THE ORGANIZATION AND POLARITY OF POLLEN MOTHER CELLS OF TRITICUM AESTIVUM

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SUMMARY
Colchicine has been applied to young developing anthers of Triticum aestivum at varying stages of maturity from the last premeiotic mitoses of the archesporial and tapetal cells to the second meiotic divisions of the pollen mother cells. The developmental stage of the archesporium at which colchicine took effect was determined by cytological examination of the ploidy levels of the nuclei of the adjacent tapetal cells. The type of pollen abnormality induced depended on the time of application and the concentration of colchicine. Uninucleate monads with 4 randomly positioned pores and uninucleate monads without pores were obtained with 0.5% colchicine. Multipored polyads and multipored uninucleate monads were observed together in anthers treated with 0.01% colchicine. Naturally occurring aberrant pollen types in hybrids of Triticum aestivum x Aegilops mutica or T. aestivum x Aegilops sharonensis have revealed a constant relationship between the disposition of the meiotic spindles and the siting of the pollen pores. The colchicine-induced abnormalities have further clarified the nature of this relationship leading to the interpretation that both the positioning of the spindles and the siting of the pores are predetermined by events taking place in the premeiotic interphase at a time just after the last mitosis of the pollen mother cells and the penultimate mitosis of the tapetum. A reorganization of the archesporial cells (sensitive to colchicine) possibly occurs at this time. Various subsequent meiotic events are dependent on the reorganization. Two of these events – the organization of meiotic spindles and the establishment of pollen symmetry – are discussed.

INTRODUCTION
Much recent evidence points to the likelihood that the species-specific wall patterning of pollen grains is determined not by the activity of the haploid genomes of the spores but by the diploid mother cell nucleus. Experiments in which colchicine or centrifugation have been applied during meiotic prophase in anthers of Lilium spp. have shown that despite the prevention or partial disruption of nuclear divisions, pollen wall patterning remains normal, although pollen polarity is disturbed or absent in unreduced and tetrakaryotic cells (Heslop-Harrison, 1971b). The programme for wall formation appears to be virtually independent of nuclear behaviour from late prophase onwards. Similarly, observations in interspecific hybrids in Linum in which microspores with incomplete chromosome complements developed into pollen grains with essentially normal exine and germ pores has led to the conclusion 'that the genetic control for exine deposition rests, not with the haploid genome, but with the spore cytoplasm' (Rogers & Harris, 1969). A close correlation between the amount of exine laid down and the amount of cytoplasm in the spore would appear to substantiate the interpretation of a factor controlling wall development that is diffuse in the
cytoplasm. Finally the occurrence of enucleate spores with wall patterns resembling normal spores (Drahowzal, 1936; Heslop-Harrison, 1971 a, b) strongly suggests that the information for wall development resides in the cytoplasm and is independent of the haploid genome of normal spores or aneuhaploid nuclear fragments of microspores.

It should be noted, however, that there is good evidence to show that processes other than wall-pattern formation in developing spores of flowering plants are under the control of the haploid gametophyte and that this genetic independence results from the isolation conferred on the spores by the callose walls. Examples of haploid control of specific processes are found in the sugar-starchy segregation in pollen of *Zea mays* (Skvarla & Larson, 1966) and also the segregation of incompatibility substances in spores of *Oenothera organensis* in which a gametophytic system of S-gene alleles is functioning (Lewis, Burrage & Walls, 1967). Furthermore, cytochemical and immunofluorescence techniques have shown that the formation of antigens within the intine of pollen grains of many species takes place at a time in the early vacuolated period soon after the release of the spores from the meiotic tetrads (Knox & Heslop-Harrison, 1970; Knox, Heslop-Harrison & Reed, 1970).

If wall pattern is determined by the translation of genetic information in the diploid pollen mother cell nucleus it is of interest firstly to determine the time during the premeiotic interphase when this information passes from the nucleus to the cytoplasm and secondly to determine the nature of the cytoplasmic factors that are primarily responsible for pollen grain symmetry. Early light-microscope observations of Wodehouse (1935) and Drahowzal (1936) not only showed the high regularity of patterning but also the overall polarity revealed in the grain by the constant positions of pollen apertures (according to species). The positions of the apertures were seen to be spatially related to the planes of divisions of the 2 spindle axes constituting the second meiotic division. Later, ultrastructural observations of Heslop-Harrison (1963) and Echlin & Godwin (1968) have shown that the positions of grain apertures are determined early in the differentiation of the primexine by the close apposition of plates of endoplasmic reticulum to the plasmalemma at the sites of the future apertures, before the dissolution of the special callose wall surrounding the tetrads.

