

Opinion Article

Origin and early evolution of land plants

Problems and considerations

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Key words: alternating generations, bryophytes, evolution, green algae, pteridophytes, sporophyte origin

The origin of the sporophyte in land plants represents a fundamental phase in plant evolution. Today this subject is controversial, and scarcely considered in textbooks and journals of botany, in spite of its importance. There are two conflicting theories concerning the origin of the alternating generations in land-plants: the “antithetic” theory and the “homologous” theory. These have never been fully resolved, although, on the ground of the evidences on the probable ancestors of land plants, the antithetic theory is considered more plausible than the homologous theory. However, additional phylogenetic dilemmas are the evolution of bryophytes from algae and the transition from these first land plants to the pteridophytes. All these very large evolutionary jumps are discussed on the basis of the phyletic gradualist neo-Darwinian theory and other genetic evolutionary mechanisms.

Considerations on the Embryophyte Evolution

Many aspects of difficult interpretation exist in the phylogenetic history of land plants. Already on the origin of plant lineage, we still have no safe dating for the endosymbiotic event, i.e., the integration of a cyanobacterium to form the precursor of chloroplasts. Similarly, we have no knowledge of the kind of eukaryote cell partner that participated in this symbiosis (as well as for the ancestor cell of animal lineage).¹

In the present paper transitional events of plant evolutionary biology considered to be of great interest will be reviewed and discussed. The origin of the sporophyte in land plants (Embryophytes) is a fundamental evolutionary problem. All organisms, except prokaryotes, undergo regular sexual reproduction that involves a regular alternation between meiosis and fertilization. In land plants it is expressed by two alternating generations.

In animals, development normally starts from a zygote, which undergoes a series of mitoses to produce a diploid organism. When this organism is mature, it directly forms haploid gametes from determined diploid cell lines by means of meiosis. In this case the meiosis is called gametogenic or, less properly, gametic. The term

“terminal meiosis” is also used because this process occurs at the end of the life cycle of these cell lineages in an organism which is otherwise wholly occupied by diploid individuals, the haploid state being represented only by the gametes. In contrast, in all land plants, life cycle is constituted by an alternation of generations, the diploid sporophyte and the haploid gametophyte. The sporophyte does not form gametes but haploid spores by meiosis. These spores divide by mitosis and develop into gametophytes, which directly produce gametes. Fertilization between male gamete and female gamete forms a diploid cell, the zygote, which restores the sporophyte generation. Therefore the land plant life cycle is of diplo-haplontic type with an intermediate or “sporic” meiosis. Although this diplo-haplontic life cycle shows great variations in the different taxonomic groups with regard to the dominance of the gametophyte (in Bryophyta *s.l.*) or sporophyte (from Pteridophyta *s.l.*, to seed-plants), these two generations remain a fundamental component of land plant life cycle. In fact, also in the current seed plant taxa gymnosperms and angiosperms, where the gametophyte had dramatically decreased in the extent of its duration and size, it is still present within the mother plant, even if consists of several thousand to two cells. Moreover, land plants, except bryophytes and many pteridophytes, are heterosporous species, i.e., the sporophyte forms two different types of sporangia (microsporangia and megasporangia). These structures produce micro and macrospores that develop into male and female gametophytes, respectively. In all the embryophytes the gametophyte and sporophyte have distinct morphologies and degree of development (alternating heteromorphic generations).

The gametophyte generation is considered to be older than the sporophyte generation, since, in evolution, the development of sex surely preceded the alternation of generations.²⁻⁴ This statement is supported by the relationship between gametophyte function and the need of water for sperm motility.

