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Prometaphase and Anaphase Chromosome Movements in Living Pollen Mother Cells

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Summary

Chromosome movements during meiosis in three plant species are described. Mean prometaphase and anaphase chromosome velocities were approximately 0.5 $\mu m \cdot min^{-1}$. Culture conditions had little effect on these velocities, but they may have delayed the onset of prometaphase or anaphase. Spindle elongation was zero during anaphase I in *Allium triquetrum* and in anaphase II in *Iris spuria*, but in both species elongation occurred during the other division of meiosis. Variations in anaphase I chromosome velocity at different positions across the *Allium* half-spindle were also observed.

Keywords: Anaphase; Chromosome movements; Meiosis; Prometaphase; Pollen mother cells.

1. Introduction

It is possible to culture pollen mother cells (PMCs) from early prophase I through the meiotic cycle, especially if cells are extruded from an anther as a coherent filament and are grown in a defined culture medium (ITO and STERN 1967, TAKEGAMI *et al.* 1981). This method is useful for biochemical studies but is not applicable to direct observation of meiosis in the light microscope (LM). For the latter, PMCs must be extruded into a slide culture chamber as a monolayer rather than as a coherent filament. But as ITO and STERN (1967) note, free cells survive poorly in culture. Despite this fact, meiosis in living PMCs has been studied in a number of species (review RYAN 1982) and PMCs can be excellent material for the study of cell division (GAROT *et al.* 1968). To date there have been no quantitative analyses of cell division in PMCs comparable to the analyses by BAJER and MOLÈ-BAJER (1954) and FUSELER (1975a and b) in mitotic endosperm cells.

Improved techniques for the culture of living PMCs have been recently developed and are described in detail in RYAN (1982). These techniques are utilized in the present paper to provide data on chromosome velocities, spindle elongation and duration of meiosis *in vitro* in the PMCs of three higher plant species.

2. Materials and Methods

Pollen mother cells of *Iris spuria* L. (2 n = 24), *Allium triquetrum* L. (2 n = 18) and *Tradescantia flumenensis* Vell. (2 n = 60) were studied. A detailed account of culture methods and ciné-microscopy are described elsewhere (RYAN 1982), but a brief description may be useful. PMCs were cultured on a slide chamber, which consisted of a glass slide with a 18 mm diameter hole drilled in the centre and with a coverslip sealed over one side of the hole. The PMCs were extruded from an anther and gently spread over a thin agar/sucrose coating on the inside surface of the coverslip. The cells were quickly covered with an inert oil and examined on an inverted microscope. The culture temperature was maintained at 20 ± 1 °C (*Iris* and *Tradescantia*) or 24 ± 1 °C (*Allium*). Time-lapse ciné films were analysed frame-by-frame using a Lafayette AAP-200 16 mm ciné projector. The projected image was reflected onto a bench top with a 45° inclined mirror and the magnification adjusted so that 1 mm on the image was equivalent to 1 μm in the cell. Relevant parts of the cell were traced onto paper and measurements recorded later. It should be noted that cellular detail was more clearly visible during film projection than in a still frame and analyses were facilitated by projecting the sequence backwards and forwards several times before tracing a particular frame. Frames two minutes of real time apart were usually chosen for analysis, giving about 20 measurements for a typical anaphase.

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A spindle pole was defined as a line drawn parallel to the approaching anaphase kinetochores, and positioned at the point of the change in contrast between the spindle and the cytoplasm (Fig. 1 *b*, 0 and 10 minutes and Fig. 1 *c*, 0 minutes, arrows). This definition was used because the pole in anastral spindles consists of an aggregation of membrane (HEPLER *et al.* 1981) and the assignment of a single point as the pole would be meaningless. Kinetochores were assumed to be the most poleward portions of the chromosomes in each half-spindle. Measurements from the pole line to the kinetochores were made along the actual paths of movement, which were found by projecting each anaphase sequence several times.

Chromosome velocities were measured from the graphs over the period of near constant velocity during mid-anaphase. Mid-anaphase was defined as the point at which the chromosomes had moved one-half of the total distance and the velocity was measured as the chromosome displacement over a 5 minute (*Allium*) or 10 minute (*Iris* and *Tradescantia*) period about this point. This period accounted for about 80% of the movement and gave a representation of velocity that was more realistic than "mean velocity" (CARLSON 1977), which is described by CARLSON as the slope of the line intersecting kinetochores at the beginning and end of a graph of anaphase.

Data for all cells analysed are included in Table 1. Many other anaphase sequences were also photographed but could not be analysed because either the plane of division was not parallel to the plane of focus, or the sequence was insufficiently clear for accurate analysis, or an incomplete record was obtained. The number of sequences *not* analysed were as follows: *Allium* anaphase I, 11, anaphase II, 1; *Iris* anaphase I, 5, anaphase II, 2; *Tradescantia* anaphase I, 5.

