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Early Seed Development in the Triticeae

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EARLY SEED DEVELOPMENT IN THE TRITICEAE

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[Plates 1–3]

CONTENTS

The rates of early seed development were compared in several species in the Triticeae which play a major role in human nutrition, and in several related genotypes whose reproductive development is of current interest to plant breeders. Embryo and endosperm development during the first five days after pollination was studied in plants of 22 genotypes grown at 20 °C with continuous light. Spikes were emasculated before anther dehiscence and then pollinated once full female receptivity was reached. The numbers of embryo and endosperm nuclei or cells in individual florets were ascertained by using large samples of fertilized florets fixed at various known times after pollination.

The pattern of early seed development was essentially the same in wheat, rye, *Triticale* and barley, although some interspecific variation in the rate between genotypes was noted. Fertilization occurred in some florets of several genotypes studied within 40–60 min after pollination. Mitosis in the primary endosperm nucleus was completed about 6–7 h after pollination. During the next 24–48 h the number of endosperm nuclei increased geometrically, doubling about every 4–5 h. The endosperm was coenocytic at first but usually at about 72 h after pollination it became cellular. The rate of nuclear development in the endosperm declined on each successive day, the greatest fall occurring at the time of cell wall formation.

Mitosis in the zygote occurred about 18–30 h after pollination which was later than mitosis in the primary endosperm nucleus. The cell cycle time in the embryo varied between species from about 12 to 18 h, and was similar to its duration in cells of other meristematic tissues in the same species. Cell cycle time in the embryo remained fairly constant during the first 5 days of seed development unlike the rate of nuclear development in the endosperm. Thus, at first the rate of embryo cell development was very slow compared with that of the endosperm nuclei, however, by the end of the fifth day the cell cycle time in the endosperm had increased to become equal to or longer than that of the cell cycle in embryo cells. The nature and possible cause(s) of rapid nuclear development in coenocytic endosperm is discussed.

While embryo volume increased steadily over the period studied, the mean volume of embryo cells decreased about tenfold. This was because at first the rate of increase in embryo volume was lower than the rate of increase in embryo cell number. Eventually these two rates became similar and thereafter further development gave rise to embryo cells whose volume was constant and roughly equivalent to that of other meristematic cells in the same species.

The rates of embryo and endosperm development were as a rule much faster in wheat species than in rye. By comparison, the rates in hexaploid *Triticale* genotypes were usually much slower than in wheat, and sometimes even slower than in rye. Results for wheat-rye chromosome addition lines, disomic for each rye chromosome, show that most rye chromosomes apparently had a pronounced effect on slowing both embryo and endosperm development. Indeed, rye chromosomes VI and VII apparently had an effect equal to that of the presence of a whole rye genome.

Comparison of the maximum rates of endosperm development in diploid and related polyploid species shows that there was no effect of polyploidy during the first 48 h of the coenocytic phase of endosperm development. Concurrently, during development of the cellular embryo there was a clear effect of ploidy level, with a positive relation between ploidy level and developmental rate. These results are compared with the effects of polyploidy on the rate of development in other tissues in the same species.

The rates of embryo and endosperm development in *Hordeum vulgare* were much faster than in diploid *H. bulbosum*. This result is discussed with reference to the mechanism of chromosome elimination from embryo and endosperm tissues of F_1-hybrids between these two species.

The present results provide a detailed picture of the course of normal early seed development in a wide range of cereal genotypes which varied with respect to several characters known to affect rate of development in other tissues. They provide, therefore, a baseline for comparative studies which aim both to describe abnormal

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early seed development and to quantify its extent, in for instance *Triticale* with shrivelled grain. At the same time they provide some indication of the factors which apparently influence or control the rate and extent of early embryo and endosperm development in these important crop species.

Introduction

The grain of wheat, rye and barley (all species in the tribe Triticeae) plays a major role in human nutrition. In 1971, for instance, the crop with the largest area under cultivation (217.2 million hectares) and the largest production (343.1 Mt (million metric tons)) in the world, was wheat (Erus 1972). Crops with the second and third highest production were two closely related species in the Gramineae, namely, maize (307.8 Mt) and rice (307.4 Mt). The embryo and endosperm tissues of these and related species in the Gramineae form the staple diet of the majority of human beings. It is perhaps surprising, therefore, how little is known about the factors which influence or determine the rate and extent of development in these tissues.

Little comparable data is available for wheat, rye and barley regarding the rate and pattern of their seed development at the level of the individual cell, tissue or floret. It is important to study development at these levels *first*, because the floret is the basic unit of grain production, and *second*, because the production of each grain depends upon the normal developmental behaviour of several highly specialized single cells or small groups of cells. In each floret fertility is always either 0 or 100% depending upon the success or failure of several vital processes including, female meiosis, embryo sac development and fertilization. Each floret contains a single female meiocyte which at meiosis gives rise to a single functional megaspore. This cell gives rise to an embryo sac, which at maturity contains an egg cell, two synergids, a central cell and a few antipodal cells. Double fertilization depends upon events involving several of these individual or unique embryo sac cells and their nuclei. Thereafter the formation of a functional endosperm and embryo depends upon an orderly sequence of cellular events in the various tissues.

Recently, a detailed study was made of cell behaviour during early seed development in *Triticum aestivum* grown under carefully controlled environmental conditions (Bennett, Rao, Smith & Bayliss 1973), and a parallel investigation was made with five hexaploid *Triticale* lines (Kaltsikes 1973). Several detailed studies of the rate and sequence of early seed development have previously been made in species from the *Triticeae* (Percival 1921; Pope 1937; Brink & Cooper 1944; Beaudry 1951; Hakansson & Ellerström 1950). However, these studies were made with plants grown under different, and sometimes undefined, environmental conditions. Consequently, it is impossible to make meaningful comparisons from results obtained by the different workers.

There are still major uncertainties both as to whether the antipodal cells have a function in early seed development, and if so, as to the precise nature of their role (Brink & Cooper 1944; Beaudry 1951; Pritchard 1964; Heslop-Harrison 1972). Similarly, there is disagreement whether there is a coenocytic stage in the development of endosperm of wheat or not, and regarding the manner in which the aleurone and inner endosperm layers arise (Brenchley 1909; Gordon 1922; Buttrose 1963; Evers 1970). Recently developmental behaviour, of a type not noted in any other higher plant species, was reported in cells of the proembryo of *Hordeum distichum* (Mericle & Mericle 1970). They claimed that the mean DNA content per cell

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decreased from about 12*C* in the zygote to about 3*C* in mid proembryo cells, however, our preliminary investigation with *Hordeum vulgare* did not confirm their observations. It was partly because of these uncertainties and contradictions that we decided to undertake a wide ranging reinvestigation of early seed development in several economically important species in the Triticeae.

Recent studies of anther development in many species (including ten from the Triticeae) have resulted in a greatly increased understanding of the factors affecting the rate and duration of meiotic and pollen grain development in higher plants (Bennett 1971; Bennett & Smith 1972; Bennett 1973; Bennett, Dover & Riley 1974). These studies have shown the value of investigating the same developmental process in several species all grown in identical environmental conditions. We decided, therefore, to adopt the same approach in our studies of seed development. By examining several genotypes grown under constant conditions we hoped to obtain results which would allow general conclusions to be drawn regarding the factors which affect the rate and extent of embryo and endosperm development in all species alike. Consequently some species were chosen for the present study which varied with respect to those characters already known to have major effects on the rate of development in somatic and meiotic cells. For instance, related diploid, tetraploid, hexaploid, and octoploid genotypes

TABLE 1. THE WHEAT, RYE, *TRITICALE* AND BARLEY GENOTYPES USED IN THE PRESENT WORK

genotype	ploidy level	chromosome number	life cycle type	breeding system
wheat				
1. *Triticum monococcum* L.	2*x*	14	annual	inbreeding
2. *T. turgidum* ssp. *dicoccum*	4*x*	28		
3. *T. aestivum* ssp. *vulgare* var. Chinese Spring	6*x*	42		
4. *T. aestivum* ssp. *spelta*	6*x*	42		
rye				
5. *Secale cereale* L. var. Petkus Spring	2*x*	14	annual	outbreeding
triticale				
6. Hexaploid *Triticale* var. Rosner	6*x*	42	annual	inbreeding
7. Hexaploid *Triticale* var. Cinnamon				
8. Hexaploid *Triticale* line TCL 3				
9. Hexaploid *Triticale* line TCL 4				
10. Hexaploid *Triticale* line TCL 19				
11. Hexaploid *Triticale* line ITSN 30				
12. Octoploid *Triticale*	8*x*	56	annual	inbreeding
wheat-rye chromosome addition lines (Holdfast wheat disomic for the addition of a known King II rye chromosome)				
13. Rye addition I	6*x*+2	44	annual	inbreeding
14. Rye addition II				
15. Rye addition III				
16. Rye addition IV				
17. Rye addition V				
18. Rye addition VI				
19. Rye addition VII				
barleys				
20. *Hordeum vulgare* L. var. Sultan	2*x*	14	annual	inbreeding
21. *H. bulbosum* Nevski	2*x*	14	perennial	outbreeding
22. *H. bulbosum* Nevski	4*x*	28	perennial	outbreeding

were included so that the effects of polyploidy could be observed. Other species were chosen for investigation because their seed development is of known, or possible, practical significance in plant breeding. For instance *Triticale* lines were included so that endosperm development could be studied with reference to grain shrivelling, which is an important problem in *Triticale* breeding (Shealy & Simmonds 1973; Zillinsky 1974*b*). Similarly, because of the current interest in the process of elimination of chromosomes derived from *H. bulbosum* in young embryo cells of F_1 hybrids produced by crossing *H. vulgare* × *H. bulbosum*, these two species were included in the present work. This elimination process may have a practical use in plant breeding in making available large numbers of haploid barley plants (Subrahmanyam & Kasha 1973).

This paper describes the normal rate and pattern of embryo and endosperm development in the various genotypes studied. Detailed discussions of the various points of interest outlined above will be covered in later papers.

Materials and methods

(*a*) *Materials*

The 22 genotypes studied in the present work are listed in table 1. They include four wheat genotypes, rye, and fourteen genotypes containing a mixture of wheat and rye chromosomes. The wheat genotypes are members of an allopolyploid series. The diploid wheat species *Triticum monococcum* is thought to have been the donor of the A genome which is present in both the tetraploid *T. turgidum* ssp. *dicoccum*, and the hexaploids *T. aestivum* ssp. *vulgare* and ssp. *spelta* (Kihara 1924; McFadden & Sears 1946). The rye, *Secale cereale* L. var. 'Petkus Spring' is a commercial variety of German origin.

The 14 genotypes studied which contain wheat and rye chromosomes include six hexaploid and one octoploid *Triticale* lines, and seven wheat-rye chromosome addition lines. One of the six hexaploid *Triticale* lines, namely 'Rosner', is a commercially available variety of Canadian origin which was released in 1970 (Larter *et al.* 1970). The hexaploid *Triticale* breeder's line named 'Cinnamon' is of unknown parentage and was produced in Mexico by CIMMYT (Zillinsky 1974*a*). Three other hexaploid *Triticale* breeder's lines, TCL 3, 4 and 19, are also of unknown parentage. Originally produced by CIMMYT in Mexico, they were chosen by Mr J. Bingham in 1970, for study at the Plant Breeding Institute, Cambridge and subsequently selected for two generations in Cambridge by Mr R. Gregory. The remaining hexaploid *Triticale* line, ITSN 30 was from the third international *Triticale* screening nursery (1972) and was obtained from CIMMYT.

A primary hexaploid *Triticale* derived from the *T. turgidum* ssp. *durum* ($2n = 4x = 28$) × *S. cereale* ($2n = 2x = 14$), contains 28 wheat and 14 rye chromosomes. However, many *Triticale* lines with 42 chromosomes selected after crossing a primary *Triticale* with hexaploid bread-wheat have been shown to contain fewer than 14 rye chromosomes (Gregory 1974). The missing rye chromosomes have been substituted by wheat chromosomes from the D genome. For instance, both 'Rosner' and 'Cinnamon' are known to have lost a pair of rye chromosomes (T. E. Miller & V. Chapman personal communication), and the missing rye chromosome is 2R (Darvey & Gustafson 1975). The numbers of rye chromosomes in the lines, TCL 3, 4 and 19 and ITSN 30 are unknown, but may be less than 14.

The octoploid *Triticale* was made by Mr V. Chapman from the cross *T. aestivum* ssp. *vulgare* var. 'Chinese Spring' × *Secale cereale* var. 'Petkus Spring' at the Plant Breeding Institute,

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Cambridge. The amphidiploid was obtained by using the colchicine method described by Bell (1950).

The seven wheat-rye disomic chromosome addition lines all contain the 42 chromosomes of *T. aestivum* ssp. *vulgare* var. 'Holdfast' plus a different known pair of *Secale cereale* var. 'King II' chromosomes. The method by which these lines with 44 chromosomes were produced, and their characteristic appearance, has been described previously (Riley & Chapman 1958; Riley & Macer 1966).

Three barley genotypes were studied. *Hordeum vulgare* L. var. 'Sultan' is a two-rowed spring variety grown commercially in Great Britain. The diploid and tetraploid forms of *H. bulbosum* are both perennial species native in the Mediterranean (Katznelson & Zohary 1967). Material of the diploid form was kindly supplied by Dr W. Lange (Stichting voor Plantenveredeling, Wageningen), while material of the tetraploid form was supplied by Dr D. H. B. Sparrow of the Waite Agricultural Research Institute, Australia. Hybrids of diploid *H. vulgare* with diploid *H. bulbosum*, and of tetraploid *H. vulgare* with tetraploid *H. bulbosum*, selectively eliminate chromosomes derived from *H. bulbosum* in embryo cells, to produce a proportion of individuals containing only the haploid number of *H. vulgare* chromosomes (Barclay, Shepherd & Sparrow 1972).

(*b*) *Methods*

(i) *The cultivation of plants*

Plants of genotypes 1–19 (table 1) were grown in a glasshouse until development in leading tillers was about 1 week prior to meiosis in the anthers. They were then transferred to a growth room at 20 ± 1 °C and given continuous light. Genotypes 20–22 were grown in a glass-house and transferred to the same growth room environment at about 1 week prior to dehiscence in leading tillers.

(ii) *Treatment of spikes for developmental studies*

In each genotype at least 10 spikes were emasculated about 2–3 days before normal anther dehiscence. Several retarded spikelets at the base of the spike, and the entire top of the spike were excised and discarded, leaving about 8–18 emasculated florets on either side of the spike axis for developmental studies. In those genotypes whose spikelets contained more than two potentially fertile florets, the third and subsequent florets were removed, leaving only the primary and secondary florets for study. Emasculated spikes were enclosed in cellophane bags to prevent pollination. About 3–5 days after emasculation, when all the florets should have become receptive for pollination, stigmas were hand pollinated with pollen from a plant of the same species, or genotype, and the cellophane bag immediately replaced over the spike. Pollinated spikes were fixed in 1:3 acetic alcohol at known intervals of between 30 min and five days after pollination. Unless otherwise stated, two spikes from separate plants were fixed 1, 2, 3, 4 and 5 days after pollination in all genotypes studied.

(iii) *Cytological methods*

Each fixed spike was carefully dissected into its constituent spikelets and florets. The relative position of each floret was noted and the ovary was excised and placed in a numbered tube containing 1:3 acetic acid and ethyl alcohol. Thus, about 80 ovaries were obtained for study in each genotype, at each sampling time.

Ovaries were hydrolysed for 12 min in 1 M HCl at 60 °C and stained in leuco-basic fuchsin

for at least 2 h. The ovule was then dissected out from each ovary in a drop of Feulgen stain on a microscope slide under a dissecting microscope. A drop of aceto-carmine stain was applied to the ovule, and the remaining ovary tissue discarded. A small incision was made by using a scalpel at the chalazal end of ovules from spikes fixed 48 h or less after pollination. The ovular wall tissue was then pulled open partly revealing the embryo sac contents. After carefully applying a coverslip the ovule was tapped gently. This further exposed the embryo sac contents which were then examined under the microscope to see if fertilization had occurred, and if so, to see the extent of post-pollination development. The entire tissues of the developing embryo and endosperm were dissected out of fertilized ovules fixed more than 48 h after pollination. After discarding unwanted ovular wall tissue, these were further stained with either aceto-carmine or alcoholic carmine and mounted under a coverslip for cytological examination.

All observations were made from temporary slides, many of which were subsequently made permanent for reference purposes.

(iv) *Scoring of embryo sac and young seed characters*

(1) *The antipodal cells.* Antipodal cell number reaches a maximum several days prior to anther dehiscence and thereafter does not alter. The number of antipodal cells was counted in each floret fixed 48 or fewer hours after pollination, irrespective of whether or not fertilization had occurred. Occasionally an entire and undamaged embryo sac was obtained in which case the absolute number of antipodal cells was scored. More often the embryo sac was broken, in which case, several antipodal cells may have been lost. Nevertheless, the number of remaining antipodal cells was scored. A large number of florets (at least 100) was scored for each genotype. The resulting data should give accurate estimates of both the maximum and relative numbers of antipodal cells in the genotypes studied.

