicotyledons *Pisum sativum*, *Lupinus polyphyllus*, and *Helianthus annuus*, and in the monocotyledon *Asparagus pseudoscaberr* (Bajer and Molé-Bajer, 1954), shows small chromosomes that exhibit this type of poleward movement. Similar anaphase movement is seen in plant species which possess large chromosomes, for example *Haemanthus kathariniae* (Bajer, 1954; 1958 *a* and 1958 *b*; Bajer and Molé-Bajer, 1956), or chromosomes with diffuse kinetochores, for example *Luzula* (Malheiros and de Castro, 1947; Ostergren, 1949).

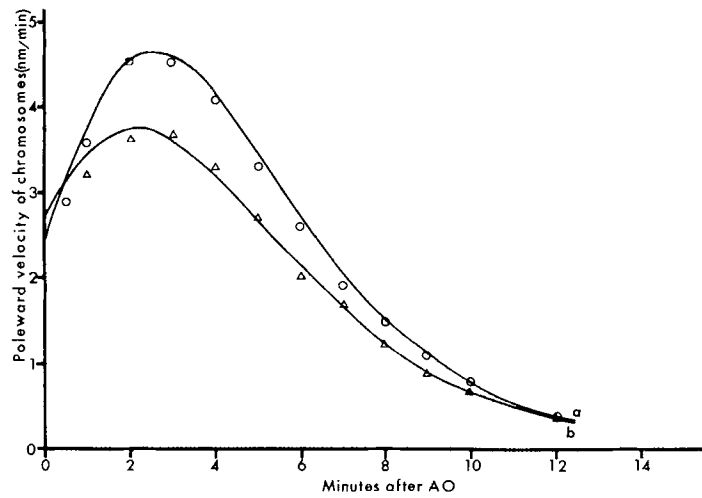


FIGURE 7 The graphically determined values of poleward chromosome velocity parallel to the spindle axis \bar{V}_{chrom} as illustrated in Fig. 6 for chromosomes at position II (open circles) and position III (open triangles) show agreement with the compound values of $\bar{V}_{(II)}$ and $\bar{V}_{(III)}$ the solid curves *a* and *b* respectively, obtained from equation (2).

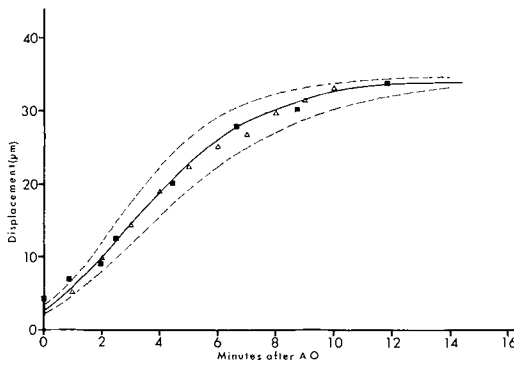


FIGURE 8 Poleward displacement of the chromosomes at position II (open triangles) calculated with the graphically determined values of $\bar{V}_{(II)}$ and equation (3) solved for:

$$y_{(II)} \text{ i.e., } y_{(II)} = \frac{V_{(II)}}{(\ln_{11} \ln r_{11})(r_{11})^t}$$

shows close agreement with the observed values of chromosome displacement (solid squares) and the least squares curve (solid curve) fitted to the observed values. The dashed-line curve denotes the 95% confidence limit of the observed data.

The numerous small chromosomes of various sea urchins have been observed to move poleward as anaphase "plates" (Mazia, 1961). Similar anaphase movement is found in the mitosis of the giant amoeba *Amoeba carolinensis* (Short, 1946; Berkely, 1948). The chromosomes of cultured

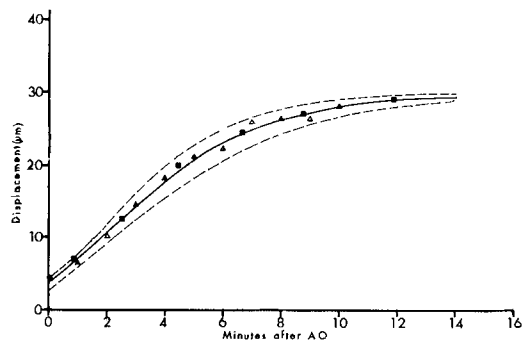


FIGURE 9 Poleward displacement of the chromosomes at position III (open triangles) calculated with the graphically determined values of

$$\bar{V}_{(III)} \text{ and } y_{(III)} = \frac{V_{(III)}}{(\ln_{11} \ln r_{11})(r_{11})^t}$$

shows close agreement with the observed values of chromosome displacement (solid squares) and the least squares curves (solid line curve) fitted to the observed values. The dashed-line curves denote the 95% confidence limit of the observed data.

chick cells (Hughes and Swann, 1948) and the autosomes of various insect spermatocytes (Ris, 1943; 1949; Forer, 1965) also show fairly synchronous poleward movements.