3. Results

3.1. Allium

Prophase and prometaphase I PMCs were difficult to culture successfully in all species studied. Prophase I was recorded in *Allium* in four cells, but only two of these entered prometaphase I. Prophase chromosome movements (RICKARDS 1975, 1981) were not seen in any of these four prophase cells (*e.g.*, Fig. 1 *a*). After the breakdown of the nuclear membrane (Fig. 1 *a*, 34 minute print), most bivalents moved to the central region of the developing spindle (50 minutes) but some moved firstly to a pole and back to the equator at approximately anaphase chromosome velocity (0.5 $\mu m \cdot min^{-1}$) before attaining bipolarity (52–90 minutes). Most bivalents underwent typical congressional movements (NICKLAS 1971) during mid- and late-prometaphase I, and metaphase I was reached 50 minutes after the breakdown of the nuclear membrane in this cell. A similar sequence of events occurred in the second prophase I PMC and both cells continued in meiosis until telophase I. Anaphase I is not shown here, however, because the chromosomes had lost contrast considerably.

Fig. 1 *b* illustrates anaphase I in an *Allium* PMC and the chromosome movements are graphically displayed in Fig. 2. Poles A and B remained a constant distance from the spindle equator (0 μm, Fig. 2) and therefore there was no spindle elongation in this cell. No spindle elongation was observed in any *Allium* anaphase I cell examined (Table 1 *a*). This is not unexpected since the spindle poles were always situated adjacent to the cell wall at metaphase I (Fig. 1 *b*, see also the electron micrographs in RYAN 1980), and thus polar separation could not occur in these cells.

In both half-spindles of the cell in Fig. 1 *b*, laterally positioned chromosomes (1 and 4) lagged behind central ones (2 and 3) and consequently had 27% slower velocity (Fig. 2). In cell 8-5 a (Table 1, 1 a) however, the anaphase chromosome groups bowed towards the poles and the lateral chromosomes moved 46% faster than central ones. In the other *Allium* cells studied (Table 1, 1 a), no differences within half-spindles were observed, but variations in mean velocities were still common between sister half-spindles.

Chromosomes converge on the poles during anaphase and if the plane of focus is not median, the observed chromosome paths will be projections of the real ones. A median plane of focus was obtained in most instances (Fig. 1 *b*) but where it was not, estimated chromosome velocities may be inaccurate. The magnitude of this error will depend on the degree of chromosome convergence. In the cell of Figs. 1 *b* and 2, the chromosome group reduced in width by 0.70 $\mu m \cdot min^{-1}$ and thus the chromosome on the edge of the spindle had a lateral velocity of 0.35 $\mu m \cdot min^{-1}$. If such a chromosome had a true poleward velocity of say 1.0 $\mu m \cdot min^{-1}$ and it was projected onto the central spindle axis (see vector diagram Fig. 3), the maximum error incurred would have been only 0.05 $\mu m \cdot min^{-1}$. Thus in practice, the velocities observed would not have been significantly different from real velocities.

No relationship was found between chromosome velocities and time in culture before anaphase I onset (Table 1, 1 a, note cell 7-1 in particular). The effect of culture temperature on chromosome velocity was not determined as all cells, with one exception, were cultured at the same temperature (cell 11-3 was cultured at 16 °C rather than at 24 °C).

Anaphase II chromosome movements are illustrated in Fig. 1 *c* and graphically displayed in Fig. 4. No relationship between chromosome position and velocity was evident. The spindle pole is located about 5 μm from the cell wall and there was approximately 45%