(2) *The egg, zygote and pro-embryo.* Whenever an unsquashed egg cell, zygote or pro-embryo was found, its maximum diameter (D) from the chalazal to the micropylar end, and its maximum diameter (d) in the plane at right-angles to this, was measured with a Vickers moving scale micrometer eyepiece. The maximum (D) and minimum (d) diameters of the nucleus and nucleolus in the egg cell; and of nuclei at prophase in pro-embryos, were also measured in suitable preparations. The volumes of individual embryos, cells, nuclei and nucleoli were estimated from the formula

$$\text{volume} = \frac{4}{3}\pi\left(\frac{D+d}{4}\right)^3,$$

which gives a good approximation for the volume of sub-spherical objects. The mean embryo volume per floret was calculated for spikes fixed at each sampling time after pollination.

Whenever possible the number of cells in each embryo was counted. Cells at all stages of mitosis were counted as one cell. The mean number of embryo cells per floret was calculated for spikes fixed at each sampling time.

(3) *The polar nuclei and endosperm.* In some genotypes the maximum (D) and minimum (d) diameters of the fused pair of polar nuclei, and of the largest nucleolus in both haploid nuclei, were measured and their volumes calculated as described above.

All or some of the following endosperm characters were scored or noted in each individual fertilized floret

(a) the number of endosperm nuclei or cells,

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(*b*) the number and location of nuclei or cells at mitosis,
(*c*) the degree of developmental synchrony between nuclei or cells,
(*d*) the number and distribution of aberrant mitoses or, interphase nuclei,
(*e*) the presence and distribution of cell walls,
(*f*) the presence and distribution of starch granules,
(*g*) the shape and general appearance of the endosperm,
(*h*) the position of the antipodal cells relative to the endosperm.

The mean number of endosperm nuclei or cells per fertilized floret in spikes fixed at each sampling time was calculated.

Results

(*a*) *The mature embryo sac prior to fertilization*

In all the 22 genotypes studied, embryo sac development is of the Polygonum type (Maheshwari 1950) and the pattern of its development is similar to that previously described for *Triticum aestivum* (Bennett *et al.* 1973). The mature embryo sac contained an egg cell, two synergid cells, two fused polar nuclei, and a variable number of antipodal cells whose modal number was characteristic of the species or genotype. The appearance of these various cell types was similar to that previously described (Morrison 1955; Bennett *et al.* 1973; Cass & Jensen 1970).

The egg apparatus, consisting of two synergids and a single egg cell, was located at the extreme micropylar end of the embryo sac. All three cells were pyriform in shape, with their smaller ends extending towards the micropyle. The egg cell, but neither of the synergids, characteristically contained a large number of highly refractile starch granules clustered around the nucleus (figure 1*c*, plate 1). The volume of the egg cell was considerably larger than that of the two approximately equal sized synergid cells. The egg nucleus, which contained a conspicuous usually vacuolate nucleolus, was located centrally in the swollen chalazal part of the cell. The chromatin in the egg cell nucleus was organized in an early prophase-like condition. This is particularly well seen in figure 1*a*, which shows an unfertilized egg cell nucleus of *Hordeum vulgare* in which the haploid number of chromosome threads (7) can be seen radiating away from the nucleolus. By comparison, the chromatin of the synergid nuclei had a more characteristically interphase-like appearance.

The two closely appressed haploid polar nuclei lay just below the egg cell at the micropylar end of the embryo sac but further from the micropyle than the egg apparatus (figures 1*a*, *b*, plate 1). Each polar nucleus was a hemisphere, and the plane of juxtaposition between the two nuclei was usually parallel to a line passing through the micropyle and thence to the chalazal end of the embryo sac. The volume of the polar nuclei was much greater than that of any other haploid cell nucleus in the embryo sac, indeed, the combined volumes of the two polar nuclei approached that of the entire egg cell. For instance, in *T. aestivum* (table 2) the volume of the polar nuclei (161389 μm^3) was about 80% of the volume of the mature unfertilized egg cell (204841 μm^3). The chromatin in the polar nuclei, like that of the egg cell nucleus, was organized in an early prophase-like condition prior to pollination. The largest nucleolus in each polar nucleus was extremely large compared with the nucleolus in the egg cell (compare figures 1*c* and *d*), for instance, in *T. aestivum* it was about nine times as large (table 2). The cell containing the polar nuclei (the central cell) was extremely large, and much bigger than the egg cell. The major volume of the central cell

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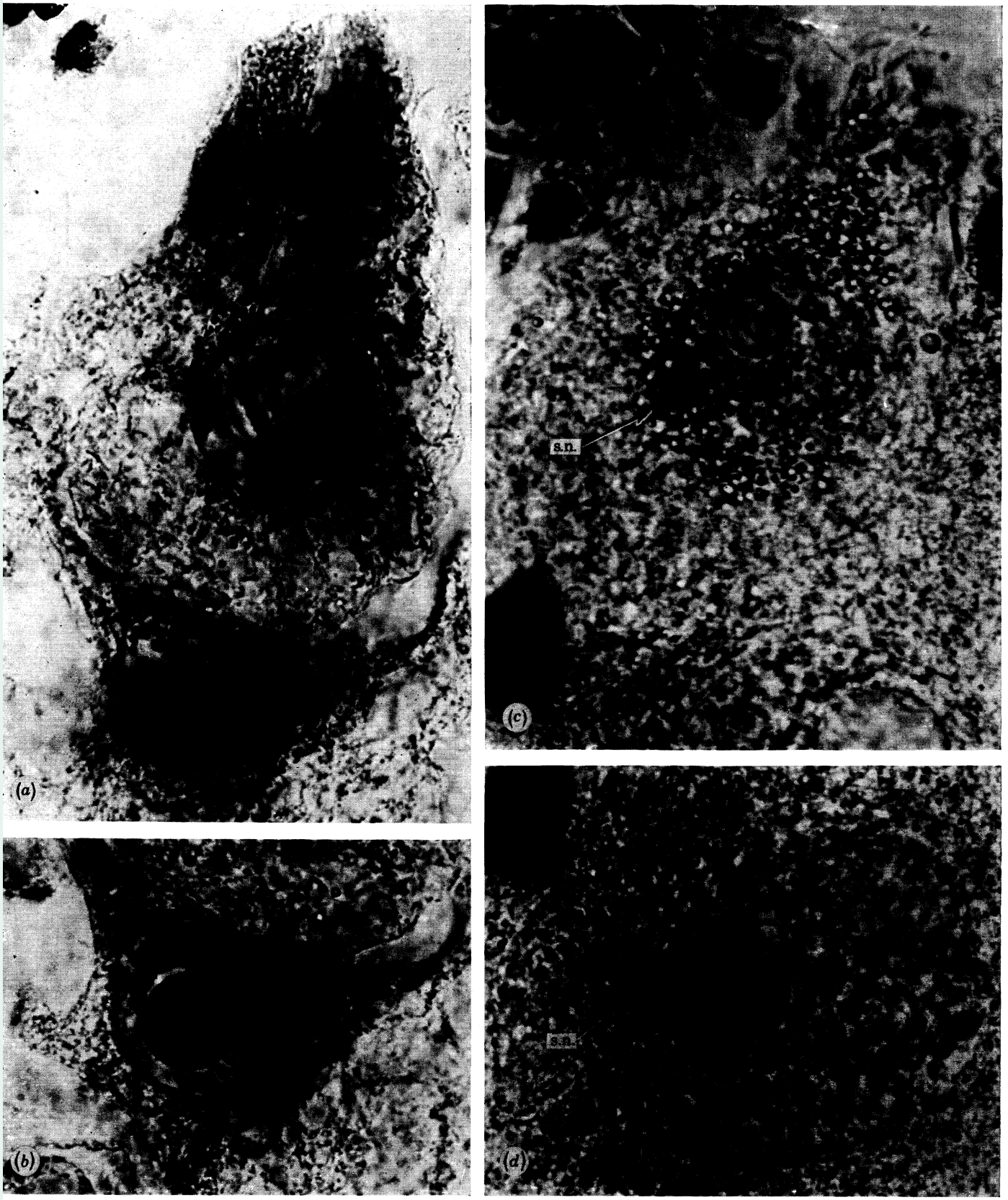

FIGURE 1 (*a*). The mature unfertilized egg apparatus and polar nucleus of *H. vulgare* cv. 'Sultan'; (*b*) an optical section showing the nucleolus of the polar nucleus out of focus in (*a*). Note the highly vacuolate egg cell cytoplasm and the highly condensed chromosome threads in both the egg cell nucleus and polar nuclei: (*c*, *d*) Sperm nuclei (s.n.) appressed to the egg cell nucleus (*c*) and the polar nuclei (*d*) in a floret of hexaploid *Triticale* cv. 'Rosner' fixed 40 min after pollination. Note the relative sizes of the nucleoli in the egg cell nuclei (*a*, *c*) and their corresponding polar nuclei (*b*, *d*). (All magn. 1000.)

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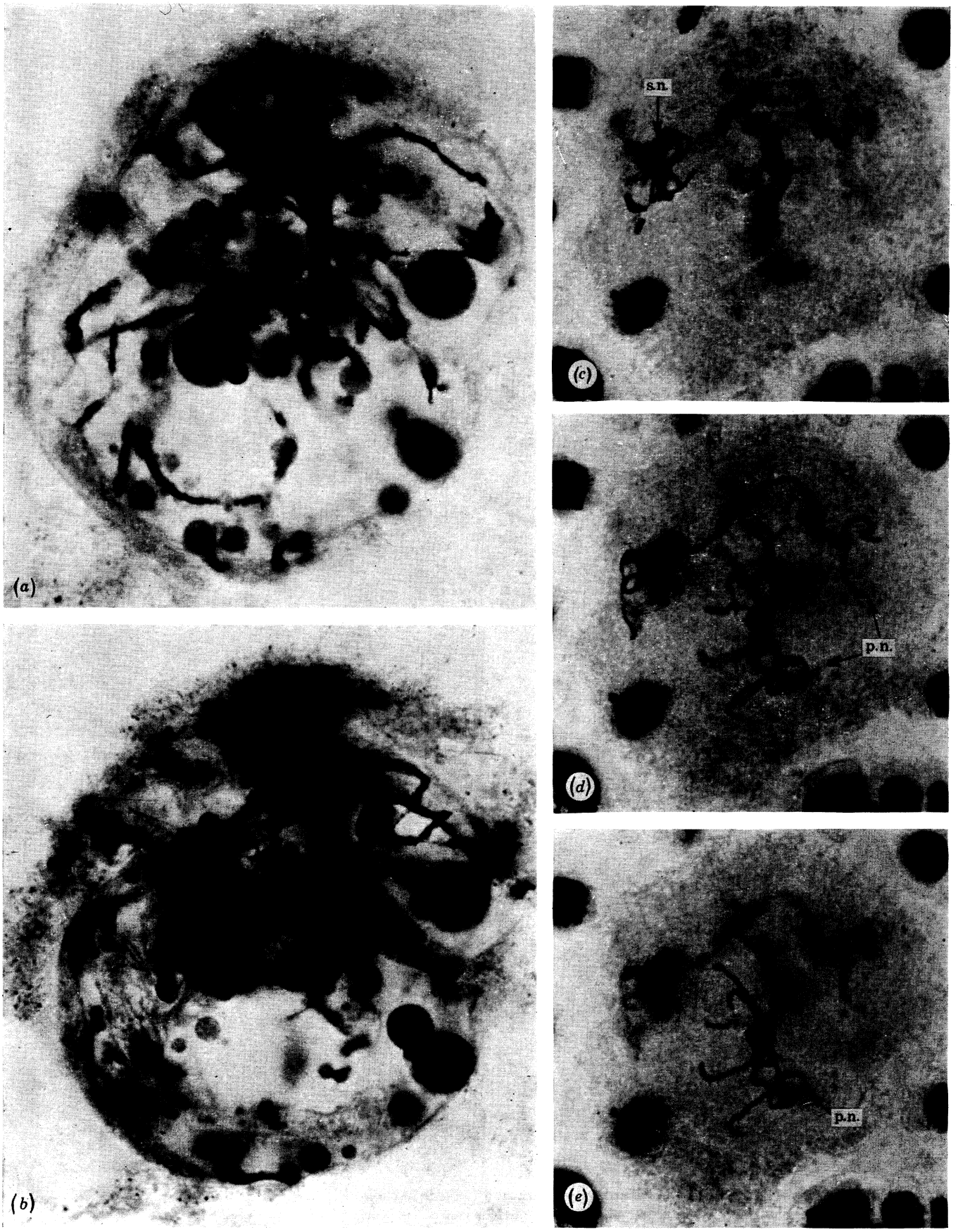

FIGURE 2. Primary endosperm nuclei of *Secale cereale* cv. 'Petkus Spring' fixed 6 h after pollination. (*a*, *b*) Optical sections through an aceto-orcein stained primary endosperm nucleus at early prophase showing condensing chromosomes and numerous micronucleoli (× 1250); (*c*–*e*) optical sections through a Feulgen stained primary endosperm nucleus at mid-prophase showing separate groups of chromosomes derived from the sperm nucleus (s.n.) and the two polar nuclei (p.n.) (× 1375).

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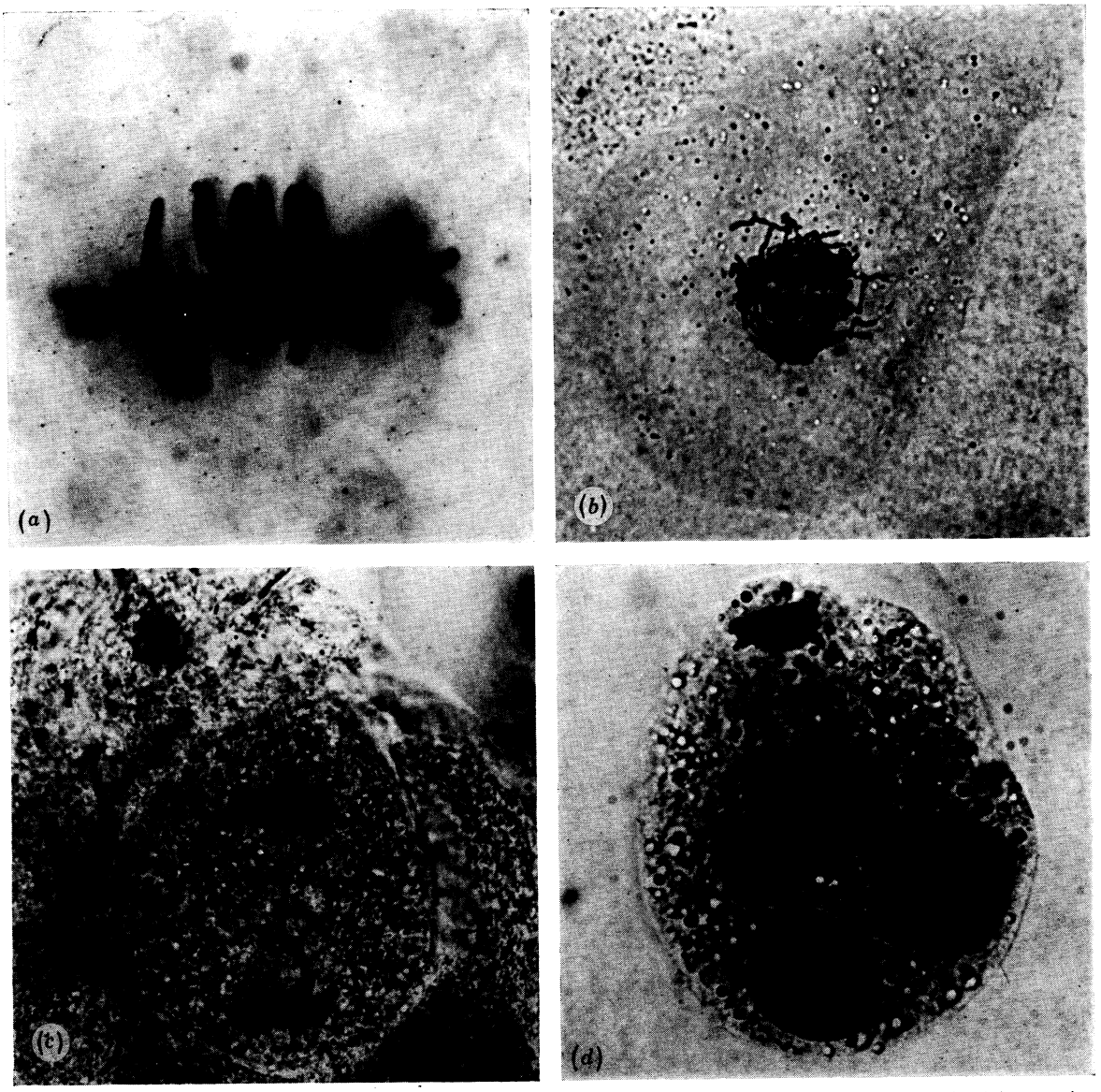

FIGURE 3. (*a*) Feulgen stained primary endosperm nucleus at metaphase in *S. cereale* cv. 'Petkus Spring' (× 2000); (*b*, *c*) Feulgen stained zygotes at mitosis: (*b*) prophase, in *S. cereale* cv. 'Petkus Spring' (× 1250), and (*c*) telophase in wheat-rye addition IV (× 850); (*d*) Feulgen stained 4-celled proembryo from hexaploid *Triticale* cv. 'Rosner' (× 1000).

