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THE ROYAL SOCIETY

The origin of alternation of generations in land plants: a focus on matrotrophy and hexose transport

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A life history involving alternation of two developmentally associated, multicellular generations (sporophyte and gametophyte) is an autapomorphy of embryophytes (bryophytes + vascular plants). Microfossil data indicate that Mid–Late Ordovician land plants possessed such a life cycle, and that the origin of alternation of generations preceded this date. Molecular phylogenetic data unambiguously relate charophycean green algae to the ancestry of monophyletic embryophytes, and identify bryophytes as early-divergent land plants. Comparison of reproduction in charophyceans and bryophytes suggests that the following stages occurred during evolutionary origin of embryophytic alternation of generations: (i) origin of oogamy; (ii) retention of eggs and zygotes on the parental thallus; (iii) origin of matrotrophy (regulated transfer of nutritional and morphogenetic solutes from parental cells to the next generation); (iv) origin of a multicellular sporophyte generation; and (v) origin of non-flagellate, walled spores. Oogamy, egg/zygote retention and matrotrophy characterize at least some modern charophyceans, and are postulated to represent pre-adaptative features inherited by embryophytes from ancestral charophyceans. Matrotrophy is hypothesized to have preceded origin of the multicellular sporophytes of plants, and to represent a critical innovation. Molecular approaches to the study of the origins of matrotrophy include assessment of hexose transporter genes and protein family members and their expression patterns. The occurrence in modern charophyceans and bryophytes of chemically resistant tissues that exhibit distinctive morphology correlated with matrotrophy suggests that Early–Mid Ordovician or older microfossils relevant to the origin of land plant alternation of generations may be found.

Keywords: alternation of generations; embryophytes; bryophytes; charophycean green algae; hexose transporter genes and proteins

1. INTRODUCTION

Alternation of generations in autotrophs is generally defined as the occurrence of a life history in which there are at least two multicellular generations, the gametophyte and the sporophyte, linked by unicellular reproductive stages, namely gametes and spores (figure 1). Spores are generated by sporic meiosis, which is the type of meiosis associated with alternation of generations (Raven *et al*. 1999). This article does not address 'alternation of generations' that may occur in various autotrophic protists that occur primarily as unicells (e.g. certain haptophyte algae), or in heterotrophs (such as foraminifera and fungi).

(a) *The occurrence of alternation of generations in autotrophs*

Life histories involving two or more alternating multicellular generations have evolved several times among photosynthetic protists (algae) (Graham & Wilcox 2000). For example, various bangiophycean red algae have a life history with two multicellular stages, and a three-stage life history appears to be a basic (plesiomorphic) feature for florideophycean red algae. Ancestors of phaeophyceans (brown algae) independently acquired alternation of two multicellular generations; so far as is known, their closest extant relatives (tribophyceans and other ochrophyte/chromophyte/heterokont algae) lack such a life history. Among modern green algae, alternation of two multicellular generations occurs only in certain orders of the class Ulvophyceae, and is lacking in the three other green algal classes that include multicellular forms (namely Trebouxiophyceae, Chlorophyceae and Charophyceae sensu Graham & Wilcox (2000)). Evidence for independent evolution of alternation of generations in red, brown and green algae suggests that it is highly adaptive. Hypothetical adaptive aspects of life history variation in autotrophs are discussed by Bell & Koufopanou (1991), Otto & Goldstein (1992), Baillard (1997) and Bell (1997). A review of alternation of generations in land plants, with an emphasis on fossil branched gametophytes that are thought to be linked to the life histories of protracheophytes and early vascular plants, is provided by Kenrick (1994).

(b) *Ploidy change and alternation of generations*

It should be noted that while textbook depictions of alternation of generations in algae and land plants

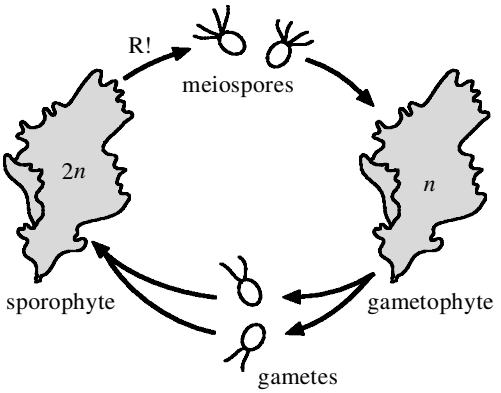


Figure 1. Diagram of alternation of multicellular generations. R! indicates the occurrence of meiosis. A life history involving spatially and temporally separate generations is characteristic of several groups of algae.

ypically describe the gametophytic generation as being aploid, and the sporophytic generation as diploid, there re many examples among the algae of life history phase hange that are not correlated with change in chromo- ome number (Graham & Wilcox 2000). For example, he nuclei of sporophytes and gametophytes of the brown eaweed *Haplospora globosa* (Tilopteridales) possess the ame number of chromosomes. However, the DNA level f sporophytic nuclei is twice that of gametophytic nuclei Kuhlenkamp *et al.* 1993). In other algae, environmental actors are thought to be as, or perhaps more, important han chromosomal level in determining the direction of fe history phase change. Environmental effects are egarded as possible explanations for cases of apogamy transition to the sporophyte phase in the absence of amete production and syngamy) and apospory (transi- ion to the gametophytic phase in the absence of meiosis nd spore production). In seedless plants, apogamy and pospory are also observed, but gene dosage effects are mportant. Maintenance of sporophytic growth depends n the presence of at least two sets of chromosomes, whereas gametophytic growth in culture does not ontinue when four or more sets of chromosomes are resent (Bell 1991).