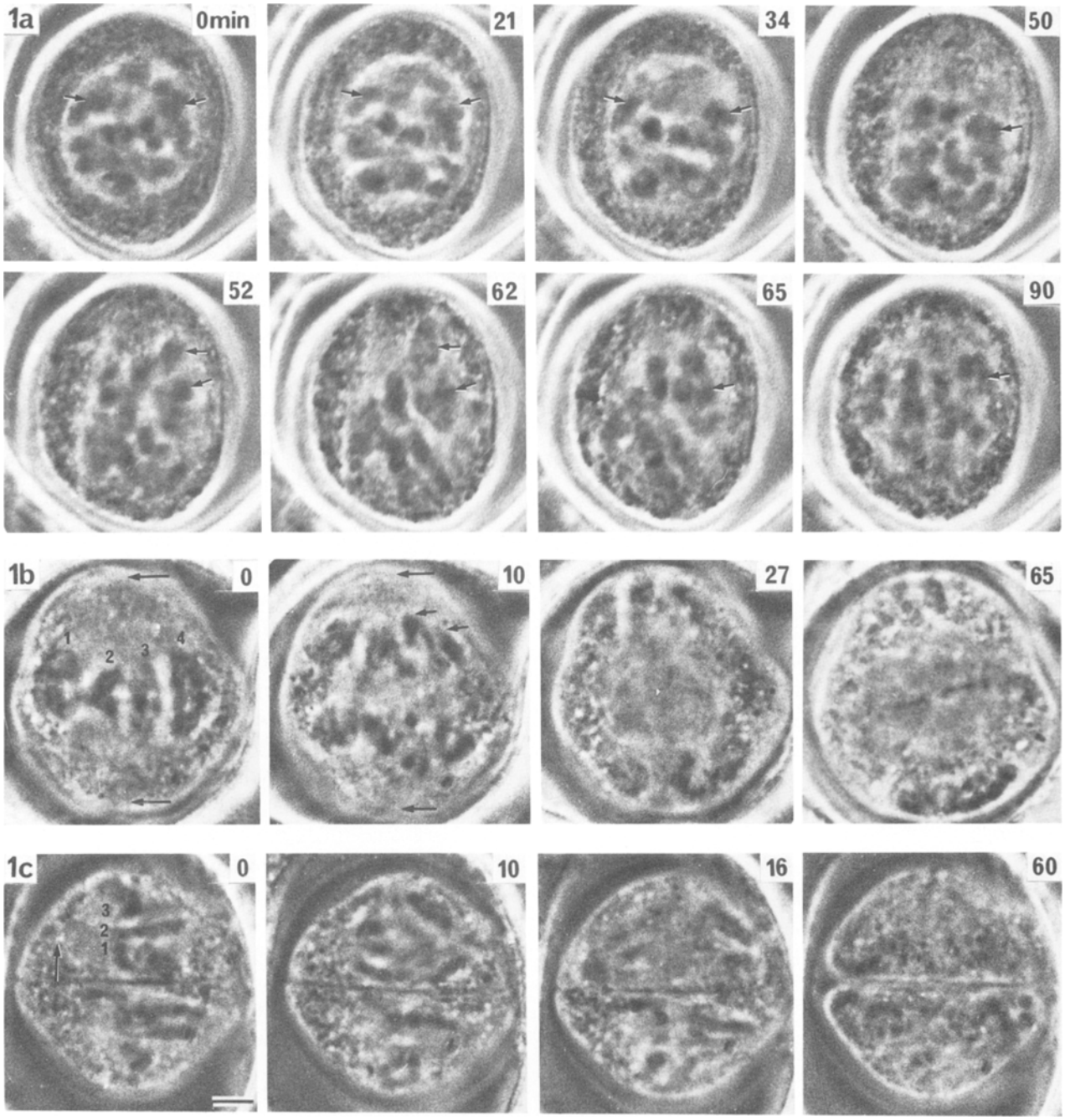
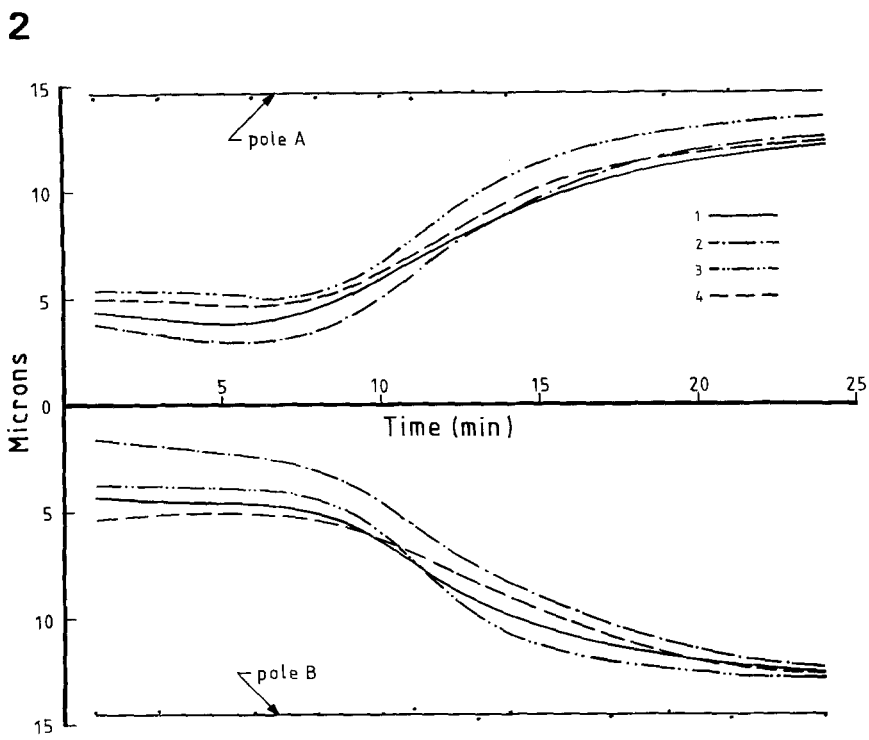
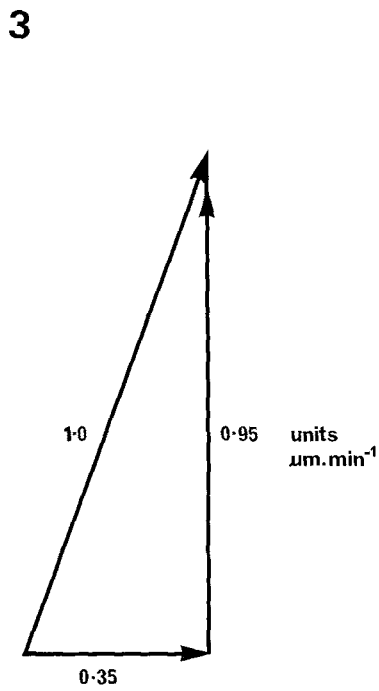