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was occupied by a large vacuole and cytoplasm was confined to a continuous layer closely appressed to its walls bounded by the egg apparatus at its micropylar end, the antipodal cells at its chalazal end, and the embryo sac wall in between. According to Cass & Jensen (1970) in *Hordeum vulgare* var. 'Atsel', the polar nuclei are usually embedded in a central thick strand of cytoplasm extending through the vacuole from the antipodal cells to the end of the egg apparatus. While this was not always clearly distinguished in squashes, nevertheless, the appearance of the central cell was compatible with this arrangement in all of the genotypes studied.

TABLE 2. THE MEAN VOLUME (μm^3) OF VARIOUS CELLS, NUCLEI AND NUCLEOLI IN THE MATURE EMBRYO SAC OF 4 GENOTYPES

organ or organelle	genotype: *Hordeum vulgare* var. 'Sultan'	'Chinese Spring' 6*x* wheat	6*x* wheat + rye Addn V	octoploid *Triticale*
polar nuclei (fused pair)	—	161 389	347 780	122 031
largest single nucleolus in a polar nucleus	—	25 589	8 097	6 849
unfertilized egg cell	115 030	204 841	238 551	248 642
nucleus of egg cell	7 349	23 599	20 929	15 697
nucleolus in egg cell	621	2 575	2 588	1 577
2-celled embryo (24 h after pollination)	134 980	201 173	241 714	—

TABLE 3. THE MAXIMUM NUMBER OF ANTIPODAL CELLS SEEN IN A SINGLE FLORET, AND THE MEAN NUMBER OF ANTIPODAL CELLS PER FLORET IN SPIKES OF 22 GENOTYPES FIXED 24 h AFTER POLLINATION, COMPARED WITH OTHER RESULTS TAKEN FROM THE LITERATURE

genotype	the present work: maximum	the present work: mean	other workers: maximum	other workers: mean	reference
1. *Triticum monococcum*	28	20	26	15	Moss (1972)
2. *T. turgidum* ssp. dicoccum	25	18	28	12	
3. *T. aestivum* ssp. vulgare	25	15.7	21	12	
4. *T. aestivum* ssp. spelta	26	17	—	—	
5. *Secale cereale*	16	12	20	8.4	
6. hexaploid *Triticale* var. Rosner	17	10.7	—	11.1	Kaltsikes (1973)
7. hexaploid *Triticale* var. Cinnamon	17	11.4			
8. hexaploid *Triticale* line TCL 3	21	13.8			
9. hexaploid *Triticale* line TCL 4	21	13.7			
10. hexaploid *Triticale* line TCL 19	18	11.4			
11. hexaploid *Triticale* line ITSN 30	20	8.5			
12. octoploid *Triticale*	16	10.9			
13. disomic addition of rye chromosome I	20	11.4			
14. disomic addition of rye chromosome II	19	11.0			
15. disomic addition of rye chromosome III	27	19.8			
16. disomic addition of rye chromosome IV	19	13.6			
17. disomic addition of rye chromosome V	21	12.5			
18. disomic addition of rye chromosome VI	12	8.9			
19. disomic addition of rye chromosome VII	17	8.2			
20. *Hordeum vulgare*	50	25.7	*ca.* 100		Cass & Jensen (1970)
21. *H. bulbosum* (diploid)	33	20.1			
22. *H. bulbosum* (tetraploid)	44	23.4			

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The antipodal cells were located at the chalazal end of the embryo sac and together usually occupied more than 50 % of its volume. Table 3 gives the maximum and mean number of antipodal cells per floret in spikes of each genotype fixed 24 h after pollination. The maximum number of antipodal cells ranged from 50 in *H. vulgare* to 12 in wheat-rye chromosome VI addition line. The number of antipodal cells varied between florets within each genotype. However, the modal number for each species was characteristic. For instance, *S. cereale* had a lower mean number of antipodal cells per floret (about 12) in each spike studied, than did the four wheat species (about 15–20), which in turn had fewer antipodal cells than the three barley species (about 20–26). The antipodal cells were highly endopolyploid and the degree of endopolyploidy varied between cells within each embryo sac in all of the genotypes studied. The chromosomes of King II rye as addition chromosomes to Holdfast wheat differed greatly in their effects on antipodal cell number (table 3). In the seven rye chromosome addition lines the mean number of antipodal cells per floret varied from 8.2 in addition VII to 19.8 in addition III. Thus, the mean antipodal cell number per floret ranged from a higher value than the mean for hexaploid wheat (17) to lower than the mean for diploid rye (12).

(*b*) *Fertilization and mitosis in the zygote and primary endosperm nucleus*

Pollen tube growth starts almost at once after the arrival of a viable pollen grain on a receptive stigma in barley (Pope 1937), rye, *Triticale* and wheat (D'Souza 1970; Meyer 1971; Zeven & Heemert 1970; Hoshikawa 1959). In the present work fertilization occurred very soon after pollination, since endosperms containing at least 16 nuclei were found in florets fixed 24 h after pollination in all the genotypes studied.

Detailed studies of events occurring in the embryo sac during the first 24 hours after pollination in *T. aestivum* var. 'Chinese Spring' were published previously (Bennett *et al.* 1973). In the present work similar observations were made only in *H. vulgare*, *Secale cereale*, hexaploid *Triticale* var. 'Rosner', and octoploid *Triticale*. The present experiments produced results very similar to those already noted in Chinese Spring. The time taken for each developmental stage varied between florets, consequently, only the fastest times for each stage are described below.

(i) Hordeum vulgare

In *H. vulgare* sperm nuclei were observed in the embryo sac 40 min after pollination. At 6 and 7 h after pollination primary endosperm nuclei were observed at prophase and anaphase respectively. At 10.5 h after pollination florets contained two interphase endosperm nuclei. Thus, mitosis in the primary endosperm nuclei was complete at about 7 h after pollination. Sperm nuclei were found in close association with the egg cell nucleus in florets fixed at 6, 7 and 10.5 h after pollination. The sperm nucleus chromatin became less dense at increasingly later times after pollination, and by 10.5 h was visibly composed of threads. At 17 h after pollination an egg cell at mid-prophase was seen, and at 22 h after pollination zygotes were regularly found at metaphase to telophase stages of mitosis. By 24 h after pollination most florets contained 2-celled proembryos. Thus, mitosis in the zygote occurred at about 23 h after pollination. These observations are in close agreement with those made previously for *Hordeum* by Pope (1937).

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(ii) Secale cereale

In *S. cereale* sperm nuclei were found associated with the egg and polar nuclei only 40 min after pollination. In spikes fixed 6 h after pollination the primary endosperm nuclei were observed at all stages of mitosis (figures 2*c–e* and figures 3*a*, *b*, plates 2 and 3). The group of chromosomes derived from the sperm nucleus were distinct both in their position, and in being more densely contracted during early prophase (figures 2*a–b*). Only at late metaphases did they become indistinguishable from those derived from the polar nuclei (figure 3*a*). At early to mid-prophase the two groups of chromosomes derived from either of the two polar nuclei could also often be distinguished (figures 2*c–e*).

At 24 h after pollination rye zygotes were still at prophase. However, mitosis was first completed at about 26 h after pollination.

(iii) Triticale '*Rosner*'

In hexaploid *Triticale* var. 'Rosner' sperm nuclei were found in both egg and central cells in florets fixed 40 min after pollination (figures 1*c*, *d*). The primary endosperm nucleus was at very early prophase in florets fixed 6 h after pollination, and mitosis must have occurred soon after, since florets fixed at 18 h after pollination were found which contained 8 interphase endosperm nuclei. No zygotes at mitosis were found in florets fixed at this time, however, some florets fixed 6 h later had already completed zygote mitosis and contained 2-celled proembryos. These results show a rate of development similar to, though faster than, that previously noted in hexaploid *Triticale* genotypes including 'Rosner' (Kaltsikes 1973).

(iv) *Octoploid* Triticale

In octoploid *Triticale* no sperm nuclei were found in embryo sacs fixed 40 min after pollination, but 4 out of 18 florets fixed 1 h after pollination contained sperm nuclei in their embryo sacs. Although numerous florets were examined, mitosis was not observed in either the primary endosperm nucleus or the zygote of octoploid *Triticale*. Mitosis in the primary endosperm nucleus must have occurred at about 6–8 h after pollination, since a floret fixed 24 h after pollination contained at least 16 endosperm nuclei. Similarly, mitosis in the zygote must have occurred at about 24 h after pollination, since an embryo consisting of 6 cells was found in a floret fixed 48 h after pollination. A very low level of fertility is characteristic of this genotype. Numerous florets containing aberrant embryo sac development were found including several with three polar nuclei instead of the normal two. Many ovules had only parenchyma cells in their embryo sacs.

(v) *Time of mitosis in the zygote*

As table 4 shows, 2-celled embryos were observed in 16 out of the 22 genotypes studied in florets fixed 24 h after pollination. No 2-celled embryos were observed in any genotype in florets fixed 18 or less hours post pollination.

From the evidence available for 'Chinese Spring' wheat, hexaploid *Triticale* cv. 'Rosner' and *H. vulgare*, it may reasonably be assumed that in all those species which had 2-celled embryos at 24 h after pollination, mitosis in the zygote was first completed at about 22 h after pollination. This division certainly first occurred in the remaining six genotypes by 30 h after pollination. This conclusion is based upon the observation in all genotypes, of

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embryos consisting of 4 or more cells in florets fixed at 48 h after pollination, coupled with a knowledge of the cell cycle times in embryo cells. Moreover, in diploid *Hordeum bulbosum*, mitosis in the zygote was observed to be first completed in florets fixed 30 h after pollination.

TABLE 4. THE MAXIMUM (MAX.) AND MEAN NUMBER OF EMBRYO CELLS PER FLORET IN SPIKES OF VARIOUS GENOTYPES FIXED 1 TO 5 DAYS AFTER POLLINATION

	time after pollination/h									
	24		48		72		96		120	
genotype	max.	mean	max.	mean	max.	mean	max.	mean	max.	mean
1. *Triticum monococcum*	2	1.2	4	4.0	16	15	43	38	135	121
2. *T. turgidum* ssp. dicoccum	2	1.15	8	5.4	33	28	70	70	156	126
3. *T. aestivum* ssp. vulgare	2	1.93	10	8.3	48	40	—	—	—	—
4. *T. aestivum* ssp. spelta	2	1.58	8	6.0	45	31	58	53	185	139
5. *Secale cereale*	1	1.0	4	3.4	10	7.4	38	26	92	55
6. hexaploid *Triticale* var. Rosner	2	2.0	4	3.7	15	9.3	70	55	153	155
7. hexaploid *Triticale* var. Cinnamon	2	1.11	6	4.7	32	28.4	58	45	84	72
8. hexaploid *Triticale* line TCL 3	1	1.0	4	3.4	18	14.5	36	32	62	50
9. hexaploid *Triticale* line TCL 4	2	1.13	4	4.0	19	13.6	69	49	104	67
10. hexaploid *Triticale* line TCL 19	2	1.02	6	4.2	17	13.9	65	44	75	64
11. hexaploid *Triticale* line ITSN 30	1	1.0	4	3.5	14	10.9	30	20	50	32
12. octoploid *Triticale*	1	1.0	8	5.0	31	20.3	—	—	140	106
13. disomic addition of rye chromosome I	1	1.0	5	3.7	18	16.8	—	—	—	—
14. disomic addition of rye chromosome II	2	1.06	8	5.3	24	19.1	—	—	—	—
15. disomic addition of rye chromosome III	2	1.75	8	7.8	28	25.7	—	—	—	—
16. disomic addition of rye chromosome IV	2	1.75	10	6.9	21	17.6	—	—	—	—
17. disomic addition of rye chromosome V	2	1.13	4	3.9	17	14.5	—	—	—	—
18. disomic addition of rye chromosome VI	1	1.0	7	4.8	18	16.0	—	—	—	—
19. disomic addition of rye chromosome VII	1	1.0	6	4.9	19	18.0	—	—	—	—
20. *Hordeum vulgare*	2	1.3	10	9.5	25	23.4	92	73	540	454
21. *H. bulbosum* (diploid)	1	1.0	4	3.0	17	10.3	54	33	70	53
22. *H. bulbosum* (tetraploid)	2	1.75	8	6.9	24	17.6	73	55	259	227

(vi) *Rate of development in different florets*

In nature the pollination of florets on a single spike is asynchronous and follows the sequence of stigma receptivity or anther dehiscence. The present technique allows receptive stigmas to accumulate, thus synchronizing florets in development. The florets on a spike were all pollinated at the same time. While the interval between pollination and the completion of zygote mitosis varied somewhat between florets on the same spike, the degree of synchrony in development was frequently quite pronounced. For instance, in *T. aestivum* var. 'Chinese Spring' where zygote mitosis is not completed in any floret until 22 h after pollination, 78 % of florets already contained 2-celled proembryos in spikes fixed 24 h after pollination. Mitosis in the primary endosperm nucleus occurred with a similar degree of synchrony between florets. Thus, in *Secale cereale* florets fixed 6 h after pollination, the primary endosperm nucleus was located in 6 florets out of 12 on one spike, and mitosis was observed in 5 of them. Further evidence of synchronous endosperm development in different florets on the same spike comes from the observation that in 16 fertilized florets of *T. aestivum* ssp. *spelta* fixed 24 h after

pollination, only one contained 8 endosperm nuclei, 2 contained 8 mitotic figures, while the remaining 13 all contained 16 endosperm nuclei. Such synchrony was typical of all the genotypes studied.

(c) *Embryo development*

(i) *Embryo cell number and cycle time*

Table 4 gives the maximum number of embryo cells observed in a single floret, and the mean number of embryo cells per floret in spikes of each genotype fixed at between 1 and 5 days after pollination. The table shows a steady increase in embryo cell number at each sampling time after pollination (figure 4). There were, however, large differences between

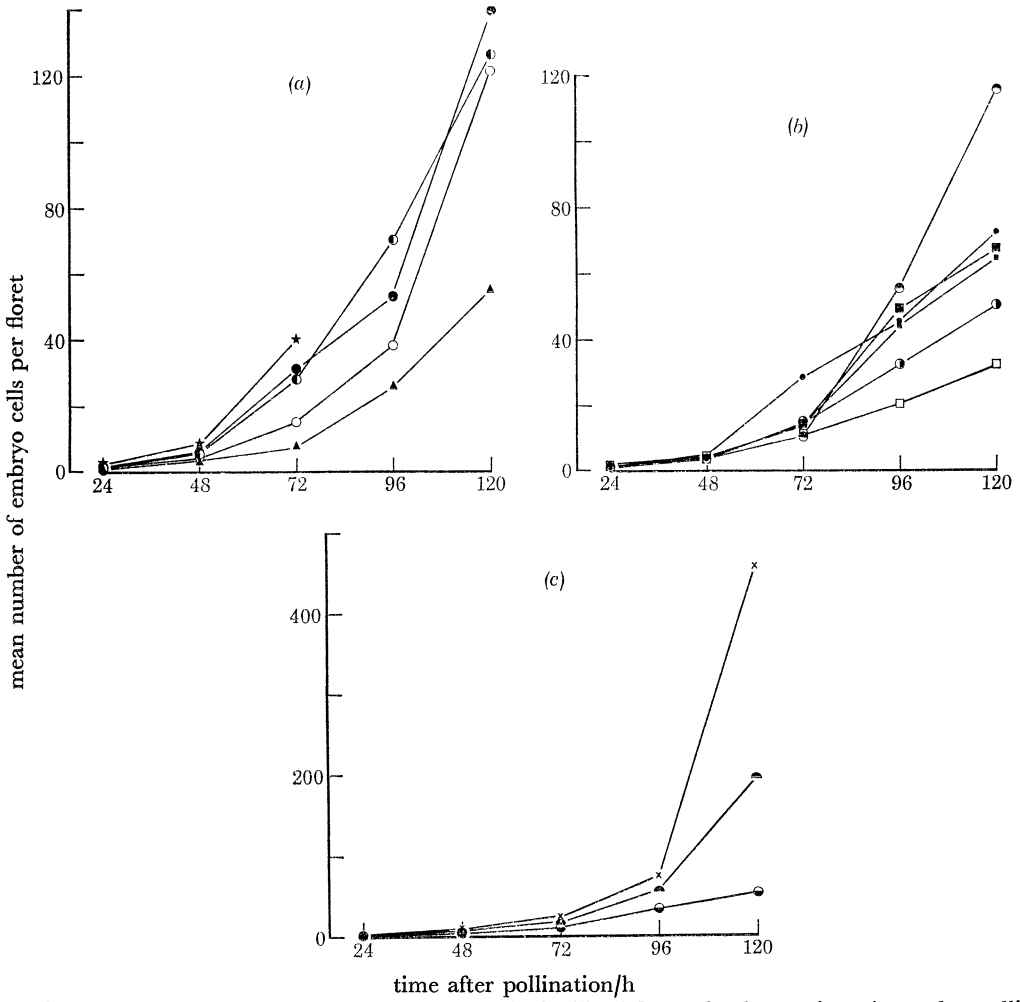

FIGURE 4. The mean numbers of embryo cells per floret in fertilized florets fixed at various times after pollination (*a*) in four wheat genotypes and rye, (*b*) in 6 hexaploid *Triticale* lines, and (*c*) in the three barley genotypes. *Key*: (*a*) ★, *T. aestivum* cv. Chinese Spring; ●, *T. aestivum* ssp. *spelta*; ◐, *T. dicoccum*; ○, *T. monococcum*; ▲, *Secale cereale*. (*b*) ◓, Rosner; ●, Cinnamon; ■, TCL 4; ▪, TCL 19; ◑, TCL 3; □, ITSN 30. (*c*) ×, *Hordeum vulgare*; ◓, tetraploid *H. bulbosum*; ◒, diploid *H. bulbosum*.

genotypes in the rate of increase in embryo cell number, and hence, in the mean cell cycle time in embryo cells. For example, the maximum number of embryo cells seen in a single floret ranged from 10 (in *S. cereale*) to 45 (in *T. aestivum* ssp. *spelta*) in spikes fixed 72 h after pollination, and from 50 (in *Triticale* ITSN 30) to 540 (in *Hordeum vulgare*) in spikes fixed 120 h after pollination.

