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The phase change in ferns

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Abstract

It is argued that the phasic alternation of sporophyte and gametophyte in ferns involves changing gene activation during oogenesis and sporogenesis respectively. The reasons for the change in activation are to be sought in the nutritional states of the cells in which the transition takes place. The readiness with which apospory can be induced in ferns has enabled the features of meiosis which are essential for the switch in the form of growth to be distinguished from those which are related to the distinctive behaviour of the chromosomes. The dramatic changes in the cytoplasm of meiotic cells seem to have little morphogenetic significance.

In Lublin it is appropriate to begin a talk about the life cycle of archegoniate plants with respectful mention of Leszczyc-Sumiński. Over a hundred years ago, working with Münter in Greifswald, he was the first to recognize the sexual nature of the fern gametophyte and to see the entry of spermatozoids into archegonia. His dissertation, published in Berlin in 1848, caused considerable sensation, even disbelief. His observations, which were basically correct, were dismissed by Schleiden as fantasies attributable to bad preparations and a poor microscope. They were nevertheless soon confirmed and contributed substantially to the great work of Hofmeister in the succeeding decade.

Although the term 'alternation of generations' (which is not an accurate translation of 'Generationswechsel') is still often used to describe the life cycle of the land plants, it is more profitable to think in terms of a phasic alternation of gametophyte and sporophyte. The use of the term 'generation' suggests the necessity of some sexual or meiotic process to generate a new form of growth, whereas in fact the transition from one phase to another can often take place independent of such nuclear phenomenon. Examples are provided by the apomictic angiosperms and the apogamous ferns. The well-defined gametophytic phase, and the ability to grow both phases of the cycle in pure culture makes the ferns particularly suitable material for the experimental study of

the life cycle. It can be confidently expected that the final elucidation of what causes a gametophyte to yield a sporophyte, and a sporophyte a gametophyte, will come from the study of these plants. They provide a clear-cut example of the cyclic alternation of these two phases of growth. In the seed plants the situation is obscured by the complications of heterospory and the reduction of the gametophytes to little more than heterotrophic gametangia.

PHASE CHANGE FROM GAMETOPHYTE TO SPOROPHYTE

The normal sexual transition from gametophyte to sporophyte follows fertilization, and it is now clear that preparation for this change occurs well before the female gamete is produced (B e l l, 1979a). In ferns with cordate gametophytes (the relatively few ferns with other forms of gametophyte have not yet been studied) the archegonia are produced in a well defined region behind the apical growing point. They therefore arise at a site which is bathed in nutrients flowing to the meristem, and which is also directly in line with growth-regulating substances returning from it.

The general cytology and development of the archegonia are well known and appear to be uniform throughout the homosporous ferns. Oogenesis can be regarded as beginning in the primary cell. The volume of this cell and of its nucleus both increase about five-fold, and this growth is accompanied by substantial synthesis of ribonucleic acid and protein (Fig. 1). Vacuoles are eliminated and the cytoplasm becomes

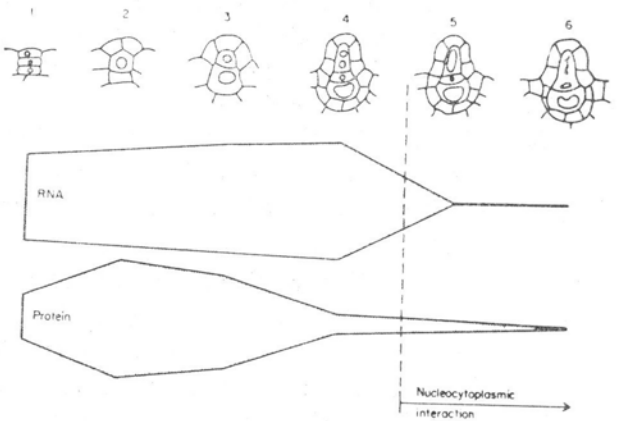


Fig. 1. A comparison of the amounts of ribonucleic acid and protein synthesis during oogenesis in *Pteridium*

1, 2 — growth of the primary cell; 3 — formation of the central cell; 4-6 — formation and maturation of the egg.

Based on pulse-labelling and quantitative autoradiography. (From C a v e, B e l l, 1974)

dense with numerous ribosomes. Much of the cytoplasm generated at this time ultimately becomes part of the egg. The binucleate neck canal cell and the ventral canal cell, also produced from the primary cell, carry little cytoplasm with them.