In most algae (*s.l.*), which are regarded as the plant taxa that preceded land plants, the gametophyte generation is the only generation of the life cycle (haplontic cycle), where the diploid zygote may be considered homologous of the sporophyte.⁵ In these algae, the development starts from a single haploid cell, a spore, which results from meiosis in the zygote. By contrast, a true diploidic sporophyte generation is found in many algae species belonging to the genera *Ulva*, *Cladophora* (green algae), *Ectocarpus*, *Dictyota* (brown algae), except for the cells involved in gamete formation (gametophyte generation). Therefore these algae have a diplo-haplontic life cycle

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Submitted: 05/23/08; Revised: 09/04/08; Accepted: 09/16/08

Previously published online as a *Communicative & Integrative Biology* E-publication: <http://www.landesbioscience.com/journals/cib/article/6987>

with a sporic meiosis. Moreover, they show an alternation between morphologically indistinguishable gametophytes and sporophytes (homomorphic or isomorphic), contrarily to other algae with a heteromorphic alternation of generations. However, a diplontic life cycle is present in some brown algae (Fucales) and green algae (*Bryopsis*, *Caulerpa*, *Codium*, *Acetabularia*). In this cycle, which is present in all animals as said, although no alternation of generations occurs, there is an alternation of two nuclear phases, one haploid (the gametes) and the other diploid (the organism which develop by mitosis from the zygote), therefore, in this case meiosis is gametic. This cycle is considered the most evolved in all the living organisms.⁶

The sporophyte, thanks to its evolutionary proven better capacity to increase gradually in size and complexity, has been a fundamental innovation for terrestrial plant development and evolution⁷. In fact, starting from the first known eukaryotic land plants, i.e., the bryophytes, the sporophyte allows a strong aerial spore dispersal, and thus with a greater genetic variation potential. These, produced in great quantity, are very resistant to desiccation because they are impregnated with sporopollenin, the most decay resistant biological material known. This process has been successively perfected in the gymnosperms and, especially, in the angiosperms (spermatophytes), where gene flow via spore dispersal has an additional mechanism by seed dispersal, completely independent of the presence of water. Starting from the bryophytes, the evolution of land plants has proceeded through a progressive reduction in the extent of the haplophase and an increasing dominance of the diplophase, considered evolutionarily favored.^{8,9} Briefly, what are the biological advantages of diploidy? One of the strongest genetic consequence of this status with a dual set of genes is the ability to mask deleterious mutations through the dominant-recessive interaction of alleles, thus allowing a large number of alleles to persist in a population or species. In fact, the vast majority of mutations that adversely affect fitness are partially or completely recessive.¹⁰ Therefore, the diploids may better adapt to unfavorable conditions because they have a pool of genes, which provides a source of genetic variability that may be beneficial in the case of negative environmental changes.¹¹ Moreover, from pteridophytes to angiosperms, most sporophytes exhibit indeterminate proliferating organs (shoots), by means of apical meristematic activity, bearing multiple sporangia. This system of less limited growth, a feature present also in the root(s), tremendously increased the contact of the plant with both water and mineral resources (soil) and light and CO₂ resources (atmosphere). This organogenic characteristic conferred to the sporophyte the capacity to increase in body size, and then to form a greater number of dispersal structures such as spores or, successively, seeds, as well as to continue growth if some stem cells were damaged or lost by biotic and a-biotic factors.¹² Therefore, sporophyte branching, together with other morphophysiological characters (cuticle, lignified cells and the differentiation of a variety of tissues), permitted the evolutionary transition toward a complete morphological and physiological sporophytic dominance upon the gametophytic phase. However, a long haploid phase can be favored by evolution under certain circumstances: in fact, selection is more efficient at eliminating deleterious mutations in haploids.⁹ Many other questions concern this subject. For example, in the diplo-haplontic cycle, the relations of the morphological and physiological characteristics of a generation to its genomic (chromosome number) constitution,⁶ or the maintenance of alternating generations

up to the angiosperms. These problems, which fall outside the scope of the present article, have no definitive answer.