In the wheat species the maximum rate of embryo cell development increased with increasing ploidy level. Thus, in spikes fixed 72 h after pollination, the maximum number of embryo cells seen in a single floret of diploid *T. monococcum*, tetraploid *T. turgidum* ssp. *dicoccum*, and hexaploid *T. aestivum* ssp. *vulgare* was 16, 33 and 48 respectively. This ranking was maintained in spikes fixed 120 h after pollination when diploid, tetraploid and hexaploid wheats contained a maximum of 135, 156 and 185 embryo cells per floret respectively. Similarly, in *Hordeum bulbosum* the diploid form contained fewer embryo cells per floret than did the tetraploid form (figure 4*c*) at each sampling time.

Secale cereale had a very slow rate of embryo cell development compared with the four wheat genotypes studied (figure 4*a*). Thus, at 120 h after pollination the mean number of embryo cells per floret in rye (55) was less than half of that found in *T. monococcum* (121), the wheat species with the lowest embryo cell number at this sampling time. A comparison of results for the six hexaploid *Triticale* lines with those for wheat and rye shows that in spikes fixed 120 h after pollination, with the exception of 'Rosner', the *Triticale* lines all had greatly reduced embryo development compared with any wheat species (compare figures 4*a*, *b*). Indeed, some lines had even slower rates of embryo development than rye, the parent species with the slower rate of development. For instance the mean number of embryo cells per floret in ITSN 30 was 32 compared with 55 in rye. In this hexaploid *Triticale* the amount of embryo cell development was less than one third of that found in any wheat species. However, the mean number of embryo cells per floret found in 'Rosner' (115) closely approached that found in the wheat species (121–139). The variation in rates of embryo cell development between the six hexaploid *Triticale* lines was greater than that found between wheat and rye species. Apparently the presence of rye chromosomes, or their interaction with wheat chromosomes, in hexaploid *Triticale* usually has a marked effect in slowing down the rate of early embryo development.

Further evidence supporting this view is provided by the results for wheat-rye chromosome addition lines (table 4). Individual King II rye chromosomes, when added to a constant wheat background, had widely different effects on the rate of embryo cell development. At 72 h after pollination the mean number of embryo cells per floret ranged from 14.5 (addition V) to 25.7 (addition III). At this sampling time, the mean number of embryo cells per floret in all seven wheat-rye chromosome addition lines was lower than in hexaploid wheat (31–40), but higher than in diploid rye (7.4). With the exception of chromosome III, every other rye chromosome had a considerable effect in decreasing the rate of embryo cell development, but, no single pair of rye chromosomes reduced the rate to that found in diploid rye.

As the time of mitosis in the zygote is known, and all embryo cells complete mitotic cycles at about the same rate, then the cell cycle time in embryo cells can be roughly estimated for each genotype using the data in table 1. Thus, during development starting at mitosis in the zygote and ending 120 h after pollination, the mean embryo cell cycle time ranged from about 11 h in *Hordeum vulgare* to about 19 h in hexaploid *Triticale* ITSN 30. During this period the mean cell cycle time in embryo cells was about 12.5 h in the three wheat species and about 16 h in rye and diploid *Hordeum bulbosum*. The mean cell cycle time in embryo cells was strikingly shorter in diploid *H. vulgare* (about 11 h) than in diploid *H. bulbosum* (about 16 h). For the corresponding period of development the minimum embryo cell cycle times in embryo cells (calculated from the data for maximum number of embryo

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cells observed in a single floret fixed at 120 h after pollination (table 4)) gives values of about 9.8 h for *H. vulgare*, 12.3 h for 'Rosner' *Triticale* and *Triticum aestivum*, and 14 h for *Secale cereale*.

(ii) *Embryo volume*

The volume of the zygote or 2-celled proembryo, in florets fixed 24 h after pollination, varied widely between the 22 genotypes studied (table 5). It ranged from about 51 000 μm^3 in *S. cereale* to about 289 000 μm^3 in wheat-rye chromosome addition line VII. In related diploid and polyploid species, embryo volume increased with increasing ploidy level (figure 5) and hence, 4*C* DNA amount. For instance, in diploid, tetraploid and hexaploid wheats the mean embryo volume was 85, 144 and 200×10^3 μm^3, respectively (table 5); similarly in diploid and tetraploid forms of *H. bulbosum* it was 107 and 197×10^3 μm^3 respectively.

TABLE 5. THE MEAN TOTAL EMBRYO VOLUME (Σv), AND THE MEAN VOLUME PER EMBRYO CELL (m.c.v) IN FLORETS FROM SPIKES OF 22 GENOTYPES FIXED AT VARIOUS TIMES AFTER POLLINATION (ALL FIGURES ARE $\times 10^2$ μm^3)

	time after pollination/h									
	24		48		72		96		120	
genotype	Σv	m.c.v	Σv	m.c.v	Σv	m.c.v	Σv	m.c.v	Σv	m.c.v
1. *Triticum monococcum*	852	710	1424	356	1024	68.2	2878	75.7	6016	49.7
2. *T. turgidum* ssp. dicoccum	1442	1254	1837	840	6508	232.4	5235	74.8	20588	163.4
3. *T. aestivum* ssp. vulgare	2012	1042	4034	487	6522	162.0	—	—	—	—
4. *T. aestivum* ssp. spelta	2030	1285	2684	443	6446	207.9	7152	134.9	17937	128.9
5. *Secale cereale*	512	512	900	264	883	119.0	2478	96.9	3729	67.8
6. hexaploid *Triticale* var. Rosner	1678	1043	2141	585	2308	247.4	7445	135.2	20682	179.7
7. hexaploid *Triticale* var. Cinnamon	2529	2279	2476	526	3311	218.9	8365	184.9	12631	174.5
8. hexaploid *Triticale* line TCL 3	1672	1672	2462	716	4456	307.7	5616	176.9	7666	153.8
9. hexaploid *Triticale* line TCL 4	2706	2394	3443	861	3422	265.3	8507	178.6	16645	248.0
10. hexaploid *Triticale* line TCL 19	2135	2093	2657	637	3610	259.3	7014	160.9	7993	123.9
11. hexaploid *Triticale* line ITSN 30	2048	2048	2653	758	3480	319.3	5551	280.3	6018	189.8
12. octoploid *Triticale*	2486	2486	4486	897	8171	402.5	—	—	21050	202.9
13. disomic addition of rye chromosome I	2336	2336	—	—	3999	238.6	—	—	—	—
14. disomic addition of rye chromosome II	2670	2547	2147	410	2966	155.0	—	—	—	—
15. disomic addition of rye chromosome III	2217	1267	2781	359	4458	173.4	—	—	—	—
16. disomic addition of rye chromosome IV	2606	1489	2866	415	4975	283.2	—	—	—	—
17. disomic addition of rye chromosome V	2417	2139	—	—	2424	167.2	—	—	—	—
18. disomic addition of rye chromosome VI	1935	1935	3094	1485	4189	261.3	—	—	—	—
19. disomic addition of rye chromosome VII	2892	2892	2686	548	5959	331.1	—	—	—	—
20. *Hordeum vulgare*	1266	938	1405	148	2357	100.7	7158	98.3	63110	139.0
21. *H. bulbosum* (diploid)	1078	1078	1440	480	2284	221.7	4450	137.2	5898	111.7
22. *H. bulbosum* (tetraploid)	1979	1131	1689	245	3546	201.5	5515	100.8	—	—

N.B. At 24 h after pollination total embryo volume is taken as the mean volume unfertilized egg cells, zygotes, and 2-celled proembryos. Thereafter only multicellular proembryos were included.

The general pattern of variation in embryo volume in the 22 genotypes studied may be summarized as follows. (A few individual results differ from this general picture. These are probably caused by the squashing of a few embryos which resulted in overestimating their volumes). Embryo volume increased steadily throughout the period starting at mitosis in the zygote and ending 5 days after pollination (figure 6). The percentage increase in embryo

volume per day was low at first, but increased later. For instance, the mean increase in total embryo volume for the six hexaploid *Triticale* lines on the 2nd, 3rd, 4th and 5th days of development was about 24, 30, 135 and 68 % respectively. Similarly in *H. vulgare*, the corresponding increases in mean embryo volume were about 10, 66, 202 and 890 % respectively. Comparing these two sets of data shows that although embryo volume increased steadily in

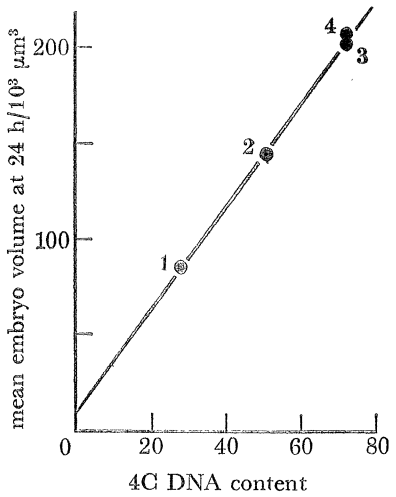

FIGURE 5. The relation between the species 4*C* DNA amount and the mean embryo volume per floret in spikes fixed 24 h after pollination. As DNA amount in diploid, tetraploid and hexaploid wheats have approximately the ratio 1:2:3 the figure also shows the relation between ploidy level and embryo volume.
Key: 1, *Triticum monococcum*; 2, *T. dicoccum*; 3, *T. aestivum* ssp. *vulgare*; 4, *T. aestivum* ssp. *spelta*.

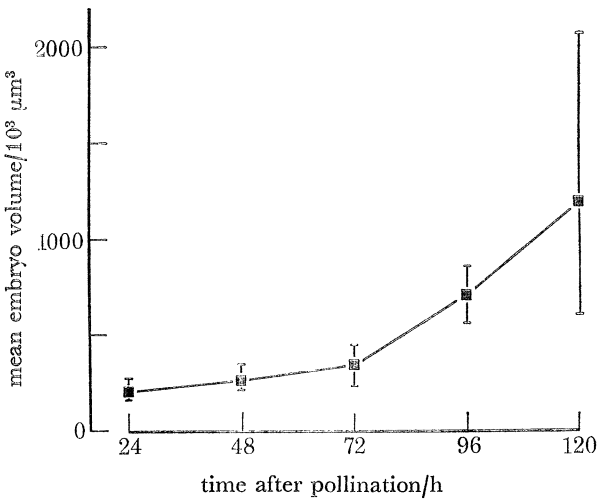

FIGURE 6. The mean and range of mean embryo volumes per floret in spikes of 6 hexaploid *Triticale* lines fixed at various times after pollination. The mean is indicated by a solid square and the range by a vertical bar.

both species, nevertheless the rate of change in total embryo volume was different in the two genotypes. Another example of this is seen from a comparison of two hexaploid *Triticale* lines. The mean embryo volume 24 h after pollination in both Rosner and TCL 3 was about 167×10^3 μm³, however, 4 days later, the mean total embryo volume in Rosner (2068×10^3 μm³) was about three times that of TCL 3 (766×10^3 μm³).

The rate of increase in the mean number of embryo cells per floret was higher during early embryo development than the rate of increase of the mean total embryo volume.

Consequently, the mean volume per cell of embryo cells was progressively decreased (figure 7). Eventually, the rate of increase in total embryo volume became directly proportional to the rate of increase in mean embryo cell number, so that the mean volume of embryo cells remained constant. Figure 7*b* shows a plot for the mean behaviour of the six hexaploid *Triticale* lines. Mean embryo cell volume decreased to one tenth during the 48 h ending at 72 h after pollination, however, during the next 48 h it remained fairly constant. The duration of the phase of decreasing mean embryo cell volume varied between genotypes. In *H. vulgare* this phase was already complete by 48 h after pollination (figure 7*c*). The duration of the phase of decreasing volume in embryo cells is determined by the rate of cell division in the embryo and the total embryo volume, and may reflect the time taken to sub-divide the latter into cells whose mean volume is equivalent to that of cells in other somatic meristems.

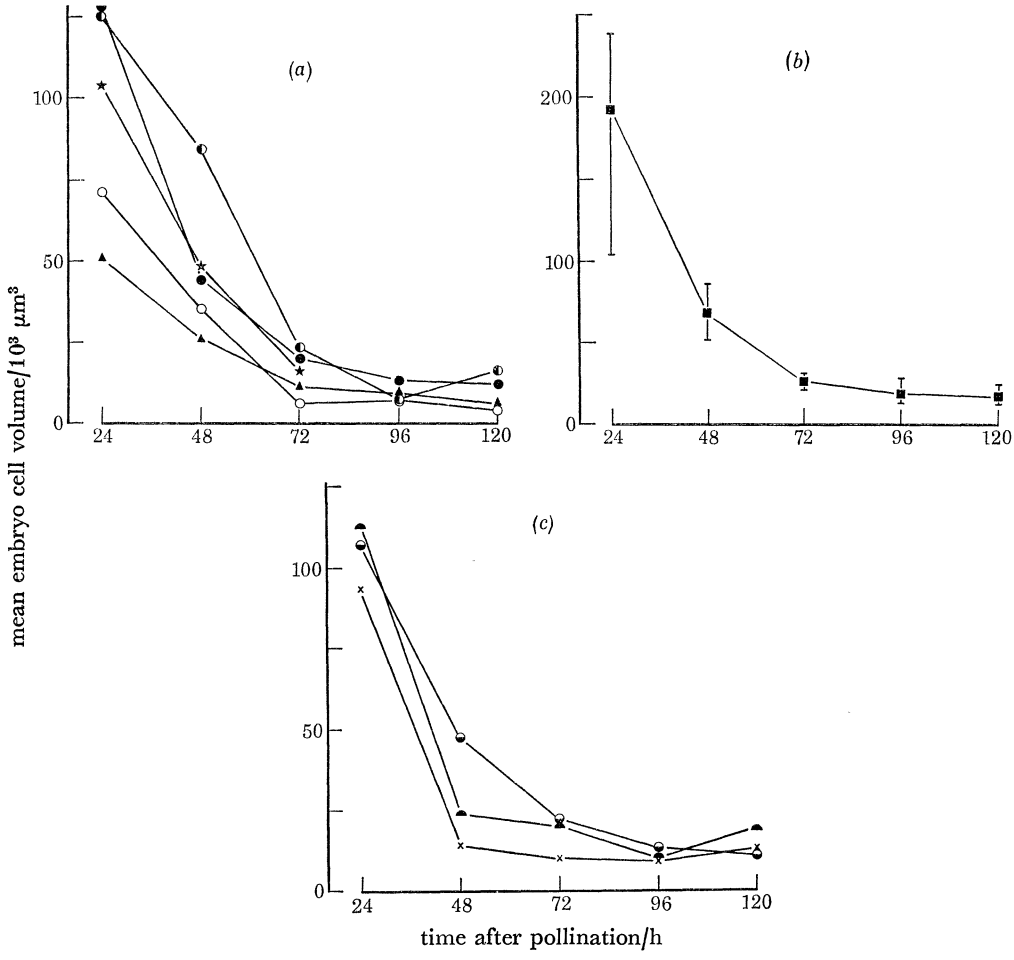

FIGURE 7. The relation between the mean volume of embryo cells and time of fixation of spikes after pollination (*a*) in four wheat genotypes and in rye (*b*) in 6 hexaploid *Triticale* lines, and (*c*) in three barley genotypes. (N.B. The key to symbols in (*a*) is the same as in figure 1*a*; in (*b*) as in figure 3, and in (*c*) as in figure 1*c*).

(iii) *Endosperm development*

The general pattern of endosperm development was essentially the same as that previously described in hexaploid wheat var. 'Chinese Spring' (Bennett *et al.* 1973). Thus, at first the endosperm was a coenocyte but later it became cellular. During the first 5 days of endosperm development the number of endosperm nuclei increased steadily, at first geometrically but

later less rapidly. Figure 8 shows typical plots for endosperm development in rye, wheat, and barley during this period.