Fig. 1. Meiosis in living *Allium triquetrum* PMCs. *a* Prophase I to metaphase I. Two bivalents (arrows) remain in the same position until the breakdown of the nuclear membrane (34 minutes). The right hand bivalent then moves to the upper pole and back to the equator. The left hand bivalent moves out of the plane of focus. *b* Metaphase I to telophase I, cell 3-1, median optical section. Four bivalents are numbered in the 0 minute print and the two short arrows indicate the kinetochores of half-bivalents 3 and 4 in the 10 minute print. The positions of the two poles are arrowed in the 0 and 10 minute print. *c* Metaphase II to telophase II, cell 5-3. Three chromosomes are numbered in the 0 minute print. The position of a pole is arrowed in the 0 minute print. Bar, 5 μm





Figs. 2 and 3. Anaphase I in *Allium triquetrum*. Fig. 2. Pole and kinetochore position in anaphase I, cell 3-1. Individual graphs for bivalents 1 to 4 of Fig. 1 *b* are shown. Individual mid-anaphase chromosome velocities for bivalents 1 to 4 are (in $\mu m \cdot min^{-1}$) 0.75, 1.10, 1.10, 0.80 respectively for the upper half-spindle and 1.00, 1.10, 1.40, 0.75 respectively for the lower half-spindle. Fig. 3. Vector diagram of anaphase movement in lateral chromosomes. See text for details

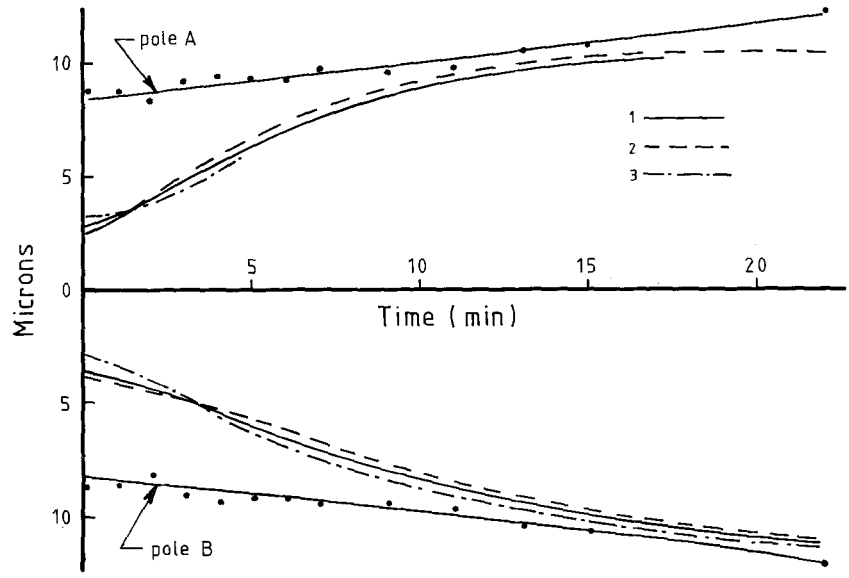


Fig. 4. Pole and kinetochore position in anaphase II of *Allium triquetrum*, cell 5-3. Individual graphs for chromosomes 1 to 3 of the cell in Fig. 1 *c* are shown. Chromosome 3 moved out of the plane of focus midway through anaphase. All chromosomes moved at approximately 0.75 $\mu m \cdot min^{-1}$ in the upper half-spindle (left half-spindle in Fig. 1 *c*) and at 0.55 $\mu m \cdot min^{-1}$ in the lower half-spindle

Table 1. *Mean mid-anaphase chromosome velocities in PMCs.* A difference in velocity between the 3-4 chromosomes in each half-spindle was not usually detected with the analysis method used. When detected, a percentage difference is given in brackets

	Spindle no.	velocity ($\mu m \cdot min^{-1}$) First half-spindle	Second half-spindle	Spindle elongation (%)	Time in culture before anaphase (minutes)
1 a	*Allium* anaphase I				
	3-1	0.95 (− 27%)*	1.05 (− 27%)*	0	14
	3-3	0.25	0.25	0	20
	7-1	0.60	0.85	0	270
	8-3	0.35	0.40	0	75
	8-5 a	0.35 (+ 50%)*	0.55 (+ 42%)*	0	3
	8-5 b	0.50	0.50	0	20
	11-3	0.50	0.40	0	2
1 b	*Allium* anaphase II				
	5-3	0.75	0.55	45	120
1 c	*Iris* anaphase I				
	10-1	0.65	0.60	15	2
	10-9	0.55	0.55	–	13
	16-10	0.45	0.55	16	30
	17-10	0.60+		–	240
1 d	*Iris* anaphase II				
	10-1	0.28+		–	600
	16-1.1	0.50	0.45	0	180
	16-1.2	0.50	0.35	0	180
	19-3.1	0.55	0.60	0	90
	19-3.2	0.85	0.80	0	90

* A plus or minus sign indicates whether lateral chromosomes moved faster or slower than central ones.
\+ Measured from a graph of the rate of kinetochore separation.

spindle elongation during anaphase II. The lower spindle of this cell was not analysed because individual kinetochores were difficult to distinguish, but it was apparent during ciné projection that anaphase II here was similar to that in the upper spindle.

