VIDEO DIGITIZER ANALYSIS OF BIREFRINGENCE ALONG THE LENGTHS OF SINGLE CHROMOSOMAL SPINDLE FIBRES

II. CRANE-FLY SPERMATOCYTE CHROMOSOMAL SPINDLE FIBRES ARE NOT TEMPERATURE-LABILE

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SUMMARY

Retardations were measured along the lengths of single chromosomal spindle fibres, from metaphase through anaphase, from video-taped images of crane-fly spermatocytes incubated at various temperatures (4—30°C). These measurements were made using a video digitizer interfaced to a microcomputer. Over most of the range of temperatures at which normal anaphase movement occurs the chromosomal spindle fibres are not temperature-labile. The non-specific and continuous fibre birefringence is temperature-labile, however. The data are discussed with respect to the 'dynamic equilibrium' model of anaphase chromosome movement. We conclude that, since single chromosomal fibre birefringence is not temperature-labile over most of the range of temperatures at which normal anaphase chromosome movement occurs, these data do not support the dynamic equilibrium model of anaphase chromosome movement.

INTRODUCTION

The 'dynamic equilibrium' model of anaphase chromosome movement was based on measurements of spindle birefringence (retardation) in metaphase-arrested oocytes of Chaetopterus pergamentaceus (Inoué, 1952a,b, 1959, 1964). As the temperature was changed the spindle retardation changed to an 'equilibrium retardation' specific for that temperature. The idea that there is a temperature-sensitive dynamic equilibrium that governs the concentration of oriented material in spindle fibres (Inoué, 1959, 1964) is based on the assumption that retardation reflects the concentration of oriented material. These retardation data were criticized as applying only to metaphase-arrested spindles (Forer, 1969); but subsequent data have shown that active (non-arrested) metaphase spindles are also temperature-sensitive and thus that the concept of dynamic equilibrium is generally applicable (e.g. see Stephens, 1973; Fuseler, 1973, 1975b; Salmon, 1975a,b).

The dynamic equilibrium concept was developed into a 'depolymerization' model for anaphase chromosome movement (Inoué, 1964, 1976, 1981, 1982; Inoué & Sato, 1967; Inoué & Ritter, 1975). The model proposes that since the polymeric components of spindle fibres are in dynamic equilibrium with monomers, chromosome movements occur by shifting the equilibrium so that the polymers depolymerize. If fibres depolymerize along their lengths (Inoué, 1964), at the kinetochores (Gruzdev,
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1972) or at the poles (Inoué & Ritter, 1975), while remaining attached to the kinetochore, polymer depolymerization could pull chromosomes poleward. This depolymerization model for force production is based on the effects of temperature on the dynamic equilibrium. By assuming that the measured retardation is proportional to the concentration of oriented polymer (microtubules), the entropy, enthalpy and free-energy changes for the temperature-sensitive polymerization reaction were calculated from data on spindle birefringence versus temperature (e.g. see Inoué, Fuseler, Salmon & Ellis, 1975; Inoué & Ritter, 1975). The calculated values for the change in free energy were used to calculate the force that could be derived from the depolymerization of microtubules (Inoué & Ritter, 1975). These calculations suggested that the depolymerization reaction could indeed produce enough force to move chromosomes.

There are several methodological problems involved in using the data in this way, however. The measurements were only semi-quantitative; they were made visually and at one spot in the spindle. That one spot is not homogeneous: it consists of both chromosomal spindle fibres (force transmitters for chromosome-to-pole movement; e.g. see Nicklas, 1975; Begg & Ellis, 1979) and continuous spindle fibres. In addition the data used for calculating the thermodynamic parameters do not necessarily reflect the concentrations of the oriented material, since the measurements did not take spindle diameter changes into account (discussed by Forer, 1976). We have overcome these problems by using cells in which single chromosomal spindle fibres can be studied. Images of these cells were recorded on video tape and objective values of birefringence were extracted from along the lengths of individual spindle fibres using a video digitizer interfaced to a computer (described by Schaap & Forer, 1983). In this paper we show that individual chromosomal spindle fibres are not in a temperature-sensitive equilibrium over most of their physiological range of temperature: the birefringence along the lengths of chromosomal spindle fibres does not change with temperature over this range. Continuous fibre birefringence, however, is sensitive to temperature. Thus the concept of a temperature-sensitive dynamic equilibrium does not apply to the force-transmitting chromosomal spindle fibres, except at very low temperatures where movement is either extremely slow or does not occur.