During the two mitoses which lead from the primary cell to the egg (Fig. 2) there are two other notable events. The first is a period of substantial cytoplasmic autophagy which appears in the central cell and continues into the young egg (Fig. 3). Regions of cytoplasm, including some of the mitochondria, are digested away. As the vesicles containing fragments of cytoplasm move to the periphery and finally disappear an extra lipoid membrane is secreted on to the surface of the maturing egg (Fig. 4). The second notable event is the striking interpenetration of nucleus and cytoplasm which occupies the remaining two-thirds of the period of maturation of the egg. This nucleo-cytoplasmic interaction appears to be a feature of oogenesis in all homosporous ferns, but its form varies. In *Lygodium* (Fig. 5), representative of an ancient line, and in *Pteridium*, a more recent fern, the nucleus produces principally vesicular evaginations. In *Pteridium* (but not seen as yet in *Lygodium*) some of these evaginations contain membranes. In *Histiopteris* (Fig. 6), probably allied to *Pteridium*, the membranous component of the evaginations is frequently extensive (B e l l, 1980). In *Dryopteris* (Fig. 7), although simple vesicular evaginations occasionally occur, they are more usually associated with extensive nuclear sheets. At present the only other genus in which this kind of evagination has been found is *Polypodium*. In all ferns so far examined many evaginations (and possibly all) contain an aggregate of dense material, reaching up to 2.0 μm in diameter (Fig. 5). Bodies of apparently similar composition occur within the nucleus, and they may arise at or near the nucleolus. They should not however be referred to as micronucleoli because they quite clearly differ in composition. There is yet no convincing evidence that they contain either ribonucleic or deoxyribonucleic acids. High-resolution autoradiography has given ambiguous results. All that is known for certain is that they are highly acidic.

PHASE CHANGE FROM SPOROPHYTE TO GAMETOPHYTE

The basic phenomena of sporogenesis in homosporous ferns have now been revealed (S h e f f i e l d, B e l l, 1979), although many aspects of cytochemistry have yet to be investigated. There are clear similarities with microsporogenesis in flowering plants. The meiocytes (spore mother cells) become surrounded by thickened walls, but there is no convincing evidence yet of the presence of callose. As the nucleus enters meiosis there are striking changes in the cytoplasm. The plastids

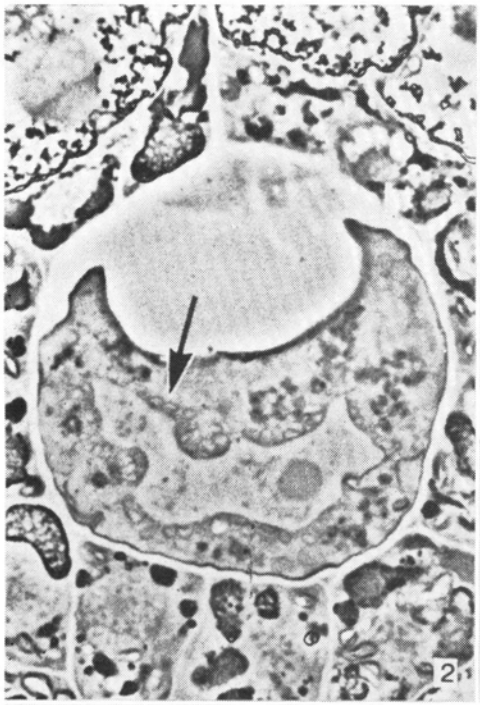
Fig. 2. Median action (1.5 μm) of an egg of *Thelypteris palustris*
Note the irregular nucleus and prominent nucleolus. The circular profiles (as at arrow) adjacent to the nucleus are nuclear evaginations. × 1260
Fig. 3. Autophagic vesicle containing remains of a mitochondrion in very young egg of *Histiopteris*. × 20 000
Fig. 4. The secretory phase in a young egg of *Lygodium* leading to the formation of the extra lipoid membrane around the mature egg. × 50 000
Fig. 5. Nucleocytoplasmic interaction in a maturing egg of *Lygodium*
A nuclear body is entering a newly forming evagination. N — nucleus. × 60 000

become dedifferentiated and their boundaries indistinct. The cytoplasm diminishes in density and the frequency of the ribosomes falls dramatically, reaching its lowest value at the end of prophase. Also at this time cytoplasmic continuities between meiocytes, and between meiocytes and tapetal cells, are broken. Following meiosis the young spores begin to lay down exine before rupture of the original meiocyte wall.