Although the siting of the apertures by ER places the problem one stage further back in the sequence of events, we still lack evidence as to the primary cause for the establishment of polarity and pattern in the pollen grain. If, however, there is a causal relationship between the symmetries in exine patterning and the disposition of spindle axes, then this is open to experimental alteration with the use of spindle inhibitors. It was shown by the work of Heslop-Harrison (1971 b) in which colchicine has been used to disturb the formation of the meiotic spindles in anthers of *Lilium* spp. that the pollen mother cells continue to develop as spores with respect to exine formation but that the cells fail to become polarized in that the aperture sites are either not defined or are distributed randomly. These results have been schematically summarized in Fig. 1 (taken with permission from Heslop-Harrison, 1971 b) to show that pollen mother cells, initially without polarization, are polarized following the establishment of the spindle axes and that the aperture sites in their turn are a reflexion of the tetrad cleavage planes.
In the experiments described below in which colchicine has been applied to developing anthers of hexaploid wheat, the resultant pollen abnormalities lead to the interpretation that wheat pollen symmetry is not a direct consequence of the disposition of the meiotic spindle axes but that both these events, the positioning of the spindles and the siting of the pores, are dependent on the postulated existence of self-replicating
cytoplasmic determinants. These determinants are established and polarized early in the premeiotic interphase of the pollen mother cells. At the same time the overall cell organization during this phase predetermines subsequent events, one of which is the pairing of the meiotic chromosomes (G. A. Dover, in preparation).

Observations on the tetrads and young pollen grains of \textit{T. aestivum} show that the 2 second meiotic divisions are in the same plane and that the longitudinal axes of the 2 spindles are parallel. Such an arrangement results in the tetrad being in the form of a planar ring. About 4 hours after the break-up of the tetrad the young pollen grains show the pores for the first time, whilst the shape of the grain reflects its former position within the tetrad ring. The constant position of the pore in relation to the walls allows for easy determination of the position of the pore in relation to the first and second axes. The pore is in a constant position adjacent to the pole of the spindle of the second meiotic division (Fig. 2). The simplicity of the single-pore system makes the wheat pollen grain suitable material for an investigation into the causal relationship responsible for pollen symmetry.

\textbf{METHODS}

All plants were grown in continuous light at 20 °C. Two millilitres of aqueous 0.5 \% or 0.01 \% colchicine were injected with a hypodermic syringe into the space enclosed by the leaf sheaths surrounding the immature flowering spikes. Tillers were sampled at daily intervals after the time of treatment. All anthers prior to the development of pollen were fixed in 1:3 acetic-ethanol, stained in basic fuchsin and mounted and post-stained in equal parts of 45 \% acetic acid and propionic orcein. Older anthers containing pollen grains were mounted and stained in acetic orcein.

\textit{Reference times in anther development}

An exact knowledge of the developmental stage of the meiocytes at the time of application was obtained by cytological examination of the ploid levels of the nuclei of the pollen mother cells and the tapetal cells. The duration of meiosis in hexaploid wheat var. Chinese Spring from the beginning of leptotene to the end of telophase II is about 24 h (Bennett, Chapman & Riley, 1971). The duration from the last premeiotic mitosis to the beginning of leptotene is about
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Time of application of 0.5% colchicine

Developmental stage of anther

Last pre-meiotic mitosis of P.M.C.

Penultimate mitosis in tapetal cells

Leptotene in P.M.C. and synchronous division in tapetal cells

Consequences of colchicine application

Metaphase I in P.M.C.

(a) 2N-binucleate
(b) 4-pore monad
(c) Unchanged

(a) 2N+4N
(b) 4-pore monad
(c) Unchanged

(a) 4N+8N
(b) Poreless monad
(c) Asynapsis

Fig. 3. Sequence of stages in the development of the archesporial cells and tapetal cells from the last premeiotic mitoses to metaphase I in T. aestivum. The time of application of 0.5% colchicine is indicated at 3 separate points in the sequence with the consequences of colchicine application noted for (a) ploid level of tapetal nuclei at metaphase I, (b) pollen type, and (c) degree of meiotic pairing.