Synchrony between nuclei during the coenocytic phase, was usually complete for several doubling cycles but thereafter became progressively decreased. Endosperm nuclei all at a single stage of mitosis were frequently found in florets fixed 24 h after pollination, although occasionally the nuclei in a single endosperm showed a range of mitotic stages even at this early stage. In florets with a range of stages there was a developmental gradient with precocious mitotic stages at the embryo end of the endosperm and retarded stages at the chalazal end. In florets fixed 48 h after pollination endosperm nuclei invariably displayed a range of developmental stages which usually extended over only a part of a cell cycle, for instance, from G2 to anaphase, or from prophase to G1. All the nuclei in one area of the endosperm were, however, at the same stage of development. Interestingly, the developmental gradient in florets fixed 48 h after pollination was the opposite to that found in florets fixed 24 h after pollination. Thus, in florets fixed 48 h after pollination precocious development occurred at the chalazal end of the endosperm, and the most retarded nuclei were located near the embryo. This type of developmental gradient persisted until evidence of synchrony could no longer be detected. In all genotypes, synchrony was usually pronounced until the time of cell wall formation. Small groups of adjacent cells still showed pronounced synchrony for about 1 day after cell wall formation, but by the 5th day after pollination little, or no, synchrony was seen.

TABLE 6. THE MAXIMUM (MAX.) AND MEAN NUMBER OF ENDOSPERM NUCLEI OR CELLS PER FLORET IN SPIKES OF VARIOUS GENOTYPES FIXED 1–5 DAYS AFTER POLLINATION

	time after pollination/h									
	24		48		72		96		120	
genotype	max.	mean	max.	mean	max.	mean	max.	mean	max.	mean
1. *Triticum monococcum*	16	16	275	235	1521	1045	4502	3859	10482	9491
2. *T. turgidum* ssp. *dicoccum*	16	14.4	306	266	2965	2262	8880	5641	21000	16391
3. *T. aestivum* ssp. *vulgare*	16	15.1	301	268	3178	2057	—	—	—	—
4. *T. aestivum* ssp. *spelta*	16	15.4	307	252	4081	2682	8046	6231	22292	14189
5. *Secale cereale*	16	13.5	291	197	2108	1302	6830	3883	11248	6840
6. hexaploid *Triticale* var. Rosner	16	9.6	120	94	905	591	6448	3414	—	—
7. hexaploid *Triticale* var. Cinnamon	16	14.5	384	213	2447	1007	5552	3139	10176	7826
8. hexaploid *Triticale* line TCL 3	16	11.3	199	138	1196	840	4194	2794	6620	3898
9. hexaploid *Triticale* line TCL 4	16	15.7	307	171	1690	1108	6628	4675	11744	8034
10. hexaploid *Triticale* line TCL 19	16	14.7	248	145	1121	648	5082	3470	10930	5632
11. hexaploid *Triticale* line ITSN 30	16	6.6	256	147	1216	842	3886	2662	7744	5465
12. octoploid *Triticale*	16	16	280	120	1522	1035	—	—	8798	6442
13. disomic addition of rye chromosome I	16	15.2	274	204	1856	1523	—	—	—	—
14. disomic addition of rye chromosome II	16	14.9	330	263	1852	1529	—	—	—	—
15. disomic addition of rye chromosome III	32	20.0	596	276	2793	2045	—	—	—	—
16. disomic addition of rye chromosome IV	32	17.4	512	293	1385	1109	—	—	—	—
17. disomic addition of rye chromosome V	16	8.4	277	217	1635	1037	—	—	—	—
18. disomic addition of rye chromosome VI	16	14.9	256	150	1078	907	—	—	—	—
19. disomic addition of rye chromosome VII	16	13.3	264	194	1002	824	—	—	—	—
20. *Hordeum vulgare*	32	11.2	937	632	3168	2346	7726	5722	>25000	—
21. *H. bulbosum* (diploid)	32	10.9	126	109	2483	888	9576	5088	24386	19319
22. *H. bulbosum* (tetraploid)	32	29.2	583	452	3251	2134	9325	7454	24000	23333

In all the genotypes studied, cell wall formation in the endosperm began in the neck region, immediately adjacent to the embryo. In most genotypes cell wall formation began at about 72 h after pollination and was complete about 24 h later. In a few instances (e.g. in several wheat-rye chromosome addition lines) cell wall formation had already begun in the neck of the endosperm in florets fixed 48 h after pollination, while, in a few other genotypes endosperm cell wall formation was still incomplete 96 h after pollination (e.g. in *T. monococcum*).

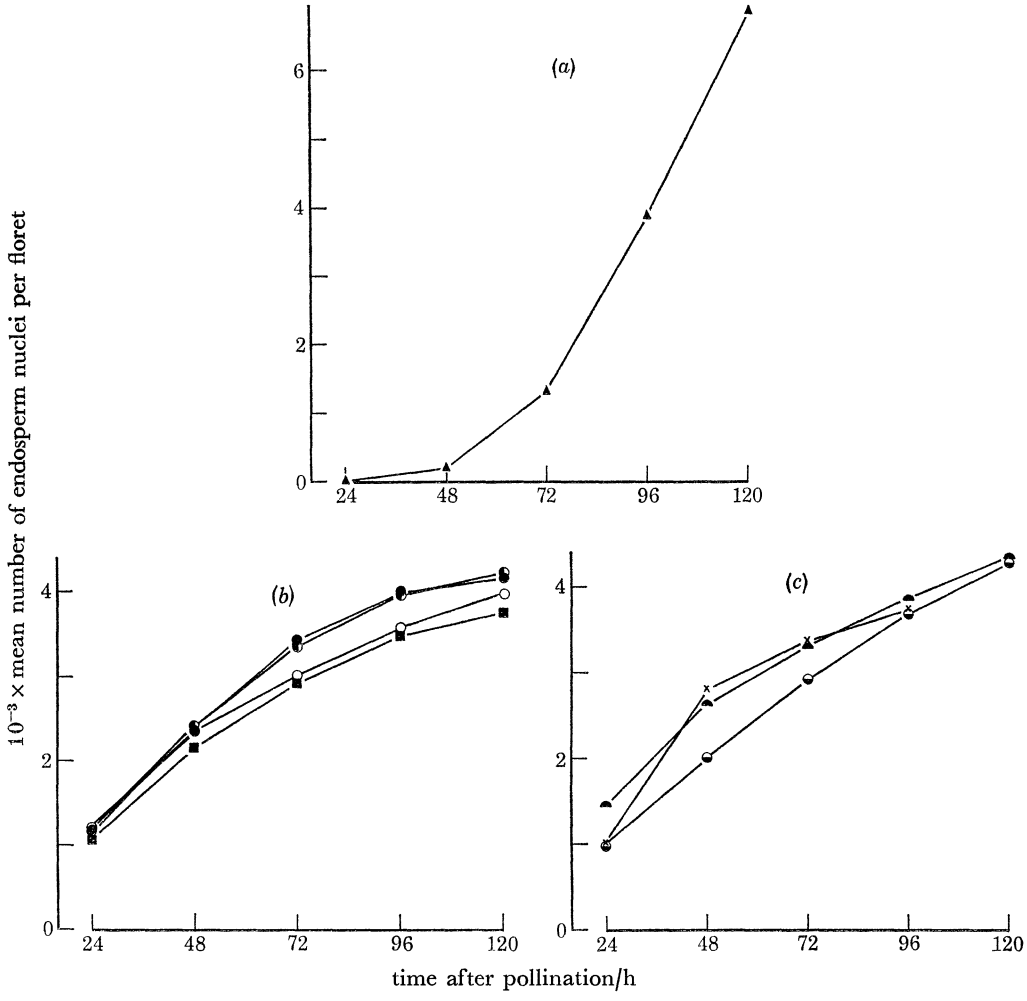

FIGURE 8. The relation between the number of endosperm nuclei per floret and time after pollination in plants grown at 20 °C. (*a*) Diploid rye (▲). (*b*) *Triticum monococcum* (○); *T. dicoccum* (◐); *T. spelta* (●); and the mean for six hexaploid *Triticale* genotypes (■). (*c*) *Hordeum vulgare* (×) and the diploid (◒) and tetraploid (◓) forms of *H. bulbosum*.

Not only did the time of endosperm cellularization after pollination vary between species, but so also did the mean number of endosperm nuclei per floret at the time of endosperm cell wall formation. The mean number of endosperm nuclei per floret at the onset of cell wall formation ranged from 150 in wheat-rye addition VI to over 2000 in three wheat species and in two barley species. Starch grain formation began in all the genotypes studied by 120 h after pollination. Numerous starch grains were first visible within about 24 h after the start of cell wall formation in the endosperm.

Mitosis in the primary endosperm nucleus was completed between 5–7 h after pollination

in all the genotypes studied. (N.B.: This mitosis is counted in the present work as ending the first nuclear cycle in the endosperm.) The second nuclear cycle in the endosperm lasted about 4.5 h in *T. aestivum* (Bennett *et al.* 1973), and about 4.0 h in *H. vulgare* in the present work. The maximum number of endosperm nuclei observed in a floret fixed 24 h after pollination was 16 or 32 in all the genotypes studied. Consequently, the mean nuclear doubling time during this period was about 4.8 h in those genotypes with 16 nuclei, and about 4.0 h in those with 32 nuclei.

In all the genotypes studied there was a progressive decrease in the rate of endosperm development on each of the five successive days after pollination. In most genotypes the rate of maximum endosperm development remained almost constant until about 48 h after development. Thus, the increase in nuclear or cell cycle time was not marked until the third day after pollination. Subsequently, it increased dramatically at the time of cell wall formation in the endosperm. For instance, in the endosperm of *T. monococcum* the maximum number of nuclear or cell cycles completed on the first 5 successive days of development after pollination was about: 5.0, 4.5, 2.5, 1.5, 1.25 respectively. Thus, the mean nuclear doubling time on days 1 and 2 was about 5.5 h; but rose to about 9.5 h on the third day prior to cell wall formation. On days 4 and 5 it rose to about 16 and 19.5 h respectively, during and after cellularization. Data for the maximum and mean number of endosperm nuclei of cells per floret both show the same trend (table 6).

The rate of maximum endosperm development was similar in all the genotypes studied. Thus, at 24 h after pollination florets containing either 16 or 32 nuclei were found in all the genotypes studied. The maximum number of endosperm nuclei observed in a single floret fixed 72 h after pollination ranged from 1002 in wheat-rye chromosome addition line VII, to 4081 in *T. aestivum* ssp. *spelta*. The corresponding range in florets fixed 120 h after pollination was from 6602 cells in hexaploid *Triticale* TCL 3, to 24386 in diploid *H. bulbosum*. Thus, the range of nuclear or cell numbers in florets fixed at 72 and 120 h after pollination represented development requiring only 2 doubling cycles, and was respectively the difference between 9 and 11 nuclear doublings in florets fixed 72 h after pollination, and between 13 and 15 nuclear doubling cycles in florets fixed 120 h after pollination.

The results for mean number of endosperm nuclei or cells per floret show a similar, but slightly greater, range of inter-genotypic variation than those for maximum endosperm development (table 6). The mean number of endosperm nuclei per floret at 24 h after pollination ranged from 6.6 in hexaploid *Triticale* ITSN 30, to 29.2 in tetraploid *H. bulbosum*. At 72 h after pollination mean endosperm cell number ranged from 648 in hexaploid *Triticale* TCL 19 to 2681 in *Triticum spelta*; while at 120 h after pollination the range was from 3898 in hexaploid *Triticale* TCL 3 to 22821 in tetraploid *H. bulbosum*. Thus, at each of these three sampling times the range of mean endosperm nuclear or cell numbers corresponded to development requiring 2.0–2.5 doubling cycles. In general the ranking of genotypes remained fairly constant throughout both the coenocytic and cellular phases of endosperm development, and irrespective of whether maximum or mean endosperm development was compared.

The highest number of endosperm nuclei observed in single florets of diploid, tetraploid and hexaploid wheat fixed 72 h after pollination was 1521, 2965 and 4081 respectively. At the same stage diploid rye contained 2108 nuclei, that is, more than diploid wheat but fewer than either tetraploid or hexaploid wheats. The corresponding results for *Triticale* lines and all seven wheat-rye addition lines show that in six out of the seven *Triticale* lines, and six

out of the seven wheat-rye chromosome addition lines, the effect of the rye chromosomes was to depress the rate of endosperm development to less than that found in rye, the parent species with the lower rate. Comparison of data for the wheat-rye chromosome addition lines shows that individual rye chromosomes had widely different effects on endosperm development during the coenocytic phase. Moreover, the presence of just one pair of rye chromosomes decreased the rate of endosperm development almost as much as the presence of the whole diploid rye genome. Thus, wheat-rye addition III (2793) had almost as many endosperm nuclei as *T. dicoccum* and *T. aestivum* var. *vulgare*, while addition VII (1002) had only half as many as diploid rye (2108), and almost as few as 'Rosner' (905), the *Triticale* with the lowest number of endosperm nuclei per floret at this stage.

The data for mean numbers of endosperm nuclei in florets fixed 72 h after pollination show the same general pattern as that described above for maximum endosperm nuclear number.

The mean number of endosperm nuclei in florets fixed 72 h after pollination in diploid *H. bulbosum* was only about one third of that found in diploid *H. vulgare*. Thus, the rate of endosperm development during the coenocytic phase was slower in *H. bulbosum* than in *H. vulgare*. However, comparison of results for florets fixed at 96 hours after pollination shows a higher number of endosperm cells in *H. bulbosum* than in *H. vulgare*. The explanation of this result is unknown. Perhaps, endosperm development in *H. bulbosum* is not slowed as much as in *H. vulgare* by the formation of cell walls in the endosperm. It is also possible that spikes fixed at different times were pollinated by plants from different clones. *Hordeum bulbosum* is a slow growing perennial outbreeder. Consequently different plants may differ for genes controlling the rate of endosperm development.

(iv) *A comparison of the rates of embryo and endosperm development*

During the first 48 h after pollination the rate of nuclear doubling in the endosperm greatly exceeded the rate of cell doubling in the embryo in all 22 genotypes studied. In *T. monococcum*, for example, the maximum embryo cell number and endosperm nuclear number in florets fixed 48 h after pollination was 4.0 and 276 respectively. As a rule, the nuclear doubling time in the endosperm (about 5 h) was about one third that of the cell cycle time in embryo cells (about 16 h) during this period. Such a difference between the rates of early endosperm and embryo development has often been noted previously in species in the Triticeae (Percival 1921; Pope 1937; Moss 1972; Bennett *et al.* 1973; Kaltsikes 1973).

During the period from 48 to 120 h after pollination, the nuclear doubling time in the endosperm was progressively increased, while the cell cycle time in embryo cells remained constant or decreased slightly. On the third day after pollination the nuclear doubling time in the endosperm approached, and in some genotypes equalled, the cell cycle time in embryo cells. During the fourth and fifth days of seed development the mean cell cycle time in the endosperm was similar to, and sometimes exceeded, that of the mean cell cycle time in the embryo. For example, in *T. monococcum* the mean cell cycle time during the third to fifth days of seed development in embryo cells remained constant at about 14.5 h, while the mean cell cycle time in the endosperm was about 10.1, 13.5 and 19.5 h on the third, fourth and fifth days respectively. During the same developmental period, the mean embryo cell cycle time in embryo cells of tetraploid *H. bulbosum* was about 15 h, while the mean cell cycle time in the endosperm was about 10.0, 13.5 and 16.0 h on the third, fourth, and fifth days of

seed development. Thus, in tetraploid *H. bulbosum* the rate of development in embryo cells exceeded the rate of development in endosperm cells by the fifth day after pollination, while, in diploid *H. bulbosum* endosperm development remained faster than embryo until the end of the fifth day after pollination.

Comparing the mean embryo cell number per floret and the mean endosperm cell number per floret at 72 h and at 120 h after pollination (tables 4 and 6) shows a highly significant ($P < 0.001$) relation between these two seed characters at both times. Clearly, therefore, the factor(s) which determine the rate of embryo cell development also affect the rate of endosperm development.

Discussion

(*a*) *Comparison of the rates of cell and nuclear development in the young seed and other cell types*

It is interesting to compare the cycle times in embryo cells with those in other cellular meristematic tissues. The mean cell cycle time in embryo cells in the present work was estimated to last about 10.8 h in *Hordeum vulgare* cv. 'Sultan', 12.3 h in *Triticale* cv. 'Rosner' and *T. aestivum* cv. 'Chinese Spring', and about 14 h in *Secale cereale*. By comparison, the mean cell cycle time also at 20 °C in root-tip meristem cells has been estimated to last 12.4 h in *H. vulgare* cv. 'Sultan' (Bennett & Finch 1972*a*); 10.8 h (Bennett, unpublished) and 12.2 h (Kaltsikes 1972) in *Triticale* 'Rosner'; 12.5 h in *T. aestivum* cv. 'Chinese Spring' (Bayliss 1972); 11.5 h in *S. cereale* cv. 'Prolific' (Kaltsikes 1972) and 12.0 h in *S. cereale* cv. 'Petkus Spring' (Bennett, unpublished). Clearly, therefore, the cell cycle times in embryo cells are very similar to those already noted in root-tip meristem cells of the same species. Presumably the rates of cell development in both types of meristematic tissue are subject to a similar type of control.