Now, on the ground of these considerations, the first appearance of the sporophyte generation in plants remains a very intriguing question. Two different hypotheses have been proposed about this event. One is the “antithetic theory” (“interpolation theory” or “heterologous theory”), proposed by Bower.¹³ Bower¹³ and other authors^{4,5} suggested that the first multicellular diploid phase or sporophyte in land plants appeared when a bryophytean gametophyte bearing gametes evolved an archegonium in a gametophyte of algal origin. Technically, the archegonium is a multicellular organ ontogenetically derived from a single (haploid) cell on the gametophyte. This structure consists of a tubular-shaped neck through which sperm swim in a fluid-filled canal to reach the egg contained in a globose base (venter). The diploid sporophyte “developed” from a zygote retained within this gametophyte archegonium by delay of the zygotic meiosis. In this case the zygote divided mitotically rather than meiotically to form a mass of diploid cells, the ancestral sporophyte. Successively, a group of cells in this diploid phase divided meiotically to form tetrads of spores; thus a larger sporophyte evolved through an increasing sterilization of the sporogeneous tissue and delayed meiosis. Later, Bower¹⁴ preferred to refer to the “antithetic theory” as the “interpolation theory”, indicating that the sporophyte was indeed “added” to the life cycle.

The other theory is known as the “homologous theory” (“transformation” or “modification” theory), proposed initially by Pringsheim,¹⁵ and successively elaborated by Fritsch.¹⁶ According to this hypothesis, the sporophyte originated by a direct modification of the gametophyte which assumed the specific function of spore production. This hypothesis is based on the life cycle of certain algae, such as *Ulva*, *Cladophora*, *Ectocarpus*, mentioned above, which have similar but distinct photosynthetic gametophyte and sporophyte (homomorphic generations). These structures are considered “homologous”, except for their chromosome number,^{5,17-19} diploid the sporophyte, haploid the gametophyte. Therefore the land plant ancestors would have been green algal organisms with a diplo-haplontic life cycle (sporic meiosis) prior to land invasion. Consequently, in the first land plants the alternating generations were isomorphic and independent, but gradually the sporophyte became attached to the gametophyte with reduction in its complexity. Apogamy (formation of a sporophyte by the gametophyte without fertilization) and apospory (formation of a gametophyte by the sporophyte without the function of meiospores) have been alleged as evidence for the “homologous theory” of alternating generations, since in some cases gametophyte and sporophyte seem interconvertible.^{5,20,21} However, this hypothesis must account for the strong shift from homomorphic generations (*Ulva*) to two different heteromorphic life cycles, one with a dominant gametophyte (Bryophyta *s.l.*) and one with a dominant sporophyte (all other land plants).^{20,22} Another important evolutionary “jump” in the transition from such algae to bryophytes is to switch from the two free-living gametophyte and sporophyte phases, present in these algae, to sporophyte dependency on the gametophyte as found in bryophytes. A further problem of this hypothesis is the homomorphic alternating generations of these algae, which is completely in contrast with the heteromorphic generations of land plants.

Nevertheless, some clarifications are needed on the phenomena of apogamy and apospory mentioned above, on a possible morphological and anatomical similarity and independence between gametophyte

and sporophyte of the first land plants, supported by recently discovered fossil gametophytes of *Lyonoophyton rhyniensis*.⁷ This plant, comprised in the Rhynie chert organisms found in Aberdeenshire (Scotland), dated 396 ± 12 m.y.a., is now known as *Aglaophyton major*. The development of its gametophyte has been well documented from the germination of spores to the formation of mature gametangia on sexually dimorphic or unisexual gametophytes.²³ Moreover, free-living gametophytes are known also for other plants of Rhynie chert such as *Rhynia* and *Horneophyton*. *Aglaophyton*, like all of the Rhynie chert sporophytes is interpreted as homosporous. These unisexual gametophytes were part of a reproductive strategy that optimized fertilization vs. benefit of out breeding like some actual bryophytes and other terrestrial plants.²³