Two events which are probably of great significance in establishing the gametophytic phase are seen in the young spore (Fig. 8). Regions of cytoplasm rich in ribosomes and surrounded by membranes ('pseudo-nucleoloids'), which first appear in midprophase and which are distributed between the four spores, begin to disperse. Concurrently the nucleus produces tubular evaginations which branch and run out into the cytoplasm (Fig. 9). The extent and contents of these nuclear tubes are not yet known, but their production is clearly analogous to that of evaginations in the maturing egg. While these events are occurring the frequency of ribosomes in the cytoplasm steadily rises and ultimately reaches a level approaching that at the beginning of prophase. It can be inferred that by the time the tetrad breaks open the morphological nature of the spore is fully determined, and that all subsequent growth will be unambiguously gametophytic.

Sporogenesis in the heterosporous ferns is yet little investigated by modern techniques. In *Marsilea* the first stages of megasporogenesis are almost identical with sporogenesis in homosporous ferns. Spores are produced in tetrahedral tetrads as normally, but from this point on there is a striking difference. As the tetrads break open one spore of each of the eight (or sixteen) tetrads begins to grow (Fig. 10). Of these growing spores only one survives. This continues to grow at the expense of the degenerating tapetum, ultimately filling the whole sporangium. It remains a single cell with the nucleus and most of the cytoplasm adjacent to the proximal pole and the remains of triradiate scar, which below a large vacuole fills with nutrients and food reserves. During the growth and maturation of this surviving megaspore there is extensive production of nuclear tubes (Fig. 11).

PLATE I



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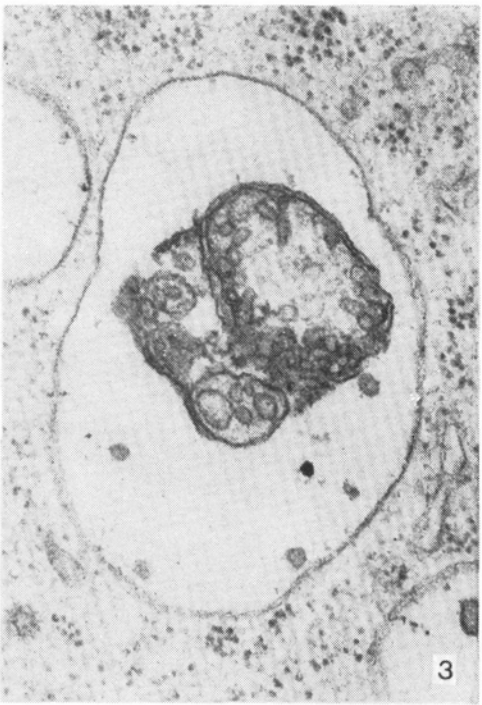

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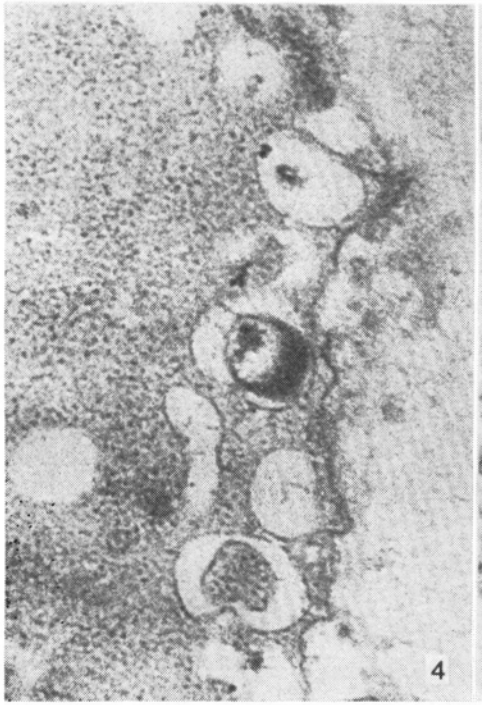