In higher plants, there are many examples of produc- ion of young sporophytes (embryos) from cells other han zygotes (e.g. microspore embryogenesis, somatic mbryogenesis and apomixis) (Harada *et al.* 1998), and he genetic basis for such variants from the expected life istory cycling is becoming clearer. For example, the rabidopsis gene *LEAFY COTYLEDON 1* (*LEC1*), which ncodes a transcription factor, is sufficient to induce mbryo-like development from vegetative cells (Lotan *et al.* 1998).

(c) *Importance of sporophyte/gametophyte interactions*

The above variations having been noted, a life history nvolving alternation of multicellular gametophyte and porophyte generations characterizes all groups of extant and plants, which comprise the Kingdom Plantae, as efined by Raven *et al.* (1999). Members of the extant lant kingdom constitute a monophyletic group that ncludes multiple lineages of early-divergent bryophytes

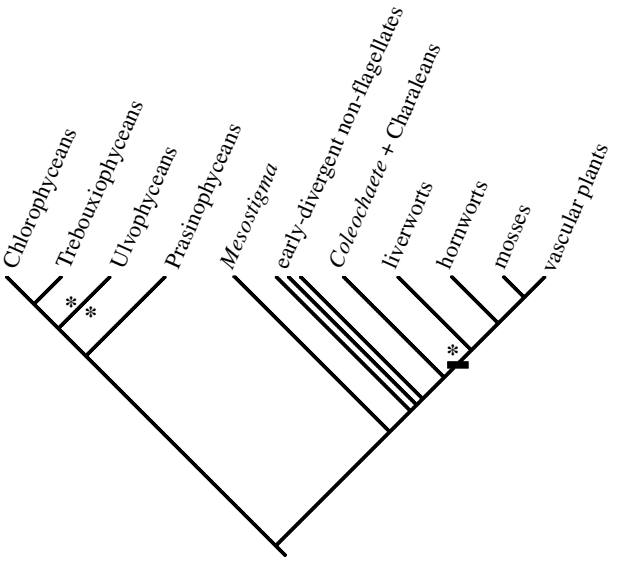
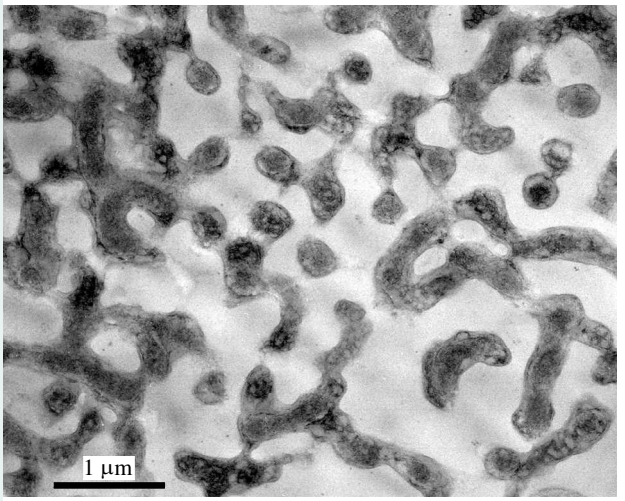


Figure 2. Diagrammatic representation of phylogenetic relationships among various groups of the green algae and embryophytes. Asterisks indicate cases of presumed independent origin of a life history involving alternation of two multicellular generations. The bar indicates the only known case among green autotrophs of the occurrence of a dependent, multicellular sporophyte (alternation of generations that are not separated temporally or spatially).

Table 1. *Matrotrophy and associated life history change have led to the origin of three high-diversity, long-lived clades*

clade	reproductive innovation	mechanism
florideophycean red algae	carposporophyte	*n*/2*n* cell fusions
embryophytes	dependent embryo	placental transfer cells
eutherian mammals	viviparity	complex placenta

and later-divergent tracheophytes (Kenrick & Crane 1997). It is important to recognize that alternation of generations in the Kingdom Plantae is distinctive in that embryonic sporophytes occur in close spatial and temporal association with female (or bisexual) gametophytes. Plant embryos, including those of the simplest liverworts as well as derived angiosperms, seem generally to be nutritionally and developmentally dependent on parental gametophyte tissues for at least some period of time in early development. The presence of a dependent embryonic stage is the basis for the term embryophytes, commonly used as a synonym for Kingdom Plantae. The occurrence of alternation of multicellular generations coupled with dependent embryos in all groups of land plants suggests that these features are autapomorphic (unique and defining) features of embryophytes (figure 2). Multicellular sporophytes do not occur in the charophyceans, the green algal lineage most closely related to embryophytes (Graham 1993), and dependence of the embryonic sporophyte is lacking in most other algae that have alternation of generations. An exception to this generality is the floridophycean red algae, whose



igure 3. A transmission electron micrograph of a portion of placental transfer cell of the liverwort *Conocephalum conicum*, nowing extensive development of wall ingrowths. The reparation was high-pressure frozen, a method that facilitates reservation of cell membranes.

arposporophyte generation is nutritionally and evelopmentally dependent on the female gametophyte.

In florideophyceans and land plants, the embryonic porophyte's dependency has been described as matro-rophy (literally 'mother feeding'), by analogy to mbryonic nutritional and developmental dependence in utherians and other viviparous metazoans (Graham, . E. 1996). Matrotrophy was a key innovation in the iversification of these three major clades (florideophy-eans, embryophytes and eutherians), its adaptiveness in ach case hypothetically related to increases in fecundity table 1). In each case, there is a morphological correlate f matrotrophy—the placenta, a region of specialized ells (tissues in the cases of embryophytes and euther-ans) that facilitates maternal to embryonic nutrient ransfer.

