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ONTOGENETIC ALTERATION OF THE SPINDLE APPARATUS: POSSIBLE RELATION TO CHROMOSOME PAIRING *

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INTRODUCTION

It is not understood how homologous chromosomes come initially into association for the events of meiosis. Because meiotic synapsis is typically complete, it is difficult to conceive how such regular success can be achieved if synapsis is initiated between chromosomes randomly scattered through the prophase I nucleus (MAGUIRE 1967; BROWN and STACK 1968). If the chromosomes are not randomly scattered through the nucleus, by what means are they moved into more intimate association? The only time chromosomes are known to be moved actively by cells is during mitosis or meiosis under the influence of microtubules of the spindle apparatus.

It has been shown in the monocot *Ornithogalum virens* that as the plant matures, chromosome centromeres (kinetochores) lie progressively closer together on mitotic metaphase plates (STACK 1971) (Fig. 1 A, B, C). It is suggested that the closer association of centromeres on metaphase plates might be a means of facilitating chromosome pairing in premeiotic mitoses. All six chromosomes of the diploid complement of *O. virens* are subtelocentric and centric end associations of homologous chromosomes were observed in squash preparations of some premeiotic mitoses. Since chromosomes seem to be moved to the metaphase plate through the action of spindle microtubules attached to centromeres, the question arises whether the spindle itself becomes more compact at the metaphase plate and thereby draws the centromeres together. We have found this to be the case in *O. virens*.

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MATERIAL AND METHODS

Plants at various ontogenetic stages were used in this study. The three stages of maturity included germinating seedlings, young bulbs five to ten mm. in diameter (approximately one month old), and flowering bulbs which were typically in excess of three cm. in diameter (four or more months old). Seeds were planted in sandy loam and grown to these stages of maturity in a greenhouse

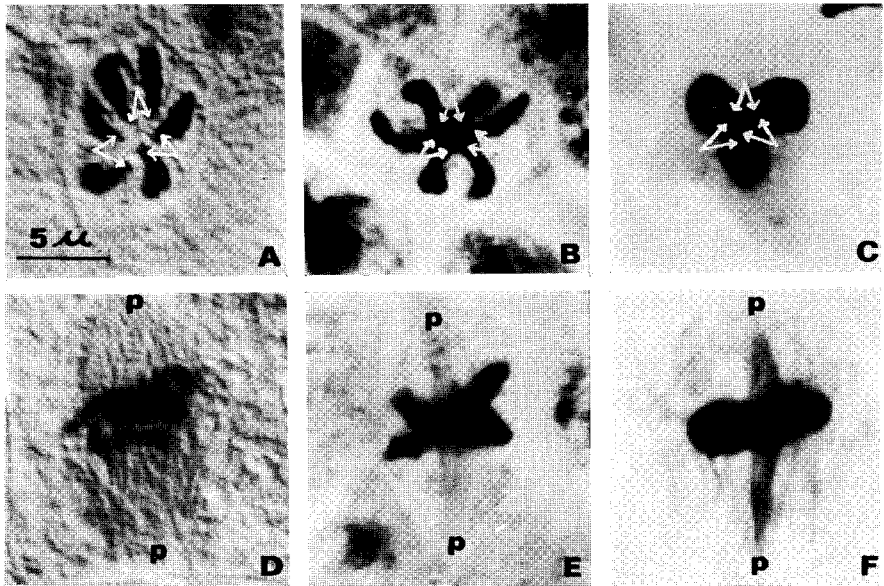


Fig. 1. — Mitotic and meiotic metaphases in *Ornithogalum virens*. Centromeres are indicated by arrows. Poles are indicated by *p*. (A) Polar view of metaphase in the root tip of a germinating seedling. Note the widely separated centromeres of the subtelocentric chromosomes compared to B and C. (B) Polar view of mitotic metaphase in flower bud tissue. (C) Polar view of the meiotic metaphase I in a microsporocyte. (D) Equatorial view of metaphase in the root tip of a germinating seedling. Note the width of the spindle apparatus in the plane of the metaphase plate and size and distinctness of the poles compared with E and F. (E) Equatorial view of early mitotic metaphase in flower bud tissue. (F) Equatorial view of meiotic metaphase I in a microsporocyte ($\times 2000$).

under natural lighting. Mature plants will bloom at any time of the year, i.e. *O. virens* is not photoperiodic. Shoot meristems from young bulbs, buds from flowering bulbs, and root tips from germinating seedlings and flowering bulbs were fixed in Navashin's fluid, dehydrated, embedded in paraffin, and sectioned. The material was stained with iron hematoxylin and examined with both bright field and Normarski differential interference microscopy. Only equatorial views of metaphase plates (Fig. 1 D, E, F) were used in making measurements of both the spindle diameter at the metaphase plate and the cell diameter on the same

optical section. Measurements were analyzed using the Student-Newman-Keuls multiple range test for significant differences between means (SOKAL and ROHLF 1969).

OBSERVATIONS

There is a progressive tendency for the spindle to be reduced in diameter at the metaphase plate as the plant matures (Table 1). The root tips from germinating seeds have the largest spindle diameters with a highly significant ($p = .01$) reduction occurring in young bulbs and root tips of blooming plants. Young flower bud tissue (prior to most tissue differentiation) and microsporocytes at metaphase I of meiosis exhibit another highly significant ($p = .01$) reduction in spindle diameters. It may be of considerable impor-

TABLE 1

Means and standard deviations of means for spindle diameters and cell diameters taken from: root tips of germinating seedlings (RTGS), root tips of blooming bulbs (RTBB), shoot meristems of young bulbs (SMYB), flower buds (FB), and microsporocytes in metaphase I of meiosis (M1) of Ornithogalum virens. Results of a Student-Newman-Keuls multiple range test for significant differences between means is indicated by a line underscoring any two means not significantly different at the 1% level.

	Spindle diameter					Cell diameter				
	RTGS	RTBB	SMYB	FB	MI	FB	RTGS	RTBB	MI	SMYB
Mean (μm)	8.7	7.2	7.0	<u>6.2</u>	<u>6.2</u>	<u>16.4</u>	<u>17.1</u>	<u>21.8</u>	<u>22.4</u>	25.3
S.D.* (μm)	.13	.17	.20	.13	.16	.54	.44	.57	.41	.87
n ⁺	100	100	75	100	63	74	87	89	53	71

* Standard deviation of the mean. + Number of observations.

tance that these latter two tissues should have the same spindle diameters. The correlation of cell diameter with spindle diameter ranged from a rather low correlation in root tips of germinating seedlings ($r = .56$) to insignificance in microsporocytes at metaphase I of meiosis ($r = .10$), thus there is generally a rather small tendency for small cells to have narrow spindles and vice versa (Table 2).

Another aspect of the alteration of spindle shape during ontogeny concerns the size and distinctness of the poles. Generally in non-bud tissues the poles are comparatively diffuse (indistinct) areas into which spindle microtubules converge (Fig. 1 D). In bud tissue, however, the poles are

TABLE 2
Correlation between cell diameter and spindle diameter in five stages of development in Ornithogalum virens

	RTGS	RTBB	SMYB	FB	MI
r*	.56 (.69, .40)	.30 (.47, .09)	.29 (.50, .06)	.39 (.57, .18)	.10 (.36, -.17)
n [†]	87	89	71	74	53

* Coefficient of correlation. 95% confidence limits are shown in parenthesis.

† Number of observations.

far more compact and spindle microtubules converge into a much smaller area (Fig. 1 E). The most distinct poles with the most extreme convergence of spindle microtubules are seen in microsporocytes at metaphase I (Fig. 1 F). PALMER (1971) made similar observations on spindle poles in maize, but he suggested that pole variability was related to differences in cell size. We definitely do not find a correlation between pole shape and cell size. At present it is impossible to say whether there is a relation between the concomitant reduction in spindle diameter and pole size, but it is tempting to speculate that there may be because both are aspects of a more compact spindle.

DISCUSSION

The reduction in spindle diameter which occurs in *O. virens* flower buds compared to the root tips of germinating seeds results in a decrease by one-half in the area (πr^2) available for chromosome centromeres on the metaphase plate. This mechanical drawing of the chromosomes into closer proximity could allow random contact of homologous parts for the initiation of pairing. Additionally or alternatively, reduced spindle diameters could be a means of maintaining paired chromosomes in close proximity during metaphase when chromosome pairing seems to least intimate (METZ 1916; WAGENAAR 1969). A relation of spindle diameter to chromosome pairing is suggested by the practically identical average spindle diameters which we observed in flower bud tissue (where pairing may be initiated) and microsporocytes at metaphase I (where pairing is obviously complete).

Both genetic and artificially induced alterations of spindles and spindle microtubules have been reported to be associated with altered patterns of chromosome pairing and synapsis. AVIVI *et al.* (1969, 1970a, 1970b) have presented evidence that in wheat the genes which are thought to be involved

with premeiotic chromosome pairing may actually have their primary effect on spindle microtubule subunits. In maize hemizygotes and homozygotes for a recessive, asynaptic gene (BAKER and MORGAN 1969) and in *Triticum* × *Aegilops* hybrids carrying B chromosomes (VARDI and DOVER 1972), meiosis is more or less asynaptic and the spindles of both archesporial and meiotic cells are probably abnormal. However, spindles at other points in the life cycle appear to be normal, which suggests that there may be genetic alterations in spindle form and function in the mitotic divisions immediately preceding meiosis (REES 1961). DRISCOLL *et al.* (1967) and DRISCOLL and DARVEY (1970) have demonstrated that colchicine injected into wheat several days before meiosis inhibits meiotic chromosome pairing and the subsequent formation of the synaptonemal complex. Because of the known disruptive effect of colchicine on microtubules, they suggested that microtubules might somehow be involved in the pairing of chromosomes. The only microtubules known to be associated with eukaryotic chromosomes are part of the spindle apparatus. EGOZQUE (1968) found that if colcemid is used to collect chromosomes in primate tissue culture, somatic crossing over is greatly reduced. COHEN *et al.* (1972) reported a lack of chromosome pairing in colcemid-treated Indian muntjak cells in tissue culture, while HENEEN and NICHOLS (1972) reported chromosome pairing (primarily by centromeric association) in tissue cultured cells of the same species, which had not been treated with colcemid. EGOZQUE (1968), COHEN *et al.* (1972) and HENEEN and NICHOLS (1972) all conclude that colcemid disturbs the usual spindle-mediated disposition of the chromosome on the metaphase plate and thereby interferes with pairing.

In conclusion, a reduction in the size of spindle apparatuses may be involved in chromosome pairing in preparation for meiosis. However, because of the non-specific nature of chromosome-microtubule-pole attachments and the generalized movements of chromosomes first to the metaphase plate and then to the poles, it is unlikely that the spindle could function specifically to draw homologous chromosomes into association. It appears more probable that the spindle functions to draw all of the chromosomes into close association so that homologous chromosomes are likely to come into contact and pairing can occur.

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SUMMARY

As the monocot *Ornithogalum virens* matures from seed germination to flowering there is a progressive reduction in the diameter of mitotic spindle apparatuses at the metaphase plate, and poles become more distinct. Apparently one result of this constriction of the spindle is a closer association of chromosomes on the metaphase plate which could be a factor in the eventual pairing of chromosomes for meiosis.