3.2. *Iris*

Ciné-micrographs of anaphase I chromosome movements in an *Iris* PMC are presented in Fig. 5 *a*. For clarity, the motions of only two of the bivalents are graphically displayed in Fig. 6. Curves describing the motion of the other four bivalents coincided with these two and thus in *Iris* all chromosomes moved at the same velocity within each half-spindle regardless of their position. No position effect on chromosome velocity was seen in other *Iris* PMCs studied (Table 1, 1 c), and chromosome groups did not bow towards or away from the poles. As in *Allium* there were differences in chromosome velocity between sister half-spindles. In two of the cells of Table 1, 1 c there was approximately 15% spindle elongation during late anaphase I, while the extent of elongation could not be estimated in the other two cells. The time in culture was not a source of variability in chromosome velocity. Similarly, an alteration in preparation technique did not affect anaphase I events: cell 16-10 was prepared in an agar sandwich (a technique normally used for correlative living cell and electron microscope studies, RYAN 1982) and chromosome velocities in this cell were normal for *in vitro* cells.

Anaphase II in a living *Iris* PMC is shown in Fig. 5 *b* and graphs of the positions of a central and a lateral chromosome from this cell are given in Fig. 7. Differences in chromosome velocity within each half-spindle could not be detected, either in this spindle or in

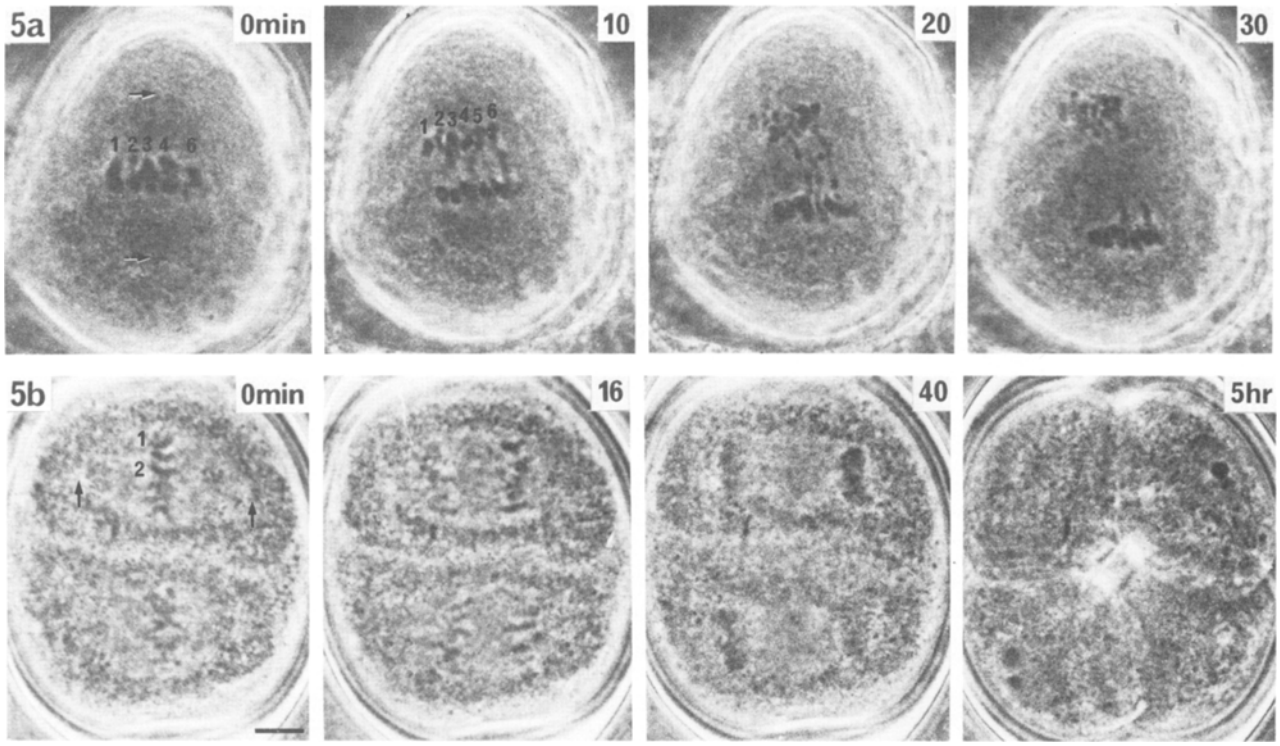


Fig. 5. Meiosis in living *Iris spuria* PMCs. *a* Metaphase I to telophase I, cell 10-1. Six bivalents are numbered in the 0 and 10 minutes prints and the positions of the poles are indicated by arrows. *b* Metaphase II to telophase II, cell 16-1. Two chromosomes are numbered in the 0 minute print and the positions of the poles are indicated by arrows. Bar, 10 μm

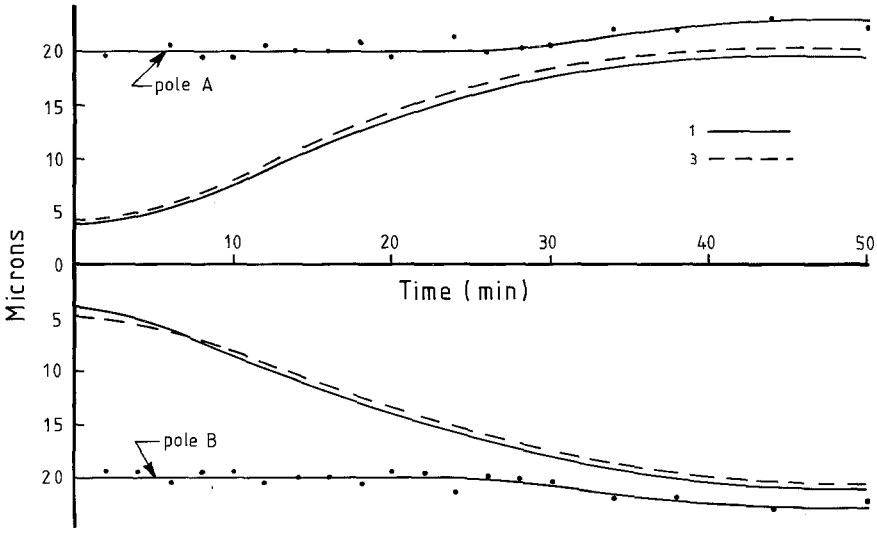


Fig. 6. Pole and minetochore position in anaphase 1 of *Iris spuria* cell 10-1. For clarity, the movement of only two of the six bivalents observed are graphed. The mid-anaphase velocities for each half-bivalent are 0.65 $\mu m \cdot min^{-1}$ in the upper half-spindle and 0.60 $\mu m \cdot min^{-1}$ in the lower half-spindle

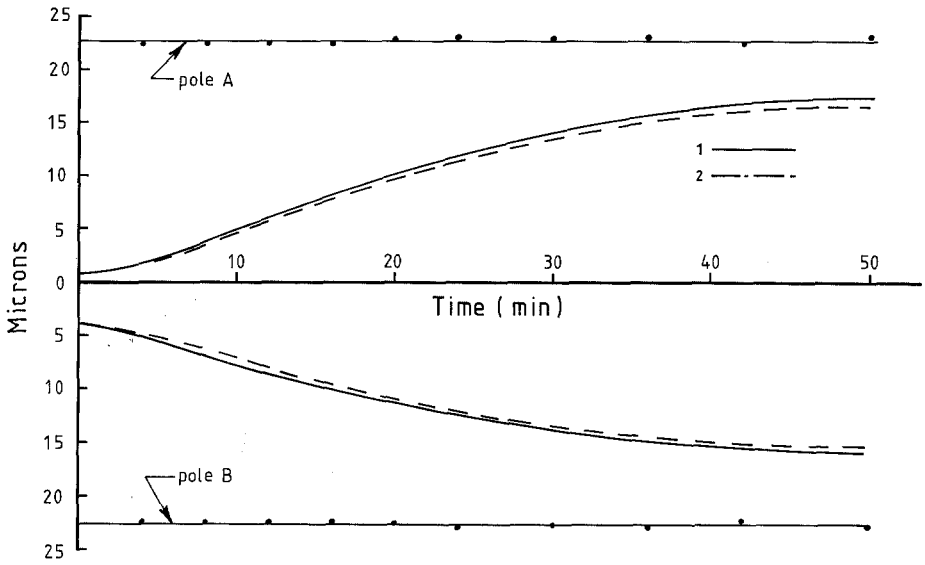


Fig. 7. Pole and kinetochore position in anaphase II of *Iris spuria*, cell 16-1.1. The movement of two of the six chromosomes observed are illustrated. All chromosomes moved at 0.50μm·min^{-1} in the upper half-spindle and at 0.45μm·min^{-1} in the lower half-spindle

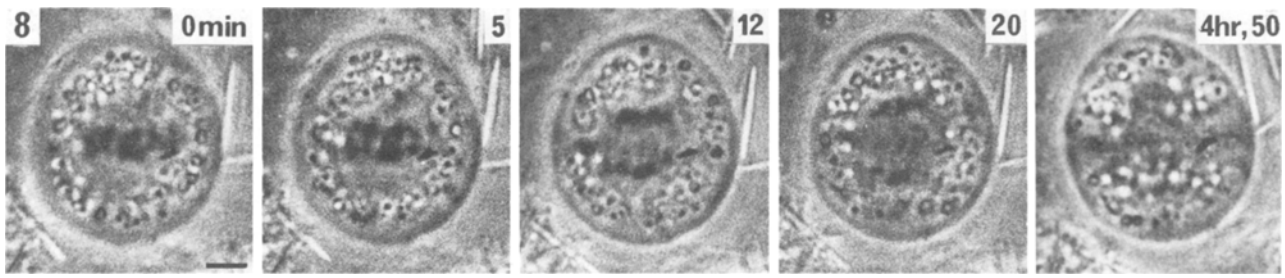


Fig. 8. Anaphase I in *Tradescantia flumenensis*, cell 14-5. Note the phase bright granules surrounding the spindle area. The dark spot to the right of the spindle equator is a speck of dust on the camera lens. Bar, 5 μm

any other studied (Table 1, 1 d). However, minor differences in velocity between sister half-spindles were common. In general, anaphase II chromosomes moved at a rate similar to but more variable than those of anaphase I and no polar elongation was observed. The time in culture usually had no effect on chromosome velocities. Only in cell 10-1, which was cultured for 10 hours prior to anaphase II, was the mean velocity considerably less than in other cells.

3.3. *Tradescantia*

This species was not particularly suitable for living cell study because of its small chromosomes and the presence of large cytoplasmic granules. Anaphase I is illustrated in Fig. 8. The graph (Fig. 9) gives an estimate only of chromosome velocity (0.25 μm · min^{-1}) because individual kinetochores could not be distinguished. This estimate may be close to the real velocity since observations in another cell gave a similar mid-anaphase I rate. Anaphase II was not studied in this species.

3.4. *Duration of Meiosis*

Table 2 summarises observations on meiotic duration in the three species studied here and utilizes data both from Table 1 and from observations on other cells (see Materials and Methods). Data derived from LAMBERT (1978) and GAROT *et al.* (1968) are also included. Interpretation of this table must take account of the fact that PMCs are sensitive to culture conditions (RYAN 1982). For example, if the culture medium was anisotonic the cells quickly died and in many cases apparently healthy cells did not enter anaphase (unpublished observations). Also, many cells did not

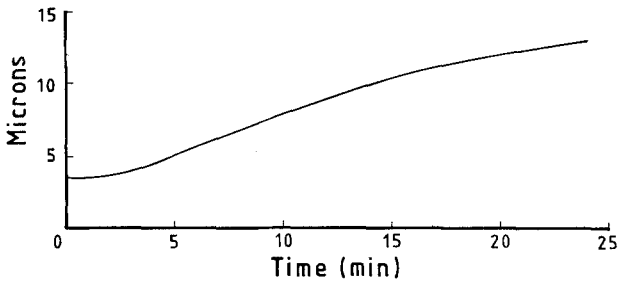


Fig. 9. Interkinetochore distance in *Tradescantia flumenensis*. Data from four bivalents pooled. The rate of chromosome separation is approximately 0.50 $\mu m \cdot min^{-1}$

progress in meiosis further than telophase I. Thus, there was considerable variation within and between the different species over those phases where chromosomes do not move greatly. Note particularly that one *Iris* cell, which was initiated in culture at metaphase I, took five hours from telophase II to tetrads; while another, initiated at metaphase II, took only one hour to progress from telophase II to tetrads. Also, the difference in time taken to progress from anaphase I to tetrads between *Iris* and *T. paludosa* may be almost entirely attributed to differences at stages where chromosome movement was minimal.

4. Discussion

The observation that "stationary" phases of meiosis were more variable in duration than those where chromosomes moved (Section 3.4. and Table 2), at first appears to contradict BENNETT (1977). He states that interspecific "changes in the duration of meiosis in higher plants usually result from proportional alterations of all the stages rather than large changes in one or just a few". However, the sensitivity of PMCs to culture conditions suggests that the variations in the duration of these stationary phases are artifacts of culture. Thus, changes in the tonicity of the medium can sometimes arrest or kill cells (RYAN 1982, SHIMAKURA 1934), but arrested metaphase I cells can be induced to enter anaphase by increasing slightly the tonicity of the medium (SHIMAKURA 1934). Also relevant is the observation that the optimum tonicity for PMC culture increases during meiosis (RYAN 1982, SHIMAKURA 1934). Therefore, culture conditions may have considerably affected the duration *in vitro* of some meiotic stages studied here, especially those stages where chromosome movement was minimal.

A comprehensive summary of mean anaphase velocities in many different mitotic and meiotic cells is given by CARLSON (1977, Table 1). As my criteria for determining velocities were different from CARLSONS (see Materials and Methods) the two sets of data are not directly comparable. For this reason mid-anaphase velocities for several representative species were recalculated from the original graphs and presented in Table 3.