In the most common manner of anaphase chromosome movement, all chromosomes enter anaphase at the same time and move poleward

with equal velocities. There are exceptions to this generality. Various chromosomes, especially the sex chromosomes in certain insect spermatocytes (Schrader, 1935; Ris, 1949), begin to move poleward at a time different from that of the other chromosomes in the spindle. The poleward velocity of certain chromosomes, especially the marginal autosomes in grasshopper spermatocyte meiosis, may be characteristically different (Nicklas, 1965). The anaphase movement of the chromosomes of *Tilia* follows the general pattern in which all the chromosomes enter anaphase at the same time and move toward the poles at the same velocity.

The phenomenon of rows of chromosomes bowing away from the spindle pole in midanaphase was previously described in the mitosis of the rhizopod *Pamphagus hyalinus* (Belar, 1921). The concave configuration attained by the chromosomes was, however, attributed to their fusion into solid daughter anaphase plates. Also, in a single example of a living *Colchicum autumnale* endosperm cell in anaphase (Bajer and Molé-Bajer, 1956), I observed rows of small chromosomes bowing away from the spindle poles. A description of the bowing of chromosome rows in *Colchicum* was not presented in the text of the Bajer and Molé-Bajer (1956) article. In pollen mitosis of *Orchis* (Hagerup, 1938) and of the massulate orchid *Dactylorhiza* (Heslop-Harrison, 1968), the chromosomes which form the vegetative nucleus, when seen in late anaphase or telophase, have approximately the same shape as the condensing chromosomes in late anaphase of *Tilia*. These observations were made on fixed cells in late anaphase to telophase. Cells in early stages of

anaphase were absent. Since the information in these papers is severely limited, it can only be speculated that these anaphases resemble the anaphase of *Tilia*.

In their general behavior the chromosomes as they move poleward in mid to late anaphase bow towards the spindle pole. In the position they take, the various kinetochores form an arc and converge toward the pole which acts as the arc's focus. Nicklas (1965) points out that this condition is the general one, and demonstrates that in *Melanoplus* spermatocyte meiosis, bowing or convergence of the autosomes toward their respective poles is due to the fact that the marginal autosomes move toward the poles with a 25% greater velocity than those on the spindle axis. Similar behavior of autosomes is found in meiosis in other insect spermatocytes (Nicklas, 1963; Forer, 1966), in *Ostracod* meiosis (Dietz, 1958), in mitosis in cultured newt (Boss, 1954) and chick (Hughes and Swann, 1948) cells, in mitosis in various endosperm (Bajer and Molé-Bajer, 1954), including *Haemanthus* (Bajer, 1957; 1958 a and 1958 b; Inoué and Bajer, 1961), and in meiosis in *Lilium* pollen mother cells (Inoué, 1964). Holokinetic chromosomes in plants, for example *Luzula* (Ostergren, 1949), and animals, for example various coccids (Ris, 1943; Cooper, 1939; Hughes-Schrader, 1940), which move poleward perpendicular to the spindle axis also bow toward the pole in late anaphase. When the long holokinetic chromosomes of *Steatococcus* (haploid male, $n = 2$) are fragmented by X irradiation, the individual fragments as well as the entire row of chromosome fragments bow toward the pole (Hughes-Schrader and Ris, 1941).

The temporal separation of the two mechanisms of chromosome movement found in *Tilia* is not generally seen in the dividing plant cells. Chromosome movement in *Haemanthus* (Bajer, 1968 b) and maize (Duncan and Persidsky, 1958) endosperm divisions is accomplished by simultaneous pole-to-pole elongation of the spindle and kinetochore fiber shortening. Pole-to-pole elongation of the spindle is absent in staminal hair and petal mitosis in *Tradescantia* (Barber, 1939). Ostergren (1949) states that in many higher plants, pole-to-pole elongation of the spindle is absent or negligible.