MATERIALS AND METHODS

Spermatocyte cultures from laboratory-reared Nephrotoma suturalis (Loew) and Nephrotoma ferruginea (Fabricius), crane flies, were prepared as described by Schaap & Forer (1979, 1983) and Forer (1982). The experimental techniques are described in detail in the preceding paper (Schaap & Forer, 1983), but also are described briefly here. The temperature of the spermatocyte culture was controlled by a temperature-control slide in which the coolant flowed directly under the coverslip on which the cells were situated. Cells were video-taped at the experimental temperature, using polarizing optics. (The photographs in this paper were taken from 'stop frame' video images using a 35 mm camera mounted on a tripod.) In most experiments suitable flat, late metaphase cells were found and were then taken to the experimental temperature by allowing coolant to enter the temperature-control slide for at least 5 min prior to the cell entering anaphase. For some experiments (the 'step-up' experiments), the cells were taken from room temperature (RT, which is 22–24 °C) to about 5 °C (in one jump) and then gradually taken back to about 20 °C in a step-wise fashion (by
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increasing the temperature of the coolant) over a period of 1–3 h. For the initial 'jump' down, coolant from the circulating water bath was allowed to enter the tubing and consequently the temperature-control slide. The cells were at about 10°C in 1–2 min and at about 6°C in about 5 min; however, the remaining drop to 4 or 5°C sometimes took an additional 5 min. The cells were maintained at the cold temperature for 10–30 min. The temperature was then increased in a step-wise fashion by changing the setting on the water-bath thermostat by several degrees. The temperature at the slide was monitored using a surface thermistor. Anaphase was usually in progress by the time the cells were back to 20°C.

The video tape recorder (VTR) that we use has an automatic gain circuit (AGC), the effects of which we described previously (Schaap & Forer, 1983); as noted in the figure legends, some of the data were taken with the AGC 'on' and some with the AGC 'off' (discussed by Schaap & Forer, 1983).

Some living spermatocytes were embedded in a fibrin clot (Forer, 1972), at room temperature, and fixed by the addition of 2% glutaraldehyde at room temperature prior to observation (detailed description of the technique will be given elsewhere). Subsequent temperature treatment was identical to that used for living cells.

Retardations along the lengths of single chromosomal spindle fibres were obtained at various times from single video fields using the previously described video digitizer system (Schaap & Forer, 1983). Single chromosomal spindle fibres in the video field were selected and scanned on several successive video fields. The intensity data were stored on floppy diskettes by the microcomputer. The computer subsequently used a calibration curve to convert the intensities to retardations. The system and method of measuring are described in detail in the preceding paper (Schaap & Forer, 1983).

RESULTS

Spermatocytes of both *N. suturalis* and *N. ferruginea* (Fig. 1) were used to study chromosomal spindle fibre birefringence. There is comparatively little continuous fibre (non-chromosomal fibre) birefringence in *N. suturalis* spermatocytes and thus when the cell is flat the single chromosomal fibres can be seen clearly. Even in cells that are not perfectly flat one or two definitely single chromosomal fibres can usually be found. On the other hand, there is considerable continuous fibre birefringence between the chromosomal fibres and over the chromosomes in spermatocytes of the closely related species *N. ferruginea* and it is often very difficult to discern single chromosomal spindle fibres unless the cells are extremely flat. (This continuous fibre birefringence fluctuates throughout metaphase and anaphase: some of it is 'diffuse',

![Fig. 1. Metaphase spermatocytes of *N. suturalis* (A) and *N. ferruginea* (B). Some fibres are indicated by arrowheads. Single chromosomal spindle fibres are clear in (A) but obscured by continuous fibres in (B). Room temperature. ×1400.](image-url)
while some of it is more localized and 'fibrous'.) For this reason most of the chromosomal spindle fibre retardations that were quantified were from *N. suturalis* spermatocytes. The shapes of the retardation profiles (retardation versus position) were the same for chromosomal spindle fibres in both species.