In embryophytes (as in eutherians), there are no inter-ellular connections linking parental and embryonic ssues, hence transport of solutes occurs by means of cell nembrane transporters (i.e. is apoplastic). Embryophytic lacental cells typically possess elaborate systems of wall ngrowths, which greatly increase the surface area of the ell membrane across which facilitated diffusion or active ransport must occur (figure 3). Such cells are known as lacental transfer cells; these may occur on one or both des of the generational gap. Placental transfer cells are ell studied at the ultrastructural level in bryophytes (see eviews by Ligrone & Gambardella 1988; Ligrone *et al*. 993) and in higher plants such as *Arabidopsis* (Murgia *et l*. 1993). Physiological studies provide substantial vidence that the placentae of bryophytes (Browning & unning 1979*a*,*b*; Renault *et al*. 1992) and flowering lants (Van Caeseele *et al*. 1996) function in apoplastic ransport.

Provision of the florideophycean embryo (carposporo-hyte) with nutrients from the maternal generation is uggested to be a strategy for amplifying the products of exual recombination where fertilization rates are limited y the absence of flagella from male gametes. (It should be noted, however, that few actual measurements of fertilization rates have been made for red algae.) A similar adaptive benefit has been hypothesized to accrue to seedless land plants, whose fertilization rates may be limited by availability of liquid water for transport of flagellate sperm (Searles 1980). The rest of this paper focuses on palaeontological, neontological and combined approaches to understanding the role of matrotrophy in the origin of alternation of generations and the dependent sporophytic embryo of land plants.

2. PALAEONTOLOGICAL EVIDENCE THAT EARLIEST-KNOWN (ORDOVICIAN) LAND PLANTS POSSESSED ALTERNATION OF GENERATIONS

Microfossils of the Mid–Late Ordovician age, described from Libyan deposits by Gray *et al*. (1982), Taylor (1995) and Strother *et al*. (1996), provide evidence that the earliest-known land plants possessed alternation of generations. These remains include spores arrayed in persistent tetrads. Persistence of the fossil tetrads suggests the presence of spore walls that were chemically resistant to the effects of microbial decomposition and diagenesis, as are the sporopollenin-impregnated spore walls of modern land plants, including those of bryophytes. Similar spore tetrads, known to have resulted from meiosis, are produced by the early-divergent (see Lewis *et al*. 1997) complex thalloid liverwort *Sphaerocarpos*. Such tetrads remained intact after high-temperature acid hydrolysis (Graham & Gray 2001), a procedure that to some extent mimics the degradative chemical and physical effects experienced by plant tissues during fossilization. Occurrence of the Ordovician spores in tetrads is strong evidence that they arose by sporic meiosis, which as noted earlier, is a hallmark of alternation of generations. So far as is known, meiosis is zygotic in all charophyceans exhibiting sexual reproduction (evidence reviewed by Graham 1993). The Ordovician spore evidence suggests that sporic meiosis was an innovation that occurred at the dawn of embryophytes.

Microfossil evidence that sporic meiosis coincided with origin of a multicellular sporophyte generation in earliest-known land plants was provided by Graham & Gray (2001), who demonstrated that the sporangial epidermis of *Sphaerocarpos*, on high-temperature acid treatment, falls apart into monostromatic cellular fragments resembling the most ancient multicellular fossils attributed to land plants. The latter, derived from Libyan deposits of Ordovician age, were associated with spores (Gray *et al*. 1982). On the basis of morphometric comparison with high-temperature acetolysed sporangial epidermis of the early-divergent moss *Sphagnum*, Kroken *et al*. (1996) suggested that the Ordovician cellular scraps represent sporangial epidermal remains, and that they are the earliest-known fossils of the sporophyte generation of land plants.

Hydrolysis-resistance of extant bryophyte sporangial epidermis was attributed to the presence of highly insoluble, wall-bound phenolic polymers, on the basis of specific autofluorescence properties (Kroken *et al*. 1996). Similar, resistant, autofluorescent materials occur in vegetative cell walls of various charophycean algae, suggesting that land plants inherited the capacity to

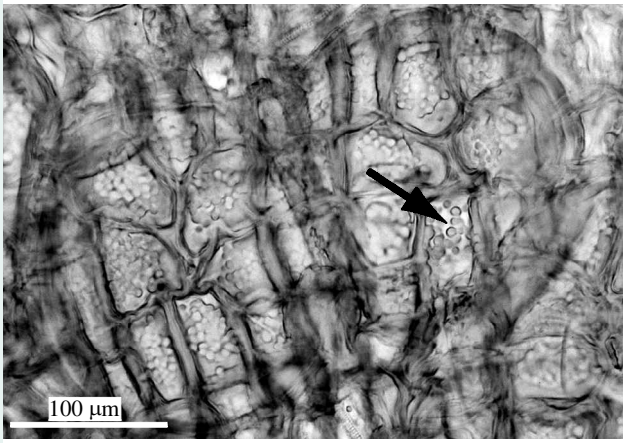


Figure 4. Light micrograph of a portion of a fertile *Coleochaete orbicularis* thallus that was subjected to high-temperature acid hydrolysis. Cell contents have been hydrolysed, but walls of the two large spherical zygotes and their enclosing cortical cells are resistant to hydrolysis. Extensive development of wall ingrowths in cortical cells (see arrow, for example) is readily discernible.

produce such compounds from charophycean ancestors. Resistant, autofluorescent walls of the charophycean green alga *Coleochaete* that are associated with zygotes have Fourier transform infrared (FTIR) spectra that are similar to those of the decay-resistant walls of another charophycean, the desmid *Staurastrum* (Gunnison & Alexander 1975*a*,*b*), but different from typical plant sporopollenin (Delwiche *et al*. 1989).