All the genotypes studied displayed a prolonged coenocytic stage of endosperm development during which several hundred or thousand nuclei were formed. During the early part of this stage, the rate of nuclear doubling was at its fastest. The shortest nuclear doubling times noted in the endosperm, which may have been as short as about 3.5 h in *Hordeum vulgare*, indicate a much faster rate of nuclear doubling than has previously been found in any cellular tissue of the species studied. Apparently, the conditions for nuclear doubling in the early coenocytic endosperm are optimal, and remain so, for a prolonged period. Moreover, the control of nuclear doubling in the endosperm appears to differ from that appertaining to the adjoining cellular embryo during the same period. There are obvious important differences between the early coenocytic stage in endosperm with early cellular development in the proembryo. Coenocytic endosperm development during the first 24 h after pollination appears to consist only of successive alternate synchronous phases of nucleo-protein synthesis and its division into two daughter nuclei at mitosis. This process occurs without any significant synthesis of cytoplasm. Consequently it may be viewed as the rapid production of nuclei to populate a large volume of preformed cytoplasm, already present in the central cell of the mature embryo sac at the time of pollination. As early endosperm development is coenocytic, it occurs without any synthesis of cell walls or of cell membranes. By comparison, development during each cell cycle in meristematic cells requires the synthesis of additional cytoplasm, cell wall material and cell membrane. In a cellular meristem, the rate of nuclear doubling is controlled at, and limited to, the rate at which essential parallel non-nuclear development is

undertaken. The absence of these restraints in coenocytic endosperm may account, at least in part, for the much faster nuclear doubling rate observed in the coenocytic endosperm than in the cellular endosperm, the embryo, and other cellular meristematic tissues.

It is interesting to compare the minimum nuclear doubling time in the endosperm with the durations of DNA synthesis (*S*) stage and mitosis (*D*) in cellular meristematic cells. In *T. aestivum* cv. 'Chinese Spring' root-tip cells at 20 °C, *S* and *D* lasted 3.8 and 1.2 h respectively (Bayliss 1972). Thus, the time taken for assembling nucleoprotein at *S*-phase and its division at mitosis in root-tip cells (5.0 h) is essentially the same as the minimum nuclear doubling cycle in early coenocytic endosperm in the same genotype (about 4.8 h). No data are available concerning the durations of individual stages of the nuclear cycle in endosperm, however, it seems possible that during early coenocytic endosperm development the cycle may be spent mainly at *S* and *D* phases, with only very short G1 and G2 stages. It also seems certain that the durations of *S*-phase and *D*-phase in such nuclear doubling cycles are shorter than in root-tip cells. Thus, while *S* and *D* in *H. vulgare* cv. 'Sultan' root-tip cells grown at 20 °C were estimated to last together 5.61 h (Bennett & Finch 1972*a*), the duration of the nuclear doubling cycle in the young endosperm of 'Sultan' was no more than 4.5 h and may have been as short as 3.5 h.

The increase in the duration of the nuclear doubling cycle noted at the end of the coenocytic stage, but before cell wall formation, may be explained by the onset of synthesis of additional endosperm cytoplasm. The further large increase in nuclear doubling time in the endosperm which occurred concurrently with cell wall formation presumably reflects both the synthesis of new cell wall material during each cell cycle, and the reduced ease of transport of all materials in a cellular as compared to a coenocytic tissue.

Starting with a single primary endosperm nucleus, up to nearly a thousand triploid nuclei were produced in *H. vulgare* within 48 h of pollination. While early coenocytic endosperm development does not involve the synthesis of cytoplasm and cell wall material nevertheless, it does require the synthesis of nucleoprotein during *S*-phase, and of spindle microtubules during mitosis. Moreover, both *S* and *D* require energy. Consequently, early coenocytic endosperm development requires a large supply of nucleotides, amino acids, and energy supplying molecules. The present results indicate clearly that all of these requirements are lavishly met. However, it is interesting to question how this is achieved. The embryo sac is surrounded by a single layer of cells containing numerous large starch grains which is almost totally broken down and absorbed after pollination but before cell wall formation in the endosperm. In a mixture of fertilized and unfertilized florets of equal maturity on a single spike fixed late during the coenocytic stage of endosperm development, the inner layer of the nucleus was entire and unaltered in the unfertilized florets but completely absorbed in fertilized florets. Thus, the cells of the innermost nucellar layer, in response to fertilization, may provide much of the energy requirements of the coenocytic stage of endosperm development. It may also be significant that the period of rapid development in coenocytic endosperm coincides exactly with the time of maximum development of the antipodal cells prior to their rapid degeneration and resorption immediately after cellularization of the endosperm.

It has frequently been claimed that antipodal cells have a secretory function (Brink & Cooper 1944; Zinger & Poddubnaya-Arnoldi 1967); indeed, their whole appearance suggests that this is so. However, the exact nature of their role has never been satisfactorily demonstrated. If the antipodal cells have a function in wheat, rye and barley, then clearly, it must

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be finished before the sudden degeneration of the antipodal cells at the end of the coenocytic phase of endosperm development. Antipodal cells become highly endopolyploid during embryo sac development prior to pollination in all the genotypes studied. In some genotypes (for instance, *H. vulgare* cv. 'Sultan') they continue to increase in size and ploidy level for about 1 day after pollination. The level of endopolyploidy in antipodal cells is directly proportional to their nucleolar volumes. This, together with the strong basiphilic nature of antipodal cell cytoplasm, implies that RNA is accumulated during embryo sac maturation, and is indicative of a function involving active protein synthesis (Heslop-Harrison 1972). It seems reasonable to suggest that the role of the antipodal cells in wheat, rye and barley, may be to supply the protein requirements of the coenocytic endosperm stage by synthesizing either amino acids, or the essential machinery required for all protein synthesis, that is ribosomal RNA, for transfer into the coenocytic endosperm cytoplasm. Experiments to test these hypotheses are being undertaken in this laboratory. Two previous observations may be relevant; *first*, the ploidy level in cells of ripening cotyledons in *Pisum sativum* was significantly correlated with the rates of both RNA and protein synthesis (Scharpé & Parijs 1973). *Second*, the number of antipodal cells in three orchid species was significantly correlated with both mean seed weight and mean embryo size (Zinger & Poddubnaya-Arnoldi 1967).

(*b*) *Interspecific effect of polyploidy on embryo and endosperm development*

Table 6 shows clearly that there was no interspecific effect of polyploidy on the maximum rate of nuclear doubling during the first 24 h of endosperm development. Thus, in florets fixed 24 h after pollination a maximum of 16 nuclei were observed in diploid, tetraploid and hexaploid wheat, and in octoploid *Triticale*. Results for florets of diploid, tetraploid and hexaploid wheat, and of octoploid *Triticale* fixed 48 h after pollination also show no effect of ploidy level. The most developed endosperms in these four genotypes contained between 277 and 306 nuclei (table 6); and therefore, some nuclei in each had completed the same number of doubling cycles (i.e. nine). These results showing no effect of polyploidy on maximum rate of early endosperm development were obtained by comparing species forming an allopolyploid series. Using *Secale cereale*, Hakansson & Ellerström (1950) observed early endosperm development in $2x \times 2x$, $4x \times 4x$, and in reciprocal crosses between $2x$ and $4x$ plants. Thus, endosperm development was observed in plants whose endosperm nuclei were $3x$, $4x$, $5x$ and $6x$. Endosperms fixed 48 h after pollination contained the same maximum number of endosperm nuclei (namely 256) irrespective of their ploidy level. There was, therefore, no effect of autoploidy on developmental rate. Similarly, in the present work, florets of diploid and tetraploid forms of *H. bulbosum* fixed 24 h after pollination, both contained a maximum of 32 endosperm nuclei.

Whereas there was no interspecific effect of ploidy level on the rate of development in the young coenocytic endosperm, there was a clear effect on rate of cell development in the young embryo. Increasing the ploidy level increased the rate of cell development in the embryo. Thus, the maximum numbers of embryo cells observed in single florets of diploid, tetraploid and hexaploid wheat fixed 72 h after pollination were 16, 33 and 48 respectively (table 4).

The present results may be compared with the known effects of polyploidy on the rate of cell development in other tissues. Increasing the level of either auto- or allopolyploidy has the effect of increasing the rate of cell development of meiocytes or microspores of wheat,

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rye and barley (Bennett & Smith 1972; Bennett & Finch 1972*b*). Thus, the effect of polyploidy on developmental rate in these cells is similar to its effect on the rate of cell development in young embryos noted in the present work.

There has been considerable disagreement on the consequences of polyploidy on the rate of development of root-tip cells. Some workers have reported that the duration of DNA synthesis did not increase with increasing ploidy level (Van't Hof 1966; Troy & Wimber 1968; Yang & Dodson 1970; Friedberg & Davidson 1970), while others reported that it did (Alfert & Das 1969; Evans *et al.* 1972). Moreover, examples have been cited in which the duration of the cell cycle in polyploids was longer (Skult 1969; Alfert & Das 1969; Evans, Rees, Snell & Suns 1972), the same as (Yang & Dodson 1970), or shorter (Gupta 1969; Kaltsikes 1972) than in related diploid species. All three results have been obtained by one of the present authors (Bennett, unpublished) in comparisons of the cell cycle time in root-tip meristem cells of wheat, barley and *Triticale*.

It seems unreasonable to expect all polyploid cells to behave in the same way. While polyploidy inevitably involves an increase in gene dosage per cell, the effect of the increase might be expected to vary depending upon the nature of the genes whose dosage is increased. The evidence of the present work, showing different consequences of polyploidy in the endosperm and the embryo, together with those results for root-tip meristem cells listed above (showing different consequences of polyploidy in the same cell type in different species) show clearly that the consequences of polyploidy can and do vary. There are, therefore, no inevitable consequences of polyploidy *per se* on rate of cell development.

(*c*) *Instability of hybrid nuclei*

(i) *Chromosome elimination in hybrid nuclei*

Hordeum bulbosum chromosomes are selectively eliminated in both embryo and endosperm tissues of F_1 hybrids between *H. vulgare* and *H. bulbosum* (Lange 1971; Kao & Kasha 1971; Subrahmanyam & Kasha 1973).

Chromosomes of one parent have often been observed to be lost preferentially from mammalian interspecific hybrid cells (Weiss & Ephrussi 1966; Weiss & Green 1967; Handmaker 1971; Rao & Johnson 1972). There is evidence that the major chromosome losses probably occur during the early cell divisions (Nabholz, Miggiano & Bodmer 1969). The mechanism responsible for rapid elimination of chromosomes is obscure. Chromosomes derived from different parents are known to retain considerable autonomy when combined in a hybrid nucleus (Graves 1972). Chromosome elimination and instability in cells of interspecific hybrids of mammals (Rao & Johnson 1972) and plants (Gupta 1969) have been claimed to be due to differences in the rates of cell development in the parental forms.

A similar hypothesis was advanced to explain one aspect of meiotic chromosome in wheat-rye amphidiploids (*Triticale*) which are meiotically unstable and have several univalents at meiosis. Such univalents are predominantly rye chromosomes (Müntzing 1956; Pieritz 1970; Thomas & Kaltsikes 1974). It has been shown that the duration of meiosis and its stages is longer in rye than in wheat (Bennett, Riley & Chapman 1971; Bennett & Smith 1972; Bennett & Kaltsikes 1973). Consequently it was suggested that differences between the rates of meiotic development in the parent species and *Triticale* hybrids may be a cause of chromosome pairing failure and the selective loss of rye chromosomes (the parent species with the slower

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rate of meiotic development) in *Triticale* hybrids (Bennett *et al.* 1971; Bennett & Kaltsikes 1973).

It seems reasonable to suggest, therefore, that selective chromosome elimination in F_1 hybrids between *H. vulgare* and *H. bulbosum* might be caused by different rates of development in the two parent species. This notion may be supported by the present observation that the rates of early endosperm and embryo development are indeed much slower in *H. bulbosum* than in *H. vulgare*. Thus, the rate of development in their F_1 hybrid, as yet unknown, must differ from that of one if not both parent species. The selective elimination of *H. bulbosum* chromosomes might be due to their relative inability to adapt to a different rate or pattern of development in F_1 hybrid cells.

Chromosome elimination is affected by the ratio of *H. bulbosum* (B) to *H. vulgare* (V) genomes. When this ratio is 1:1 or 1:2 elimination occurs, but when it is 2:1 little or no elimination takes place (Kao & Kasha 1971; Subrahmanyam & Kasha 1973). These observations might be explained if the rate of development in hybrid cells approximated more closely to that of the *H. bulbosum* parent as the ratio of B to V genomes is increased. It has already been suggested that variation in meiotic stability in *Triticale* at different ploidy levels might be explained on a similar basis. On average, the higher the ratio of rye to wheat genomes in *Triticale*, the more stable is the meiotic behaviour of the rye chromosomes (Krolow 1973). Moreover, the rate and pattern of meiotic development in *Triticale* approaches more closely that of the rye parent as the ratio of rye to wheat genomes is increased (Bennett & Kalsikes 1973). It was suggested that these two sets of observations may be causally related (Bennett & Kaltsikes 1973).

(ii) *The combination of wheat and rye chromosomes*

Plant breeders have devoted a considerable effort, with some success, to selecting wheat-rye amphidiploid hybrids (*Triticale*) which combine the desirable qualities of both parent species. While cell development in *Triticale* is stable during vegetative growth, nuclear instability is both common and pronounced at meiosis (Riley & Miller 1970) and during early seed development (Zillinsky 1974*b*). As explained above, developmental instability in hybrid cells may be caused by different rates of cell development in the parent species. The durations of the cell cycle in root-tip cells of wheat, rye and *Triticale* are very similar (Bennett 1974). Consequently, differences between the developmental rate of wheat and rye chromosomes are unlikely to cause instability during vegetative growth. However, meiotic development in rye was slower than in corresponding wheat species (Bennett & Smith 1972). The present results show that the mean and maximum rates of embryo cell development in rye were slower than in all four wheat species examined. Similarly, the maximum and mean rates of endosperm development in rye were slower than in the wheat species except for *T. monococcum*. Thus, it has been shown that the rates of cell development in wheat and rye are similar during vegetative growth when cell development is stable in their hybrids, but dissimilar during meiosis and early seed development, when nuclear development is unstable in *Triticale* hybrids. These results may be interpreted, therefore, as supporting the notion that instability in *Triticale* hybrids is caused by different rates of nuclear development in the two parent species. The present results show that five of the seven rye chromosomes, when present as disomic additions to hexaploid wheat, had a pronounced effect in slowing embryo cell development to a rate below that of the wheat parent species. If developmental instability in

Triticale nuclei is caused by different rates of development in the two parent species which are determined by many chromosomes, then, this may be difficult to overcome in *Triticale* breeding programmes.

Conclusion

The present results provide a detailed picture of the pattern and rate of normal early embryo and endosperm development in a wide range of related cereal genotypes. They therefore provide the baseline for comparative studies which seek to either describe the nature of abnormal early seed development, or to quantify its extent. The present results are being used for this purpose in a study of abnormal endosperm development in those genotypes with a mixture of wheat and rye chromosomes listed in table 1, and in detailed studies of the time and frequency of chromosome elimination in F_1 hybrids between several types of *Hordeum vulgare* and *H. bulbosum*.

The present work provides strictly comparable data for a large number of genotypes varying with respect to several characters known to affect developmental rate in other tissues of the same species. They indicate several factors which may influence or determine the rate and extent of early embryo and endosperm development. A detailed analysis of these factors will be made in a later paper, however, the main areas of certain or possible interest are listed below. *First*, comparison of, for instance, the results for hexaploid wheat with those for wheat-rye chromosome addition lines indicates that individual chromosomes may carry genes with large effects on endosperm development. Attempts should be made to map any such genes and to define their individual effects on early grain development. *Second*, the observation that, despite its higher ploidy level, hexaploid wheat produced embryo cells and endosperm nuclei at rates equal to, or faster than, its diploid and tetraploid relatives seems important and may pinpoint an important facet of the agronomic success of hexaploid wheat. *Third*, it is suggested in the present work that the antipodal cells may function by supplying the protein needs of endosperm nuclear development during the coenocytic phase. If so, then their size and number should play an important role in early seed development. Experiments to test this hypothesis are being made.