The effect of temperature on the birefringence along the lengths of single chromosomal fibres was studied over a temperature range from 4°C to 30°C. The physiological range of temperatures at which divisions occur normally is about 6°C to 28°C for *N. ferruginea* and about 8°C to 30°C for *N. suturalis*. (In *N. ferruginea* spermatocytes there are abnormal divisions at 30°C; below 6°C there is either no movement, it is too slow to measure, or it is delayed beyond the limits of the experimenter's patience. In *N. suturalis* spermatocytes anaphase movements at temperatures above 30°C or below 8°C have not been studied. We expect that 30°C is near the upper limit for normal *N. suturalis* spermatocytes because the two species are very similar and because the animal colonies do not survive when laboratory conditions are above 27°C for extended periods (Schaap & Forer, 1979; unpublished observations).

We first studied cells that were taken to the experimental temperature shortly before anaphase and kept at that temperature from metaphase through late anaphase. Over the physiological range of temperatures (at which normal movement occurs) there was very little (if any) effect of temperature on retardation: when average retardations at the kinetochores of spindle fibres in both metaphase and anaphase of *N. suturalis* spermatocytes are plotted against temperature there is very little effect of temperature between 10°C and 25°C (Fig. 2). (To obtain the data given in Fig. 2, chromosomal spindle fibre retardations were calculated at several times from late metaphase through anaphase; the kinetochore retardations in this figure are average kinetochore retardations of several repeat measurements at the same time and are representative of the retardations at that stage. High or low retardations resulting from jumps in birefringence (Schaap & Forer, 1983) were not used for these averages.) It is relevant to point out that the effects of temperature on the birefringence at the kinetochore reflect effects of temperature on the birefringence of the rest of the fibre (Schaap & Forer, 1983). Similar data for *N. ferruginea* spermatocytes and some second meiotic divisions are given in Table 1. Though chromosomal fibre birefringence does not change much between 10°C and 25°C, it does change outside this range (Fig. 2).

Whereas there seems to be little change in chromosomal spindle fibre birefringence

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Fig. 2. Effect of temperature on the retardation at the kinetochore (*N. suturalis*) during late metaphase (a) and early anaphase (b). A representative kinetochore retardation value was taken at each of these times and averaged with those from other fibres. The averages and their standard deviations (vertical bars) are graphed. The number below the error bars represents the number of fibres used in the average while the number in parenthesis represents the number of cells. Data were grouped together so that the temperatures are ±1 deg. C. These cells were subjected to a constant temperature after the shift from the normal environmental temperature (22–24°C). (▲) and (●) were calculated from data taken with the AGC on or off, respectively.
Temperature effects on spindle fibres

Fig. 2
Table 1. Kinetochore birefringences

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Retardation (±S.D.) (nm)</th>
<th>No. of fibres</th>
<th>No. of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. ferruginea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.11 (±0.25)</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>RT</td>
<td>1.38 (±0.20)</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>RT</td>
<td>1.33 (±0.26)*</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Early anaphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. ferruginea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.27 (±0.21)</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>RT</td>
<td>1.55 (±0.22)</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>RT</td>
<td>1.33 (±0.29)*</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td><em>N. suturalis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1.43 (±0.23)*†</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

The retardations are given as average kinetochore retardations (and standard deviations) of the number of fibres measured. These data were not included in Fig. 2. All the data were recorded with the ACC ‘off’ except for those marked †. The average retardations marked * are from second meiotic division cells and includes data from both sex-chromosomal and autosomal spindle fibres as the two types of chromosomes could not be distinguished between.

with temperature, within most of the physiological range of temperatures, it is difficult to decide from the data (Fig. 2) whether there might indeed be a small change in birefringence with temperature: there are large spreads in the values of birefringence for fibres at any given temperature, as indicated by the large standard deviations. The comparisons are further complicated by the fact that the birefringence of an individual fibre can vary by as much as 25 or 30% during metaphase and anaphase (Schaap & Forer, 1983) and by the fact that the data are derived from different numbers of fibres from different cells. Use of Student’s t-test indicates that there is most likely no effect of temperature, but the results are ambiguous. To decide how much the birefringence of individual chromosomal spindle fibres might vary with temperature, we studied individual spindle fibres as the temperatures were changed.