Occurrence of similar, resistant compounds in walls of both charophyceans and bryophyte sporangial epidermis suggests the possibility that the Ordovician cellular remains cited earlier could be of charophycean origin. However, the only known example of resistant multicellular tissue in charophyceans is that of the thallus cells, which form a cortical layer that nearly completely encloses mature zygotes of *Coleochaete orbicularis* (Delwiche *et al*. 1989). In such an instance, the zygotes are quite conspicuous and much larger in diameter than the surrounding cells (figure 4); no such enlarged cells occur with the Ordovician cell scraps. Resistant materials in bryophyte sporangial epidermal cell walls may be adaptive in protecting developing spores from microbial attack; earliest land plants may also have derived such a benefit from their presence (Graham & Gray 2000). If Ordovician microfossil scraps do represent sporangial epidermis, the plants from which they were derived possessed a multicellular sporophyte and, hence, alternation of generations. However, fossil remains of the placental region, representing evidence that plants had acquired matrotrophy by the Ordovician, are so far lacking.

3. HYPOTHETICAL STAGES IN THE ORIGIN OF ALTERNATION OF GENERATIONS IN EMBRYOPHYTES

Several stages (A–E) in the evolutionary origin of land plant alternation of generations (figure 5) can be deduced by comparison of the reproductive features of charophyceans and bryophytes.

(a) *Stage A*

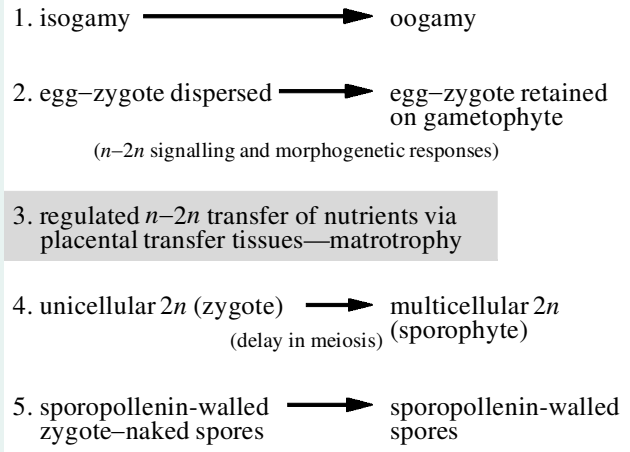
An early step, occurring in charophyceans, is hypothesized to have been transition from isogamy to oogamy (production of flagellate sperm and larger, non-flagellate eggs), which also occurs in land plants. Determination of the direction of character transition is dependent on the existence of a robust phylogeny for charophyceans but, to date, phylogenies of charophyceans that are inferred from various nucleic acid sequence data have been highly incongruent. This state of affairs has most likely resulted from extinction, leaving us with a sparse extant representation of the group. Consequently, Graham, L. E. (1996) and Graham & Gray (2001) recommended reliance on molecular architectural data, such as intron insertion events, gene rearrangements or movement of genes between cellular genomes, as is also advocated by Qiu & Palmer (1999). Such data indicate that isogamous Zygnematales are earlier-divergent than oogamous *Coleochaete* and Charales (Graham & Gray 2001; Graham & Wilcox 2000).

However, Sluiman & Guihal (1999) reported that 18S rDNA sequence analysis suggests that the oogamous genus *Chaetosphaeridium*, which is frequently linked with *Coleochaete* on the basis of morphological similarities (see Graham & Wilcox 2000) and *rbcL* sequence data (Chapman *et al*. 1998), may not be closely related to *Coleochaete* but, rather, relatively early-divergent within charophyceans. Other small subunit rDNA sequence analyses indicate surprisingly early divergence of charaleans (Huss & Kranz 1997), this differing dramatically from *rbcL* data indicating that a clade including both Charales and *Coleochaete* is sister to embryophytes (McCourt *et al*. 1996). Further analysis of the evolutionary origin of oogamy in the charophycean–embryophyte lineage may depend on our ability to map isogamy to oogamy transition(s) on to a robust phylogeny.

Aspects of the transition to oogamy that require further study include: (i) origin of the complex, multicellular gametangia of Charales and transition from single-celled antheridia in some species of *Coleochaete* to multicellular aggregates of spermatangial and sterile cells that occur in other *Coleochaete* species (Graham 1993); (ii) changes in flagellate gamete anatomy that may be linked to transition to oogamy (Duncan *et al*. 1997); (iii) loss of flagellar development at the origin of egg cells; (iv) origin of the enlarged eggs, filled with food reserves, of charaleans; (v) origin of the trichogyne, a tubular protuberance of eggs, whose distal wall undergoes controlled hydrolysis, allowing release of sperm attractants and sperm entry in *Coleochaete* (Graham 1993); and (vi) the origin and chemical character of sperm attractants in oogamous charophyceans and bryophytes. A possible approach to the final issue might be to examine *Coleochaete* and charaleans for compounds that are known to coordinate mating in zygnemataleans (Sekimoto *et al*. 1993).

(b) *Stage B*

Retention of eggs (and zygotes that develop from them) on the maternal (or bisexual parental) thallus is the next hypothesized step (figure 5), as this is an essential precedent for the development of nutritional and developmental interactions between generations. Among charophyceans, examples of egg/zygote retention occur in



igure 5. Hypothesized stages in the evolutionary origin of natrotrophic alternation of generations as it occurs in modern mbryophytes. Some proposed mechanisms are shown in arentheses. Shading of no. 3 indicates the area of research hat is described most fully in this paper.