48 h (Bennett & Smith, 1972). There is one division in tapetal cells sometime during the meiotic interphase and a synchronous division of tapetal nuclei occurs at the beginning of leptotene. With this knowledge it is possible to determine the earliest developmental stage affected by colchicine in a sampled anther. Reference to Fig. 3 shows the ploid levels observed in the tapetum at metaphase I at various intervals after the application of colchicine. Parallel observations have been made on the behaviour of the meiotic chromosomes during the various treatments.

The interpretations of the results have been made both from colchicine-induced errors and from pollen abnormalities that arose in hybrids between Triticum aestivum and the related diploids Aegilops mutica and A. longissima.

OBSERVATIONS

Colchicine-induced aberrations

By varying the concentration of colchicine injected into the tillers, situations were created in the pollen mother cells in which either (1) all meiotic divisions had been prevented so that one tetraploid mononucleate monad resulted, or (2) multipolar divisions occurred such that the nucleus of the pollen mother cell divided into a number of separate micronuclei.

Uninucleate monads. When 0.5% colchicine was applied to immature tillers there was a complete failure of segregation of homologous chromosomes during first meta-
phase; and in a similar manner segregation of sister chromatids was prevented at second metaphase so that one tetraploid restitution nucleus was formed in place of the 4 haploid nuclei of the tetrad. The tetraploid monads further differentiated into large tetraploid pollen grains each with 4 pores that were randomly distributed over the surface (Fig. 4). Four-pored uninucleate monads (Fig. 4) were obtained when colchicine was applied during the period between the penultimate division of the tapetum (see Fig. 3) and first metaphase of the pollen mother cells.

Polyads. With more dilute colchicine (0.01 %) the normal bipolar spindle of the first meiotic division was replaced by a multipolar spindle, and the metaphase I chromosomes moved randomly to each pole. There was no reduction division between pairs of homologous chromosomes but instead the aggregation of chromosomes around the poles often resulted in both members of a homologous pair being present at the same pole. Each chromosome then underwent a normal bipolar division, with a separation of sister chromatids, the result being a multinucleate polyad made up of several pairs of cells (Figs. 5, 6). The polyads further differentiated into multicelled pollen grains in which a pore developed at the polar extremity of each cell (Fig. 7).

Multipored uninucleate monads. Many of the anthers sampled after treatment with 0.01 % colchicine contained not only multipored polyads but also large multipored (i.e. more than 4) uninucleate grains (Figs. 8, 9).

Poreless pollen. When 0.5 % colchicine was applied to anthers at a time deduced from the ploid levels of the tapetum to lie between the last premeiotic mitosis of the pollen mother cells and the penultimate division of the tapetum (Fig. 3), the resulting monads developed into apparently fully developed pollen grains but without pores (Fig. 10). The stage of development just after the last premeiotic mitosis, at which poreless pollen can be induced, is also the stage at which colchicine-induced asynapsis of chromosomes at metaphase I can be induced by colchicine treatment (G. A. Dover, in preparation). The significance of this early phase during the premeiotic interphase, in which both the pairing of meiotic chromosomes and the symmetry of pollen grains appear to be predetermined, is brought out in the Discussion.

Aberrant pollen grains in wheat/Aegilops hybrids

Quadrinucleate 4-pored pollen. Plants of Triticum aestivum var. Chinese Spring that contained alien chromosomes of Ae. mutica as additions to the wheat chromosome complement, frequently gave rise to pollen grains in which there was defective development of the tetrad cross-walls although the quadrinucleate condition of the pollen indicated that there had been orderly functioning of the 2 second-division spindles. In this instance the 4 pores of the grain showed a regularity of distribution that was an exact reflection of the positioning of the products of the nuclear divisions within the monad (Fig. 11).

Pollen having varying numbers of micronuclei. Hybrids between Ae. mutica and T. aestivum showed meiotic irregularities and there was no longer a clean segregation and separation of 4 haploid genomes because of irregular chromosome pairing. Many laggard univalents at anaphase I and II appeared as micronuclei in the pollen. Often a micronucleus was sufficiently distant from the macronucleus during the first division
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to divide bi-polarly at the second division, eventually leading to the formation of micrograins each complete with a pore. At the same time many grains developed that contained the macronucleus along with varying numbers of micronuclei that had divided at the first meiotic division. Such pollen grains have 1, 2 or 3 pores (Fig. 12). No correlation has been found between the number of micronuclei in a grain and the number of pores.