Now, the problem concerning these two conflicting hypotheses, and in particular the “homologous” one, resides at a fundamental question: the ancestor(s) of land plants. Considerable evidences support the hypothesis that green algae (Chlorophyta *s.l.*), and in particular, the Charophyceans or Charophyta (*Coleochaete*, *Chara*, *Nitella*) are the most representative group of immediate land plant ancestors.^{4,24} Particularly interesting are species of the modern genus *Coleochaete*, and in particular *C. orbicularis*, which appear to offer developmental clues to embryophyte origin.²⁵ This genus comprises small fresh-water algae, some of which with branched filamentous plant body, others with cells growing in radiating rows attached together to form a flat disc, forming a distinct thallus. This “dorsiventral bifacial” thalloid structure is considered interesting in relation to the ancestors of land-plants.²⁶ In fact, gametophytes of liverworts and some vascular plants (*Equisetum*, lycopods, certain ferns) retain a similar pattern of parenchymatous growth.²¹ *Coleochaete* has the most highly developed system of sexual reproduction in the Charophyceans. Most species are oogamous (see below), similarly to embryophytes. Vegetative cells near the margin of the thallus become greatly enlarged and form oogonia; each oogonium (a single cell) includes one nonmotile female gamete or egg. The oogonium develops a long neck, the trichogyne. Other vegetative cells become antheridia which by division form a number of smaller cells, each of which produces an apically biflagellate sperm. These sperms are consistent with sperms of land plant.⁴ Fertilization by these swimming sperms occurs within the oogonium through the trichogyne. Thereafter, the zygote enlarges and remains in the gametophyte, becoming surrounded by a layer of parenchymatous cortical protective cells. These cells permit the transfer of nutrients from the mother organism to the zygote (matrotrophy). After a resting period the zygote undergoes meiosis inside the oogonium, giving rise to a group of eight or more cells, which becomes biflagellate zoospores. These, after escaping from the zygote wall, develop into new haploidic plants. Algae such as *Coleochaete* (and also *Chara* and *Nitella* as well as other green algae) exhibit a life cycle of haplontic type where one generation, i.e., the individual or gametophyte in the cycle is haploid, the only diploid phase being the zygote (zygotic meiosis).

No group in the plant kingdom has presented greater difficulties in classification than the Charophyceae. Some authors consider Charales and Coleochaetales distinct orders and believe that the Coleochaetales are monophyletic and less closely related to land plants than the Charales.²⁷ In fact, in the case of *Chara* and *Nitella* the reproductive organs may be considered more similar to those of bryophytes. The female reproductive organ, the oogonium consists

of a large central cell, in which the egg is produced, surrounded by a sterile jacket of elongated spiral cells extending beyond the fertile cell and forming a crown at the tip. A sterile jacket tissue is characteristic of male and female sex organs (gametangia) in the embryophyta where encloses and protects the gamete producing cells. The antheridia of the Charales are very complicated; they produce sperm, which fertilize the egg cell within the oogonium. These algae produce only gametes and never flagellated spores. The complete absence of spore flagellation is a peculiar character of primitive and modern land plant spores.²¹ The zygote of Charophyceans is very resistant to dry and cold conditions. This adaptation is attributed in part to a wall formed by sporopollenin.

In any case, the monophyletic origin of embryophytes, and that Charophyceae (Charophyta), especially Coleochaetales and/or Charales, are the closest living algal relatives of land plants is agreed.^{4,28-31} This single origin of land plants from charophycean algae is based on a great number of shared common morphological, cytological, ultrastructural, biochemical and, especially, molecular attributes, the existence of which has been demonstrated by numerous studies. These principal key characters are: the capacity to produce sporopollenin; a phragmoplast cytokinetic system of microtubules indistinguishable from that of higher plants, including an open mitosis with a interzonal spindle; a cell plate which forms centrally and progress centrifugally; primary plasmodesmata that provide intercellular links, structurally comparable to plant plasmodesmata; complex branched thalli resulting from acquisition of the capacity for a 90° change in the polarity of cell division; apical meristematic (stem) cells similar to those of land plants but lacking more than two cutting faces; tissues consisting of three-dimensional arrays of related cells; asymmetric cell division; cellular specialization within the multicellular body; the placenta, manifested in the form of specialized transfer cells at the base of the oogonium (archegonium venter, in the bryophytes), connecting the maternal gametophyte and retained diploid generation or nuclear phase;^{4,7,12,32} a flagellar structure of motile cells similar to that found in sperms of embryophytes.^{33,34} Very important are the biochemical evidences, i.e., chlorophylls (a and b), carotenoids, starch, the photorespiratory enzyme glycolate oxidase found in peroxisomes, cellulose-synthesizing complexes (rosettes), and flavonoids (in the Charales).⁴ In particular, molecular studies, together with statistical sophisticated analyses, identify the charophytes as the closest living relatives of the land plants. These studies considered different parameters such as the DNA sequence data from four genes representing three plant genomes: *atpB* and *rbcL* (plastid), *nad5* (mitochondrial), the small subunit (SSU) rRNA gene (nuclear),^{4,35-37} and also tRNA introns.³⁸⁻⁴⁰