'oleochaete and Charales, facilitated by mitotic production f a layer of corticating cells or elongation of spirally wisted tube cells, respectively. In both cases, the nclosing cells belong to the parental generation Graham 1993). In Charales, tube cells undergo extension t the same time as the egg cell enlarges, such that nature eggs are thought to be near the maximal size eached by zygotes, and fully enclosed before fertilization ccurs (Pickett-Heaps 1975). In contrast, in *Coleochaete*, he enclosing layer of parental cells does not develop until fter fertilization, and zygotes (rather than eggs) undergo nlargement and storage accumulation. In both cases, as et undefined cell–cell signalling processes probably co-rdinate development.

Only in *Coleochaete* could initial development of corti-ating cells be said to result from intergenerational zygote/parental thallus) communication, but zygote/ arental cell signalling that influences maturation of the *n*/*n* cell complex may occur in both *Coleochaete* and Char-les. Hence these are the taxa of choice for analysis of ypothesized communication systems, diffusible signal-ng molecules and their receptors. Some *Coleochaete* pecies produce zygotes that are less completely corticated han others (Szymánska 1989); comparison of expression atterns at critical developmental stages might reveal enetic differences relevant to the zygote retention issue. ggs are reportedly not retained in *Chaetosphaeridium* Thompson 1969); if *Chaetosphaeridium* is sister to *'oleochaete*, differential expression studies of these taxa ould be very informative.

Molecules known to have signalling functions in igher plants that could also operate in charophyceans nd early-divergent bryophytes include hydrogen eroxide, a diffusible and relatively long-lived molecule whose production is in part regulated by peroxisomal atalase (Karpinski *et al.* 1999); secreted peptides or mall proteins that act as hormones (Gehring 1999; letcher *et al.* 1999); and sugars (Koch 1996; Graham, . A. 1996). The role of sugars is discussed below in ore detail.

(c) *Stage C*

A third postulated step in origin of the life history of embryophytes (figure 5) is the origin of matrotrophy, transfer of nutrients and/or morphogenetic factors from parental tissues to zygotes. Such a step is dependent on the earlier evolution of retained eggs/zygotes (see above), because diffusive or degradative loss of parentally secreted nutrients or other compounds would greatly reduce their supply to progeny (zygotes) that are spatially separate, particularly in an aquatic environment. Contiguity of *Coleochaete* parental cells and zygotes, localized development of elaborate ingrowths on the walls of cortical cells that are in direct contact with zygotes, and conspicuous post-fertilization nutrient storage (in the form of starch and lipid) provide circumstantial evidence for occurrence of matrotrophy. Close spatial association of parental tissues whose cells possess typical placental transfer morphology (wall ingrowths) and physiological evidence for solute transport across the apoplastic, inter-generation junction (Browning & Gunning 1979*a*,*b*; Renault *et al.* 1992) provide evidence that matrotrophy occurs in bryophytes. These data suggest that embryophytes could have acquired matrotrophy from charophycean ancestors, and that matrotrophy preceded the origin of multicellular sporophytes. Molecular strategies for testing these possibilities are discussed in a later section of this paper.

(d) *Stage D*

Transition from a unicellular zygote (as produced by charophyceans) to production of a multicellular diploid sporophyte generation (as in embryophytes) by repeated mitotic division of the zygote is the next postulated step in the origin of land plant alternation of generations (figure 5). The mechanism most often hypothesized for this transformation is 'delay in meiosis'. This means that a phase of mitotic proliferative growth would occur between zygote formation and spore production by meiosis. An alternative idea, that the land plant sporophyte originated in green algal ancestors that had first acquired alternation of multicellular generations, is currently less favoured, as it is not supported by modern phylogenetic analysis. Extant green algae that exhibit alternation of generations are not closely related to the ancestry of land plants, and charophycean green algae, which are closely related to embryophytes, lack alternation of generations, so far as is known. Hypothetical origin of embryophytes from green algae having temporally and spatially separate generations also does not provide a satisfactory explanation for the origin of matrotrophy.

The finding of an extant or fossil 'charophycean' that had intercalated even a single mitotic division between syngamy and meiosis would mean discovery of a possible transitional form, a descendant of such a transitional form or a close parallel to the simplest possible (two-celled) land plant sporophyte. The adaptive advantage of having a multicellular sporophyte is that greater numbers of genetically diverse meiospores could result, facilitating both colonization effectiveness and increase in population genetic variability, and hence greater evolutionary flexibility. Evidence that such adaptive advantage exists is provided by progressive increase in sporophyte size during the course of embryophyte evolution.

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Reconstruction of the critical molecular events involved n delay of meiosis may be possible by probing the enomes of extant charophyceans and early-divergent mbryophytes for genes that are involved in the regula- ion of meiotic initiation. Such genes have been identified n metazoans (e.g. Barton & Kimble 1990; de Vries *et al.* 999), yeasts (e.g. Englebrecht & Roeder 1990; Matsura *t al.* 1990; Printz *et al.* 1995; Iino *et al.* 1995; Colomina *et l.* 1999; de los Santos & Hollingsworth 1999; Edelmann *t al.* 1999; Ohno & Mattaj 1999; Sagee *et al.* 1999), and lowering plants (e.g. Bouchard 1990; Bai *et al.* 1999); a ecent review of meiotic chromosome organization and egregation in plants is provided by Dawe (1998). Given uch information, it may ultimately be possible to ransform charophyceans so that their zygotes recapitu- ate the meiotic delay postulated to have occurred during he origin of embryophytes.