We studied individual *N. suturalis* and *N. ferruginea* metaphase spermatocytes that were rapidly taken from room temperature to 4°C or 5°C; then the temperature was increased gradually (‘step-up’ experiments) to about 20°C (Figs 3, 4, 5). (Spindle fibre retardations do not change through metaphase and anaphase (Schaap & Forer, 1983), and thus any one chromosomal spindle fibre should be of constant birefringence except for the effects of temperature and random jumps.) Figs 6, 7, 8 illustrate retardations along the lengths of three fibres treated in step-up experiments. At 4°C or 5°C the chromosomal spindle fibres were usually only barely visible (this was variable, however), so their retardation profiles were very noisy. The birefringences of individual chromosomal spindle fibres recovered rapidly by 7°C or 8°C and were back to room temperature levels by about 10°C. Above 10°C and up to at least about 22°C there was no further change in chromosomal spindle fibre birefringence in the 18 *N. suturalis* or 20 *N. ferruginea* spermatocyte chromosomal spindle fibres (from 4 cells of each species), although, as with cells held at a constant temperature (Schaap & Forer, 1983), fluctuations in birefringence sometimes occurred.
Glutaraldehyde-fixed cells were studied to see if the effect of temperature on chromosomal spindle fibre birefringence was somehow an artefact of our analysis procedure. Two cells were analysed in detail (11 chromosomal spindle fibres); the cells at room temperature were fixed with glutaraldehyde at room temperature and the fixed cells were then placed at different temperatures. In both cells chromosomal spindle fibre birefringence was about the same as that in living cells and birefringence...
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Fig. 6. Step-up experiment retardation profiles of a chromosomal spindle fibre of a *N. suturalis* spermatocyte. The temperature causes the fibre to be shorter at low temperatures (compare 4.5 °C and 10 °C). P and K in this and subsequent figures represent the pole and kinetochore regions, respectively. Each + in these and subsequent fibre profiles represents the retardation data obtained from one pixel. Depending on the angle of the fibre on the TV monitor, there are five to nine pixels per μm (see Schaap & Forer, 1983a). A. Metaphase; B, very early anaphase; C, anaphase. AGC off.

did not change at all when individual fibres were studied at various temperatures between 4 and 22 °C (Fig. 9). Thus our technique would seem to work, and effects of temperature on chromosomal fibre birefringence *in vivo* reflect changes in spindle fibres and not artefacts of the technique.

The effects of temperature on interzonal birefringence were studied to see if the temperature sensitivity of spindle birefringence observed by other workers (e.g. see Inoué, 1952a, 1959, 1964; Fuseler, 1973, 1975b; Stephens, 1973; Salmon, 1975a)
could be contributed primarily by the continuous fibres; the birefringence in the interzone of crane-fly spermatocytes was used as a measure of continuous birefringence. However, because different cells have different thicknesses, the measured retardations are not directly comparable between cells; thus without correction for variable cell thicknesses the data are not directly proportional to the concentration of oriented material.