Questions remain as far as which type of algae (*Coleochaete*, *Chara*, *Nitella* or others) are actually the closest phylogenetically to bryophytes and all other land plants. Some conflicting results are reported in the literature. Katana et al.,⁴¹ placed *Coleochaete* and also the genus *Klebsormidium*, on the ground of nuclear SSU rDNA sequences, closer to primitive land plants than *Chara* and *Nitella*. On the other hand, the work of Nakayama et al.,⁴² utilizing nuclear-encoded SSU rRNA sequences, demonstrated that these last algae are more closely connected to land plants than *Coleochaete* and *Klebsormidium*. However, the presence of group II introns in two different tRNA genes of land plants suggested that *Chara/Nitella* are the closest living relatives to land plants.^{38,40}

A more recent investigation carried out with the aim to resolve the relationships among the four major lineages of land plants (liverworts, mosses, hornworts and vascular plants), and including also *Coleochaete*, *Chara* and *Nitella* (and some other green algae) through the analysis of chloroplast (cp; cp-*atpB*, cp-*rbcL*, and cp-SSU and cp-LSU rDNAs), mitochondrial (mt; mt-LSU rDNA) and nuclear (nu; nu-18S rDNA) genes, with additional data obtained from GenBank, provided a further confirmation of the close phylogenetic link between these algae and all the other land plants.³¹

However, a strong and abrupt evolutionary jump exists between these algae, where the diploid generation is represented only by the zygote, and the land plants, which present already in the bryophytes a pluricellular and organized sporophyte. In none of these living algae (or fossils, as far as it is known), an alternation of generations, i.e., presence of a sporophyte, exists: all advanced charophytae have only the gametophyte.

Therefore, it is very difficult to be in favor either of the “antithetic” or the “homologous” theory. The “antithetic theory” of sporophyte origin is considered the more plausible and based on rational concepts, given also the preponderance of all the evidences found on *Coleochaete*, *Chara* and *Nitella* as the representative of the ancestral forms of land plants, and considering also the possibilities offered by their life cycles.⁴ An argument strongly unfavorable to the “homologous theory”, which is based on algae with homomorphic and independent gametophytes and sporophytes, as reported before, is that these organisms are definitively considered not among those green algae most closely related to land plant ancestors.²⁶ Moreover, no genetic explanation for this hypothesis has been proposed up to now.

If we consider that the bryophyte sporophyte originated by a delay of zygote meiosis with zygotic mitoses by which a dependent sporophyte might have formed and persisted, another interpretation of this process is possible. The course of meiosis is the product of a definite temporal sequence under the action of different genes. Therefore, a block of the meiotic stages due to mutations might have occurred. Many cases of a genotypic control of the meiosis have been reported in plants.⁶ The delay of zygotic meiosis might result in a phenomenon comparable to apomixis in plants, a kind of diplospory. Certainly, the diploid cell or spore did not form an unreduced (2n chromosomes) gametophyte but a sporophyte.

Unfortunately no relationship exists between the current green algae with diplontic cycle and land plants. Otherwise, it would be possible to imagine another scenario with regard to the origin of the alternating generations of land plants (but this is a speculative idea). Considering gametes directly formed by meiosis by an organism with only the sporophyte generation, could these haploid cells form, by a delaying fertilization process and through mitoses, a gametophyte-like organism? In this case, mechanisms similar to those hypothesized for delayed meiosis, i.e., a mutation or several mutations⁴ could be also sound.