The sequence of occurrence of the two mechanisms of chromosome movement seen in *Tilia* is the reverse of what is found normally in animal cells. Generally, in animal cells the chromosomes

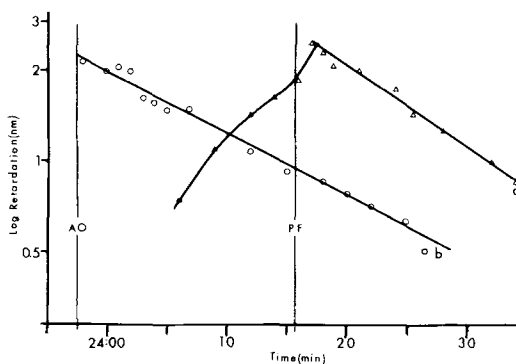


FIGURE 10 The decay of phragmoplast fiber retardation (curve a, triangles) and of anaphase spindle fiber retardation (curve b, circles) exhibit similar linear curves when log retardation is plotted vs. time. AO, anaphase onset. PF, phragmoplast formation complete.

are moved poleward initially by shortening of the kinetochore fibers, then they are separated farther by pole-to-pole elongation of the spindle. This type of chromosome movement is found in mitosis in cultured newt (Boss, 1954) and chick (Hughes and Swann, 1948) cells. Similar behavior is also observed in the poleward movement of the autosomes in spermatocyte meiosis in various insects (Ris, 1943; 1949; Nicklas, 1963; 1965; Jacques and Biesele, 1954; Makino and Nakanishi, 1955; Forer, 1966). Pole-to-pole elongation of the spindle and shortening of the kinetochore fibers occur simultaneously in some *Orthopteran* spermatocytes (Izutsu, 1960).

The formation of the phragmoplast and the behavior of the interzonal fiber birefringence observed in *Tilia* are unlike those seen in other plant cells. The birefringence of the interzonal fiber increases as the chromosomes move poleward, and it reaches a maximum value of retardation before midanaphase with the completion of the phragmoplast. As the cell plate forms in midanaphase with the completion of the phragmoplast and begins increasing in width, the birefringence of the phragmoplast fibers begins a slow decay, becoming very weak by telophase. In the endosperm mitosis of *Haemanthus* (Inoué and Bajer, 1961), the interzonal fiber birefringence decreases during anaphase and becomes very weak or undetectable by late anaphase. As the phragmoplast begins forming at the end of anaphase, the birefringence of the interzonal fibers increases. These phragmoplast fibers become stronger in retardation as the cell plate forms, in early telophase. The phragmoplast fiber birefringence decreases centrifugally along the axis of the cell plate as it is completed. In *Lilium* (Inoué, 1953) the phragmoplast forms later in telophase than is observed in *Haemanthus*. Generally, phragmoplast formation in other plant species takes place in late anaphase to telophase (Wilson, 1928).

Presented to the faculty of the University of Pennsylvania in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

This work was supported in part by National Institutes of Health grant CA10171, National Science grant GB37139X awarded to Dr. Shinya Inoué, and training grant 5T01 HD00030.

Received for publication 7 February 1974, and in revised form 7 October 1974.