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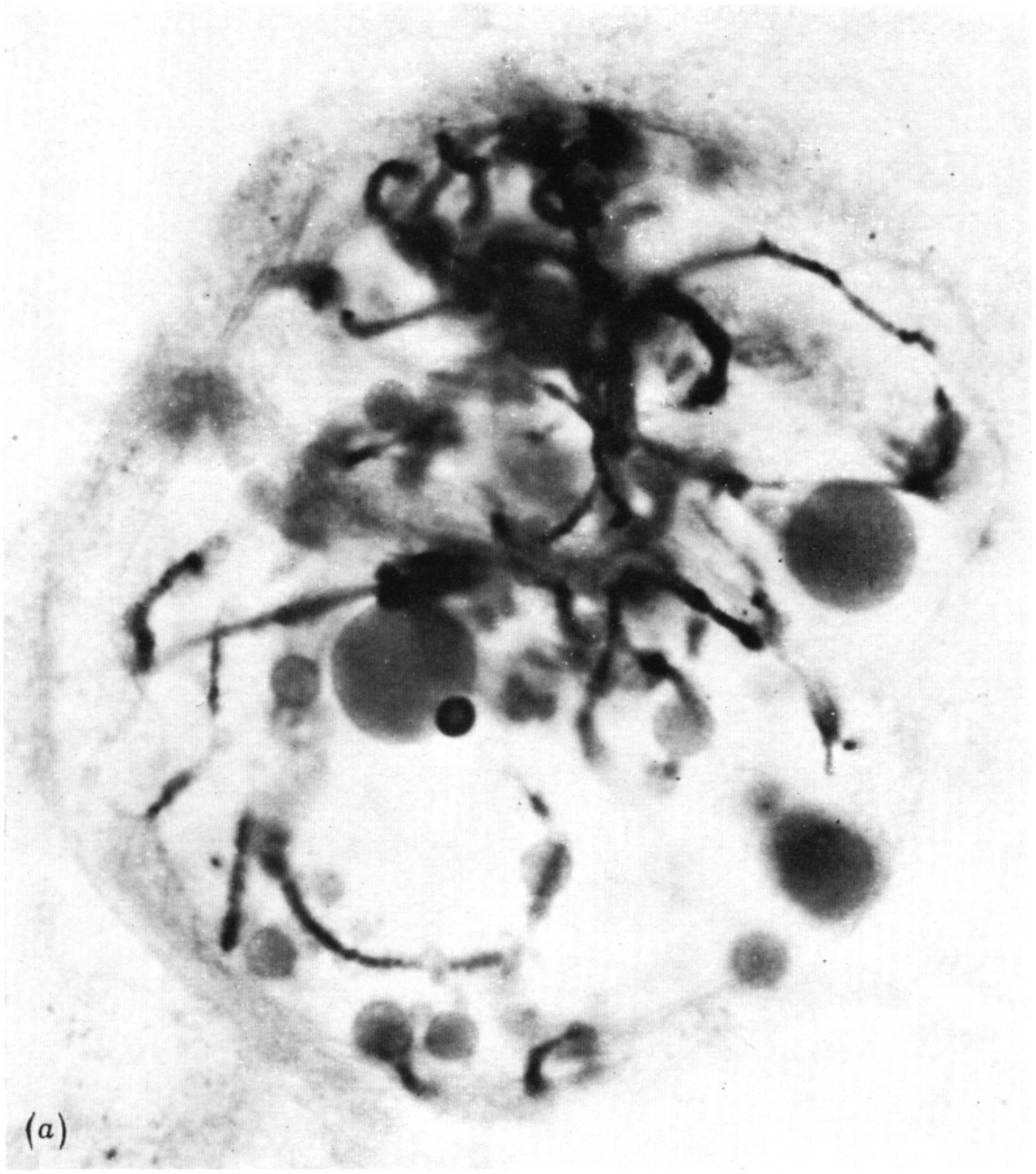
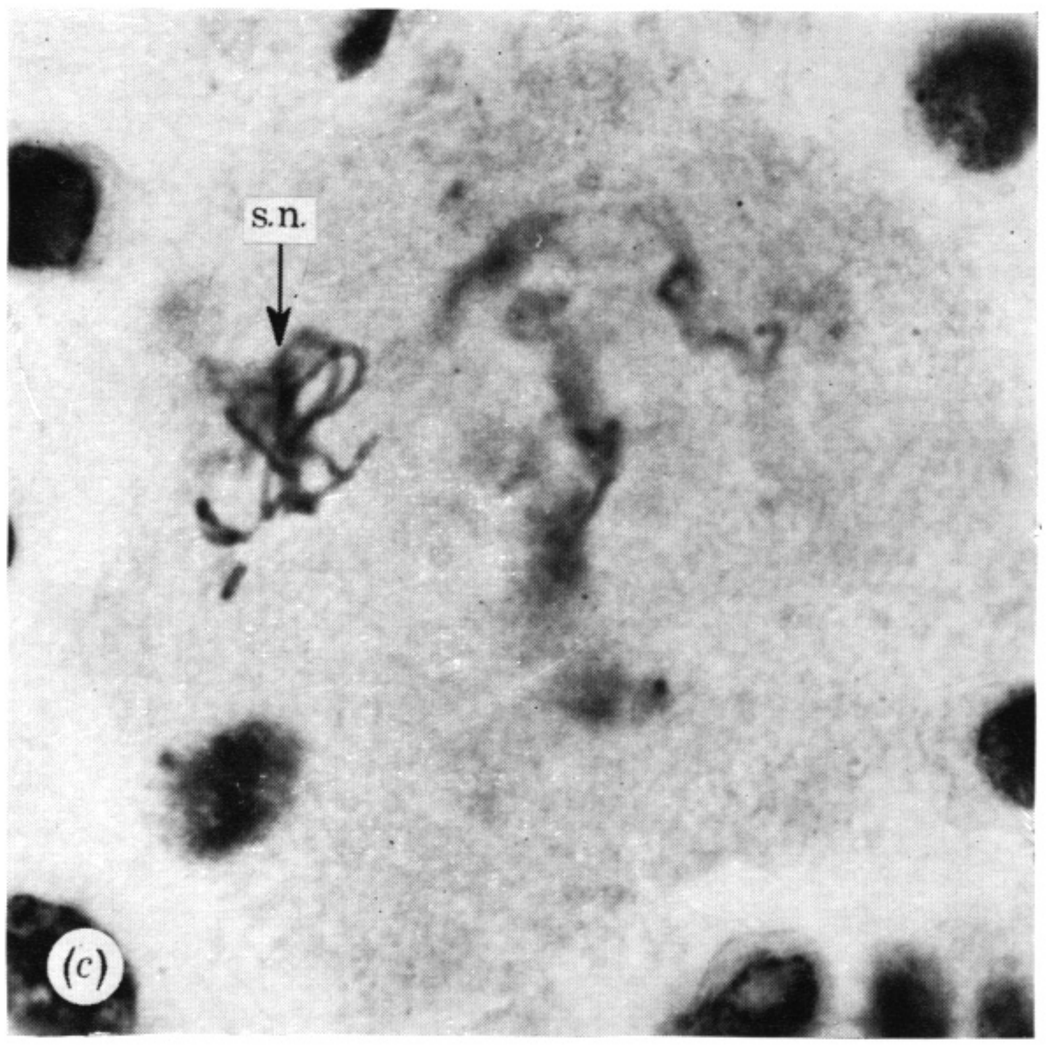
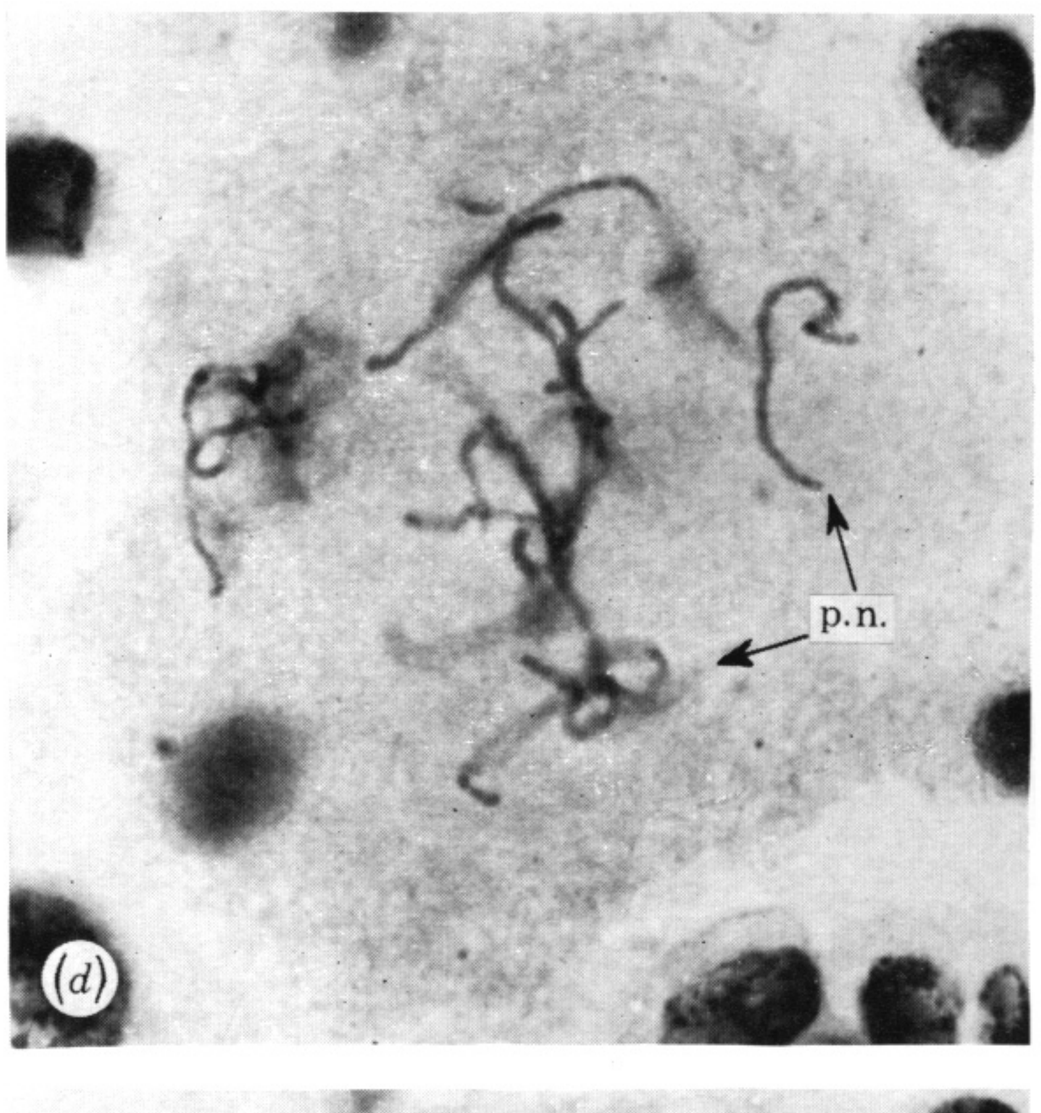
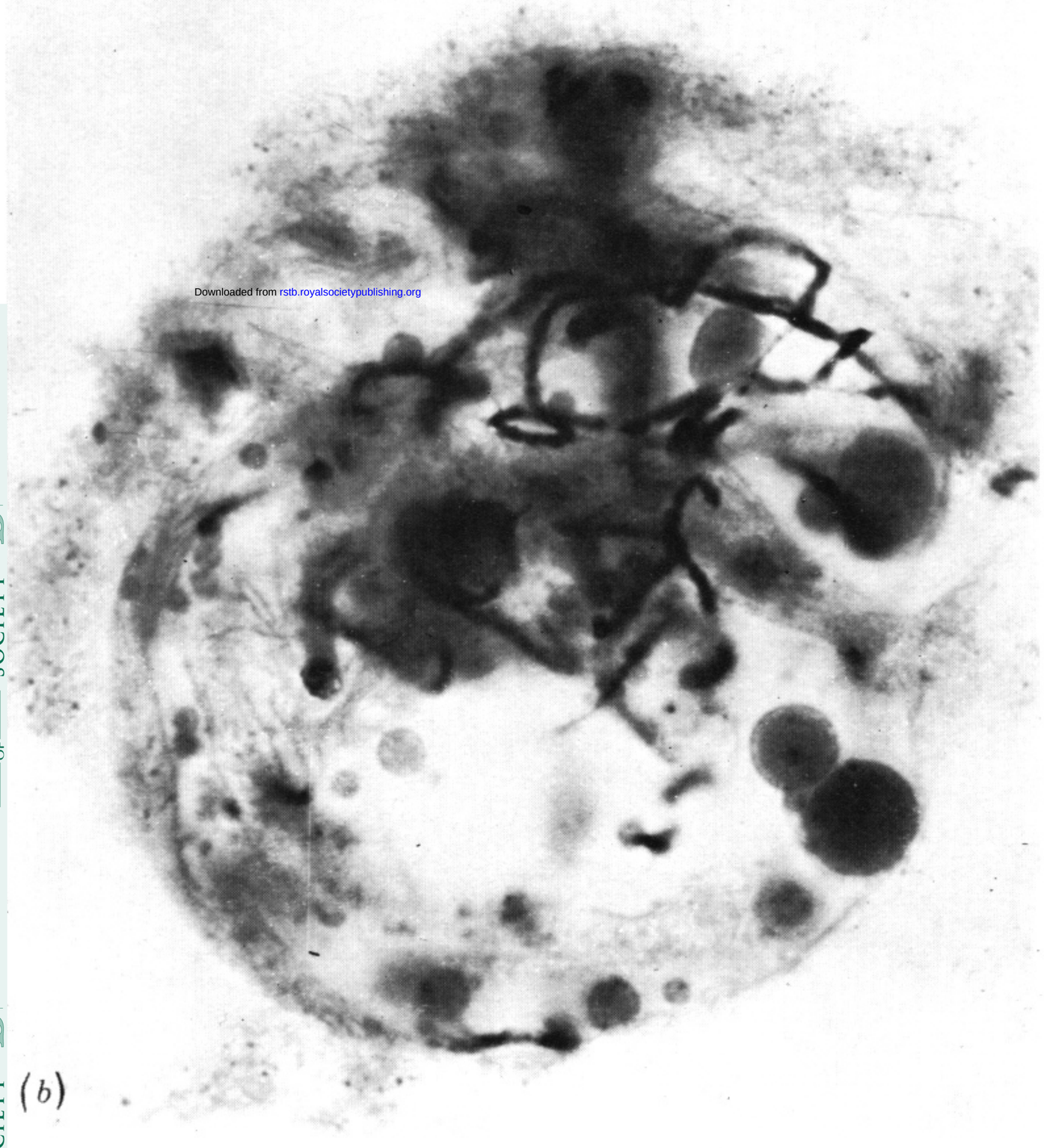
FIGURE 1 (*a*). The mature unfertilized egg apparatus and polar nucleus of *H. vulgare* cv. 'Sultan'; (*b*) an optical section showing the nucleolus of the polar nucleus out of focus in (*a*). Note the highly vacuolate egg cell cytoplasm and the highly condensed chromosome threads in both the egg cell nucleus and polar nuclei: (*c*, *d*) Sperm nuclei (s.n.) appressed to the egg cell nucleus (*c*) and the polar nuclei (*d*) in a floret of hexaploid *Triticale* cv. 'Rosner' fixed 40 min after pollination. Note the relative sizes of the nucleoli in the egg cell nuclei (*a*, *c*) and their corresponding polar nuclei (*b*, *d*). (All magn. 1000.)









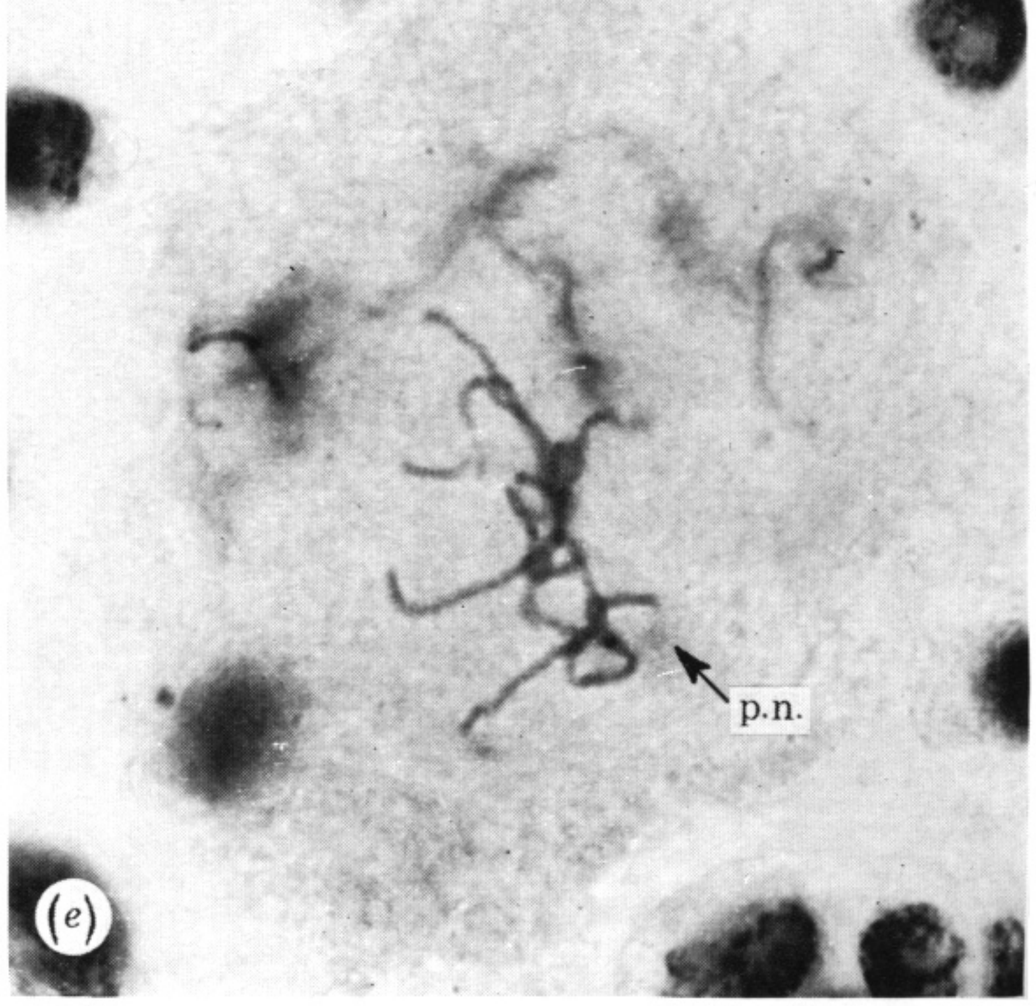
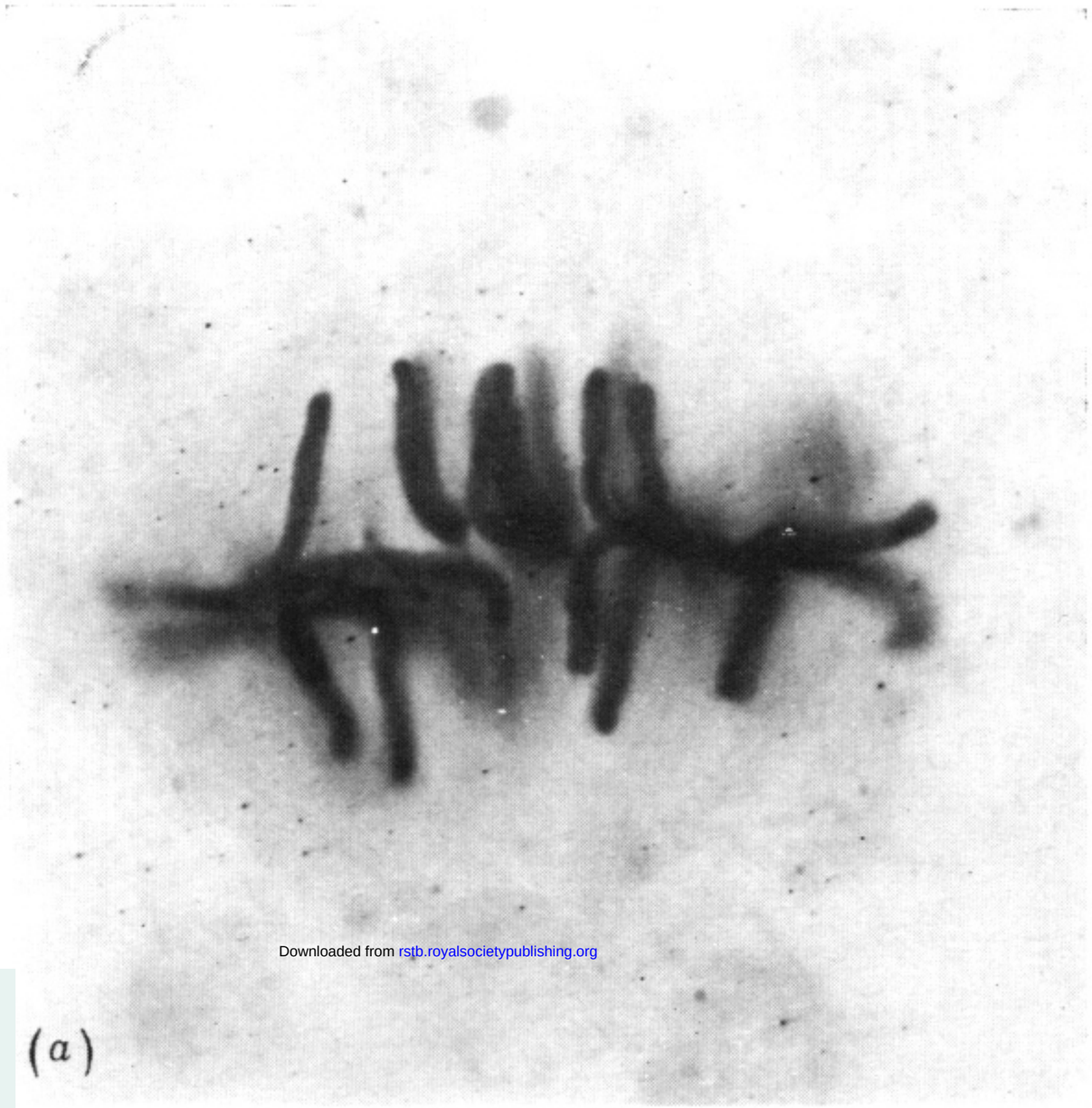
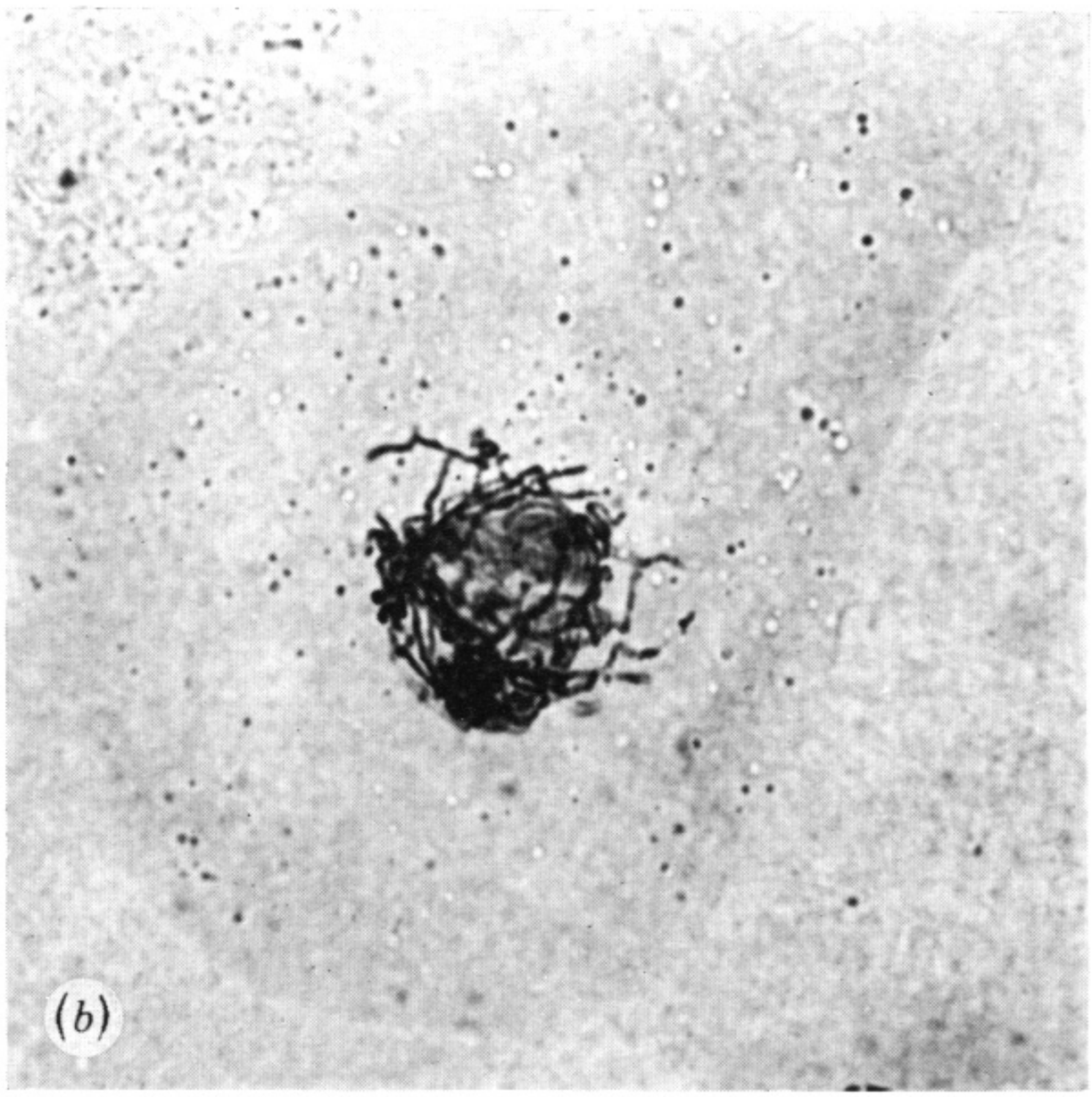
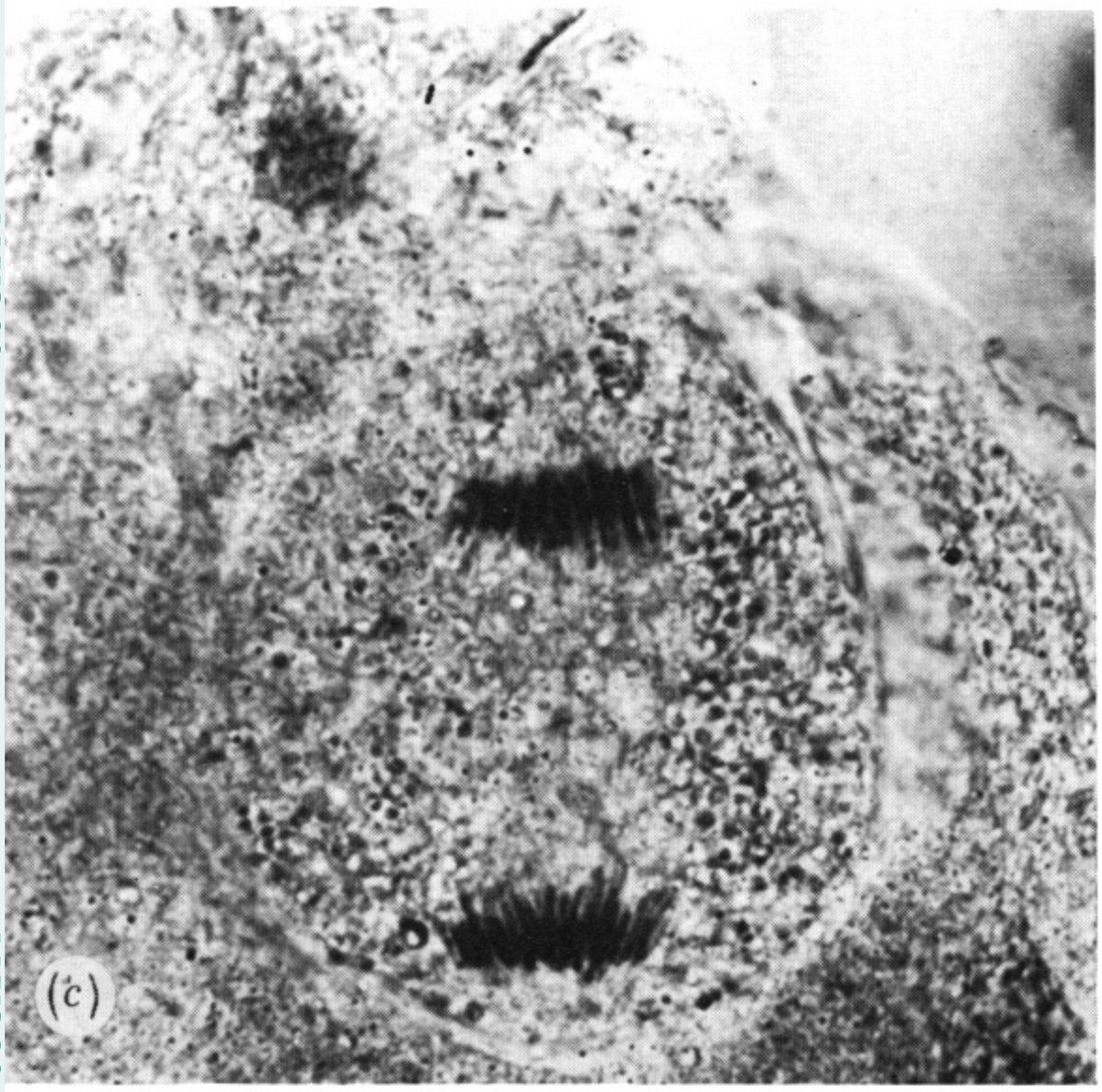


FIGURE 2. Primary endosperm nuclei of *Secale cereale* cv. 'Petkus Spring' fixed 6 h after pollination. (*a*, *b*) Optical sections through an aceto-orcein stained primary endosperm nucleus at early prophase showing condensing chromosomes and numerous micronucleoli (× 1250); (*c*–*e*) optical sections through a Feulgen stained primary endosperm nucleus at mid-prophase showing separate groups of chromosomes derived from the sperm nucleus (s.n.) and the two polar nuclei (p.n.) (× 1375).







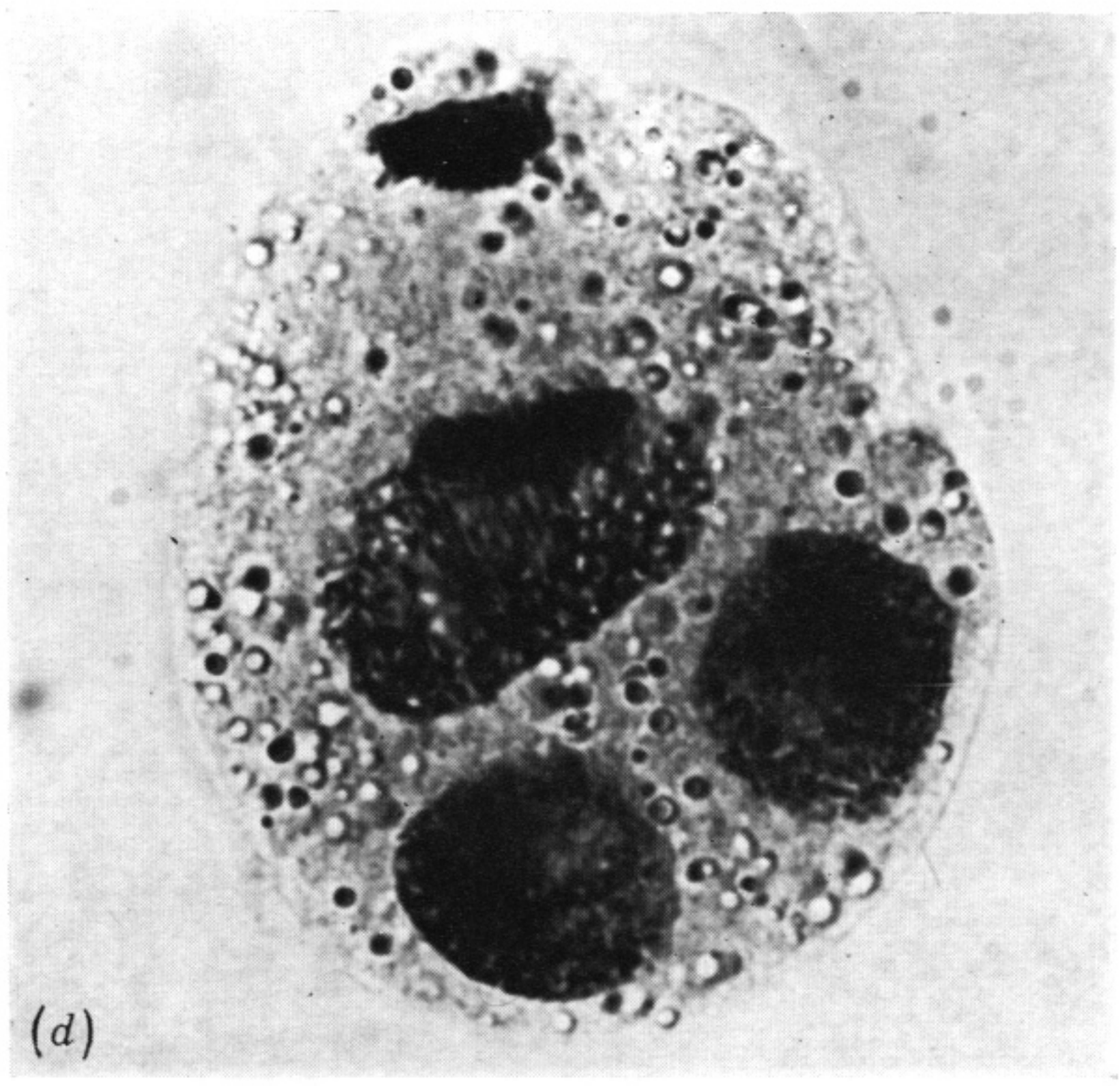


FIGURE 3. (*a*) Feulgen stained primary endosperm nucleus at metaphase in *S. cereale* cv. 'Petkus Spring' (× 2000); (*b*, *c*) Feulgen stained zygotes at mitosis: (*b*) prophase, in *S. cereale* cv. 'Petkus Spring' (× 1250), and (*c*) telophase in wheat-rye addition IV (× 850); (*d*) Feulgen stained 4-celled proembryo from hexaploid *Triticale* cv. 'Rosner' (× 1000).