The origin of the quadripolar microtubular system QMS) characteristic of embryophytic sporocytes, which rovides an essential scaffolding for proper spatial segre- ation of plastids and nuclei into the four daughter roducts (Brown & Lemmon 1997), is unknown. Whether his structural component of meiotic cells is present in an arlier form in charophyceans is not known, and would equire ultrastructural and fluorescence immunolocaliza- ion studies of germinating zygotes. Correlative analysis f nuclear DNA levels would also be valuable but has een difficult because large amounts of storage materials, utofluorescent sporopollenin-like zygote wall layers and utofluorescent cortical cell walls (in the case of *Coleochaete*) can interfere.

Microarray studies in yeast systems have revealed that loidy change regulates the expression of genes involved in he cell cycle (such as G1 cyclins) and actin control of olarized growth. However, the mechanism by which loidy change is sensed is not as yet understood (Galitski *et l.* 1999). Since homologous cell cycle and actin-regulation enes are likely to occur in plants, it is possible that ploidy hanges occurring at the transitions between gametophytic nd sporophytic phases of the life history are similarly mportant in determining differences in the morphology of he two stages.

(e) *Stage E*

A final step, from production of non-walled, flagellate eiospores, as occurs in modern *Coleochaete*, to production f nonflagellate, sporopollenin-walled meiospores, as ccurs in all embryophyte groups, is also postulated to ave been involved in the origin of plant alternation of enerations (figure 5). This is because the relative fficiency of walled-spore dispersal and germination uccess on land may have driven evolutionary transition o increasingly larger multicellular sporophytes capable of roducing many meiospores from a single fertilization vent. The alternative, illustrated by charaleans, is roduction of many large, resistant zygotes (representing ultiple fertilization events), each of which produces probably only) one, non-resistant, meiotic product. Such reproductive strategy would not be adaptive if fertiliza- ion frequency is limited by water availability. If spores acking protective walls were unable to survive to ermination following terrestrial dispersal, the multi- ellular sporophyte likewise would not be adaptive. The

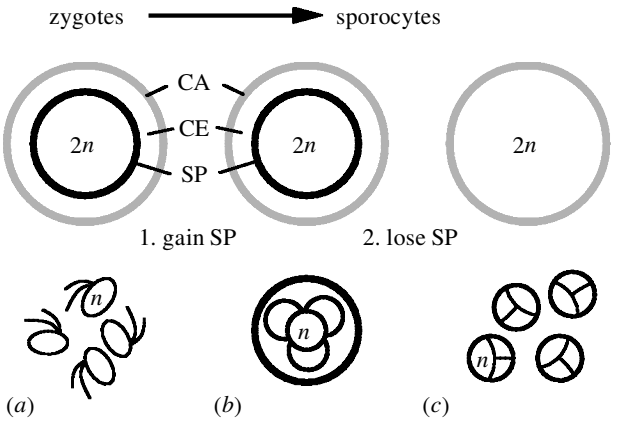

Figure 6. Hypothetical transformations involved in the origin of walled, non-flagellate spores, as they occur in modern embryophytes by gain of sporopollenin (SP) in spore walls (1), then loss of sporopollenin from sporocyte walls (2). (*a*) The extant charophycean *Coleochaete* produces zygotes having callosic (CA), cellulosic (CE) and SP layers, within which develop flagellate, non-walled meiospores. (*b*) A hypothetical intermediate stage in which diploid sporocytes (that are homologous to charophycean zygotes) have resistant walls, and produce walled, non-flagellate meiospores. Persistence of the resistant sporocyte wall forms an envelope around meiospore tetrads, preventing their dissociation. (*c*) Sporopollenin-walled, dissociated meiospores as they occur in most embryophytes.

sporopollenin-coated wall of embryophytic spores has been regarded as having an adaptive function in the terrestrial environment, by providing structural stability and retarding microbial degradation during dispersal (Graham & Gray 2001).

It is as yet unclear whether multicellular sporophytes appeared prior to the origin of walled meiospores, or vice versa. It is possible that earliest embryophytes (defined by presence of a multicellular sporophyte, however small) might have lived in water or very moist environments in which selection did not operate heavily against unwalled, flagellate meiospores. Production of greater numbers of or more genetically diverse meiospores could have provided sufficient adaptive advantage for delay in meiosis to have occurred prior to origin of walled spores. Amplification (apparently by reduplication) of zygotic DNA levels and subsequent production of 8–32 meiospores in *Coleochaete* (Hopkins & McBride 1976) provides evidence of the adaptive value that a multicellular sporophyte could have in nearshore waters.

An inner wall layer of sporopollenin-like material is typically produced during zygote maturation in charophyceans. The transition to walled meiospores is postulated to have involved a change in the timing of sporopollenin production, such that it occurs at a later developmental stage, during spore maturation (figure 6). Transitional forms may have produced sporopollenin both at the spore mother cell stage (hypothesized to be homologous to charophycean zygotes by Graham 1990) and during spore maturation. Information regarding regulation of sporopollenin synthesis and deposition derived from analysis of higher plant mutants may be useful in deducing the genetic transformations that were involved in the production of sporopollenin-walled spores.