Other aberrant grain types from hybrids between T. aestivum and Ae. longissima. Fig. 13 shows one macrograin adjacent to a fully developed micrograin complete with pore. To the left of the other macrograin there has been incomplete segregation of the protruding portion of the wall and no pore is visible.

Fig. 14 is a 'dumbbell' pollen grain in which 2 nuclei are present in one half, which has a pore; the other, anucleate, half contained cytoplasm but no pore.

In the unusually elongated pollen grain in Fig. 15 the axis of the first division was in the direction of the longitudinal axis of the grain. The 2 axes of the second division can be deduced (first on the right of the grain) by the cytoplasmic continuity that exists (not visible in the plane of focus) and (secondly on the left) by the small corresponding lock-and-key wall segments between the 2 grains on the left. There are pores corresponding to all the 4 pole sections of the original tetrad, but not in the middle section isolated by 2 cross-walls from the rest of the cell. Such a poreless area fell between 2 poles.

DISCUSSION

Observations on young pollen grains indicate that the siting of the pore is constant in relation to the positions of the spindle axes of the second meiotic division (Fig. 2). The constancy of this relationship has long been known from the work of Drahowzal (1936) and Wodehouse (1935); although it is only recently that a common causal relationship between the positions of the spindle axes and pollen symmetry has been established experimentally in Lilium spp. (Heslop-Harrison, 1971b). A further indication of the precision of this relationship is seen in quadrinucleate grains (Fig. 11) in which the 4 pores exactly overlie the 4 nuclei of the monad. Other aberrant grain types from hybrid wheat/Aegilops genotypes also seem to substantiate a causal relationship between the disposition of the spindles and the siting of the pores. Fig. 13 shows a fully developed micrograin, arising presumably as a result of a normally functioning spindle, complete with a pore; whereas in the other pollen grain in which there has been incomplete separation of the protruding portion of the wall, no pore is visible. The ‘dumbbell’ grain of Fig. 14 has a pore only in the half containing the 2 nuclei, the presence of only a single pore presumably resulting from a malfunctioning spindle that has also prevented a complete separation of the 2 nuclei to both halves of the dyad. The poreless enucleate half of the grain would indicate that there has not been a regular spindle at this point. Fig. 15 seems to indicate that pores developed only at the polar ends of the second division spindles and not in parts of the aberrant grain wall that were removed from the polar regions, as for example the middle poleless section.

Such observations on normal and aberrant pollen types also seem to rule out the possibility that the control of pollen wall differentiation depends on gene action in the
haploid spore. Moreover, they indicate that the governing factors cannot be dispersed uniformly throughout the cytoplasm (Rogers & Harris, 1969). The nuclei of many micrograins can consist of single chromosomes and it would be theoretically difficult to postulate genetic messages independently capable of specifying pore development on many chromosomes of the complement. Similarly, there is no correlation between the number of pores and the number of micronuclei in any one grain (Fig. 12). At the same time many of the aberrant pollen formations show that protruding wall sections containing cytoplasm do not form pores (Figs. 13-15). This evidence is not in agreement with the notion that there might be a long-lived message diffuse in the cytoplasm. However, it is consistent with the existence of an extranuclear pore-determining factor which is discrete and is divisible in a way that correlates with the number of spindle poles.

Is it possible, however, to be able to conclude that a direct causal relationship exists between the disposition of the spindle axes and the siting of the pores?

The pollen grain types obtained from anthers treated with colchicine seem to indicate an indirect relationship. Four types of pollen abnormalities were induced with colchicine depending on the developmental stage of the archesporium at the time of application and the concentration used. They arose as follows: (1) using 0.5% colchicine applied immediately prior to meiotic prophase – monad pollen grains are obtained with 4 randomly placed pores (Fig. 3); (2) using 0.5% colchicine applied at a time between the end of the last premeiotic mitosis of the pollen mother cells and the penultimate division of the tapetum – poreless monads developed (Fig. 10); and (3), (4) 0.01% colchicine applied any time between the last premeiotic mitosis of the pollen mother cell and meiotic prophase – 2 types of grain abnormalities in the same anthers, multipore polyads (Figs. 5–7) and multipore monads (Figs. 8, 9).

The appearance of 4 randomly positioned pores in uninucleate monads, in which spindle formation has been completely inhibited by colchicine, suggests that the number of pores is related to the potential number of spindle poles but that the spindles need not be formed. Disruption of spindle formation thus did not influence the number of pores but removed the normal restrictions on their positioning. The proliferation of pores following dilute colchicine treatment is not dependent on the concomitant development of a multipolar spindle. The increase in the pore number may be associated with an increase in spindle-pole determinants without the formation of multipolar spindles. If the determination of a pore and the establishment of a pole of a spindle axis depended upon a common cause the development would be explained both of 4-pore uninucleate monads with 0.5% colchicine and multipore uninucleate monads with 0.01% colchicine.

There is very little evidence on pole-determining factors in the cells of higher plants although the search has been extensive (for review see Luykx, 1970). Paraffin-sectioned anthers of wheat show that the first division spindles of pollen mother cells are mostly parallel to the long axis of the anther and that the second division spindles are transverse to this. The spindles of the premeiotic mitoses are variously oriented. At some point, therefore, in the development of the anthers of wheat an overall polarity appears to be laid down which predetermines the orientation of the spindle axes and the
positioning of the pollen pores. After the establishment of polarity, despite the prevention of spindle formation by colchicine, because pore number has been determined already, 4-pore monads develop. However, the appearance of pollen monads without pores when colchicine is applied between the last mitosis and the onset of meiosis suggests prevention of the establishment of polarity. Other events that would normally occur in meiosis are also disrupted by this treatment, particularly the pattern of meiotic chromosome pairing (G. A. Dover, in preparation). However, in Lilium spp. and in Compositae, having a massive archesporium, no premeiotic polarization of pollen mother cells has been observed (J. Heslop-Harrison, personal communication) corresponding to the premeiotic determination of polarity observed in wheat and its relatives. The long premeiotic interphase in wheat anthers may, therefore, be of critical importance for the release of genetic information that is responsible for the control of subsequent development.

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REFERENCES


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Figs. 4–10. Effects of 0·5 %, or 0·01 % colchicine applied to developing anthers of _T. aestivum_ var. Chinese Spring.

Fig. 4. Uninucleate monads of _T. aestivum_ with 4 randomly positioned pores (after treatment with 0·5 % colchicine). × 400 approx.

Fig. 5. Bipolar second metaphase divisions in nuclear fragments originating from a prior multipolar first metaphase division in pollen mother cells of _T. aestivum_ (after treatment with 0·01 % colchicine). × 1000 approx.

Fig. 6. Polyad showing cells in pairs as a result of second anaphase bipolar division following multipolar first division, cf. Fig. 5. 0·01 % colchicine. × 400 approx.

Fig. 7. Multicelled pollen grains with cells in pairs and pores at polar extremities (not all visible). 0·01 % colchicine. × 400 approx.

Fig. 8. Multipored uninucleate monad. 0·01 % colchicine. × 1000 approx.

Fig. 9. Multipored monads together with polyad. 0·01 % colchicine. × 400 approx.
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Fig. 10. Poreless monads. 0.5% colchicine. x 400 approx.

Figs. 11–15. Aberrant pollen types from wheat/Aegilops hybrid genotypes.

Fig. 11. Quadrinucleate monad with 4 pores overlying the 4 nuclei in plant of T. aestivum having one additional chromosome derived from A. mutica. x 400 approx.

Fig. 12. One-, 2- and 3-pored pollen in amphiploid of T. aestivum × A. mutica. x 400 approx.

Fig. 13. Micrograins and normal pollen grains in T. aestivum × A. sharonensis hybrids. Micrograin to the right is fully separated from the adjacent macrograin and is complete with a pore. Macrograin on the left has a protruding wall section not fully separated and without a pore. x 400 approx.

Fig. 14. 'Dumbbell' pollen grain in T. aestivum × A. sharonensis. The right-hand half contains both nuclei and has a single pore (not visible in plane of focus). Left-hand half, containing cytoplasm, is enucleate and poreless. x 1000 approx.

Fig. 15. Aberrant pollen grains from T. aestivum × A. sharonensis. All 4 segments derived from original tetrad have pores (see text). Middle section of elongated grain is poreless. x 400 approx.
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