Control of Pollen Fertility through the Agency of the Light Regime in the Grass *Dichanthium aristatum*

By

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With 16 Figures

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Introduction

In a previous paper (Knox & Heslop-Harrison 1963) we have described the cytological mechanism of apomixis in *Dichanthium aristatum* (Poir.) C. E. Hubbard (*Andropogoneae*), and given evidence to show that the incidence of apomixis may be controlled by the light regime in which plants are grown. In the course of these experiments it became clear that the photoperiodic treatments given not only affected the apomictic system, but also modified pollen fertility in a systematic way. The present paper provides an account of these effects on pollen formation.

Materials and Methods

Plants of a tetraploid race of *D. aristatum* (2n = 40) of Australian origin (C. S. I. R. O. Accession No. 14366) were grown in a peat-sand mixture. A standard ration of a nutrient solution was provided every third day, and the plants were watered to run-off on intervening days. The experiment was conducted in growth chambers with air temperature regulated above 22° C. Long-day (LD) treatment was provided by exposing the plants to natural daylight supplemented with incandescent light at c. 100 f. c. to give a day-length exceeding 16 hours. During short-day (SD) treatment they were illuminated by batteries of warm-white fluorescent tubes, giving an intensity of 900—1000 f. c., for periods of 8 hours daily.

Material for cytological study was collected as previously described (Knox & Heslop-Harrison 1963). Florets were fixed in acetic alcohol or Langlet's modification of Navashin's solution. After wax-embedding and serial sectioning, tissues were stained in standard cytological stains, in-

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cluding methyl-green pyronin. The bulk of the basiphilic material in the cytoplasm of meiocytes and tapetal cells is ribonuclease digestible, and in these tissues intensity of pyronin staining under standard conditions provides a good guide to amounts of cytoplasmic RNA present in free or membrane-attached ribosomes.

Experimental Observations

Photoperiod and reproductive behaviour. — The race of *D. aristatum* employed remains vegetative indefinitely under long day conditions, flowering being dependent on exposure to days of less than 12 hours illumination. In the experiment, plants were grown to an age of 135 days under LD, and then sorted into groups of four, each matched for stature, tillering and number of leaves. Groups were then exposed to 5, 10, 20, 40 and 60 SD before restoration to LD, while a further group was retained continuously on SD. Only those plants receiving more than 40 SD produced flowers, indicating that under these conditions the minimum induction period lies between 20 and 40 SD.

Under continuous SD and with 80 SD, the minimum period to flower was 72 days from the beginning of induction, and with 40 SD, 85 days. The distribution of inflorescences in the three flowering groups is given in Fig. 1. Under continuous SD, inflorescences were initiated almost throughout the plant, from the terminal node down to the 12th below it. In the minimally induced group, flowering was restricted practically entirely to the terminal shoot and the two nodes below it, while an intermediate type of distribution appeared in the group receiving 60 SD.

The amount of SD induction experienced affected also the structure of the inflorescences. Under continuous SD, only the terminal inflorescences produced lateral branches; the remainder, down to the 12th node below, were simple. Both the 40 SD and 60 SD group inflorescences customarily carried one or two lateral branches throughout the flowering zones.

Male fertility. — Fertile anthers in *D. aristatum* are recognisable by their plump appearance and purple colour; sterility in varying degrees is indicated by yellowness or greenness, coupled with reduced size or distinct shrivelling. At approximately thirty-day intervals from the onset of flowering in the continuous SD group, records of the state of the anthers in all inflorescences in flower at the time of observation were taken. The results, expressed as percentage fertile anthers, are given in Fig. 2.

The differences revealed are striking. With the minimal induction treatment of 40 SD, the first emergent inflorescences were at least partly male fertile, and complete fertility was attained in a further 60—70 days under LD conditions. With an induction period of 60 days, the first differentiated inflorescences were highly sterile, and with increasing LD exposure fertility progressively increased but never to better than 70%. With

continuous SD treatment, which produced the highest level of florifery, anther sterility was almost complete throughout the experiment.