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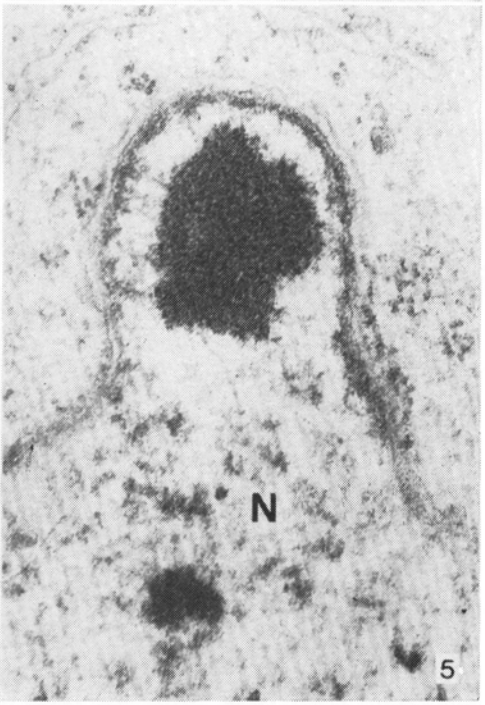

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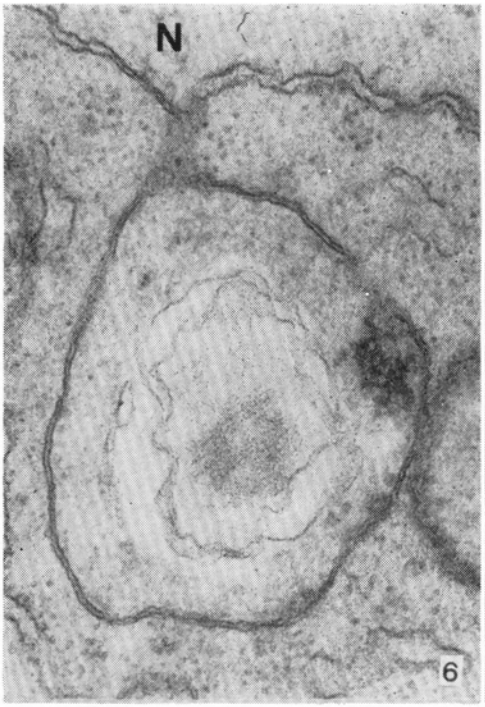
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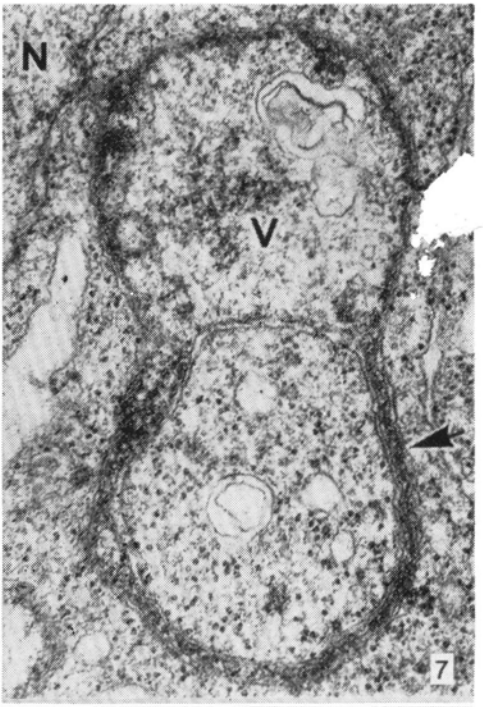
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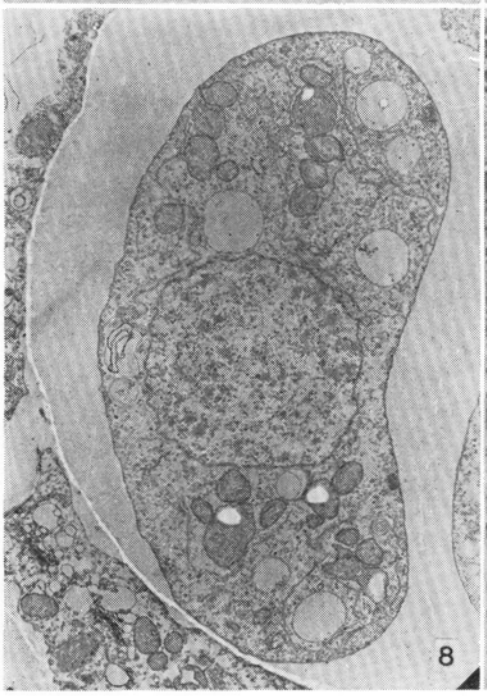
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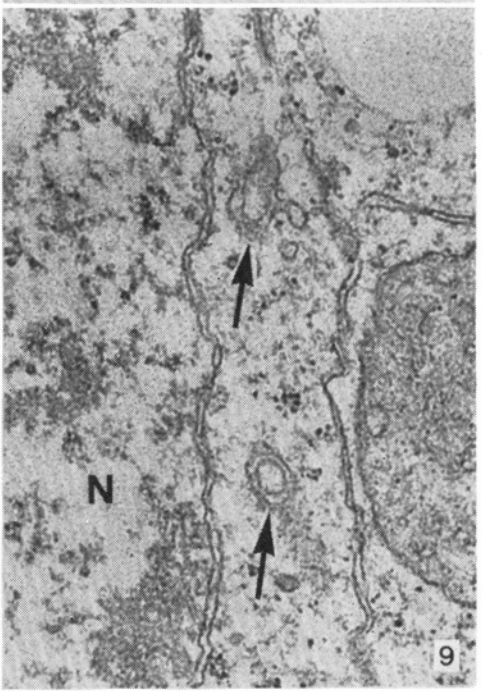
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Fig. 6. A nuclear evagination in *Histiopteris* containing extensive membranes N — main body of nucleus. × 68 000

Fig. 7. A nuclear evagination in *Dryopteris filix-mas*

Part is a vesicle (V) and the remainder a nuclear sheet (arrow). The vesicular portion contains membranes. N — main body of nucleus. × 60 000

Fig. 8. A young spore of *Pteridium* still in the tetrad, the stage at which nuclear tubes are produced. × 8000

Fig. 9. Nuclear tubes (arrows) in a young spore of *Pteridium* N — nucleus. × 60 000

There is no obvious explanation of why only one spore develops in each tetrad and the remainder regress, since initially all four spores are similar in size and ultrastructure. The mechanisms may be genetic, based on obligatory crossing over between two genes maintained in a heterozygous condition. If only one combination of these four alleles were viable in the environment of the megasporangium, then a 1 : 3 ratio would be regularly maintained. The fact that only one of these growing spores comes to maturity may depend on nothing more than random competition. It is however striking that the mature megaspore always has its proximal pole directed towards, and close to, the attachment of the sporangium.

THE RELEVANCE OF APOGAMY AND APOSPORY TO THE NORMAL CYCLE

The transition from gametophyte to sporophyte, and its converse, are the morphological manifestations of changes in gene activation. Although in homosporous ferns fertilization is essential for further development of the egg, the spermatozoid, consisting of little more than a highly condensed nucleus and a motile apparatus, probably does not influence the nature of the growth from the zygote. Fertilization appears to be essential for further development in the homosporous ferns since the nucleocytoplasmic interaction during the maturation of the egg involves extreme despiralization of the chromatin, leaving the nucleus in a state in which it cannot divide without fusion with the condensed chromatin of the male (Bell, 1979b). The indications are therefore clear that activation of the sporophytic genes begins in oogenesis, and the cause should be sought in the flux of metabolites and growth-regulating substance to which the archegonial region is subject. Apogamy, the transition from gametophyte to sporophyte without sexual fusion, whether natural (as in *Dryopteris borreri*) or induced experimentally (as in *Pteridium* (Whittier, 1964) and many other ferns) takes place in a similar situation behind the apical meristem. In naturally apogamous ferns as *Dryopteris remota* and *D. borreri*, chromosomal uniformity is main-

Fig. 10. A spore in the megasporangium of *Marsilea* just released from the tetrad The spore is beginning to grow. The proximal pole is indicated by the cutinized excrescence at the top of the spore. × 8000

Fig. 11. Nuclear tubes in a maturing megaspore of *Marsilea* N — nucleus. × 68 000

Fig. 12. Young spore of *Dryopteris borreri* produced in an 8-celled sporangium Bodies peculiar to these spores are shown at the arrow. × 4500