REFERENCES

- BAJER, A. 1954. Cine-micrographic studies on mitosis endosperm. I. *Acta Soc. Bot. Pol.* **23**:383-412.
- BAJER, A. 1957. Cine-micrographic studies on mitosis in endosperm. III. The origin of the mitotic spindle. *Exp. Cell Res.* **13**:494-502.
- BAJER, A. 1958 a. Cine-micrographic studies on mitosis in endosperm. IV. The mitotic contraction stage. *Exp. Cell Res.* **14**:245-256.
- BAJER, A. 1958 b. Cine-micrographic studies on mitosis in endosperm. V. Formation of the metaphase plate. *Exp. Cell Res.* **15**:370-383.
- BAJER, A. 1968 a. Fine structure studies on phragmoplast and cell plate formation. *Chromosoma (Berl.)*. **24**:383-417.
- BAJER, A. 1968 b. Chromosome movement and fine structure of the mitotic spindle. Aspects of cell motility. *Symp. Soc. Exp. Biol.* **22**:285-310.
- BAJER, A., and R. D. ALLEN. 1966. Structure and organization of the living mitotic spindle of *Haemanthus* endosperm. *Science (Wash. D.C.)*. **151**:572-574.
- BAJER, A., and J. MOLÉ-BAJER. 1954. Endosperm, material for study on the physiology of cell division. *Acta Soc. Bot. Pol.* **23**:69-109.
- BAJER, A., and J. MOLÉ-BAJER. 1956. Cine-micrographic studies on mitosis in endosperm. II. Chromosome, cytoplasmic, and brownian movements. *Chromosoma (Berl.)*. **7**:558-607.
- BAJER, A., and J. MOLÉ-BAJER. 1972. Spindle dynamics and chromosome movements. *Int. Rev. Cytol.* **34**:271.
- BALLENTINE, R. 1954. High efficiency still for pure water. *Anal. Chem.* **26**:549-550.
- BARBER, H. N. 1939. The rate of movement of chromosomes on the spindle. *Chromosoma (Berl.)*. **1**:33-50.
- BECKER, W. A. 1938. Recent investigations in vivo on the division of plant cells. *Bot. Rev.* **4**:446-472.
- BELAR, K. 1921. Untersuchungen über Thecomben der Chlamyoclophrys-Gruppe. *Arch. Protistenkd.* **43**:287-354.
- BELAR, K. 1929. Beiträge zur Krausalanalyse der Mitose. III. Untersuchungen an den Staubfarbenharrzellen und blattmeristenzellen von *Tradescantia virginica*. *Z. Zellforsch. Mikrosk. Anat.* **10**:73-134.
- BERKLEY, E. 1948. Spindle development and behavior in the giant amoeba. *Biol. Bull. (Woods Hole)*. **94**:196-175.
- BOSS, J. 1954. Mitosis in cultures of newt tissues. III. Cleavage and chromosome movements in anaphase. *Exp. Cell Res.* **7**:443-456.
- COLLINGWOOD, G. H., and W. D. BRUSH. 1964. Knowing Your Trees. American Forestry Association, Washington, D. C.
- COOPER, K. W. 1939. The nuclear cytology of the grass mite, *Pediculopsis graminum* (Reut.), with special

- reference to karyomeiokinesis. *Chromosoma (Berl.)*. **1**:51-103.
- DERMEN, H. 1932. Chromosome numbers in the genus *Tilia*. *J. Arnold Arbor. Harv. Univ.* **13**:49-51.
- DIETZ, R. 1958. Multiple Geschlechtschromosomen bei den Cypriden Ostracoden ihre Evolution und ihre Teilungsverhalten. *Chromosoma (Berl.)*. **9**:359-440.
- DUNCAN, R. E., and M. D. PERSIDSKY. 1958. The achromatic figure during mitosis in maize endosperm. *Am. J. Bot.* **45**:719-729.
- FORER, A. 1965. Local reduction of the spindle fiber birefringence in living *Nephrotoma suturalis* (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol.* **25**(1, Pt. 2):95-118.
- FORER, A. 1966. Characterization of the mitotic traction system, and evidence that birefringent spindle fibers neither produce nor transmit force for chromosome movement. *Chromosoma (Berl.)*. **19**:44-98.
- FUSELER, J. 1972. Unusual aspects of chromosome movement and phragmoplast formation in *Tilia americana*. *Biol. Bull. (Woods Hole)*. **143**:462 (Abstr.).
- HAGERUP, O. 1938. A peculiar asymmetrical mitosis in the microspore of *Orchis*. *Hereditas*. **24**:96-98.
- HARLOW, W. M. 1957. Trees of the Eastern and Central United States and Canada. Dover Publications Inc., New York. 1-288.
- HESLOP-HARRISON, J. 1968. Synchronous pollen mitosis and the formation of the generative cell in massulate orchids. *J. Cell Sci.* **3**:457-466.
- HUGHES, A. F. W., and M. M. SWANN. 1948. Anaphase movements in the living cell. A study with phase contrast and polarized light on chick culture cells. *J. Exp. Biol.* **25**:45-70.
- HUGHES-SCHRADER, S. 1940. The meiotic chromosomes of the male *Llaveiella taenechina* Morrisson (Coccidae) and the question of the tertiary split. *Biol. Bull. (Woods Hole)*. **78**:312-337.
- HUGHES-SCHRADER, S., and H. RIS. 1941. The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragment. *J. Exp. Zool.* **87**:429-456.
- INOUE, S. 1953. Polarization optical studies of the mitotic spindle. I. The demonstration of spindle fiber in living cells. *Chromosoma (Berl.)*. **5**:487-500.
- INOUE, S. 1959. Motility of cilia and the mechanism of mitosis. *Rev. Mod. Phys.* **31**:402-408.
- INOUE, S. 1964. Organization and function of the mitotic spindle. In *Primitive Motile Systems in Cell Biology*. R. D. Allen and N. Kamiya, editors. Academic Press, Inc., New York. 549-598.
- INOUE, S., and A. BAJER. 1961. Birefringence in endosperm mitosis. *Chromosoma (Berl.)*. **12**:48-63.
- INOUE, S., G. W. ELLIS, E. D. SALMON, and J. W. FUSELER. 1970. Rapid measurement of spindle fiber birefringence during controlled temperature shifts. *J. Cell Biol.* **47**(2, Pt. 2):95 a-96 a. (Abstr.).
- INOUE, S., and H. SATO. 1967. Cell motility by labile association of molecules: the nature of mitotic spindle fibers and their role in chromosome movement. *J. Gen. Physiol.* **50** (6, Pt. 2):259-296.
- IZUTSU, K. 1960. Phase-contrast cinematographic studies on meiosis in Orthopteran spermatocytes. II. Chromosomal movement in the first meiotic anaphase. *Cytologia (Tokyo)*. **25**:293-304.
- JACQUES, J. A., and J. BIESELE. 1954. A study of Michel's film on meiosis in *Psophus stridulus* L. *Exp. Cell Res.* **6**:17-29.
- MAKINO, S., and Y. H. NAKANISHI. 1955. A quantitative study on anaphase movement of chromosomes in living grasshopper spermatocytes. *Chromosoma (Berl.)*. **7**:439-450.
- MALHEIROS, N., and D. DE CASTRO. 1947. Chromosome number behavior in *Luzula purpurea* Link. *Nature (Lond.)*. **160**:156.
- MAZIA, D. 1961. Mitosis and the physiology of cell division. In *The Cell*. Vol. 3: Meiosis and Mitosis. J. Brachet and A. E. Mirsky, editors. Academic Press, Inc., New York. 77-412.
- NICKLAS, R. B. 1963. A quantitative study of chromosome elasticity and its influence on chromosome movement. *Chromosoma (Berl.)*. **14**:276-295.
- NICKLAS, R. B. 1965. Chromosome velocity during mitosis as a function of chromosome size and position. *J. Cell Biol.* **25** (1, Pt. 2):119-136.
- OSTERGREN, G. 1949. *Luzula* and the mechanism of chromosome movement. *Hereditas*. **35**:445-468.
- PEKARIK, J. 1932. Ein vergessendes Object für das Studium der Kern und Zellteilungvorgänge in Teken. *Planta (Berl.)*. **16**:788-802.
- PETERS, C. C., and VAN VOORHIS, W. R. 1940. Statistical procedures and their mathematical bases. 1st edition. McGraw-Hill Book Company, New York.
- RIS, H. 1943. A quantitative study of anaphase movement in the aphid *Tamalia*. *Biol. Bull. (Woods Hole)*. **85**:164-174.
- RIS, H. 1949. The anaphase movements of chromosomes in the spermatocytes of the grasshopper. *Biol. Bull. (Woods Hole)*. **96**:90-106.
- SCHRADER, F. 1935. Notes on the mitotic behavior of long chromosomes. *Cytologia (Tokyo)*. **6**:423-430.
- SHORT, R. B. 1946. Observations on the giant amoeba, *Amoeba carolinensis*. (Wilson, 1900). *Biol. Bull. (Woods Hole)*. **90**:8-18.
- WADA, R. B. 1950. The mechanism of mitosis based on the studies of the submicroscopic structures and of the living state of the *Tradescantia* cell. *Cytologia (Tokyo)*. **16**:1-26.
- WILSON, E. B. 1928. *The Cell in Development and Heredity*. 3rd edition. The Macmillan Company, New York. 1-232.