4. APPROACHES TO AN UNDERSTANDING OF THE EVOLUTIONARY ORIGIN OF MATROTROPHY AND PLACENTAL FUNCTION IN EMBRYOPHYTES

Combined neontological and palaeontological approaches, nd comparative studies of extant charophyceans and ryophytes are recommended for elucidation of the evolu- ionary process surrounding the origin of embryophytic natrotrophy and placental function. An example of a ombined neontological/palaeontological approach is the omparison of acid-hydrolysis resistant, autofluorescent nature placentae of *Coleochaete orbicularis* (the only charo- hycean known to produce placental transfer-like cells) figure 4) and those of bryophytes (Kroken *et al*. 1996). nalogously, morphology of the placental interface of the xtant pteridophyte *Tmesipteris elongata* was used by Frey *et al*. (1997) to postulate the occurrence of placentae in *Horneophyton lignieri*, based on fossils that were originally ublished by Taylor & Taylor (1993).

Deposition of resistant polymers into the walls of lacental cells occurs during late development of zygotes *Coleochaete*) or sporophytes (bryophytes). These materials re not present (as judged by absence of specific wall auto- uorescence and lack of resistance to high-temperature cid hydrolysis) in vegetative thalli or during early zygote evelopment, including the period of presumed solute ransport. Thus deposition appears to be under regulatory ontrol. The function of resistant wall compounds is not roven but could include shutting off solute flow or rotection from microbial attack (Kroken *et al*. 1996; Graham, L. E. 1996). Wall ingrowths of *Coleochaete* are lso heavily impregnated and consequently resist acid ydrolysis. As a result, cell wall ingrowths, which repre- ent a physical manifestation of matrotrophy, are readily isible at the light microscopic level, both in bright field figure 4) and UV-V fluorescence excitation. This suggests hat if ancestral charophyceans related to *Coleochaete*—rganisms transitional between charophyceans and arliest embryophytes—or early embryophytes also ossessed such features, as is likely, they should have left esistant remains in the form of distinctive microfossils. Thus it is possible that microfossils younger than those resently known may yet be found that will be informa- ve regarding the earliest events in the history of mbryophytes and their acquisition of alternation of enerations. We are currently cataloguing high- emperature, acid-hydrolysed fertile thalli of various *oleochaete* species and placentae of modern bryophytes ith the hope that they may provide useful search images or palaeontologists as they seek remains of earliest mbryophytes or fossil evidence of matrotrophy.

Neontological approaches include comparative analyses f putative nutrient transport from maternal cells to ygotes of *Coleochaete* and between gametophytic and porophytic tissues of early-divergent embryophytes. We ave focused on comparative study of the hexose trans- orter gene family in charophyceans and early-divergent mbryophytes (described in more detail below). Parallel fforts could be made to trace evolutionary change in mino acid transporters (Fischer *et al*. 1998) and H^+-TPases. Genetic analysis of the regulation and biosynth- sis of wall-bound resistant polymers that characterize nature charophycean and bryophyte placentae is also desirable. It may be possible to determine if parental imprinting affects allocation of resources at the plant placenta (reflecting intragenomic, i.e. paternal–maternal, conflict), as is believed to occur in eutherians (Haig 1996*a*,*b*, 1997) and, if so, when this might have evolved.

5. COMPARATIVE STUDY OF CHAROPHYCEAN/EMBRYOPHYTE HEXOSE TRANSPORTER GENES AND PROTEINS

Physiological studies by Renault *et al*. (1992) in the early-divergent moss *Polytrichum* strongly implicate hexose sugars (rather than sucrose) as the major carbohydrate species that is moved from gametophyte to sporophyte. Because the intergenerational junction is devoid of symplastic connections (plasmodesmata), cell membrane hexose transporter proteins are implicated. Such transporters are thought to facilitate diffusion and/or actively cotransport sugars and protons; homologous proteins in bacteria, fungi, protists, higher plants and higher animals are members of a major family of transmembrane facilitators (Marger & Saier 1993).

It is postulated that glucose, once imported into zygotes/sporophytes, is transformed into starch or lipid storage, or used more immediately for embryo and sporophyte development and spore production. Both processes act as sinks that would tend to promote increased apoplastic transport from gametophytic sources. In higher plants, sugars not only serve as energy sources and building blocks for structural elements such as cellulose but are also potent regulatory molecules. Sugars function as signalling molecules that activate or repress genes involved in cell cycle regulation, photosynthesis and pigment production, glyoxylate metabolism, respiration, starch and sucrose synthesis and degradation, nitrogen metabolism, pathogen defence and wounding responses, and senescence (Ehness *et al*. 1997; Koch 1996; Graham, I. A. 1996; Truernit *et al*. 1996). For example, chlorophyll *a*/*b* binding protein genes and *rbcS* genes are sugar-repressible, whereas the gene encoding nitrate reductasel is sugar-inducible. Further, genes essential for biosynthesis of ethylene are activated by sugars, and those for brassinolides are repressed. Receptors are hypothesized to be integral cell membrane hexose transporter proteins (Lalond *et al*. 1999).

Plants also possess sugar sensors. These are molecules that detect the presence of imported sugars and transduce the signalling responses. The sugar phosphorylation enzyme hexokinase (HXK) (which also functions at an early step of cytoplasmic glycolysis) is one example of a sugar sensor, because the sugar responses of higher plants require only that imported sugar is phosphorylated by this enzyme (Jang *et al*. 1997). It is thought that the signalling HXK is spatially associated with membrane hexose transporters (Smeekens & Rook 1997). HXK is highly conserved from bacteria to yeast and higher plants and animals, and is thus likely to occur in charophyceans and bryophytes, and to have similar sensing functions in these organisms.

