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6 · Chromosome movements in the meiosis of insects, especially crane-fly spermatocytes

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I write as a cell biologist interested in the mechanisms of chromosome movements during mitosis and meiosis: studies on insect cells have contributed greatly to our understanding of these processes (for example, see review by Schrader 1953). Much of my work has utilized primary spermatocytes from crane-flies (Diptera: Tipulidae); I will try to explain here some of the characteristics of these cells which make them useful as objects of study. To see how studies on crane-fly spermatocytes fit into the general picture it is necessary to first describe the general state of our understanding of chromosome movements.

Chromosome movements during various stages of mitosis and meiosis have been well described from studies of fixed and of living cells under the light microscope. Such studies give good estimates of the timing of stages, chromosome velocities, distances travelled, and so forth. From these data one can calculate the force and energy requirements for moving chromosomes (reviews in Forer 1969; Nicklas 1971); very small forces and very little energy are required. The cytological component responsible for transmitting the force to the chromosome is identified at the light-microscopical level as the chromosomal spindle fibre, which extends between the chromosome and the spindle pole (see Cornman 1944; Schrader 1953; Mazia 1961; Forer 1969, 1974; Nicklas 1971, 1975). Studies with the electron microscope show that microtubules, 25 nm-diameter, hollow-looking cylinders, are attached to the chromosome's centromere (i.e. the spindle fibre attachment region of the chromosome), and thus would seem to have *some* important role or roles in mitosis and meiosis. But it is not known what microtubules do. One of the important problems at present is to identify chemically and ultrastructurally those chromosomal spindle fibre components that produce the force for chromosome movement. Eventually one wants to understand how force production is regulated, but it is difficult to deal with this question until one knows what the 'motor' is. Hence, a prime concern at present is to identify the force producers, and to see how they work, chemically.

Choice of material

Which type of cell does one choose to try to understand what produces the force for chromosome movement? In the scientific tradition I have been brought up with, one assumes that basic mechanisms are similar in different cell types, and then one chooses the cell type which is most appropriate for the particular questions being asked. If, for example, one wants to isolate spindles in order to do chemical analyses, one chooses a cell type that is synchronous (i.e. all the cells enter metaphase and anaphase at the same time)

and in which cells exist in sufficient quantities to do chemical analysis of the isolated spindles. Sea-urchin zygotes are often used for such isolations (Mazia & Dan 1952; review in Forer 1969). Sea-urchin zygotes are not so suitable for other experiments, however. Crane-fly spermatocytes have several characteristics which are extremely valuable in studying mechanisms of chromosome movement during cell division.

First, these insects can be reared in the laboratory, throughout the year, so that cells are available at all times. Methods for rearing crane-flies are described by Forer (1981). Availability of cells is an important criterion, for it is more difficult (and hectic) to do experiments when the experimental material is restricted to a short time of the year than when it is available daily. Other reasons for using crane-fly spermatocytes are cytological and are perhaps most easily explained by describing how preparations are made, and by describing chromosome movements during meiosis.

Preparations of primary spermatocytes are made from testes dissected from fourth-instar larvae. Cells within any given testis are more-or-less synchronous, in that all the cells pass through stages between prophase-I and telophase-II within a 1–2 day period. Because of this synchrony, larvae are dissected only when they have reached the 'proper' age; for *Nephrotoma suturalis*, this is about 7–9 days into the fourth instar. With practice, larvae of the 'proper' age can be recognized from morphological characteristics, without keeping precise records of their age (see Forer 1981).

The main principle followed in keeping spermatocytes alive while dissecting the larvae and removing the testes is to prevent evaporation; the testes are $\lesssim 1\ \mu l$ in volume, and evaporation occurs rapidly in such small volumes. To prevent evaporation I use a heat filter on the viewing lamp and cover the larvae with a non-toxic halocarbon oil, so that the testes are covered with oil at all times. In the final step, a testis that is free from fat and other tissue is placed on a coverslip (under oil) and pierced. The cells come out and become flattened against the coverslip by the oil (for details see Forer & Koch 1973; Schaap & Forer 1979; Forer 1981). Such a 'smear' of living cells can be observed with either an inverted or a normal microscope, using whichever optical system is preferred.

Smear preparations are relatively simple but they are not suitable for perfusion of test chemicals; the perfusion causes the cells to be dislodged from the coverslip. In order to perfuse spermatocytes, they are held in place in a fibrin clot (Forer 1972), surrounded by a buffered insect Ringer's solution (Ephrussi & Beadle 1936). They survive for many hours under these conditions. Other, more complex Ringer's solutions can also be used (Begg & Ellis 1979).

Chromosome movements in living crane-fly spermatocytes, viewed using phase-contrast microscopy, are illustrated in Fig. 6.1. At the end of the prophase the nuclear membrane breaks down. During prometaphase, the next stage, chromosome movements are variable, but chromosomes often move up and back in the direction of the spindle axis. The chromosomes gradually approach the equator, which they all reach at metaphase, about 80–90 min after nuclear membrane breakdown. There are only three autosomal bivalents, plus two unpaired univalents, the X- and Y-chromosomes, and all are visible throughout. The distances between the centromeres at each end of a bivalent are 4–7 μm, and the pole-to-pole distances at metaphase are over 20–25 μm in well-flattened cells.

There are two flagellae (precociously-formed sperm tails) associated with each centriole. A flagellae-plus-centriole complex can be seen light-microscopically at each aster in prophase, and at each spindle pole in prometaphase through late anaphase.