Although the “antithetic” or “homologous” hypotheses try to explain the origin of the sporophyte of embryophytes from algae, fossil evidences did not help to resolve this evolutionary puzzle because intermediary organisms have not found. The earliest known fossil land plants presented a well-formed sporophyte.³⁰

In this frame a questionable speculation may be whether tracheophyte and bryophyte sporophytes have had any direct evolutionary connection.^{21,43} In fact, the problem is the great difference between bryophytes and pteridophytes, the latter with distinct and indepen-

dent gametophyte and sporophyte (except for the initial sporophyte developmental phase). The question concerning the Anthocerotae bryophyte group (hornworts) as possible direct ancestors of the pteridophytes will be discussed below.

In the history of the evolution of land plants, a further significant phylogenetic dilemma concerns the transition from bryophytes to pteridophytes.

Most botanists consider bryophytes (liverworts, hornworts and mosses) and basal pteridophytes to be the oldest living remnants of the eukaryotic plants that colonized the land.^{24,28} This flora exhibits phenotypic characters or innovations in both the gametophyte and sporophyte, which enabled them to be adapted to the existence on land, and in particular, to optimize their reproductive capacity by spore dispersion.

If we consider bryophytes as the first land plants derived directly from a green algal progenitor, a large evolutionary gap exists between these two types of organisms not only in life cycle, but also at the structural/developmental level. The gametophyte, which for its dominance upon the sporophyte distinguishes the bryophytes from all other embryophytes, is the most elaborate of the land plants. The principal characteristics of the bryophyte gametophytes are the growth forms constituted by a flattened prostrate thallus (hornworts and thalloid hepatics), and an erect or creeping cylindrical leafy shoot (leafy liverworts, simple thalloid liverworts and mosses). These growth habits correspond to the optimal life forms to maximize light capture for photosynthesis and enhance water conservation or providing maximum surface area to volume for gas exchange (liverworts and mosses), and with upright growth (mosses) to facilitate spore dispersal.⁷ Another new evolutionary character is represented by a well-developed apical growing region with a single apical cell that is responsible for gametophyte growth. Leaf primordia overarch and protect the apical cell and immediate derivatives. Mucilage-secreting hairs accompany leaf development in mosses and liverworts and furthermore afford protection against damage and desiccation.²⁸ The geometry of this apical cell may be one of four fundamental shapes such as: wedge-shaped (cuneate), lens-shaped (lenticular), tetrahedral (pyramidal) and hemidisoid.^{44,45} Pyramidal systems are typically associated with leafy habits, lenticular systems with highly flattened thalli, often with thickened midrib and monostromatic wings, cuneate and hemidisoid apical cells are responsible of thalloid growth forms, including those of complex thalloid liverworts and hornworts. In mosses, pyramidal systems predominate.⁴⁴ In particular, evolutionary adaptations enabled gametophyte mosses to acquire more extensive and specialized conducting tissues,⁴⁶ and the capacity to have desiccation and thermal tolerance.²⁸ Moreover, these gametophytes show remarkable powers of vegetative reproduction by different means and to produce clones.^{47,48} In the evolution of these first land plants the sudden appearance of multicellular sex organs with a sterile jacket of cells around the gametes (antheridia and archegonia) was another most remarkable events.⁴⁸ The evolution of these gametangia and, in particular, of female sex organs or archegonia, necessary for embryo development, constituted a complex evolutionary process with morphogenetic characters that required a close coordination with physiological and ecological signals. For example, production of sex organs must be timed appropriately and must occur rapidly in response to fluctuating seasonal conditions.²⁸ Although the parallel evolution of antheridia was also required, the

formation of archegonia was fundamental because these structures protect and nourish the young sporophyte (embryos) influencing its development. The archegonium, already mentioned above, apparently simple, is an extremely sophisticated reproductive device. In fact, at the interface with gametophyte tissue (archegonium venter) there are specialized transfer cells with highly branched or convoluted walls that increase the surface area through which the nutrients necessary to egg cell development first, to zygote maintenance and later embryo growth, pass.²⁴ Gametangia of mosses and liverworts, unlike those of pteridophytes are stalked and extend from the epidermal surface²¹ and various mechanisms and structures evolved to protect them.²⁸ In contrast, among bryophytes, only hornworts gametangia are contained within the thallus, like those of pteridophytes.⁴⁹ However, the development of archegonia of pteridophytes is different of that of hornworts.²⁸