A comparison of Tables 1 and 3 reveals that mid-anaphase velocities are similar in mitotic and meiotic spindles of plant cells although there may be a trend for mitotic mid-anaphase velocities to be higher than those for meiosis (compare especially *I. aphylla* and *T. virginiana* mitotic cells with *I. spuria* and *T. flumenensis* PMCs). This trend has been observed by CARLSON

Table 2. *Duration of meiosis in PMCs*

	Allium	*Iris*	*T. flumenensis*	*T. paludosa*	
				a	b
Late prophase I	4 h				
Prometaphase I	30 min				
Metaphase I	0-4 h	0-4 h			
Anaphase I	15-25 min	40 min	30 min	30 min	25 min
Telophase I-prophase II		4 h 30 min		2 h 40 min	
Prometaphase II		30 min		30 min	
Metaphase II		1-4 h		20 min	
Anaphase II	15 min	30 min		30 min	20 min
Telophase II-tetrads		5 h (1 h)		40 min	70 min
Anaphase I-tetrads		15 h 30 min (cell 10-1)		5 h 10 min	

a Estimated times from LAMBERT (1978).
b Estimated times from GAROT *et al.* (1968).

Table 3. *Anaphase chromosome velocities* ($\mu m \cdot min^{-1}$)

Species	Cell type	Anaphase mitosis	Anaphase I	Anaphase II
T. paludosa[1]	PMC		0.4	0.4
Melanoplus sp.[2]	spermatocyte		0.65-0.87	
Chortophaga sp.[3]	spermatocyte		3.0	
Iris aphylla[4]	endosperm	1.0		
Haemanthus katherinae[5]	endosperm	0.6		
T. virginiana[6]	endosperm	1.2		
Tilia americana[7]	endosperm	2.3		

1 GAROT *et al.* (1968).
2 NICKLAS (1965).
3 CARLSON (1977).
4,5 BAJER and MOLÈ-BAJER (1954).
6 BARBER (1939).
7 FUSELER (1975 a).

(1977) for animal cells but the lack of data with the same plant species precludes more definite conclusions here. It is also evident from the two tables that mid-anaphase chromosome velocities in animal meioses are spread over a wider range than in PMCs but this trend may be spurious because of the low number of plant species studied.

It is interesting to note that in an *Allium* anaphase I cell cultured at 16 °C (cell 11-3, Table 1, 1 a), the mid-anaphase velocity was similar to the other cells cultured at 24 °C. This does not accord with the observations of FUSELER (1975 b) who noted that chromosome velocities decrease with decreasing temperature. However the range of velocities observed in *Allium* anaphase I was wide (Table 1, 1 a) and the cell 11-3 may represent simply a cell at the upper end of a different range.

Anaphase movement can usually be resolved into two components: spindle elongation; and chromosome-to-pole movement. Spindle elongation has been described either as being negligible or absent in plant cells (ÖSTERGREN 1949), or as being present in all cell types studied to date (CARLSON 1977). No spindle elongation was found during anaphase I in *Allium triquetrum* in the present study (Fig. 2). Furthermore, the PMCs of this species cannot change their shape or size during anaphase I because there is a thick, rigid callose wall around the cell and observations in both the LM (Fig. 1 *b*) and electron microscope (RYAN 1980) have shown that the spindle poles are adjacent to the cell membrane at metaphase I. These results are the first unequivocal demonstration of anaphase movement without spindle elongation in plant cells. In anaphase II the spindle pole is located about 5 μm from the cell wall and this indicates that spindle elongation is at least possible. Indeed, approximately 45% spindle elongation was observed in *Allium* anaphase II. These observations suggest that spindle elongation may always occur unless elongation is physically impossible as in *Allium* anaphase I. This possibility is negated in the general case, however, since in *Iris* anaphase II, where there is also no spindle elongation, the spindle pole is located in the cytoplasm. While the amount of spindle elongation during anaphase is known to be variable between different types of spindle in other species as well (RIS 1943, MAZIA 1961), the significance of these variations remains uncertain. Perhaps they simply emphasize that different, but equally effective, means can achieve the separation of daughter chromosome groups.

In most *Allium* anaphase I cells studied, the half-bivalents in each half-spindle moved polewards at the same velocity, although some differences between sister anaphase groups were common (Table 1, 1 a). In one spindle (spindle 3-1) the chromosome groups bowed away from the pole while in another (spindle 8-5 a) they bowed towards the poles. Large differences in velocity between lateral and central chromosomes of -27% and $+46\%$ respectively were the major reasons for this bowing and where only small such differences were observed no bowing occurred. More definite position effects have been reported during anaphase by NICKLAS (1965) in *Melanoplus* spermatocytes and FUSELER (1975 a) in *Tilia* endosperm. In *Melanoplus* bowing towards the pole was due to a 25% increase in velocity of lateral chromosomes while in *Tilia* bowing away from the pole was due to lateral chromosomes moving

at the same velocity as median ones but having further to travel. Neither of these situations appear to apply routinely to anaphase I in *Allium* and the reasons for the observed differences in this species are not known at present.

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