Individual inflorescences revealing partial sterility showed a characteristic pattern, the fertile florets being restricted to the base; in all cases it

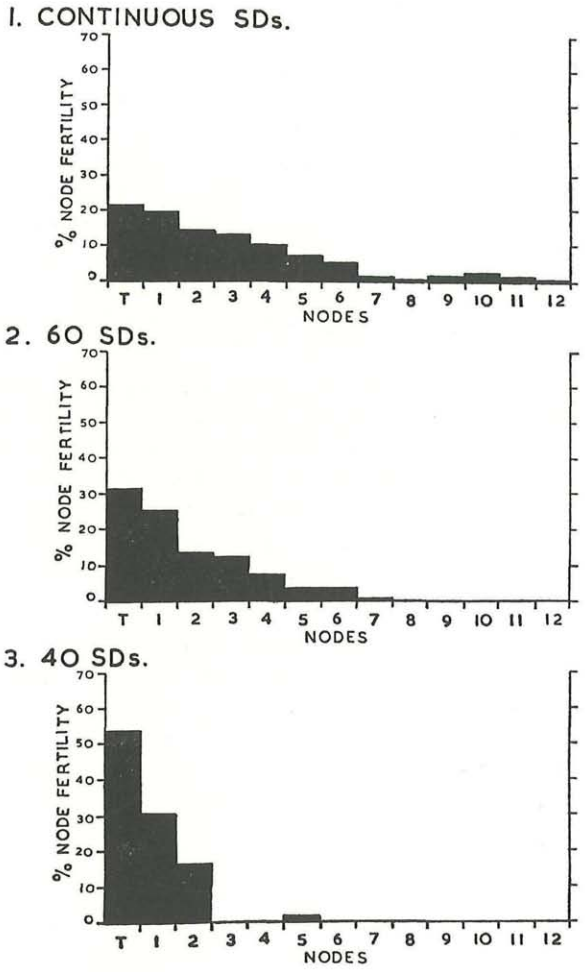


Fig. 1. Distribution of inflorescences in three groups each of four plants flowering after 40 short days, 60 short days and in continuous short days. Percentage per node counted basipetally from the terminal inflorescence (T).

is evident that the apical parts of the inflorescence are most susceptible to conditions inducing male sterility. The small amount of fertility observed at the second scoring of the continuous SD plants was accounted for by

fertile anthers formed at the base of the first lateral inflorescence emergent below the terminal one.

In general, it seems that inflorescences initiated during or immediately after SD induction are likely to be male sterile to a greater or lesser degree. Fertility seems contingent on development under long day conditions, although it is noteworthy that complete fertility is not necessarily attained, even within a six month LD period, where induction has been protracted, as in the 60-SD group.

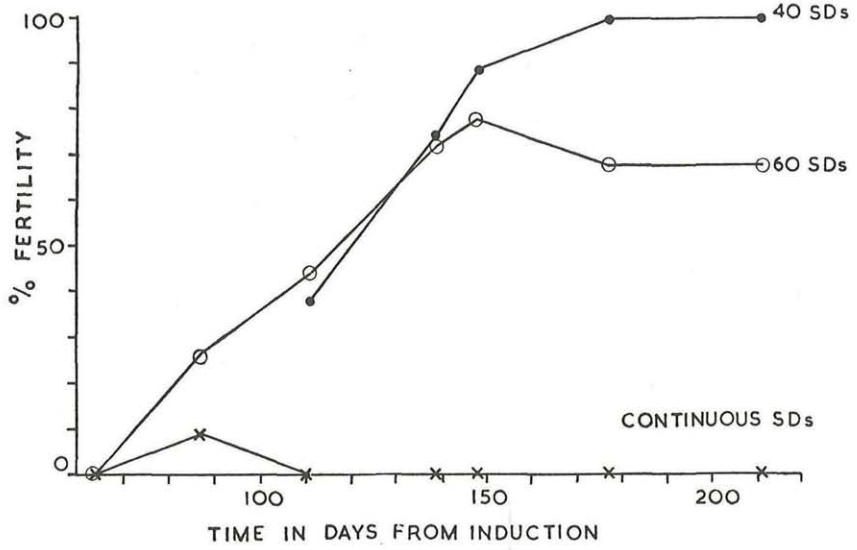


Fig. 2. The effects of varying periods of short-day treatment on male fertility. Data obtained from records made at approximately 30 day intervals during the flowering period. „% Fertility" refers to the percentage of fertile anthers in all the inflorescences in flower at the time of each observation. Time is from onset of SD induction.

Pollen Development

The normal pathway. — Development in the fertile anther follows the standard pattern of the grasses (Arber 1934; Maheshwari 1950). Four distinct cell layers are normally present in the anther wall: the epidermis, the underlying endothecium, the middle layer, and the innermost layer which differentiates as the tapetum. Four, five, or occasionally six ranks of archesporial cells are established by the final divisions preceding meiosis.