In regard to the bryophyte sporophyte some important remarks can be made. Given the basal position of these plants in embryophyte phylogeny,^{24,27} to consider this sporophyte less complex than those of pteridophytes is an oversimplification.²⁸ Although in bryophytes each sporophyte is practically a solitary sporangium designed to make and release spores, this sporangium, in its different aspects, shows a particularly complicated structure that is not found in other land plants (tracheophytes). In fact, particular characters are present such as the peristome of true mosses for spore dispersion, the cellular elaters of liverworts, and the highly complicated sporangium of hornworts that continues to elongate throughout the growing season. Such intricate sporangial complexity is not present in the tracheophytes, because its role has been taken by the vegetative sporophyte as a whole, not by the sporangium itself.²⁸ Another peculiar characteristic of the bryophyte sporophyte, in contrast with the sporophyte of tracheophytes, which is polysporangiate, is the formation of a multitude of sporophytes that may be produced by the single gametophyte.²⁸ If out crossing occurs, each sporophyte is genetically different from the other sporophytes originated on the same gametophyte. This ability to generate multiple sporophytes strongly increases the genetic variability of bryophyte gametophytes. In the tracheophytes, only basal homosporous plants have sometimes the capacity to form multiple sporophytes in longer-lived subterranean gametophytes (except the case of primary and secondary polyembryony present in Gymnosperms, whose gametophyte is not subterranean).^{28,49} In addition, in bryophyte sporophyte different mechanisms for elevating the sporangium and increasing spore dissemination have evolved, along with other characters related to better sporophyte development and survival: photosynthetic capacity, presence of the placenta with specialized cells at the sporophyte/gametophyte interface, food-transporting cells.^{50,51} The sporangial dehiscence may involve several specialized mechanisms. Hornwort sporophyte dehisces in a particular way along two sutures on either side of the sporangium, while many liverworts have capsules that split into four valves, and mosses form an operculum with an underlying elaborate peristome, as reported before. Complex structures as stomata occur in many mosses (for example *Sphagnum*) and in two genera of hornworts.²⁸

Starting from the assumption that bryophytes are the earliest and simplest land plants, and thus the ancestors of pteridophytes (and by that of all other tracheophytes), numerous significant gaps exist also between these two early groups of land plants. It is noteworthy

that some authors raised the theoretical possibility that bryophytes derived from basal pteridophytes as a simplified form of their more complicate sporophyte structure.⁷ The pteridophyte sporophytes are responsible for vegetative growth and are provided with true xylem and phloem elements and mechanical tissues. The appearance of true water- and food-conducting tissues in pteridophytes represented an event of enormous consequences for the evolution of land plant sporophyte. Although this particular topic is beyond the aims of the present paper, let us remember that these tissues conditioned all tracheophyte (sporophyte) evolution. The selection pressure favored the diploidic organisms with conducting tissues, i.e., the sporophyte generation of vascular land plants (from pteridophytes to angiosperms) at the expense of the gradual but dramatic decrease of their gametophyte generation. Moreover, contrarily to bryophytes which produce only homosporous spores that can develop sexually as dimorphic gametophytes, a significant number of living (and extinct) pteridophytes are heterosporous, forming male and female gametophytes. Heterospory appears to have conferred a selective advantage under terrestrial conditions.²⁸ A very recent paper of Fujita et al.⁵² evidenced an interesting difference in shoot development between bryophytes such as mosses and vascular plants. Their results indicate that polar auxin transport is not involved in shoot development (the haploid gametophyte) in mosses, in contrast to shoots (the diploid sporophyte) of vascular plants. Consequently, these two plant groups seem to use a different molecular mechanism during their development.