Interzonal retardations were quantified from *N. suturalis* spermatocytes kept solely at one temperature and from *N. ferruginea* spermatocytes in step-up experiments. Interzonal retardations are weak in *N. suturalis* spermatocytes, but they are indeed temperature-sensitive (Fig. 10). (The retardations in Fig. 10 are averages of several linear scans through the interzone of different cells, each of which was kept solely at one experimental temperature.) Interzonal birefringence is stronger in *N. ferruginea*
spermatocytes than in *N. suturalis* spermatocytes; thus temperature sensitivity of non-chromosomal fibre birefringence should be readily observable in *N. ferruginea* spermatocytes. (Indeed metaphase cells with barely distinguishable chromosomal fibres at room temperature have single fibres at lower temperatures, when the non-chromosomal fibre birefringence is almost eliminated (Fig. 5), as also reported by Salmon & Begg (1980).) Data on interzonal retardations for two cells are given in Fig. 11, from which it is concluded that interzonal birefringence is clearly temperature-dependent. Since any one cell maintains approximately the same width until at least mid-anaphase, and the measurements are always made through the same part of the cell, interzonal retardations at different temperatures within the same cell are comparable, although the exact relationship to concentration of oriented material (coefficient of birefringence) is unknown because the thickness of the cell is unknown. Progression through metaphase and anaphase alone cannot account for the observed retardation changes because, though there may be considerable fluctuations in birefringence in the interzone during anaphase, there is no predictable increase or decrease in interzonal retardation at constant temperatures (Fig. 12). Thus, unlike chromosomal spindle fibres, continuous spindle fibres (interzonal fibres) are in a temperature-sensitive dynamic equilibrium throughout most of the physiological range of temperatures, in both species. Qualitative observations suggest that astral birefringence also varies with temperature in the same way as interzonal birefringence (i.e., aster diameter and birefringence decrease as the temperature decreases).

Spindle pole-to-pole distances were altered by temperature. In some of the step-up experiments cells were filmed prior to the initial drop in temperature. In six out of seven such cells the pole-to-pole distances decreased about 20% during the initial temperature drop. (These were five *N. ferruginea* and two *N. suturalis* spermatocytes.) In the seventh cell (from *N. suturalis*) the pole-to-pole distance decreased by about 10%. The spindles increased in length as temperature was stepped up and generally reached their original lengths by the time that the cells were at 10°C. (The increases in retardation usually started before the increase in length.) Decreases in pole-to-pole distances due to large drops in temperature were noticed previously in grasshopper spermatocytes (Barkas & Nicklas as cited by Nicklas, 1975) and metaphase-arrested *Chaetopterus* oocytes (Inoué, 1952a), although they were not seen in crane-fly spermatocytes by Salmon & Begg (1980). In the crane-fly spermatocytes we studied, the shortening of the pole-to-pole distances occurred when the retardations of the chromosomal spindle fibres became temperature-labile.

The retardation profiles of individual spindle fibres were studied during the step-up experiments to see if we could determine a 'growth end' for a chromosomal spindle fibre. That is, if the birefringent material is added primarily at one end of a spindle fibre, as in current models of spindle microtubule organization (e.g. see Bergen & Borisy, 1980; Margolis, Wilson & Kiefer, 1978), then birefringence might

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Fig. 8. Step-up experiment retardation profiles of a *N. ferruginea* spermatocyte chromosomal spindle fibre showing the build up of retardation after cold treatment. AGC off.
Temperature effects on spindle fibres

Fig. 10. Average interzonal retardations of different *N. suturalis* spermatocytes during early anaphase. Each point represents one cell kept at that temperature throughout anaphase. AGC on.

be expected to grow from one end. We were unable to detect such local changes in birefringence, but rather we saw only increases along the entire length of the chromosomal spindle fibres (e.g. see Fig. 8). Perhaps such local changes could be detected with better time resolution (e.g., using the video tape recorder at 60 frames/s instead of the 1 frame/s we used) or by using a TV camera that would give less electronic noise in the recorded image.

DISCUSSION

Inoué’s pioneering work with the polarizing microscope established without doubt the existence of fibrous elements in the mitotic apparatus of living cells (Inoué, 1952a, b). These fibres can be visualized because they are composed of oriented material and thus are birefringent.