We began our analysis of hexose transport by determining that at least some charophycean algae and bryophytes (e.g. *Coleochaete*, Graham *et al*. 1994; the charophycean desmid *Closterium*, Lewitus & Kana 1994;

nd several *Sphagnum* moss species, P. Bilkey, unpublished ata) are capable of importing and using exogenous exose sugars, as deduced by growth experiments. This as necessary because a number of green algae are nown to be incapable of utilizing hexose sugars. For xample, all of the (chlorophycean) *Chlorococcum* species ested by Parker *et al*. (1961) were incapable of utilizing ugars for growth. The model-system organism *Chlamydo-onas reinhardtii* (also chlorophycean) is likewise unable to tilize exogenous sugars (Harris 1989). Forsberg (1965) oncluded that axenic cultures of *Chara* were also unable o utilize exogenous sugars; however, Smith (1967) btained evidence that *Nitella translucens* can take up and netabolize ^{14}C-labelled glucose.

We found that hexose utilization by charophyceans and ryophytes increased under conditions in which dissolved norganic carbon (DIC) was limiting to photosynthesis. his suggests a likely adaptive advantage of hexose-ransport expression in vegetative cells or tissues. We then ypothesized that hexose transporter genes were brought nder increased regulation in derived charophyceans aving retained zygotes, such that hexose sugars can be noved from green cells of the parental generation to zygote torages, even under conditions when DIC is not limiting to egetative growth. *Coleochaete* produces greater numbers of eiospores than any other green alga lacking alternation of enerations, and we hypothesize that matrotrophy explains his difference. We suggest that matrotrophy was inherited y embryophytes from ancestral charophyceans and used as means of supporting amplified spore production, and in spects of sporophyte morphogenesis.

Because rapid diversification of tissue-specific members f gene families is involved in the origin of functional nnovations and evolutionary radiation in other organ-smal groups (Iwabe *et al*. 1996; Henikoff *et al*. 1997), we re exploring the possibility that gene duplication, diver-ence and tissue-specific expression play important roles n the evolutionary origin of plant matrotrophy. We are urrently testing these hypotheses by cataloguing equences of hexose-transporter gene family members in elected charophyceans and bryophytes, then using the nformation to study expression patterns. We postulate hat specific gene family members may occur only in haraphyceans having retained zygotes, and that related, lacenta-specific gene family members may occur in ryophytes and other embryophytes.

Hexose transporter genes of the green alga *Chlorella* Sauer & Tanner 1993) and *Arabidopsis* (as well as other owering plants) have been well characterized at the nolecular level, allowing identification of conserved egions. We have used such genetic database information o design primers for use in polymerase chain reaction mplification, cloning and sequencing of portions of everal members of hexose transporter gene families in a umber of charophyceans and bryophytes. We were uccessful in obtaining multiple, partial, putative hexose-ransporter gene sequences from all three mosses nvestigated, *Sphagnum*, *Andreobryum* and *Mnium*, using the orward primer 5′-GATGGTACCGGATCCTTYTTY CARCARYTIACIGGIATHAA-3′ and the reverse rimer 5′-GATCTGCAGTCGACTCDATIGGIAYICCY TTIGTYTCIGG-3′. 5′ portions of the primers contain estriction sites to facilitate directional cloning.

Partial sequences of similar genes were also obtained from the hornwort *Megaceros*, the liverwort *Conocephalum* and several charophycean green algae with the same primers, but amplification product was not consistently obtained for liverworts and charophyceans. Evidence that our primers amplify portions of hexose transporter proteins includes high similarity in primary sequence data and in deduced hydropathy profiles of charophycean and bryophyte/amino acid sequences inferred from our DNA sequences and those of homologous regions of *Chlorella* HUP1 (hexose uptake protein 1), and *Arabidopis* STP 1 (sugar transporter protein 1).

In conclusion, it appears that the use of (i) neontological approaches such as molecular analysis of matrotrophy in charophyceans and bryophytes, and (ii) combined neontological/palaeontological approaches in which resistant morphology provides links between extant charophytes and bryophytes and microfossils, are likely to illuminate the evolutionary origin of the embryophytic life cycle to a degree previously thought impossible. Such studies provide not only insight into evolutionary mechanism, but also have implications for understanding the effects of earliest plants on Ordovician biogeochemistry and may be useful in deducing how the complex regulatory systems operating in higher plant reproductive development have been constructed over evolutionary time.

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Discussion

P. Kenrick (*Department of Palaeontology, Natural History Museum, London*). You suggest that fecundity was a major driving force behind the origin of the sporophyte generation in land plants: rarity of fertilization on land is compensated by the multiplication of the products of fertilization (spores) made possible by the development of sporophytes. Can similar arguments be made for the development of sporophytes in other algal groups (e.g. Ulvales)?

L. K. E. Graham. The most comparable situation occurs in red algae, where there is a parallel temporal–spatial association between the sporophyte (carposporophyte) and female gametophyte. At least theoretically, the frequency of red algal fertilization may be limited by the absence of flagella from male gametes. It has been argued (Searles 1980) that fecundity was the major driving force in the origin of the carposporophyte of red algae; origin of the land plant sporophyte was cited as a parallel example. Some physiological and anatomical work on red algal female gametophyte–carposporophyte associations (Hommersand & Fredericq 1990) seems to support Searles's conjecture.

The hypothesis may reasonably be extended to Ulvales, hough intimate association of sporophytes and gameto- hytes is lacking. Multicellular ulvalean sporophytes can learly produce greater numbers of meiospores than can resumed ancestral forms (modelled by Ulotrichales whose diploid stage is unicellular), and this could be dvantageous in the marine habitat (Graham & Wilcox 000). It is possible that mating frequency in ulvaleans ould be limited by the ability of gametes to locate each other in a big ocean, but few studies have focused on mating success rates in algae. Such data could be helpful in testing the hypothesis.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES