J. Cell Sci. 49, 51–67 (1981)
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AUTOSOMAL SPINDLE FIBRES INFLUENCE SUBSEQUENT SEX-CHROMOSOME MOVEMENTS IN CRANE-FLY SPERMATOCYTES

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SUMMARY

In meiosis-I in crane-fly spermatocytes 3 autosomal half-bivalents move to each pole in anaphase while the 2 sex-chromosomal univalents remain at the equator. The sex chromosomes move to opposite poles only after the autosomes reach the poles; the sex chromosomes start to move polewards about 25 min after the autosomal half-bivalents have begun to move.

We irradiated portions of single autosomal spindle fibres with an ultraviolet microbeam and found that these irradiations altered the subsequent sex-chromosome movements. Two effects were observed. In one, one of the sex chromosomes did not move at all; the sex chromosome that remained at the equator would normally have moved to the pole associated with the irradiated autosomal spindle fibre. In the second, both sex chromosomes moved to the same pole, always that of the non-irradiated side. These effects occurred whether or not autosomal anaphase movement was blocked by the irradiation. There was no wavelength dependence for altering sex-chromosome movements.

Sex-chromosome movements were altered only when at least one sex-chromosomal spindle fibre was adjacent to the irradiated autosomal spindle fibre; when neither sex chromosome had a spindle fibre adjacent to the irradiated autosomal spindle fibre the sex chromosomes always moved normally. Irradiation of sex-chromosomal spindle fibres during sex-chromosomal anaphase showed short blockages of movement (usually 5–8 min), and then complete recovery. Direct irradiations of sex-chromosomal spindle fibres (without irradiating autosomal spindle fibres) when the autosomes were in anaphase but the sex chromosomes were in metaphase never caused abnormal sex-chromosome movements. These results eliminate the possibility that when we irradiated autosomal spindle fibres that were adjacent to sex-chromosomal spindle fibres the sex-chromosomal spindle fibres were irradiated inadvertently and were unable to recover from the damage.

We suggest that the irradiations of autosomal spindle fibres alter a *control* system involved in 'turning on' the sex-chromosomal spindle fibre motors, rather than directly altering the motors. We suggest that interactions between spindle fibres are somehow involved in this control system.

INTRODUCTION

The mechanisms involved in the movements of chromosomes from the equator to the poles during anaphase are not yet fully understood. Chromosome movements often appear to be *independent* of each other: during anaphase, half-bivalents within a cell can move polewards with different velocities (Schaap & Forer, 1979), individual chromosomes can stop moving independently of others moving to the same pole (Forer, 1966), or single chromosomes can be manipulated without affecting the other

chromosomes (Nicklas & Staehly, 1967). On the other hand, some chromosome movements are *not-independent*, and there appears to be 'co-ordination' between the movements of different chromosomes with respect to both *timing* and *direction* of movements (e.g. Mazia, 1961; Hughes-Schrader, 1969; Camenzind & Nicklas, 1968; Forer, 1980).

The work reported here was done on mechanisms of co-ordination between the autosomes and the sex chromosomes in crane-fly spermatocytes. In anaphase-I in crane-fly spermatocytes the 3 autosomal half-bivalents move to each pole while the 2 sex-chromosome univalents remain at the equator (Bauer, Dietz & Röbbelen, 1961; Dietz, 1969, 1972*b*). The sex chromosomes move to opposite poles only after the autosomal half-bivalents have reached the poles; at room temperature the sex chromosomes begin to move polewards about 20–30 min after the autosomes start to move polewards (Schaap & Forer, 1979).

The movements of the autosomes are co-ordinated with those of the sex chromosomes in several ways (e.g. see discussion by Forer, 1980). For example, if 4 autosomes go to one pole and 2 autosomes go to the other, only 1 sex chromosome moves poleward; the other sex chromosome does *not* move (Dietz, 1969; Forer & Koch, 1973). Dietz (1969) felt that this co-ordination was achieved because all spindle fibres were in equilibrium with a pool of tubulin subunits; Forer & Koch (1973), on the other hand, felt that the co-ordination was due to autosomal spindle fibres interacting with sex-chromosomal spindle fibres.

As we report here, sex-chromosome movements in crane-fly spermatocytes are often altered after irradiation of portions of autosomal spindle fibres (by means of an ultraviolet microbeam apparatus). Control experiments show that the effect on sex-chromosome movements is not due to inadvertent irradiation of sex-chromosomal spindle fibres. We suggest that the irradiations of autosomal spindle fibres alter a system that controls whether the sex-chromosomal spindle fibre 'motors' will be turned on, rather than altering the spindle fibre motors directly.

MATERIALS AND METHODS

Animals and living spermatocyte preparations

Crane flies (*Nephrotoma ferruginea* Fabricius) were reared in the laboratory (Forer, 1964, 1981). Living spermatocyte preparations were made essentially as described by Schaap & Forer (1979) with minor modifications as follows. Testes were removed from 4th instar *N. ferruginea* larvae under halocarbon oil (oil 10–25; Halocarbon Products, Hackensack, N.J.) and transferred to Voltalef oil (Huile 10S; Ugine Kuhlmann, Divisions Plastiques; 11, bd. Pershing; 75017 – Paris) that was in a 'well'. The well was a 20 mm diameter hole in a 3 in × 3 in × 1 mm brass plate; a quartz coverslip (0·35 mm thick) covered the hole and acted as the bottom of the well. (Our lens is corrected for 0·35 mm thick quartz, and focal shifts occur if coverslips are different from this; some suppliers provided coverslips of varying thickness, so we measured the thickness of each coverslip, and used only those that were 0·35 mm thick.) The coverslip was attached to the brass plate using Scotch brand Magic Transparent Tape. This gave a tight seal and there was no leakage; by bending back the end of the tape (to put sticky side against sticky side) we had a 'handle' to remove the tape and recycle the expensive quartz coverslips. For recycling, quartz coverslips were first washed with soap (Alconox) in hot water. Then, after rinsing with distilled water, the coverslips were dried

thoroughly with lens paper (to avoid scratching). The transmissions of the coverslips were periodically checked in a spectrophotometer.

After a testis was placed under Voltalef oil on the quartz coverslip in this well, a preparation was made as described previously (Forer & Koch, 1973; Schaap & Forer, 1979; Forer, 1981) for use on an inverted microscope. We let the cells flatten for approximately 45 min before starting to observe them.

Time-lapse apparatus and photography

Cells were photographed before, during, and after irradiations using 16 mm film (Kodak Plus X Negative Film 7231). We used a Bolex camera (H-16-J) controlled by a time-lapse control panel (R. J. Matthias and Associates, Houston, Texas, U.S.A.). Pictures were taken at 8 frames per minute. Exposure times were in the range of 0·3–0·5 s. Negatives were developed in Diafine developer using a Cramer automatic processor (Sarasota, Florida, U.S.A.). Positive work prints, used for analysis, were obtained from a commercial laboratory.

Ultraviolet microbeam apparatus

A Nikon (model M) inverted microscope was modified for ultraviolet (u.v.) microbeam irradiations, which were done through the objective. A diagram of the apparatus is shown in Fig. 1. The light source was a 100 W mercury arc lamp (Illumination Industries Inc.) in a Schoeffel LH150 lamp housing powered by a Schoeffel (Westwood, N.J.) LPS251 Universal

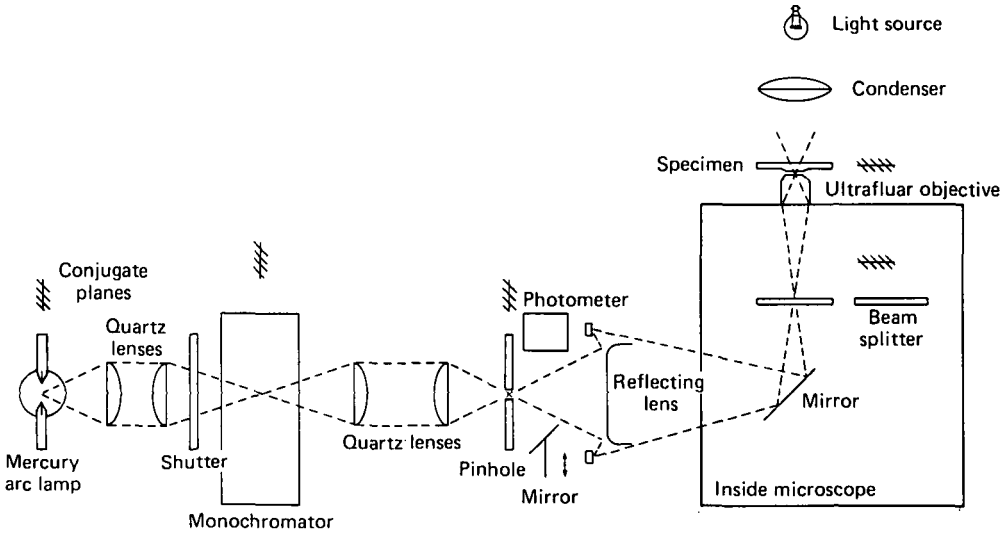

Fig. 1. A diagram illustrating the optical arrangement used in the ultraviolet microbeam apparatus.

compact-arc lamp power supply. We modified the lamp housing to incorporate a mechanical shutter; the shutter is driven by an adjustable timer, and provides accurate exposure times from 0–180 s, in 0·25-s units. The light leaving the lamphouse passes through the shutter, passes through a quartz lens (the Schoeffel LHA150 focussing sleeve), and is then focussed onto a Schoeffel GM100 miniature grating monochromator, fitted with equal-sized (1·4 mm) entrance and exit slits. (With these slits the spectral half-bandwidth is 12 nm.) After leaving the monochromator the light passes through 2 adjustable quartz lenses (Canadian Instrumentation and Research, Ltd, Cawthra Road, Mississauga, Ontario) and is focussed onto a pinhole or slit (Energy Technology Inc., San Luis Obispo, California). (The pinholes are 100, 200 and 400 μm in diameter corresponding to 1·05, 2·1 or 4·2 μm diameter in the cell; we used only the 400-μm pinhole for the experiments reported herein.) After leaving the pinhole, the light takes

1 of 2 possible paths, either to a detector (with a mirror pushed *in*) or to the specimen (with the mirror pulled *out*). The mirror is a front-surface mirror that, when pushed in, is 45° to the light path.

When the mirror is pushed in, the light from the pinhole is reflected onto the surface of a P1100 silicon detector probe (Alphametrics, Winnipeg, Manitoba, Canada). The probe is connected to a model 1010 photometer (Alphametrics) that reads digitally in amps; the detector probe was calibrated (for wavelengths between 250 and 1100 nm) by the manufacturer, so that the reading in amps can be converted directly into light intensity. Intensity readings were taken immediately before each irradiation and the irradiation times were adjusted (using the shutter timer) to give the total energy required. For each wavelength used we measured the transmission of the optical system between the pinhole and the specimen by measuring the intensity with the mirror pushed in, as just described, and then by pulling the mirror out and placing the detector above a clean quartz coverslip (placed at its usual position on the stage) and measuring the intensity in this plane. Thus we are able to convert intensities with the mirror pushed in, read immediately before irradiation, to intensities incident on the cells or – using the calibrated irradiation times – to total energy incident on the specimen for each irradiation.

When the mirror is pulled out, the light leaving the pinhole passes through a catoptric (reflecting) lens (designed by Canadian Instrumentation and Research, Ltd, Dunwin Drive, Mississauga, Ontario and fabricated by Applied Physics Specialities Ltd, Don Mills, Ontario), is reflected from a front-surface mirror, and then passes through an adjustable beam-splitter. [The beam-splitter has 2 positions: one position, used at all times except during irradiations, is a green-reflecting, red-transmitting dichroic filter; the second position, used during irradiations, is a u.v.-transmitting, green-reflecting filter. (Both filters were supplied by Applied Physics Specialities Ltd, Don Mills, Ontario.) The reflected light is that used for observing the specimen; the transmitted light is that used as the irradiating ultraviolet light or the red light that marks the position of the focussed pinhole, as will be described below.] The light then passes through a Zeiss Ultrafluar phase-contrast glycerol-immersion lens (100×, N.A. 0·85) and the image of the pinhole is focussed into the plane of the specimen.

We have used 2 methods for confirming the location and focus of the microbeam prior to and/or following the irradiations. In the first, a scratched front surface mirror was placed at the specimen plane, on top of a quartz coverslip. The microscope was focussed on the scratches, the monochromator was adjusted to 546 nm, and the green image of the pinhole was observed (see Uretz & Perry, 1957). The Nikon model M microscope has an epi-illumination 'data projection' system, which allows one to superimpose onto the field of view any image that one wants; we used this system to project into the field of view an image in red that was coincident in size and position with the reflected green image of the pinhole. Thus, when specimens were viewed (without a mirror) we could use this 'data projection' system to project into the field of view (and onto the films) an image of the pinhole.

The second (and more convenient) method was to aluminize a corner of the quartz coverslips on which the preparations were made. This did not harm the cells, and allowed us to observe a reflected image of the pinhole without removing the slide from the stage, by moving the slide over to the aluminized region. Using such slides we confirmed after each irradiation that the reflected image of the pinhole matched that projected into the field using the data illuminator.

The Zeiss Ultrafluar lens used for irradiations is nominally achromatic for visible and ultraviolet light. We checked this (and at the same time checked the alignment of the microbeam) in the following way. Working in red light, we placed a green and ultraviolet-sensitive/red-insensitive emulsion (Kodak high-contrast process ortho film 4154) onto a quartz coverslip. The microbeam was aligned, and, using different regions of the emulsion, we 'irradiated' the film with green light and with various ultraviolet light wavelengths (from 240 to 300 μm). We confirmed that the focus was the same for green and for ultraviolet light (and that the alignment of the microbeam was correct). This was true only when coverslips were 0·35 mm thick; we used this method (together with thickness measurements) to select coverslips of the proper thickness from those nominally 0·35 mm, supplied by ESCO. Others (from Applied Physics Specialities Ltd, Don Mills, Ont.) were correct as supplied.

Film analysis

Work prints were studied by analysis of single frames using an Athena projector (model 224 by L-W Photo Inc., Van Nuys, California, U.S.A.). The velocities of chromosomes were determined as described by Schaap & Forer (1979).

RESULTS

In normal meiosis-I in crane-fly spermatocytes, at the start of anaphase the 3 autosomal half-bivalents move towards each pole while the 2 sex-chromosomal univalents remain at the equator; the sex chromosomes move to opposite poles after the autosomal half-bivalents reach the poles, and the sex-chromosomal anaphase begins about 25–30 min after the beginning of autosomal anaphase (Fig. 2). We irradiated single autosomal spindle fibres with varying doses and varying wavelengths of u.v. light; these irradiations caused altered sex-chromosome movements.

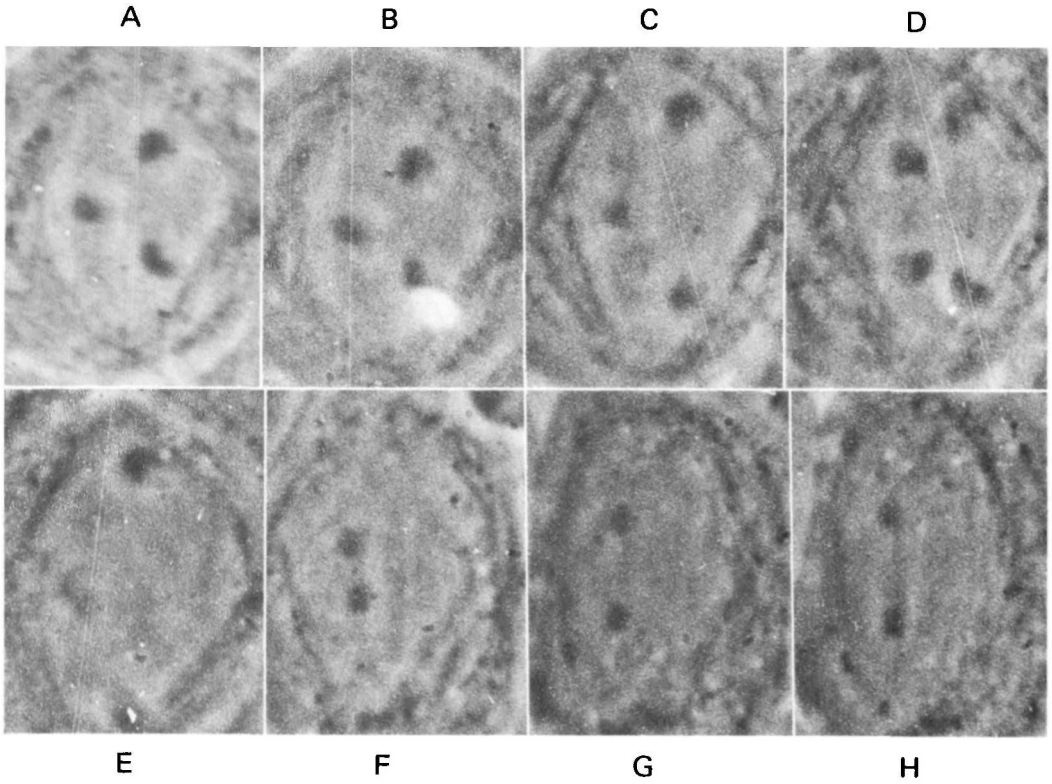

Fig. 2. Normal sex-chromosome movement in a *N. ferruginea* spermatocyte following u.v. microbeam irradiation (using 300 nm wavelength u.v. at a total incident energy of $7{\cdot}5 \times 10^{-3}$ ergs per μm^2 at the focussed spot). A, B, one focal plane; with C, 1 autosomal half-bivalent moving to each pole; D is in another focal plane, that of the other two pairs of autosomal half-bivalents. The region irradiated, shown in the white circle in B, was photographed immediately before irradiation. The sex chromosomes move poleward normally (E, F, G, H). Times of photographs: A, 0 min; B, 1·4 min; C, 7·3 min; D, 7·4 min; E, 15·7 min; F, 35·2 min; G, 41·6 min; H, 47·0 min. × 2000.

Sex-chromosome movements were altered only when at least one sex-chromosomal spindle fibre was adjacent to the irradiated autosomal spindle fibre; when neither sex chromosome had a fibre adjacent to the irradiated autosomal spindle fibre the sex chromosomes *always* moved normally. Autosomal spindle fibres were classified

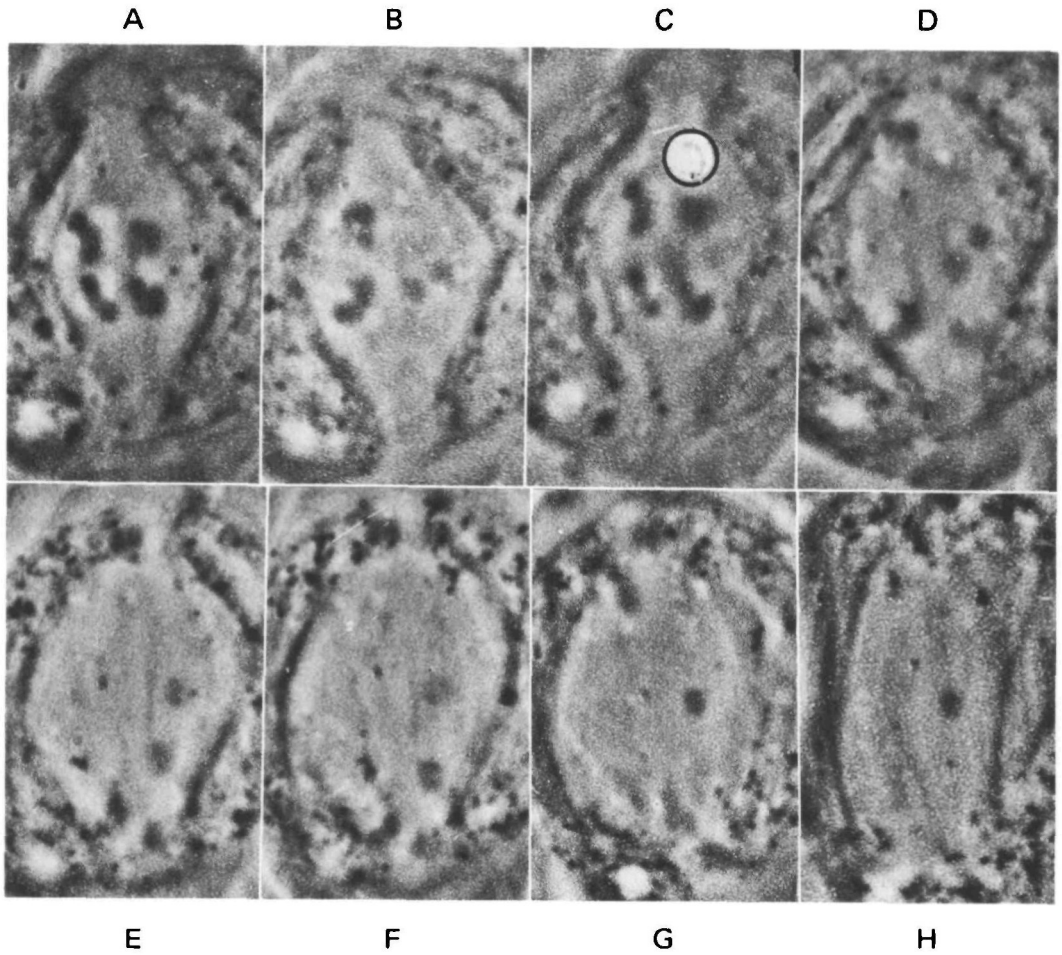

Fig. 3. Altered sex-chromosome movement in which one sex chromosome remains at the equator throughout anaphase after irradiation of an autosomal spindle fibre (using u.v. of 280 nm wavelength, at a total incident energy of 0·15 ergs per μm^2 at the focussed spot). A, C are in one focal plane, whilst B, D are in another. The irradiated region, shown in white in C, was photographed immediately before the irradiation. Sex-chromosome movement is seen in E–H; the 'near' sex chromosome moves to the lower pole whilst the 'far' sex chromosome remains at the equator. Times of photographs: A, 0 min; B, 1·04 min; C, 1·82 min; D, 6·8 min; E, 18·0 min; F, 22·1 min; G, 26·5 min; H, 43·4 min. × 2000.

as *adjacent* or *not-adjacent* to sex-chromosomal spindle fibres without knowledge of the results of the irradiation, as follows. The positions and focal planes of the autosomes and of the sex chromosomes were drawn on index cards that were coded. Then each

of us independently went through the cards and decided whether the sex-chromosomal spindle fibres might be adjacent to the irradiated autosomal fibre, or whether there was an autosomal spindle fibre between the irradiated fibre and any sex-chromosomal spindle fibre, in which case it was not-adjacent (see Fig. 5). (Ambiguous cases were

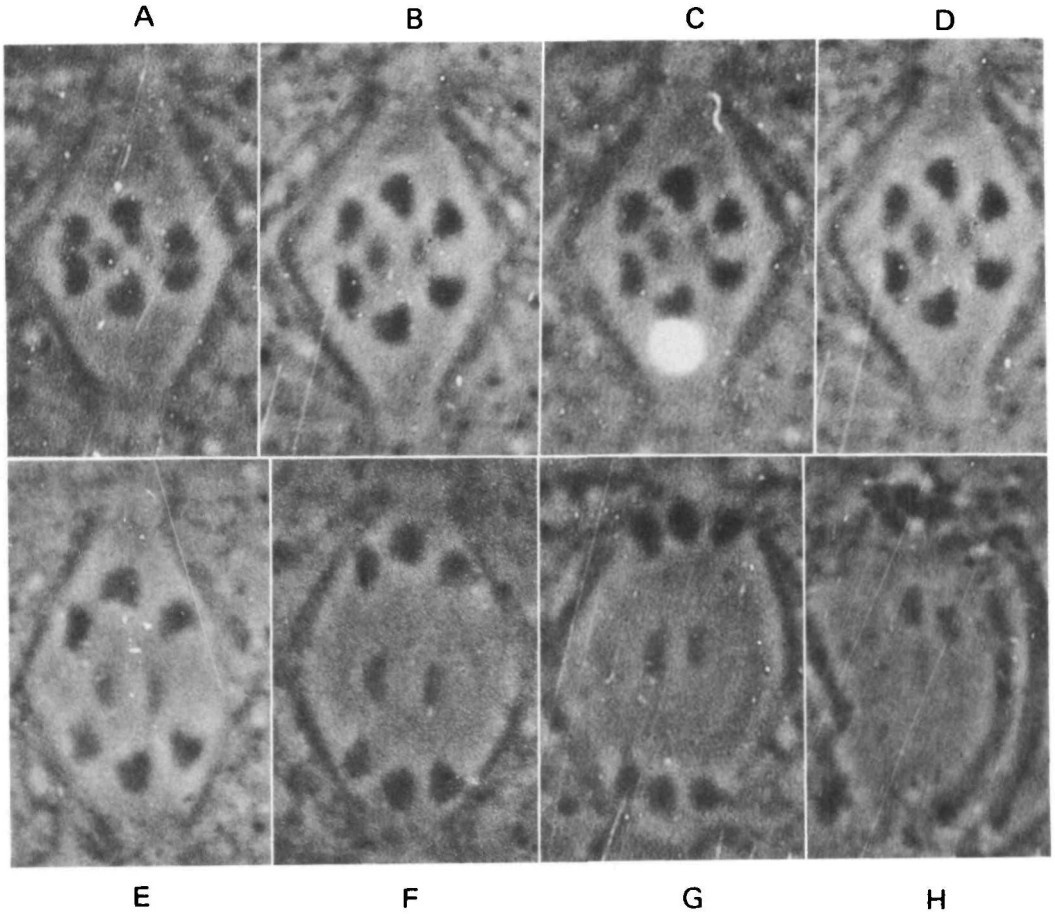

Fig. 4. Altered sex-chromosome movement in which both sex chromosomes move to one pole after irradiation of an autosomal spindle fibre (using u.v. of wavelength 275 nm, at a total incident energy of 15×10^{-2} ergs per μm^2 at the focussed spot). The irradiated region, shown in white in C, was photographed immediately before the irradiation. Both sex chromosomes move to the same (upper) pole (F–H). Times of photographs: A, 0 min; B, 11·0 min; C, 11·7 min; D, 12·2 min; E, 14·0 min; F, 18·6 min; G, 39·8 min; H, 68·8 min. × 2000.

discarded.) We disagreed in only a few cases, which we then resolved by re-looking. There were 55 cases of irradiation of *adjacent* fibres, that resulted in 30 alterations of sex-chromosome movement, and there were 15 cases of irradiation of *not-adjacent* fibres, in which there was *no* case of altered sex-chromosome movement. Similar results were obtained with a smaller number of spermatocytes from another species of crane-fly, *Nephrotoma suturalis* (Loew).

Primarily 2 kinds of altered sex-chromosome movements were produced by irradiations of an adjacent autosomal spindle fibre. In one kind of alteration, one sex chromosome did not move at all; it stayed at the equator while the other sex chromosome moved normally (Fig. 3). (The non-moving sex chromosome that remained at the equator was pushed randomly into either new cell at telophase.) If one designates the autosomal spindle fibre that was irradiated as being associated with the 'irradiated pole', the sex chromosome that moved went to the *not-irradiated* pole. Hence the non-moving sex chromosome ordinarily would have moved to the irradiated pole, and the effect of the irradiation (of the autosomal spindle fibre) was to stop movement of the sex chromosome to the irradiated pole.

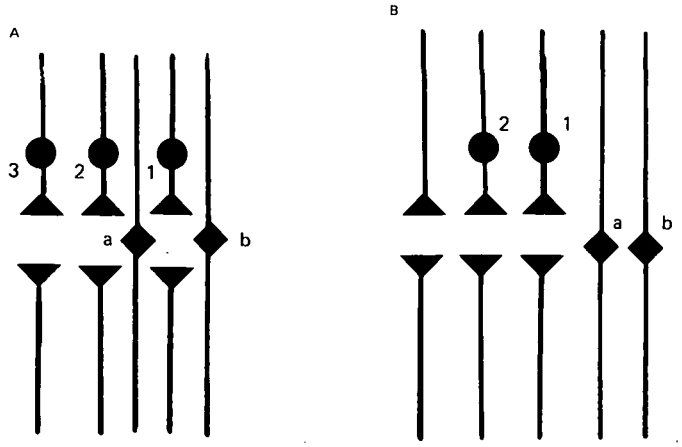

Fig. 5. A, B. Two different configurations of autosomes and sex chromosomes. In A 1, 2 and 3 are three different irradiated spindle fibres, and a and b are the sex chromosomes. Fibres 1 and 2 are adjacent to at least one sex-chromosomal spindle fibre, but fibre 3 is not-adjacent to any. Fibre 1 is adjacent to 2 sex-chromosomal spindle fibres, fibre 2 to only one. Sex chromosomes a and b are both near to fibre 1; a is near to 2, but b is far from 2. In B, 1 and 2 are two different irradiated spindle fibres, and a and b are the sex chromosomes. Only fibre 1 is adjacent to a sex-chromosomal spindle fibre. Sex chromosome a is near to fibre 1, and sex chromosome B is far from fibre 1.

In the second kind of alteration, both sex chromosomes moved, but they both moved to the same pole (Fig. 4). In these cases the 2 sex chromosomes moved to the *not-irradiated* pole. Hence here, too, the irradiations stopped movement to the irradiated pole.

The 2 different kinds of alteration of sex-chromosome movement are associated with 2 different spindle geometries. Both sex chromosomes moved to the same pole when *two* sex-chromosomal spindle fibres were adjacent to the irradiated autosomal spindle fibre; two sex chromosomes moved to the same spindle pole in 33% of the 30 irradiations in which sex-chromosome movements were altered, and in each case *two* sex-chromosomal spindle fibres were adjacent to the irradiated autosomal spindle fibre. One sex chromosome did not move poleward when only *one* sex-chromosomal spindle fibre was adjacent to the irradiated autosomal spindle fibre; one sex chromo-

some remained at the equator throughout anaphase in 53 % of the 30 irradiations in which sex-chromosome movements were altered, and in each case only *one* sex-chromosomal spindle fibre was adjacent to the irradiated autosomal spindle fibre.

In some cells (4 of the 30) alterations in chromosome movement were different from that described above. In one cell neither sex chromosome moved after irradiation of an autosomal spindle fibre. In another cell, the onset of sex-chromosomal anaphase was delayed considerably, but both sex chromosomes then moved normally. In a third cell, one sex chromosome remained at the equator and the other sex chromosome moved to the irradiated pole. In the fourth cell, both sex chromosomes moved to the irradiated pole.

Alterations of sex-chromosome movements occurred at all wavelengths, regardless of whether the irradiation caused the associated autosome to stop moving. That is to say, irradiation of the autosomal spindle fibre may cause the associated autosomal half-bivalent to stop moving (e.g., see Forer, 1966), depending on the wavelength of the u.v. light and the dose (Sillers & Forer, unpublished); these irradiations caused alterations in sex-chromosome movements, as described above, even when the irradiations had no effect on the movement of the associated autosomes. In our experiments autosome movements were unaffected in 60 % of the 30 cells in which sex-chromosome movements were altered.

Our interpretation of these results is that alterations in sex-chromosome movement occurred only when the irradiated autosomal spindle fibre was adjacent to at least one sex-chromosomal spindle fibre. But could it be that the effect is one of *dose* rather than proximity ?

Our experiments were not done with constant dose or wavelength, because most of them were aimed at obtaining an action spectrum for blocking autosomal movement (Sillers & Forer, unpublished). Doses for those irradiations in which the irradiated autosomal spindle fibre was *adjacent* to at least one sex-chromosomal spindle fibre are listed in Table 1; we can detect no consistent effect of wavelength or dose, with the possible exception that light of wavelength 270 nm seems especially efficient in inducing such alterations. Doses for irradiations in which the irradiated autosomal spindle fibre was *not-adjacent* to a sex-chromosomal spindle fibre are listed in Tables 2 and 3; these irradiations are within the range of doses in which adjacent irradiations produced alterations in sex-chromosome movement. Considering the total number of adjacent irradiations, there was no effect on sex-chromosome movement 46 % of the time. There was no effect in all 15 cases of not-adjacent irradiations. If 0·46 is the probability for 'no effect', the probability for all 15 cases being no effect is $(0{\cdot}46)^{15}$, or about 9×10^{-6}. Similar low probabilities are reached by considering separately the data for irradiations using 270 nm wavelength u.v. light (namely, $1{\cdot}2 \times 10^{-4}$) or irradiations using 300 nm u.v. (namely, 4×10^{-3}). Hence it seems unlikely indeed that the effect is one of dose.

The effect on sex-chromosome movements seems to be restricted to irradiations of autosomal spindle fibres that are adjacent to sex-chromosomal spindle fibres. But could it be that during irradiations of autosomal spindle fibres adjacent to sex-chromosomal spindle fibres we inadvertently irradiated sex-chromosomal spindle

fibres, and that it is this direct irradiation of the sex-chromosomal spindle fibres that causes the altered sex-chromosome movements ? We have done 2 sets of experiments to test this possibility.

Table 1. *Irradiations of autosomal spindle fibres that were* adjacent *to at least one sex-chromosomal spindle fibre*

	Fraction of sex-chromosome movements altered after irradiation with a relative dose of:								
u.v. wavelength, n.m.	2·5–5	12·5	25	50	75	125	175	200	275
260			1/1						
265				0/1				1/1	
270	4/5	1/1				0/1	1/1	1/1	
275	3/4			1/1		0/2			
280	0/2	0/1	0/1	0/3		0/1	1/1		1/1
290	3/6	1/2	3/3	0/1	1/1	1/1			
300	2/5	0/1	1/1	2/3			1/1	0/1	

The denominator represents the total number of irradiations at that wavelength and dose. A relative dose of 1 corresponds to about 3×10^{-3} ergs/μm^2 at the focussed spot.

Table 2. *Irradiations of autosomal spindle fibres using u.v. light of* $\lambda = 270$ *nm*

	Effects on sex-chromosome movement after irradiation of an autosomal spindle fibre that was			
	Adjacent to a sex-chromosomal spindle fibre		Not-adjacent to a sex-chromosomal spindle fibre	
Relative dose*	Effect	No effect	Effect	No effect
2·5	1	1		2
5·0	3			
12·5	1			1
25·0				1
50·0				
125·0		1		
175·0	1			
200·0	1			1
275·0				1
Totals	7	2	0	6

* See legend to Table 1.

In the first set of experiments we irradiated sex-chromosomal spindle fibres directly, after the sex chromosomes began to move polewards. Sex chromosomes in crane-fly spermatocytes are amphitelically oriented (i.e. each one has 2 chromosomal spindle fibres, one to each pole). During sex-chromosomal anaphase one spindle fibre shortens (the spindle fibre that is attached to the pole towards which the sex chromosome is moving) whilst the other spindle fibre elongates. We irradiated across

one or both spindle fibres going to one pole, after which one chromosome stopped moving, for 3–15 min (in different experiments), and then movement resumed normally. The other sex chromosome moved normally throughout (Figs. 6, 7). [It might be of interest to note that irradiations across both spindle fibres to one pole in fact irradiated both a shortening and an elongating sex-chromosomal spindle fibre; after such irradiations the sex chromosome that stopped moving was usually (7 out of 10 cases) the one associated with the irradiated *shortening* spindle fibre.]

Table 3. *Irradiations of autosomal spindle fibres using u.v. light of* $\lambda = 300$ *nm*

Relative dose	Effects on sex-chromosome movement after irradiation of an autosomal spindle fibre that was:			
	Adjacent to a sex-chromosomal spindle fibre		Not adjacent to a sex-chromosomal spindle fibre	
	Effect	No effect	Effect	No effect
2·5	1	2		
5·0	1	1		1
12·5		1		1
25·0	1			
50·0	2	1		2
125·0				1
175·0	1			3
200·0		1		
Totals	6	6	0	8

Because the effect of direct irradiation of sex-chromosomal spindle fibres on sex-chromosome movement was temporary, for 15 min at most, and because sex-chromosome anaphase did not start until about 15–20 min after irradiation of an autosomal spindle fibre, it seems quite unlikely that inadvertent irradiation of the sex-chromosomal spindle fibre would have caused the observed alterations in sex-chromosome movement. Results of a separate set of experiments confirm this conclusion.

In a second set of experiments we directly irradiated sex-chromosomal spindle fibres (shortly after the autosomes started moving polewards) *without* irradiating an autosomal spindle fibre. In these experiments the sex chromosomes were in metaphase and the autosomes were in anaphase; we irradiated sex-chromosomal spindle fibres *interzonal* to the autosomal kinetochores, thereby ensuring that no autosomal spindle fibre *could* be irradiated. In 10 such irradiations sex-chromosome movements were always normal (e.g. see Fig. 8). Hence the altered sex-chromosome movements are due to irradiations of an autosomal spindle fibre directly adjacent to a sex-chromosomal fibre, and are not due to (or mimicked by) direct irradiation of sex-chromosomal spindle fibres.

It is of interest in considering the mechanisms by which irradiations of autosomal spindle fibres influence sex-chromosome movements to consider *which* of the 2 sex

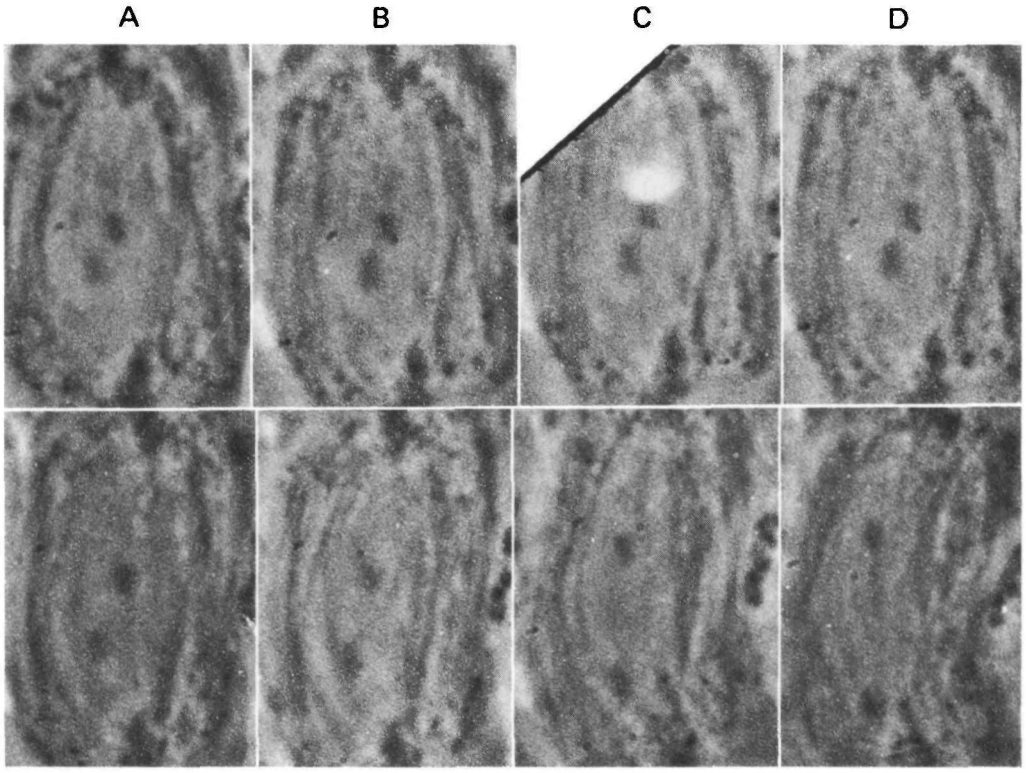

Fig. 6. Direct irradiation of a sex-chromosomal spindle fibre (using u.v. of wavelength 300 nm and a total incident energy of $7{\cdot}5 \times 10^{-2}$ ergs per μm^2 at the focussed spot). The irradiated region, shown in white in C, was photographed immediately before the irradiation. The irradiated chromosome temporarily stopped moving (for 15 min) to the irradiated (upper) pole (D–F) but then resumed movement (G, H). Times of photographs: A, 0 min; B, 1·8 min; C, 5·0 min; D, 9·8 min; E, 18·0 min; F, 22 min; G, 26·5 min; H, 43·4 min. ×2000.

chromosomes was altered by the irradiation. That is to say, was the movement altered of that sex chromosome that had a spindle fibre adjacent to the irradiated autosomal spindle fibre, or was the movement altered of the *other* sex chromosome? We used the drawings on coded file cards to judge whether the sex chromosome was *near* or *far* from the irradiated autosomal spindle fibre; if the sex-chromosomal spindle fibre was adjacent to the irradiated spindle fibre the sex chromosome was near, otherwise it was far, as illustrated in Fig. 5. In some irradiations both sex chromosomes were near (see Figs. 5, 4). (We again discarded ambiguous cases.) There were 16 irradiations that caused altered sex-chromosome movement in which one sex chromosome was near and the other was far; in 44% of these cases (7 out of 16) the far sex-chromosome was the one with altered anaphase.

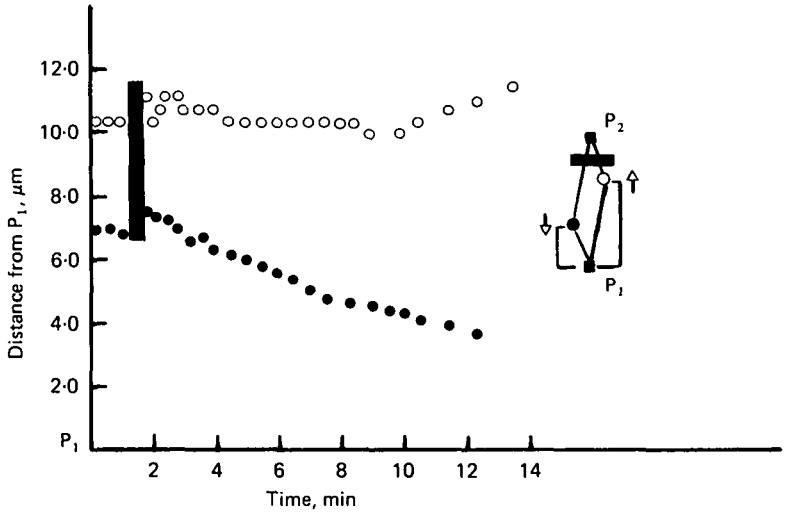

Fig. 7. A graph of position from pole P_1 (ordinate) *versus* time (abscissa) for a cell in which 2 sex-chromosomal spindle fibres were irradiated, as illustrated in the inset. (The arrows in the inset indicate the direction of movement, and the solid black rectangle indicates the irradiated region.) The sex chromosome moving to pole P_2 stopped moving temporarily, for about 8 min. (The irradiation was with u.v. of wavelength 275 nm, with a total incident energy of 0·39 ergs per μm^2 at the focussed spot.)

DISCUSSION

Irradiations of autosomal spindle fibres that alter sex-chromosome movements would seem to do so because they alter a *control* system, rather than the sex-chromosome *motors*. That is to say, if one assumes that the force for chromosome movement comes from the chromosomal spindle fibre, the irradiation would seem to alter a system that controls when the fibre is turned on and off rather than altering the force-producing systems of the fibre itself. There are several pieces of evidence that suggest this interpretation, as follows. (1) *Direct* irradiation of the sex-chromosomal spindle fibres alters movement in a way quite different from that after irradiation of the autosomal spindle fibre. Irradiation of sex-chromosomal spindle fibres prior to sex-chromosome movement has no effect; irradiation of sex-chromosomal spindle fibres after the sex-chromosomes have started to move polewards temporarily blocks movement of one sex chromosome, for 3–15 min, but movement then resumes normally. On the other hand, one main effect of irradiations of an adjacent autosomal fibre is to block movement of one sex chromosome permanently. That is to say, direct irradiation resulted in no effect, or a temporary block, whilst irradiation of the autosomal fibre resulted in a permanent block; this suggests to us that the latter irradiations alter a control system that turns on the motor, rather than altering the motor itself. (2) The other main effect of irradiating an autosomal spindle fibre is that both sex chromosomes go to the same (not-irradiated) pole; this, too, suggests that the irradiations alter a control system rather than the motor. (3) When irradiation of an autosomal spindle fibre (that is adjacent to a sex-chromosomal spindle fibre) causes

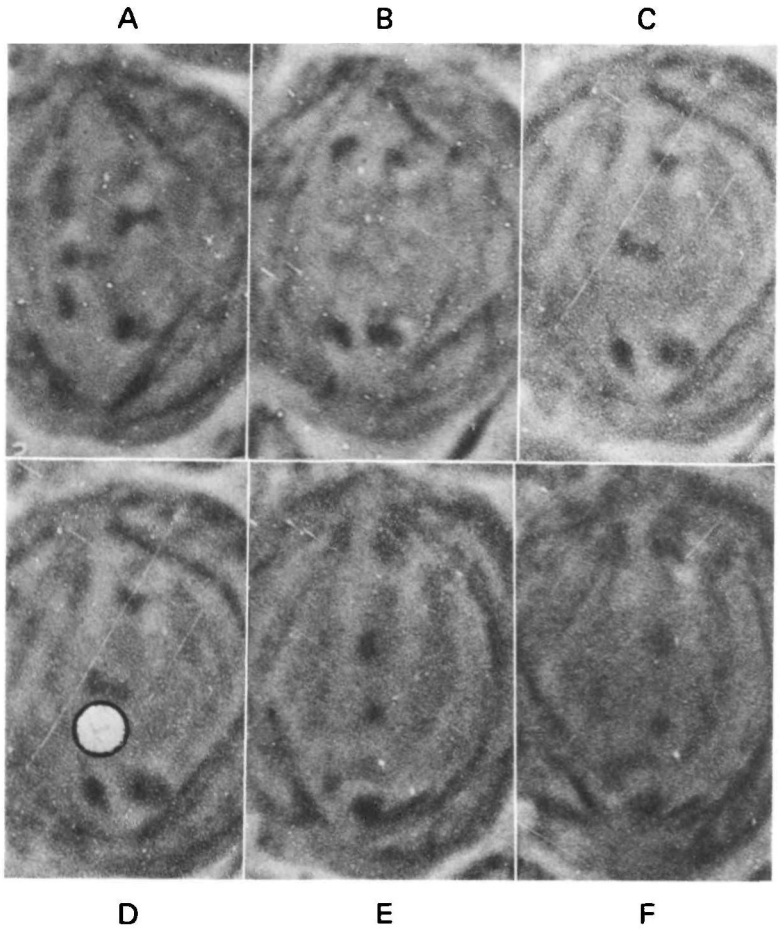

Fig. 8. Irradiation of sex-chromosomal spindle fibres without irradiating the adjacent autosomal spindle fibre; the irradiation (the white region in D) is interzonal to the autosomal kinetochores, and took place just before D was photographed. The irradiation was with u.v. of wavelength 290 nm, with a total incident energy of $1{\cdot}5 \times 10^{-2}$ ergs per μm^2 at the focussed spot; the sex chromosomes moved normally in anaphase (E, F). Times of photographs: A, 0 min; B, 4·7 min; C, 10·5 min; D, 10·8 min; E, 24·5 min; F, 27·7 min. × 2000.

one sex chromosome not to move, i.e. to remain at the equator throughout anaphase, the chromosome that does not move is not necessarily the one that is adjacent to the irradiated autosomal spindle fibre; about half the time the 'far' sex-chromosome does not move. This, too, suggests that the alteration is to a control system rather than directly to the motor, because if the effect were on a motor one would expect the adjacent sex chromosome consistently to be the one with altered movement.

We suggest that there are interactions between sex-chromosomal spindle fibres and adjacent autosomal spindle fibres that are involved in regulating sex-chromosomal spindle fibre force production, and that it is this interaction that is altered by the irradiation of the autosomal spindle fibre. (Equivalently, there may be a common control system – such as a calcium pump, and/or membrane system – that both

fibres interact with, and that is altered by the irradiation.) Other possible explanations of our results are that the radiation induces a photoproduct that diffuses to the adjacent spindle fibre; or, the effects on other movements are mediated via the pool of monomer subunits that is in equilibrium with the spindle fibres, as has been suggested by Dietz (1969, 1972*a*, *b*) for other examples in which changes in autosome movement result in subsequent alterations in sex-chromosome movements. We find it difficult to see how interactions via common pools of subunits can explain the geometrical requirements of our data; sex-chromosome movements are altered only when at least one sex-chromosomal spindle fibre is adjacent to the irradiated autosomal spindle fibre. Further, the alteration when 2 sex-chromosomal spindle fibres are adjacent to the irradiated autosomal spindle fibre is different from the alteration when only 1 sex-chromosomal spindle fibre is adjacent to the irradiated autosomal spindle fibre. Similar geometrical constraints were found in micromanipulation experiments (Forer & Koch, 1973). We are unable to explain these geometrical requirements by means of interactions between all spindle fibres and a common pool of monomer subunits (Dietz, 1969, 1972*a*, *b*), and hence we rule out this possibility.

Photoproducts may indeed be produced by the irradiations, and one might argue that the geometrical requirements arise because the photoproducts would be too diluted to have an effect if they diffused further than the adjacent spindle fibre. This seems possible, but other data argue against this possibility. First, as a direct corollary of this suggestion, one would have to say that the action of the photoproduct on autosome movement is different from that on sex-chromosome movement; the irradiations caused altered sex-chromosome movement even when the movement of the autosome that was associated with the irradiated spindle fibre was not altered. Also, such irradiations caused altered sex-chromosome movement but never altered the movements of adjacent autosomes. While these corollaries do not rule out the idea of a photoproduct, they do make it more complicated. A second set of data would seem to rule out the possibility that a diffusible photoproduct is responsible for our results, however; these are data from micromanipulation experiments (see Forer & Koch, 1973), as follows. If an autosomal half-bivalent moving polewards in anaphase is detached from its spindle fibre and is pointed towards the opposite pole in such a way that a spindle fibre forms to that pole and the half-bivalent moves there, the late anaphase cell then has 4 autosomes at one pole and 2 at the other; sex-chromosome movement is then altered in exactly the same way as described here, namely, one sex chromosome moves to the pole that has 2 autosomes whilst the other sex chromosome does not move. This alteration in sex-chromosome movement has *geometrical* requirements: sex-chromosome movements are not altered unless the spindle fibre of the manipulated (detached) half-bivalent is *adjacent* to that of a sex chromosome (Forer & Koch, 1973). Because micromanipulation experiments have exactly the same geometrical requirements as the irradiation experiments but without the possibility of photoproducts being formed, we think it unlikely that the effects of u.v. irradiation that we describe here are due to a photoproduct. Rather, we think that the results from the 2 different experimental approaches have a common basis; that is, that there is interaction between the autosomal spindle fibre and the adjacent

sex-chromosomal spindle fibre, either directly or via a common intermediate (such as a membrane-bound calcium pump), and that this interaction is involved in turning on the sex-chromosomal spindle fibre 'motor'.

Irradiations of autosomal spindle fibres had 2 main effects on sex-chromosome movement: if *one* sex-chromosomal spindle fibre was adjacent to the irradiated fibre, then one sex chromosome moved normally to the non-irradiated pole, but one did not move at all; if *two* sex-chromosomal spindle fibres were adjacent to the irradiated fibre, however, then *both* sex chromosomes moved to the non-irradiated pole. This difference seems to us to be an interesting and fundamental one, even though we are unable even to guess at mechanisms. Perhaps electron microscopy of such cells might provide some clues about mechanisms.

There seemed to be no clear-cut wavelength dependence for altering sex-chromosome movement by irradiating an autosomal spindle fibre (Tables 1–3). There *is*, however, clear-cut wavelength dependence for stopping autosome movement with similar irradiations (Sillers & Forer, unpublished), or for affecting spindle breakdown by irradiating the cytoplasm (Brown & Zirkle, 1967), for example. The presence of a clear action spectrum, with a single peak, indicates that there is one particular step which is most sensitive to the irradiation – or that there are several, but all have the same wavelength dependence. Our data (Table 1), though not definitive, suggest that there is no such single peak of efficiency for the effect on sex-chromosome movements. If so, that might suggest that there are several steps that can cause the alteration, and that the different wavelengths may affect different steps.

In our experiments sex-chromosome movement was altered by irradiation of autosomal spindle fibres even when there was no effect on the movement of the autosome that was associated with the irradiated spindle fibre. There are several possible interpretations of this result. For example, one might argue that the irradiation affects only one spindle fibre component but that different amounts of damage cause the different effects: if there are 100 myosin filaments (say) associated with each autosomal spindle fibre, destruction of $\geqslant 10$ filaments would alter sex-chromosome movement but destruction of $\geqslant 25$ filaments is necessary to stop the autosome from moving. This is possible, but the 2 effects do not seem to have the same wavelength dependence (Table 1 *versus* Sillers & Forer, unpublished). Thus, another interpretation seems more likely: alterations to sex-chromosome movements arise because a component of the spindle fibre is altered that is different from the autosomal spindle fibre motor. For example, if the postulated control system for sex-chromosome movement is different from the one that controls autosome movement, the alteration in sex-chromosome movement could be due to damage to this control system, whilst the blocking of autosome movement could be due to damage to the autosome's motor or to the autosome's control system. To us, it seems more likely that the 2 effects are due to damage to 2 different systems; that is to say, it seems likely to us that sex-chromosome movement is altered when the irradiation alters an autosomal spindle fibre component that is separate from the autosomal spindle fibre motor components.

We thank Mrs Anna Adhihetty for excellent typing of many versions of this manuscript. This work was supported by grants from the National Research Council/Natural Sciences and Engineering Research Council of Canada.

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(*Received* 10 *July* 1980 - *Revised* 16 *October* 1980)