Spindle birefringence was shown to be labile to low temperatures (e.g. see Inoué, 1952a, 1959, 1964). In this paper we have shown that over most of the temperature range at which normal movement occurs chromosomal spindle fibres are not labile to lowered temperatures, but that the continuous (interzonal) fibres are. Others have also shown that chromosomal spindle fibres and kinetochore microtubules tend to be more

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Fig. 9. Retardation profiles of a glutaraldehyde-fixed *N. suturalis* spermatocyte chromosomal spindle fibre at different temperatures. The small variations are probably due to the scans being of slightly different parts of the spindle fibre (see Schaap & Forer, 1983). AGC off.
Fig. 11. Average retardations in the interzonal regions of two *N. ferruginea* spermatocytes in a step-up experiment. (■) Retardation at RT immediately before the temperature jump down. (●) Retardations at different times during the step-up. Each point (■ or ●) is the average of three to five linear scans through the interzonal region at different times and temperatures: the different points at the same temperature are from different images, at slightly different times. AGC off.

Resistant to experimental manipulation (including cold) than non-chromosomal spindle fibres and non-kinetochore microtubules. (Stability of chromosomal spindle fibre birefringence has been demonstrated in: *Chaetopterus pergamentaceus* (Inoué, 1952b); *Dissosteira carolina* and *Hippiscus rugosus* (A. Barkas & R. B. Nicklas, cited by Nicklas, 1975); *N. ferruginea* and *Trimerotropis maritima* spermatocytes (Salmon & Begg, 1980); HeLa cells (Salmon, Goode, Maugel & Bonar, 1976). Stability of kinetochore microtubules—believed to be responsible for most of the observed chromosomal spindle fibre birefringence (e.g. see Inoué et al. 1975; Sato, Ellis & Inoué, 1975; Inoué, 1976)—has been demonstrated in: *Pales ferruginea* (*N. ferruginea*) spermatocytes (Muller, 1972); PtK<sub>1</sub> cells (Brinkley & Cartwright, 1975; Euteneuer & McIntosh, 1981; Rieder, 1981); PtK<sub>2</sub> cells (Roos, 1973); mammalian cells (Brinkley,
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The birefringence observed in the mitotic apparatus is primarily 'form' birefringence, due to the regular alignment of isotropic rods (e.g. see Rebhun & Sander, 1967; Sato, 1975; Sato et al. 1975; Forer & Zimmerman, 1976). When retardation is caused by the alignment of a relatively small volume of rods (<10%), then the coefficient of birefringence (obtained by dividing the measured retardation by the thickness of the object in the direction of observation) is linearly proportional to the concentration of rods in that volume (e.g. see Forer, 1976). Because retardation is a function of thickness, retardations can be compared to other retardations only when the dimensions of the object remain constant. (Similarly, spectrophotometric optical density readings can be directly compared to one another only if a cuvette of the same thickness is used.) Thus, retardation values can be used as measures of the concentrations of oriented material in the mitotic apparatus after experimental manipulation or throughout mitosis under different conditions only if the mitotic apparatus widths or spindle fibre widths do not change.

The chromosomal spindle fibre widths of crane-fly spermatocytes do not change with temperature (Schaap & Forer, 1983). Thus, our measured retardations are
proportional to coefficients of birefringence and to concentrations of oriented material. Since chromosomal spindle fibre retardation is not affected by temperature over most of the temperature range at which normal chromosome movement can occur (i.e. between 10 °C and 25 °C), then there is no change in the concentration of oriented material in the spindle fibres over this range. Thus the oriented material in the chromosomal spindle fibres is not in a temperature-sensitive dynamic equilibrium over most of the physiological temperature range. Since chromosomal spindle fibres transmit the force for chromosome-to-pole movement (e.g. see Nicklas, 1975; Begg & Ellis, 1979), and since the data on 'spindle fibre' lability do not apply to chromosomal spindle fibres, there is no experimental basis for the interpretation that chromosomal spindle fibre organization is regulated by a temperature-sensitive dynamic equilibrium or that chromosomal spindle fibre depolymerization causes anaphase chromosome-to-pole movement. In contrast to the chromosomal spindle fibres, the continuous spindle fibre birefringence (as measured in the interzone) is temperature-sensitive. (Salmon & Begg (1980) also found that *N. ferruginea* spermatocyte continuous fibres were temperature-sensitive whereas chromosomal spindle fibres were stable at low temperatures.) We suggest, therefore, that the results of previous workers (e.g. see Inoué, 1952a, 1959, 1964; Fuseler, 1973, 1975a; Stephens, 1973; Salmon, 1975a) on the lability to decreased temperature of overall spindle birefringence pertains primarily to continuous fibres and not to chromosomal spindle fibres. Thus although the dynamic equilibrium model could apply to continuous spindle fibres, and perhaps also to spindle elongation and shortening, it cannot apply to the chromosome-to-pole movement that is due to the temperature-insensitive chromosomal spindle fibres. This suggestion can be tested in the following way.

Data from the temperature sensitivity of spindle birefringence have been used to calculate the change in free energy, entropy and enthalpy associated with spindle polymerization (e.g. see Inoué et al. 1975). If, as we argue, this is primarily due to the polymerization of continuous fibres, then calculations of the same parameters from our interzonal birefringence data should give values similar to those reported for whole spindles. The thermodynamic parameters were calculated by Inoué and co-workers on the assumption that the birefringence values are direct measures of the concentration of oriented microtubules and that monomer subunits are in an equilibrium with the polymers so that:

\[
(A_0 - B) \xrightarrow{k} B.
\]

In this equation \(A_0\) is the maximum concentration of orientable polymer (maximum retardation), \(B\) is the actual concentration of oriented polymer (measured retardation), and \(A_0 - B\) is the remaining monomer (e.g. see Inoué, 1959, 1964; Inoué & Sato, 1967; Stephens, 1973; Fuseler, 1973, 1975b; Salmon, 1975a, b). *Average* spindle retardations are used as measures of \(B\), while the assymptote of the retardation versus temperature curve is used to estimate \(A_0\). Van't Hoff plots (of log \((B/(A_0-B))\) versus \(1/K\)) and the van't Hoff equation are used to calculate the changes in entropy, enthalpy and free energy during the polymerization reaction; the basic procedure for the calculations was outlined by Inoué et al. (1975, p. 735). For our calculations we
Table 2. Thermodynamic parameters calculated from measured retardations

<table>
<thead>
<tr>
<th>Fibre type and species</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$t_0$ (°C)</th>
<th>$\Delta S$ (e.u.)</th>
<th>$\Delta G^*$ (kcal/mol)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td></td>
<td></td>
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<tr>
<td><em>N. ferruginea</em></td>
<td>33·7</td>
<td>17·7</td>
<td>116·1</td>
<td>-0·9</td>
<td>Data Fig. 11A</td>
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<tr>
<td></td>
<td>44·2</td>
<td>10·8</td>
<td>155·6</td>
<td>-2·2</td>
<td>Data Fig. 11B</td>
</tr>
<tr>
<td><em>N. suturalis</em></td>
<td>26·6</td>
<td>19·0</td>
<td>91·0</td>
<td>-0·6</td>
<td>Data Fig. 10</td>
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<tr>
<td>Chromosomal</td>
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<tr>
<td><em>N. suturalis</em></td>
<td>99</td>
<td>6</td>
<td>354</td>
<td>-6·5</td>
<td>Representative data from step-up experiments</td>
</tr>
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</table>

$\Delta H$ is the enthalpy change in kilocalories; $t_0$ is the temperature at which $\Delta G = 0$; $\Delta S$ is the entropy change in entropy units; $\Delta G^*$ is the free energy change under standard temperature and pressure conditions.

*The following retardations were used in the calculations: $A_0$, 1·1 nm; 10 °C, 1·0 nm; 5 °C, 0·3 nm.

used the interzonal birefringence versus temperature data of the two *N. ferruginea* cells given in Fig. 11, and we used the pooled data from *N. suturalis* cells given in Fig. 10. Because the asymptote of the increasing retardations was not clear, the maximum retardation was used to represent $A_0$. The results of our calculations are given in Table 2, together with estimates of these parameters for the polymerization of a chromosomal fibre based on the temperature sensitivity of these fibres below 10 °C, as determined by step-up experiments. (Since exact kinetochore positions were often difficult to determine at the lower temperatures an approximate kinetochore retardation was used.) The values we calculated for the various thermodynamic parameters can be compared with the values in the literature. Inoué & Ritter (1975) reviewed these data and stated that the free energy change for the polymerization reaction is about $-0·7$ kcal/mol (of subunit polymerized), that the reaction is endothermic (enthalpy change is generally about 30–40 kcal/mol) and that the reaction is entropy driven (entropy change is about 100–200 e.u.). (Compilations of the previously calculated thermodynamic parameters have been published by Fuseler (1973), Stephens (1973) and Salmon (1975a).) The thermodynamic parameters we calculated for interzonal birefringence are right within the ranges of the previous values but those we calculated for chromosomal spindle fibres below 10 °C are not. This supports our interpretation that the previously published parameters pertain primarily to non-chromosomal spindle-fibre birefringence. Thus these parameters cannot be used to describe reaction equilibria for the oriented material of the chromosomal spindle fibres.

The data presented here emphasize that one cannot apply the retardation measurements of one area of the spindle to the dynamics of chromosomal spindle fibres. Birefringence measurements not specifically made on single chromosomal spindle fibres include both chromosomal and continuous fibre types and do not reflect the differential sensitivities of the fibres to experimental manipulation. From our data on the different temperature sensitivities of chromosomal and non-chromosomal spindle
fibres, it seems clear that changes in average retardations cannot be used to reflect changes in concentrations of oriented material in both fibre types. Thus birefringence measurements of one single spot in a non-homogeneous spindle can give an erroneous picture even for the mixed population.

Other erroneous conclusions can be drawn from measurements of one spot in a spindle, even if the spot contains only one fibre type. We have shown (Schaap & Forer, 1983) that there is no decay in the birefringence of a crane-fly spermatocyte chromosomal spindle fibre during anaphase. We argued that the temperature-sensitive birefringence decay seen by Fuseler (1973, 1975a,b) was probably an artefact due to the fact that birefringence was measured a few μm in front of the poleward-moving chromosomes. The temperature sensitivity of the anaphase birefringence decay is due to the fact that chromosome-to-pole velocity is temperature-sensitive (e.g. see Fuseler, 1973, 1975b; Schaap & Forer, 1979) and the spot at which the birefringence is measured moves towards the pole faster at higher temperatures than at lower temperatures.

The birefringence of individual chromosomal spindle fibres does not change with temperature over most of the physiological range of temperatures. Chromosome-to-pole velocity does indeed change with temperature, however: chromosome-to-pole movement is faster at higher temperatures than at lower temperatures (Schaap & Forer, 1979). Thus, since chromosomal fibre birefringence is constant throughout anaphase and at different temperatures, there is no correlation between the temperature-dependent chromosome-to-pole velocity and the temperature-independent chromosomal spindle fibre birefringence. The only measured birefringence that changes with temperature in crane-fly spermatocytes is the interzonal birefringence: compare Figs 10 and 11 (on interzonal birefringence) in this paper with fig. 5 (on chromosome-to-pole velocity) of Schaap & Forer (1979).

A final point concerns the identity of material giving rise to spindle birefringence. Chromosomal spindle fibre birefringence is sensitive to temperatures below 10°C. Although chromosome movement was not detected below 6°C (Schaap & Forer, 1979), there is still a 25% or so 'remnant' of the original chromosomal spindle fibre birefringence until at least 4°C is reached. Most of the spindle microtubules have disappeared at this temperature (Schaap & Forer, unpublished). Areas of reduced birefringence produced by ultraviolet microbeam irradiation of single chromosomal spindle fibres have 30–40% of the original birefringence (Sillers & Forer, 1983). 'Residual' birefringence that was about 30–40% of the original birefringence was seen in isolated sea-urchin mitotic apparatuses after the disappearance of the microtubules (Goldman & Rebhun, 1969; discussed by Forer, 1976). These residual birefringence values may reflect the presence of a spindle fibre component that contributes birefringence but has temperature and ultraviolet sensitivities different from that of the bulk of the birefringent material, the microtubules.

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Temperature effects on spindle fibres

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