The events in the archesporium and the enveloping tapetum are of primary importance in tracing the basis of pollen sterility. To establish criteria whereby the incidence of abnormality could be recognised, the normal developmental sequences in the sporogenous tissue and the tapetum

were arbitrarily subdivided into phases according to readily identifiable cytological events, and a survey was made of all the anther loculi of fertile florets of 12 sample inflorescences to establish the correlation between these phases in the two tissues.

The sporocyte sequence was as follows:

PMC. Pollen mother cells: after the final mitotic divisions in the archesporial tissue, but before the onset of the meiotic prophase.

M1. Meiotic prophase to telophase I.

M2. Interphase I to tetrad.

1. Rounding of microspores to break-up of tetrad. The cytoplasm is dense and non-vacuolate, and exine growth has not begun.
2. Beginning of exine growth. Microspores vacuolated, with the nucleus occupying a peripheral position.
3. Exine growth complete, and pollen wall conspicuously furrowed; vacuole still present.
4. Vacuole eliminated; cytoplasm increasing in basiphilia. Nucleus central, in late interphase, or undergoing pollen mitosis.
5. Pollen grains mature. Cytoplasm dense, non-vacuolated, with tube nucleus and paired generative nuclei.

The tapetal sequence was as follows:

I. Early phase; cells isodiametric, little cytoplasmic basiphilia.

II. Cells enlarging longitudinally but still quadrate in transverse section; cytoplasm finely granular with abundant basiphilia. Tapetal mitoses producing binucleate cells.

III. Cells binucleate, and now flattened in section due to lateral expansion as anther volume increases. Cytoplasm dense and heavily basiphilic.

IV. Phase of degeneration. Cell contents shrunken and usually clumped against the anther wall.

V. Dissolution complete except for a residue adherent on the inner face of the endothecium.

Although comprehensive fine-structural studies have not been made on the anther of *D. aristatum*, enough observations are available to permit the tapetal events to be interpreted in general terms and related to those established for other species (e. g. *Cannabis sativa* and *Silene pendula*, Heslop-Harrison 1962).

As mentioned above, the basiphilia observed light-microscopically is ribonuclease digestible, and its presence can be associated with the generation during normal tapetal development of a copious ribosomal endoplasmic reticulum. This reaches its maximum at a time when the tapetal cells are binucleate, and when presumably they are at the peak of metabolic activity. At this time exine material is formed. In the subsequent period of dissolution, organelles and metabolic products are released into the loculi of the anther, the nuclei totally degenerate, and the residual cytoplasm collapses irregularly against the inner face of the anther wall.

The correlation between the phases of microsporogenesis and tapetal development and degeneration in the fertile anthers examined is shown in Fig. 3. Broadly, the peak of tapetal activity as expressed in proliferation of ribosomes and development of organelles is reached during the second meiotic division and the very early life of the spores. Rapid growth of the

Tapetum class								
V	—	—	—	—	—	—	168	240
IV	—	—	—	—	245	156	36	—
III	—	—	12	20	9	—	—	—
II	—	60	10	—	—	—	—	—
I	44	—	—	—	—	—	—	—
	PMC	M1	M2	1	2	3	4	5
				Sporocyte sequence				

Fig. 3. Correlation between stages of pollen development and tapetal class in fertile anther loculi from twelve inflorescences sampled in the middle of a flowering period.

pollen is correlated with the onset of tapetal degeneration, and when the spores are finally filled with a dense cytoplasmic contents after the intermediate period of growth with vacuolation, the tapetum is wholly resorbed.

Cytological basis of sterility. — The principal difficulty in attempting to establish the cytological basis of induced male sterility is the obvious one that individual developmental sequences cannot be followed when observations have to be made on fixed and sectioned material. If there were only one type of deviation the problem would be more straightforward, but it is quite certain that pollen failure can arise in several different ways, and is not simply the product of one specifiable developmental defect. Thus there is no doubt that time-related variation occurs even within single inflorescences which reveal, macroscopically, the same general kind of sterility in all florets. Abnormality may become evident at almost any stage from the pre-meiotic onwards to the almost mature pollen, so that at the time of anthesis the tissues of different sterile anthers present a variety of appearances. Moreover, sterility is not merely the outcome of the arrestation of normal development at different points of time. Rather does it appear that there are several deviant pathways of development, characterised each by a syndrome of abnormalities and a particular type of final product.

Since piecing together the probable pathways leading to pollen failure from the evidence of fixed stages must be an exercise in deduction, there are obvious opportunities for error. A knowledge of the normal sequence and of the correlations to be expected between the key tissues — tapetum and sporocytes — does, however, make it possible to detect deviations in younger anthers which would probably have lead to sterility. Moreover, with abundant material available for sampling spanning the whole time

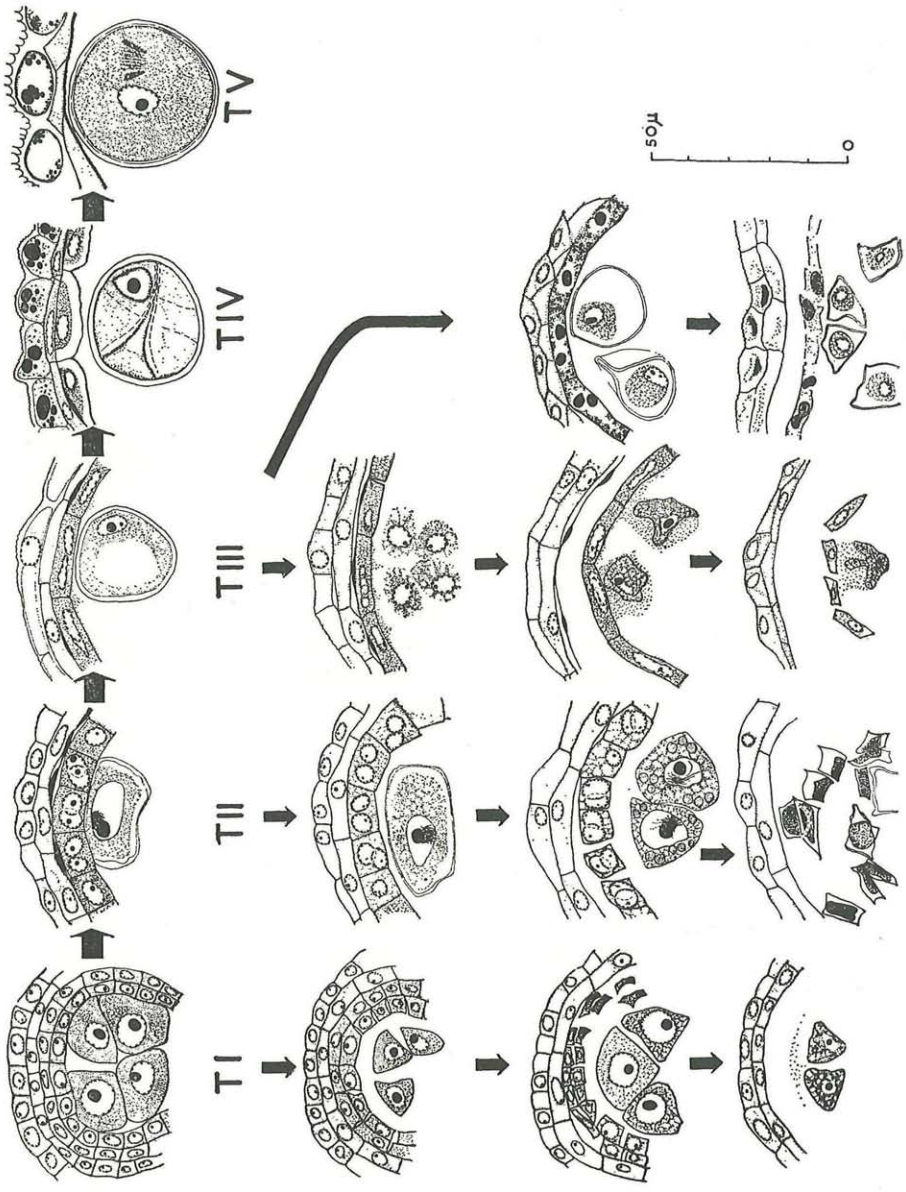
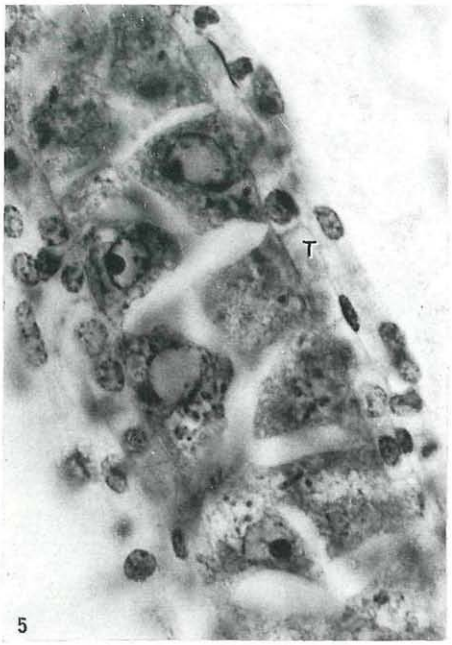
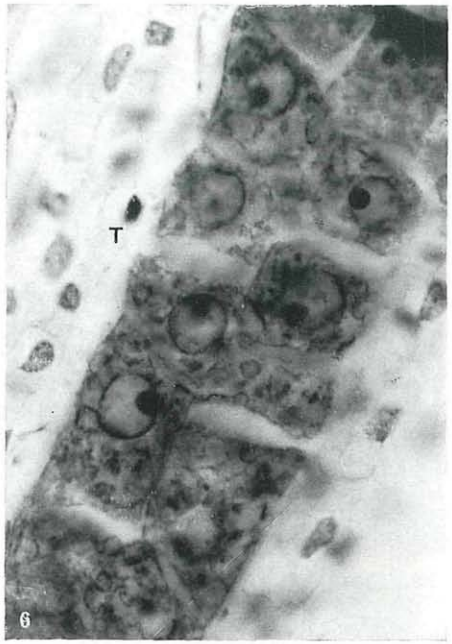
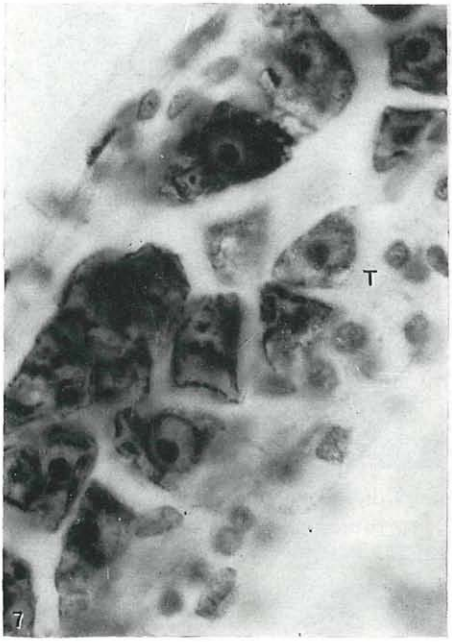
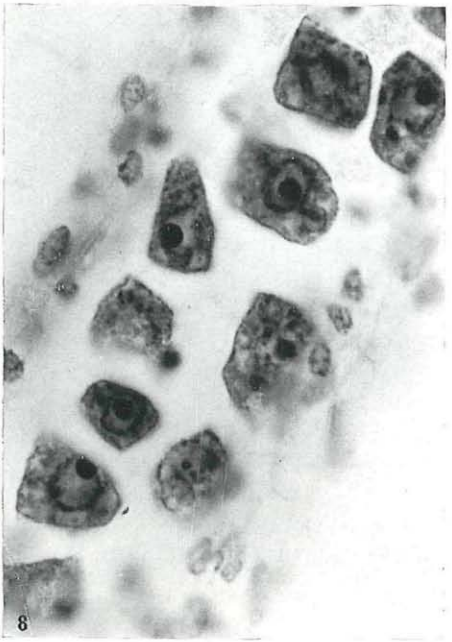


Fig. 4. Diagrammatic summary of the main aberrant behaviour patterns. TI-TV refer to the tapetal classes in the normal sequence at the top of the diagram. The arrowed sequences refer to the pathways A—D described in the text, reading from left to right.









For explanation see tab. 18.

course of development it has been feasible to attempt the task of distinguishing the principal pathways, since by no means all the conceivable kinds of aberration have been encountered.

The major aberrant behaviour patterns are summarised in Fig. 4, where they are related to the tapetal phases defined above. Representative examples of the various conditions are illustrated in the photomicrographs, Figs. 5—16. The different pathways may be briefly characterised as follows:

A. A deviation from normal becomes apparent when the tapetum is in phase I. Vacuolation begins while the cells are still quadrate in transverse section, and the cytoplasm does not become basiphilic (Fig. 5). Ultimately the tapetal nuclei are left in essentially empty cells (Fig. 6). Degeneration of the PMCs occurs more gradually. Vacuolation begins later in the meiocytes than in the tapetal cells, and the vacuoles formed are small, giving the cytoplasm a characteristic alveolar appearance. As the vacuoles enlarge, the cytoplasm aggregates on the wall and becomes pycnotic (Figs. 7 and 8). Ultimately the PMCs may collapse entirely. In this sequence meiosis fails altogether, the meiocyte nuclei advancing at the most no further than early prophase.

B. Departure from the normal occurs after the degeneration of the middle layer of the anther wall, when most of the tapetal cells are binucleate. In the fertile anther the tapetal cytoplasm would be finely granular and highly basiphilic; in anthers showing incipient sterility the cytoplasm has little basiphilia and assumes a coarsely alveolar appearance (Fig. 9). The condition is frequently accompanied by a tendency for the cells to separate along their radial walls, or to become detached in sheets from the anther wall. The actual detachment may be a consequence of cytological processing, but the readiness with which it occurs is a symptom of the condition. The cytoplasm of the tapetal cells becomes increasingly vacuolate, and basiphilia is lost, until finally there is a complete degeneration into a pycnotic, amorphous mass.

Again, meiocytic degeneration is more gradual than that of the tapetum. Most frequently, abnormality becomes apparent in late prophase of the first meiotic division, but it may be delayed until the completion of meiosis II. The cytoplasm becomes coarsely alveolar with prominent densely staining aggregations. These become very distinctive when the tapetal cells have reached maximum vacuolation (Fig. 9). The meiocytes then lose the appearance of organised cells; the nuclear envelope is no longer evident; the cytoplasm is an amorphous network of dark granules, and only the nucleoli may remain distinguishable (Fig. 10). The cell walls break down, and ultimately the contents of the anther loculi caolesce into a dark-staining disorganised mass, surrounded by the similar tapetal remnants (Fig. 11).

C. The tapetal cells progress beyond the binucleate stage, extending tangentially and becoming highly basiphilic before abnormality is manifest.

Then the cytoplasm becomes coarsely alveolar, and the cells separate along their radial walls (Fig. 12). They may remain attached to the anther wall initially, or may rapidly become detached in sheets. Vacuolation begins, and the staining capacity of both cytoplasm and nuclei declines. Ultimately all the tapetal cells become detached, and lie scattered among the degenerating microspores before final dissolution.

Meiosis is completed normally, and the microspores are released from the tetrad. Walls may or may not be formed. Where none are produced, the spores are highly irregular in form; they become finely vacuolated, the cytoplasm loses its basiphilia and degeneration follows (Fig. 13). Where walls are formed, the spores become highly vacuolated, with a sparse peripheral cytoplasm. Turgidity is then lost, the cytoplasm collapses in the centre of the grains and the walls become highly convoluted (Fig. 14). After the final dissolution of residual cytoplasm in the loculus, the empty spore walls remain as conspicuous evidence of the lateness of developmental failure.

D. In this pathway, tapetal development progresses as in **C** to a stage equivalent to III in the normal sequence (Fig. 4), but thereafter the behaviour is unique. The nuclei become densely staining, and ultimately those in each cell coalesce into an opaque central mass (Fig. 15). The cytoplasm first forms large pycnotic aggregates, and then is digested to leave no more than tenuous strands supporting the persistent dark-staining nuclear mass (Fig. 16). The cells of the tapetum do not separate radially; rather does the whole tapetal layer contract towards the centre of the loculus.

Again meiosis is completed, and the microspores are released from the tetrad. The subsequent failure of pollen formation follows the patterns described under **C**.

It is possible that sterility may arise in other ways than those outlined in **A** to **D**, and there is no guarantee that the sequences are exactly as described. However, the great bulk of the observed aberrations can be fitted into one or other of these patterns, and with the unambiguous criteria available for judging the points at which departure from normal has occurred it is difficult to see that the interpretations can be grossly in error.

Discussion

Light regime and male sterility. — *Dichanthium aristatum* may be added to the substantial list of species in which it has been shown that pollen fertility, and hence functional sex, can be affected by daylength (Heslop-Harrison 1957). In so far as the trend is towards a depression of male function in conditions promoting early and abundant flowering, it is closely comparable with that known in several other short-day plants. The depression of pollen fertility is for example essentially similar to that demonstrated in precociously flowering *Rottboellia exaltata* (Heslop-

HARRISON 1959), a grass of the *Andropogoneae* with photoperiodic responses of the same kind as *D. aristatum*.

The parallel with the behaviour of *Zea mays* is also very close. Although maize will flower in a wide range of day-lengths, its development is hastened by short days, and, after the onset of flowering, continued exposure to short days during the early differentiation of the normally wholly-staminate terminal inflorescence induces a greater or lesser degree of pollen sterility (HESLOP-HARRISON 1961). In the experiment with *D. aristatum* reported here, it has not been demonstrated unequivocally that the effect on male fertility is photoperiodic in the strict sense, since the groups of plants compared received different total amounts of light energy, and this would undoubtedly affect their nutritional status. However, it may be noted that in an experiment with maize it has been shown that the incidence of pollen sterility is lower in short-day grown plants when the "night" is interrupted by a period of low-intensity illumination, indicating that the response is of a typical photoperiodic type (MOSS, unpublished). It seems likely, then, that the very specific effect in *D. aristatum* will prove not to be due simply to general differences in carbohydrate nutrition, but also to be photoperiodic.

Cytology of pollen sterility. — So far as we are aware there has been no previous attempt to define in detail the cytological pathways concerned in pollen sterility induced by abnormal light regimes. Various stages of degeneration in the sporocytes and neighbouring tissues have, however, been described and illustrated by HOWLETT 1936 (*Lycopersicum esculentum*), NIELSEN 1942 (*Glycine soya* cultivar Biloxi), MADSEN 1947 (*Cosmos sulphureus* cultivar Klondike), and J. & Y. HESLOP-HARRISON 1957 (*Silene pendula*).

In all these cases it is apparent that pollen failure follows as a consequence of abnormality in tapetal function, and this seems true also in many investigated examples of heritable pollen sterility, genic and cytoplasmic. In most of the male-sterile mutants of *Lycopersicum esculentum* described by RICK 1948, differentiation and function of the tapetum were highly abnormal. The different mutants were characterised, within limits, by different patterns of malfunction, involving mostly a delaying or blocking of the normal developmental sequence. Similar phenomena were described by CHILDERS 1952 in male-sterile races of *Medicago sativa*. In some races of *M. sativa*, however, sterility seemed to arise from hypertrophy of the tapetal cells, which usurped the greater part of the anther loculi.

Close parallels to the aberrations observed in *D. aristatum* may also be found in the "cytoplasmic male sterile" and "semi-male sterile" types of *Beta vulgaris* described by ARTSCHWAGER 1947. In the semi-male sterile type sterility is not complete, and is seemingly influenced to some extent by environment. Again, different patterns of abnormal development were identified, including one in which the tapetum showed rapid early growth

leading to the destruction of the microspores, as in the example described by CHILDERS 1952 in *Medicago sativa*.

A fuller understanding of the basis of pollen sterility in cases such as these must await a proper elucidation of tapetal function in normal development. That the tapetum does in fact act as a nurse tissue for the meiocytes and developing pollen is beyond doubt, but it is also clear that it is responsible for several different syntheses, some simultaneous and some following each other sequentially, all of which, presumably, must be effective for pollen formation to proceed normally. The efficient transfer of tapetal products to the meiocytes is also obviously a sine qua non of pollen development. What has been described in this paper are the grosser manifestations of some types of break-down; histochemical and tracer experiments are now required to pin-point more accurately where the basic failures occur. With this information it may be possible to proceed further and elucidate how photoperiod, acting presumably through the general hormonal and nutritional milieu of the plant, modulates tapetal activity and so governs pollen fertility.

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Summary

Dichanthium aristatum (POIR.) C. E. HUBBARD, a grass of the *Andropogoneae*, previously shown to have an aposporus apomictic system susceptible to modification by the light regime, varies in pollen fertility according to photoperiodic experience.

With exposure to 40 short days at an age of 135 days, the first emergent inflorescences were at least partly male fertile, and complete fertility was attained in a further 60—70 days under long day conditions. With continuous short-day treatment from an age of 135 days, anther sterility was almost total.

An investigation of the cytological basis of the induced pollen sterility has revealed four pathways of aberrant development. These differ in the times at which deviation from the normal begins and in the kind of end-product. In all of the identified pathways the first indications of abnormality are seen in the tapetum, and it is concluded that the induced sterility is uniformly a consequence of tapetal malfunction.

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