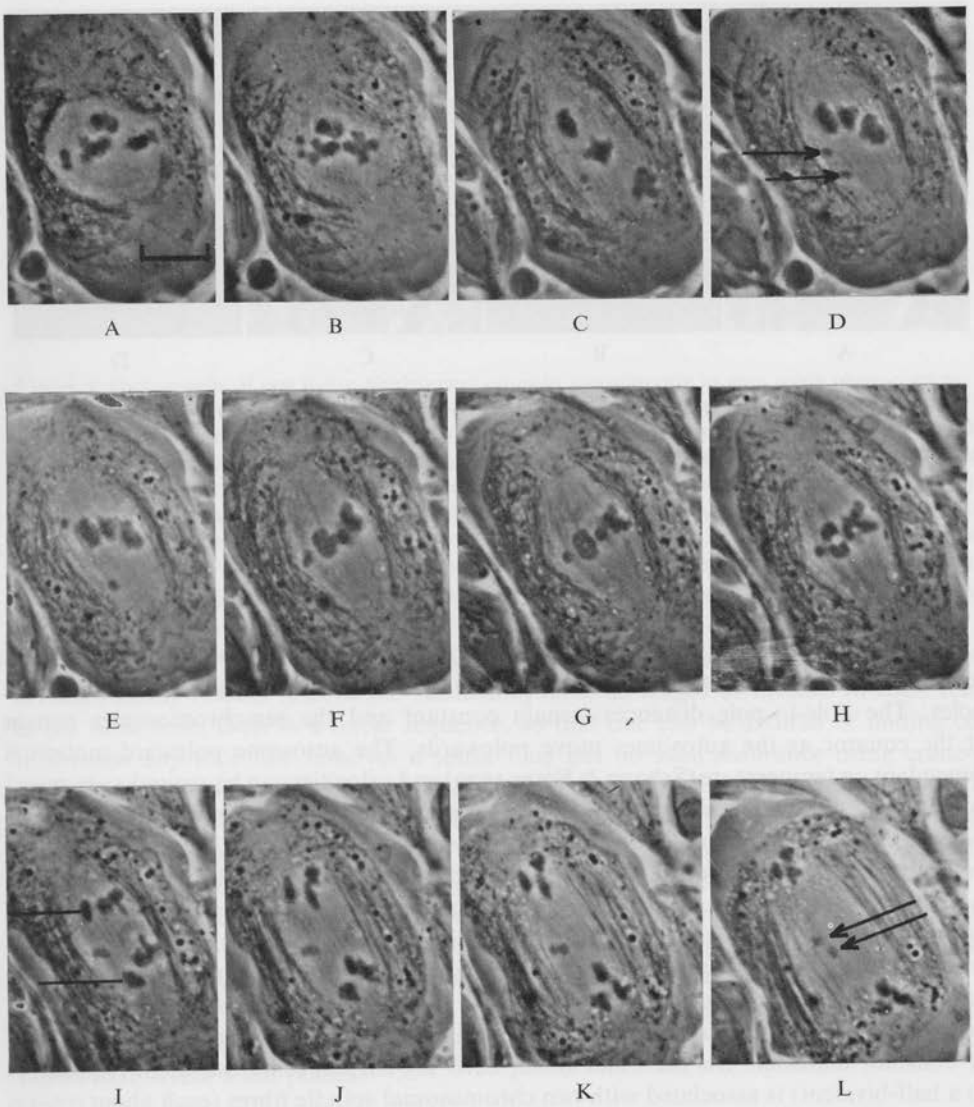

FIG. 6 1. A series of phase-contrast micrographs of one living *Nephrotoma suturalis* spermatocyte. A: shortly before nuclear membrane breakdown; the three (larger) bivalents and two univalents are seen dark against the lighter background of the nucleus. Scale = 10 μm. B: 6 min after nuclear membrane breakdown. C: 40 min after nuclear membrane breakdown; prometaphase. Each of the chromosomes has moved along the length of the spindle. D: 60 min after nuclear membrane breakdown; the two sex-chromosomes are indicated with arrows. E: 67 min after nuclear membrane breakdown; prometaphase. The chromosomes are still not at the equator. F: 102 min after nuclear membrane breakdown; metaphase. The chromosomes are more or less at the equator. G: 113 min after nuclear breakdown; anaphase has not yet started. H: 120 min after nuclear membrane breakdown; anaphase has just started. The bivalents have separated into two half-bivalents that have begun to move polewards. I: 130 min after nuclear membrane breakdown; two partner half-bivalents are indicated with lines. J and K: 141 and 145 min after nuclear membrane breakdown respectively; the two sex-chromosomes are still at the equator. L: 158 min after nuclear membrane breakdown; the two sex-chromosomes—indicated with arrows—have begun to move polewards, one towards each pole. All photographs are at 900× magnification (see the 10-μm scale in A).

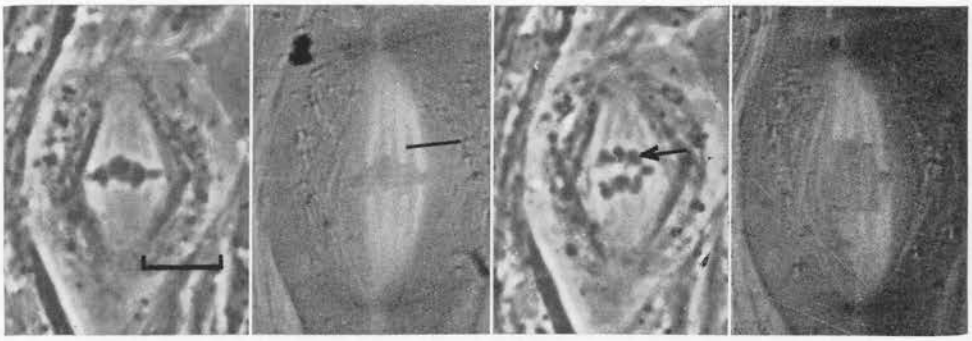

FIG. 6 2. A series of phase-contrast microscope (A and C) and polarization microscope (B and D) photographs of a living *Nephrotoma suturalis* spermatocyte. A: metaphase; the chromosomes are all at the equator. Scale = 10 μm. B: the same cell, 5 min later; the spindle fibres (one of which is indicated with a line) are visible as bright lines. The two sex-chromosomes are at the same position, in two focal planes, so the spindle fibres appear as if they were from one sex-chromosome. C: 5 min later; anaphase. The half-bivalents have separated (one is indicated with an arrow) and are moving poleward. D: 2 min later, as seen using polarization microscopy, Magnification $\simeq$1000 $\times$.

During anaphase, which begins at variable time intervals after all the chromosomes reach the equator, each autosome separates into two half-bivalents that move to opposite poles. The pole-to-pole distances remain constant and the sex-chromosomes remain at the equator as the autosomes move polewards, The autosome poleward motion is dependent on temperature (Schaap & Forer 1979) and velocities can be around 1 μm min^{-1} (which is about 50 cm yr^{-1}). The sex-chromosomes remain unpaired, and they move polewards, to opposite poles, only after the autosomes near the poles. The spindle pole-to-pole distance increases during the poleward movement of the sex-chromosomes, which is much slower than that of the autosomes, by about a factor of 5 (see Schaap & Forer 1979). As seen using polarization microscopy, each autosomal half-bivalent is associated with one chromosomal spindle fibre, about 1 μm in diameter, extending between each half-bivalent and the nearest pole (Figs 6.2 & 6.3). As the half-bivalent moves to the pole in anaphase, the associated chromosomal spindle fibre shortens whilst remaining of constant diameter. On the other hand, each sex-chromosome univalent (equivalent to a half-bivalent) is associated with *two* chromosomal spindle fibres (each about 0·5–1·0 μm), one to each pole (Fig. 6.3). As each sex-chromosome moves to a pole in anaphase, one spindle fibre shortens whilst the other one elongates by a corresponding amount.

Two main advantages of crane-fly spermatocytes are the small number of chromosomes, and the relatively small chromosome size compared to the spindle length. It is thus a relatively simple matter to follow the movements of each of the five chromosomes simultaneously, via photography or videotape. Further, as illustrated in Fig. 6.3, there are only five chromosomal spindle fibres per half-spindle (i.e. between the chromosomes and the poles). Each fibre is approximately 1 μm in diameter, with easily detectable birefringence. (Birefringence, or optical anisotropy, is an optical property that enables spindle fibres to be seen in living cells by means of polarization microscopy.) Because of the small number of chromosomes, one can be sure that one is studying single chromosomal spindle fibres, with no underlying or overlying fibres, by seeing where all the five fibres are. One simply rejects those cells in which spindle fibres overlap. These character-

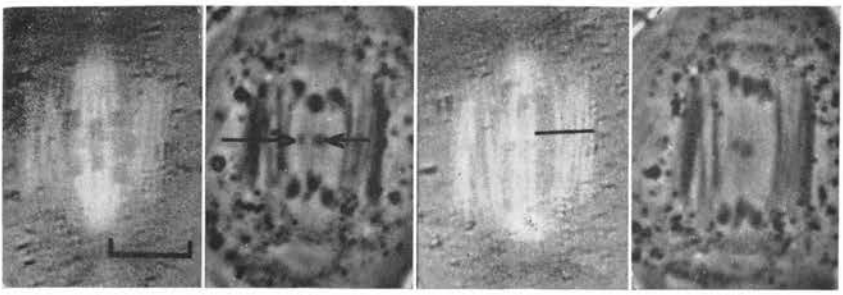

FIG. 6.3. Photographs of one living *Nephrotoma suturalis* spermatocyte as seen using phase-contrast microscopy (B and D) and polarization microscopy (A and C). A: Early anaphase; five spindle fibres (that appear as white lines) are seen going to the upper pole. Scale = 10 μm. B: 8 min later; the two sex-chromosomes, still at the equator, are indicated with arrows. C: 8 min later, the two sex-chromosomes are still at the equator. Each sex-chromosome has a spindle fibre to each pole; one such fibre is indicated with a line. D: 14 min later; the sex-chromosomes are just beginning to move to opposite poles. Magnification 1100×.

istics, together with year-round availability, make the system almost uniquely advantageous for studies on living cells in division.