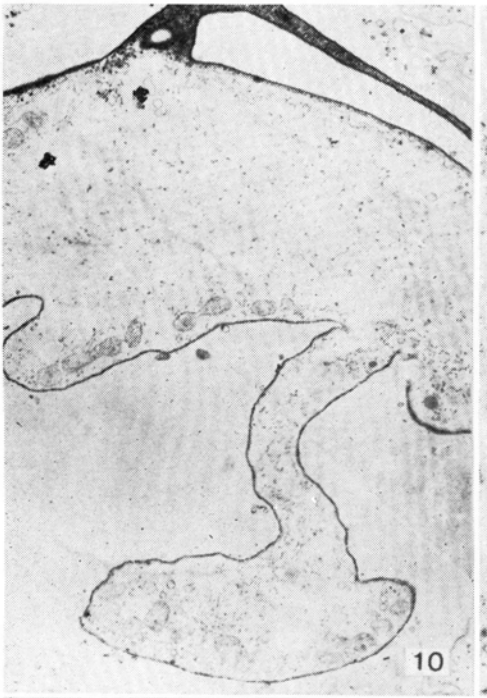
Fig. 13. Fragment of leaf of *Pteridium* regenerating aposporously one week after placing on agar medium

The new gametophytic tissue forms a border at the edge of the leaf. × 132

tained by the formation of restitution nuclei before meiosis (Döpp, 1932). Uniformity of chromosome number does not in itself explain the apogamous cycle. It is evident that the spores are already conditioned for apogamy. In this regard it is significant that the spores of *D. borreri* (Fig. 12) contain bodies not so far seen in the spores of ferns reproducing sexually. Their composition is not yet known, but it is probably largely proteinaceous. This additional complexity of the cytoplasm in a naturally apogamous fern can be regarded as the beginning of a superior nutritional state. After a relatively few cell generations, and in a region corresponding to that normally producing archegonia, the internal environment of the cells evidently reaches the critical level at which the sporophytic genes become fully activated. Since this is achieved without oogenesis, fertilization is not required for further development.

Apospory, the shift from sporophyte to gametophyte without meiosis, reveals at once the independence of chromosome number and phase of growth. There are many indications that apospory is induced by some form of nutritional deprivation. This need not amount to starvation. Recent experiments with *Pteridium* (Sheffield, Bell, in press) have shown that fragments of juvenile leaves placed on a nutrient agar surface and well illuminated produce recognizable aposporous outgrowths in as little as three days (Fig. 13), well before any signs of starvation or autolysis. There is further evidence that the act which is decisive in inducing apospory is severing vascular continuity with the main body of the plant. The inference is that deprivation of some essential metabolites or growth-regulating substances is adequate to switch gene activation from sporophytic to gametophytic. Once switched the new form of growth is quite stable and in a fern reproducing sexually aposporous gametophytes enter gametogenesis quite normally.

The readiness with which apospory can be induced in homosporous ferns provide a means of identifying those features of meiosis which are essential for the accompanying change in the form of growth. It



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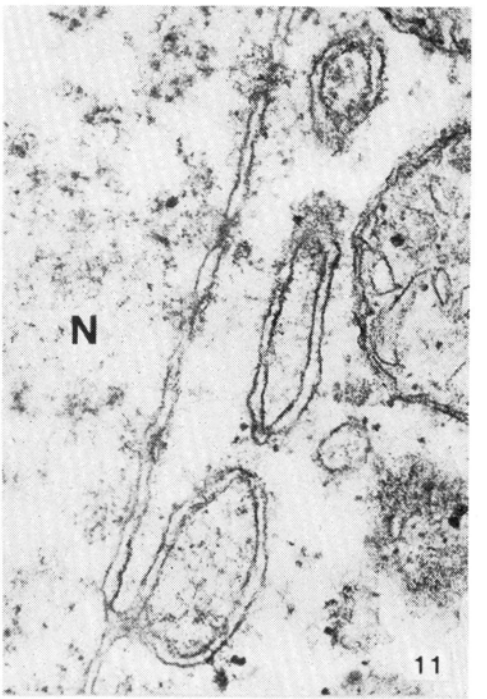

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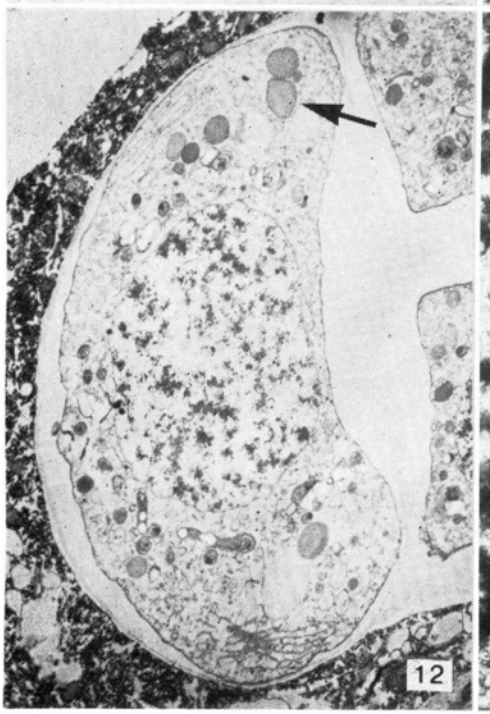

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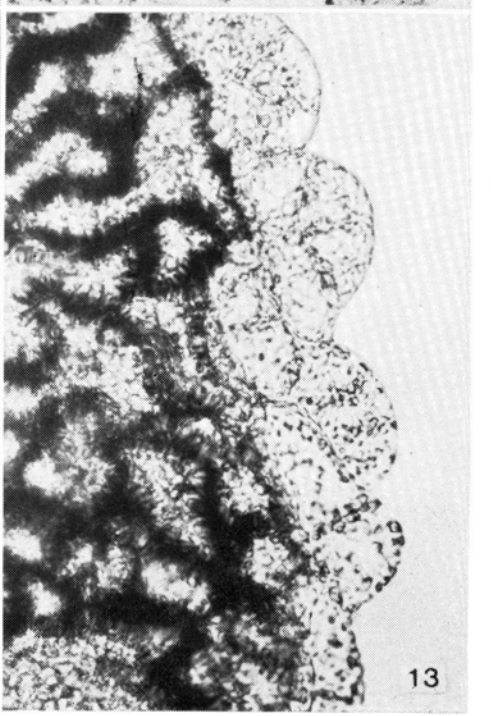

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is clear that the dramatic change in the frequency of ribosomes in the meiotic cytoplasm is not significant in this respect, and Heslop Harrison (1971) has misinterpreted this phenomenon. The depolymerisation of much of the cytoplasmic ribonucleic acid is probably intimately related to meiosis. Since the thickened wall of the meiocytes would prevent outward diffusion, the polyribonucleotides generated in the cytoplasm by the digestion of the ribosomes must enter the nucleus. It is notable in this connection that ribonucleic acid is already known to cause mitotic chromosomes to behave in a meiotic manner (Kodani, 1948; Wilson, Cheng, 1949), and the shift in ribonucleic acid may therefore be bound up with chromosome condensation and bivalent formation. Continuing investigations of apospory (Sheffield, unpublished data) have shown no corresponding elimination of ribosomes during the initiation of apospory, and the incorporation of tritiated uridine during induction was found to rise substantially. Heslop Harrison's (l.c.) interpretation of the cytoplasmic changes in meiosis provide an excellent example of how the study of flowering plants alone has led to wholly erroneous views about questions of fundamental importance in the causal morphology of the life cycle. The change in gene activation during sporogenesis seems more likely to arise from the exclusion of all but the simplest metabolites from meiotic cells by the thickened wall of the meiocytes. These cells are thus in a situation analogous to that of fragments of leaves detached from the parent plant and placed on an agar surface.

THE HETEROSPOROUS CYCLE

In heterosporous ferns (and probably in heterosporous plants generally) there is no nucleocytoplasmic interaction in the egg. Further, in contrast to homosporous ferns, where it is unknown, parthenogenesis in heterosporous ferns is well established. It seems beyond doubt that the nucleocytoplasmic interaction characteristic of the maturing egg in homosporous ferns is pushed back into the maturation of the megaspore (when the production of nuclear tubes is particularly extensive) in the heterosporous. If the reasoning in relation to oogenesis in the homosporous ferns is correct, then the megaspore in the heterosporous ferns is already predisposed towards sporophytic growth and no further nucleocytoplasmic interaction is necessary. The megaspore of a heterosporous fern (and of other heterosporous land plants) is thus equivalent to the egg of a homosporous fern, and both are analogous to the spore of an obligately apogamous fern. In all the activation of the sporophytic genes and the repression of the gametophytic has irreversibly begun (Bell, 1979b).

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