The synchrony of cells in crane-fly testes can be a disadvantage in studying cell division. Many insects (e.g. in the Acrididae) have all stages of meiosis in their testes at the same time, even in a linear sequence, so that one can be assured of finding cells in division any time one removes a testis. One has no such assurance using crane-fly spermatocytes, however, and I sometimes need to dissect 5–10 male larvae before obtaining testes which contain cells in division. However, the synchrony can also be an advantage: individual testes have of the order of 5000–10 000 cells, and preparations containing cells in division contain *many* cells in division, with a large selection to choose from.

To summarize my reasons for studying crane-fly spermatocytes: the cells are available all the year round; they can be studied in simple smear preparations or held in clots, for perfusion; they are large and contain only five chromosomes, which can easily be followed simultaneously; and there are only five chromosomal spindle fibres (each 1 μm in diameter) per spindle, so one can study individual fibres without interference from others in different focal planes.

Problems of chromosome movement

I have described chromosome movements in crane fly spermatocytes during meiosis-I. Various aspects of these chromosome movements raise interesting problems that we are far from understanding. One concerns the *timing* of meiotic processes: why do autosomal half-bivalents enter anaphase before the sex-chromosomes enter anaphase? Why is chromosome-to-pole motion separated in time from pole-to-pole elongation? Why do cells have both processes rather than just one? Why the variable timing as the chromosomes move up and back along the spindle before ending up at the equator; and indeed, what signal is there that all the chromosomes are at the equator, so that anaphase can start? With regard to the time interval between the start of autosomal anaphase and sex-chromosome anaphase, one can say that this interval is not dependent on the completion of autosomal anaphase (Forer & Koch 1973), and that the interval

is influenced by temperature (Schaap & Forer 1979). But aside from these statements of experimental 'facts', I cannot even hazard intelligent guesses to the questions just posed. Nor does it seem likely that we will be able to deal directly with questions of when and how the individual motors are turned on, until we know more about what the individual motors are, and how they work.

Another problem is that of *co-ordination* between chromosomes. I use the term co-ordination to refer to chromosome movements that are somehow *not* independent, and seem 'co-ordinated' (Forer & Koch 1973; Forer 1974), even though they are in different chromosomes; e.g. the two sex-chromosomes remain unpaired, and are sometimes even situated on opposite sides of the spindle, yet they begin anaphase at the same time, and move to opposite poles. Nicklas (1971) discusses similar cases of 'preferential distribution of unlinked chromosomes'. Another example of such co-ordination in crane-fly spermatocytes is the behaviour of supernumerary sex-chromosomes which are sometimes found in crane fly spermatocytes. When there are three sex-chromosomes, at anaphase one goes to one pole, one to the other, and the third does not move; when there are four sex-chromosomes, at anaphase two go to one pole, and two go to the other; when there are five, two go to one pole, two to the other, and the last does not move (Dietz 1969). Thus, there seems to be co-ordination both in direction of motion, and in taking an inventory of numbers of sex-chromosomes which are to segregate. As a further example, one can demonstrate using micromanipulation techniques that this co-ordination involves a *continual* monitoring of position; if a sex-chromosome moving to a pole in anaphase is pushed in the direction opposite to that in which it was moving—i.e. towards the opposite pole—until it is closer to the opposite pole than is the sex-chromosome moving originally to that opposite pole, then both sex-chromosomes reverse their direction of motion (Forer & Kock 1973). Sex-chromosome anaphase also involves co-ordination with autosomes; when *autosome* segregation in crane-fly spermatocytes is altered so that four half-bivalents move to one pole and two move to the other, one sex-chromosome does not move (Dietz 1969; Forer & Koch 1973).

Co-ordination between chromosomes is not restricted to crane-fly spermatocytes, but can be seen in cells of many other species (see Nicklas 1971; White 1973). For example, 'distance segregation' occurs in mantispid spermatocytes, in which multiple sex-chromosomes do not pair, but move none the less to opposite poles (Hughes-Schrader 1969). As another example, in *Gryllotalpa* spermatocytes the single sex-chromosome always moves to the same pole as the larger member of a bivalent that contains two half-bivalents of unequal size (Payne 1912, 1916; White 1951; Camenzind & Nicklas 1968). We know very little about the *mechanisms* by which these 'co-ordinated', interdependent movements take place, however, although there are some guesses (Camenzind & Nicklas 1968; Dietz 1969; Forer & Koch 1973; Forer 1974). I find it very difficult even to pose useful experimental questions dealing with these movements at the present state of our understanding of chromosome movement mechanisms.

The last general problem I will consider is that of chromosome *orientation*. What is the mechanism that determines that at metaphase and anaphase the half-bivalent partners in a bivalent each have *one* chromosomal spindle fibre, extending to opposite poles, whilst the non-paired half-bivalents, that is, the X and Y sex-chromosome univalents, each have *two* chromosomal spindle fibres, one to each pole? In prometaphase the sex-chromosomes often have only single chromosomal spindle fibres, to one pole, and they sometimes take repeated trips along the length of the spindle, as do sex-chromosomes in grasshopper spermatocytes (Nicklas 1961). However, by metaphase the sex-chromo-

somes in crane-fly spermatocytes each have two fibres, one to each pole. By what mechanisms is this achieved? With regard to autosomal *bivalents*, 'mistakes' are sometimes made; in early prometaphase crane-fly spermatocytes, the half-bivalents in a bivalent may be oriented to the same pole, or individual half-bivalents may have spindle fibres to both poles (Bauer *et al.* 1961). These 'errors' are usually corrected during prometaphase. In a series of elegant experiments using grasshopper spermatocytes, Nicklas and collaborators have studied the mechanisms by which bivalents reach the correct orientation; the critical element stabilizing bivalents in a bipolar orientation (i.e. one half-bivalent oriented to each pole) is *tension* in the chromosome (reviewed in Nicklas 1971). Little is known of the molecular mechanisms of this process, but identification of tension as a crucial parameter should eventually enable mechanisms to be identified *in vitro*.

For *sex-chromosomes*, on the other hand, orientation mechanisms seem to be different. For example, absence of tension on the grasshopper spermatocyte X-chromosome does not result in a stabilized orientation with fibres to both poles. I cannot even guess why the unpaired grasshopper spermatocyte sex-chromosome has only one spindle fibre and goes to a pole prior to autosome anaphase, whereas the unpaired crane-fly spermatocyte sex-chromosomes eventually become oriented with fibres to both poles, and enter anaphase after the autosomes.

In summary: Certain aspects of spermatocyte cell division in crane-flies seem to me to be of particular interest, namely, the *timing* of the different movements, the *co-ordination* of movements between different chromosomes, and the different *orientations* of the different chromosomes. Understanding these aspects is at least as important as identifying the force-producing components. However, most work to date has been done on trying to identify the force-producing components. In my particular case, that is because this aspect of the problem seems to be the one most amenable to direct experimental attack. In the remainder of this essay I want to deal with experiments on the organization of the spindle, and on trying to determine what produces the force for chromosome movement.

Forces for chromosome movement

The chromosomal spindle fibre, extending between chromosome and pole, contains a component or components that transmit force to the chromosome, and cause the chromosome to move. Evidence for this belief comes from ultraviolet microbeam irradiations of crane-fly spermatocytes during meiosis-I (Forer 1966). Small portions of the cell were irradiated, of varying size and shape in the order of magnitude of cubes of side 1–3 μm. Irradiations were either of individual spindle fibres or of equal volumes of other areas of the cell. In anaphase, irradiation of a chromosomal spindle fibre could cause the associated chromosome (half-bivalent) to stop moving, while other chromosomes going to the same pole moved normally. These results show that the spindle fibre is necessary for movement to occur and that the effect of the irradiation is not due to a general poisoning, because other chromosomes moved normally. They also show that the individual motors moving the individual chromosomes can act independently of one another. Equivalent irradiation either between the separating chromosomes or outside the spindle region did not stop chromosomes from moving. This suggests that components running from pole-to-pole or pole-to-equator are not necessary; i.e. the only necessary component for chromosome-to-pole movement is the chromosomal

spindle fibre. (Qualifications to these conclusions are discussed in Forer 1966.) Finally, irradiations at different distances along the length of the chromosomal spindle fibre show that at least 80% of the length of the chromosomal fibre is necessary; irradiations 8 μm away from the chromosome, at a chromosome-to-pole distance of 10 μm, which were the farthest irradiations attempted, are able to block chromosome movement (Forer 1969). We hope to get clues concerning the nature of the required spindle fibre components by irradiating with monochromatic light and measuring the action spectrum for blocking chromosome movement (Sillers & Forer in preparation).

It is relevant to digress and point out two other results of microbeam irradiations of crane-fly spermatocytes. One result concerns the irradiation of one autosome's spindle fibre during anaphase. When the associated half-bivalent stops moving no other half-bivalent in the cell stops moving *except for* the partner half-bivalent; i.e. the half-bivalent moving to the opposite pole which was previously paired with the 'irradiated half-bivalent'. This half-bivalent stops moving even though its spindle fibre was *not* irradiated, and even though no other chromosomes moving to that pole stop moving. This consistent result (Forer 1966, 1969) complicates the conclusions discussed above. But it does illustrate another aspect of chromosome *co-ordination* in crane-fly spermatocytes: the movements to opposite poles of formerly conjoined half-bivalents are somehow interdependent. The mechanism for these interdependent movements is a mystery, but there may be one clue. Separating half-bivalents in crane-fly spermatocytes are mechanically linked (Forer & Koch 1973), and it might be that these mechanical linkages are the mediators of the interdependent movements. Of interest in this regard are data on grasshopper spermatocytes which indicate co-ordination between movements to opposite poles when only one of the two half-spindles is heated (Nicklas 1975).

The other result concerns the irradiation of chromosomal fibres associated with the sex-chromosomes. It will be recalled that each sex-chromosome has one fibre to each pole throughout its movement. Irradiation of a small region of the *shortening* fibre blocks movement of that chromosome, but identical irradiation of the *elongating* fibre seems to have less effect on chromosome movement (Sillers & Forer 1981). This suggests that only the shortening fibre is necessary for poleward chromosome movement of the chromosome. Sex-chromosome movements are also altered by irradiation of autosomal spindle fibres, but only when the spindle fibre is adjacent to that of a sex-chromosome (Sillers & Forer 1981). This strongly suggests that the 'co-ordination' between the movements of autosomes and sex-chromosomes is mediated by interactions between their respective spindle fibres.

Formation and organization of the spindle fibres

Assuming, then, that spindle fibres produce and transmit forces to their associated chromosomes, how are the spindle fibres set up? Centrioles are not necessary for normal spindle formation in crane-fly spermatocytes; when cells are flattened, centrioles (identified by their associated flagellae and by the associated small asters) can be found separated from the spindle poles. Cell-division is normal, even when one spindle pole in primary spermatocytes has no centriole—and indeed, perfectly normal second-division meiosis occurs even when one secondary spermatocyte contains four centrioles and the other has none (Dietz 1959, 1966). Because centioles seem to be unnecessary, it would seem that the chromosomes themselves have a major role in organizing the spindle.

How are the chromosomal spindle fibres formed, and how are they 'dissolved'

during anaphase? One can study the birefringence of spindle fibres *in vivo* (Inoué 1953, 1964), and hence one can study the formation and dissolution of those spindle fibre components which contribute to the birefringence. Before describing the results, I want to point out an important qualification to the work. Birefringence is due to regularly oriented components occupying a small volume fraction of a spindle fibre—of the order of 2–3% of the spindle fibre volume. The optical property, 'birefringence', allows the fibres to be seen in living cells using the polarizing microscope. One studies the birefringent components when following the birefringence, but the non-birefringent spindle fibre components are 'invisible' and need not act in the same manner. If one built an automobile from transparent ('invisible') plastic, and painted a *few* of the components red (say the doors and the interior seats) one could see only these coloured components. One could observe and study the doors and the seats, but the motor (the pistons, etc.) would remain invisible, and conclusions about the doors and seats would not necessarily apply to the motor or to other systems of the car.

The formation of the birefringent spindle fibre components in crane-fly spermatocytes can be observed directly. These components appear first as thin, barely detectable fibres, in early to mid prometaphase, and gradually become thicker and more birefringent. They reach their maximum thickness and birefringence in metaphase (Dietz 1963; Forer 1964; Begg & Ellis 1979). How are the birefringent components 'dissolved' in anaphase? The birefringence along the length of a given autosome's chromosomal spindle fibre is not uniform. Thus, by studying the pattern of birefringence along the length of the fibre as the fibre shortens during anaphase, one can deduce where the dissolution of the birefringent components takes place. The positions of different birefringence do *not* move poleward with the chromosomes, but rather stay in place as the chromosome moves to them and past them (Forer 1976; Forer *et al.* in preparation; Salmon & Begg 1980). Thus, the dissolution of the *birefringement* components would seem to take place at the chromosomal end of the fibre (Forer 1976).

Which spindle fibre components give rise to the observed birefringence? A common view is that the microtubules alone give rise to the observed birefringence (e.g. Begg & Ellis 1979). I believe that components other than microtubules also contribute to birefringence, by adding to the birefringence of the microtubules in some regions of the spindle and subtracting from it in other regions, and for me this point is still not proved (see Forer 1976, 1978; Forer & Brinkley 1977). However, I think that my view is rather a minority one. If the majority view is correct, the described changes of birefringence *in vivo* can be interpreted directly in terms of changes in organization of microtubules.

How are the spindle fibres organized at the ultrastructural level? The most extensive observations on cell division in crane-fly spermatocytes are those of Fuge (review in Fuge 1977a, b). To summarize the main points, both centromere microtubules (i.e. those attached to the chromosomes) and non-centromere microtubules are seen primarily in short segments, of $\lesssim 1\ \mu m$. Thus, single microtubules do not extend between chromosome and pole. Centromere microtubules overlap ('intermingle' with) non-centromere microtubules near the centromeres, as in *Haemanthus* endosperm mitosis (e.g. Bajer 1968a, b; Forer *et al.* 1979). Indeed, with the possible exception of cross-sections just at the level of the centromere (La Fountain 1974), one cannot really identify clear bundles of microtubules (Fuge 1977a, b). At anaphase, the highest microtubule density (microtubules per cross-sectional area of spindle) is found just in front of the poleward-moving centromeres. Most intriguing to me, are the chromosome arms; there are regularly arranged grooves in the chromosome arms, and rows of microtubules that extend down

these grooves seem to be bridged to the chromatin (Behnke & Forer 1966; Fuge 1977a, b). The function of this arrangement is not known.

Microtubules are not the only components seen electron microscopically in spindles. I think that actin filaments found in spindles in crane-fly spermatocytes (Behnke *et al.* 1971; Forer & Behnke 1972) and in other cells are involved in force production (see Forer 1978; Forer *et al.* 1979).

In summary: I have tried to explain why I work with crane-fly spermatocytes in studying chromosome movements during cell division. I have described various intriguing aspects of chromosome movements, and various experiments dealing with force production during cell division and the organization of spindle fibres. Unfortunately, one still cannot identify with certainty the spindle fibre components which produce the forces for chromosome movement.

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