This dominant sporophyte condition seems to have already been present in the first vascular fossils (rhyniophytes) such as *Cooksonia* and *Rhynia*.^{30,53} Among tracheophytes, each (female) gametophyte of heterosporous forms has the capacity to produce only one sporophyte, except for extinct basal homosporous tracheophytes and current Gymnosperms, as mentioned above. In this character these basal pteridophytes were similar to bryophytes. Another significant innovation that appeared in the pteridophytes was the ability of the sporophyte, after its first developmental phases, to become completely independent of the gametophyte. Obviously, this capacity is correlated to the difference in structural and physiological complexity between this sporophyte and that of bryophytes, as mentioned before. The problem of the sporophyte independence evolution may be overcome only if hornwort plants, i.e., Anthocerotae, are placed at the basal position of extant pteridophytes⁵⁴ or assuming an extinct missing link. This particular evolutionary scenario got strong support from new molecular data.³¹ The broad study of these authors provided substantial support for the placement of hornworts as a sister group to vascular plants. This novel result could solve the long debated question on a direct lineage connection between tracheophyte and bryophyte sporophyte.⁴⁵ This relationship is strengthened also by several morphological and physiological characters of the hornwort sporophyte. Among these are the persistently chlorophyllous and nutritionally largely independent sporophyte, rhizoid-like present on the sporophyte foot, the longevity and large size of the sporophyte, and paleobotanic findings.³¹ The evidence on the closer relations of hornwort with vascular plants concerning sporophyte evolution and development, may override the hypothesis concerning a direct origin of pteridophytes from charophycean-like algal ancestor(s) or other algal type (with diplo-haplontic cycle) formulated by some authors.^{21,43}

The origin and early evolution of land plants or embryophytes, after all, allowed the evolution of two elaborate different phase cycles (gametophyte/sporophyte alternance in bryophytes and pteridophytes) followed by the development of complex organs and tissue systems. These phase life cycles remain two extraordinary evolutionary paths, which according to the neo-Darwinists, can be explained by the classical gradualist theory of evolution (phyletic gradualism). Unfortunately, a precise transitional fossil lineage of these vegetational changes have not been found because evidence on the origin and diversification of very early land plants have come mainly from dispersed spores and megafossils of sporophytes.³⁰ Surprisingly, in spite of the great quantity of phylogenetic studies that indicate the strong relationships between charophyceae and bryophytes and between bryophytes and pteridophytes (and all other land plants), now increasingly based on molecular data, the evolution of the entire multicellular diploid phase of land-plant life-cycle is poorly discussed in terms of evolutionary mechanisms.

Since natural selection of existing genetic variations is assumed as the mechanism responsible of these stages of plant evolution, it must be hypothesized that a significant number of continuous micro-changes or point mutations resulted in the transition from algal organisms to bryophytes and from these to pteridophytes. Therefore it was necessary that many of these phenotype changes were characterized by a well-integrated functional succession followed immediately by natural selection.^{7,55} The traditional Darwinian view of gradualism states that evolution is a continuous process requiring a long period of time. This is not yet supported enough by the fossil evidence mentioned above which would indicate that early land plants most likely evolved at the end of the Ordovician period and certainly not later than the early Silurian (about 470–425 Myr).^{24,56}

However, considering that the bryophytes were formed principally by the haploid gametophyte as the dominant phase, any mutation expressed in such organisms would have been immediately exposed to selection, which might have operated against most of them.⁹ So bryophytes, diversified by adaptive radiation in the three groups reported, became evolutionarily stable.^{9,48}

Other genetic phenomena might have been involved in the evolution of all these plant groups such as transposons, gene duplications and/or genome duplications or large chromosomal rearrangements⁷ as well evolutionary changes, due to gradual vs. saltational or punctuated equilibrium.⁵⁷

However, no serious evidence exists, at the moment, on the particular genes responsible for the great differences between algae and bryophytes and between the latter and pteridophytes.

In conclusion, I have tried to point out and discuss some critical events of land plant evolution, the understanding of which requires future studies to build a large-scale framework that includes further key factors especially at paleobotanical and molecular levels. In particular, the sequencing of several full genomes of these relevant taxa might provide an overview of their phylogenetic relationships. However, a larger vision of evolutionary processes is necessary, giving major attention to the multiplicity of relationships among endogenous genetic-molecular and epigenetic mechanisms and environmental factors.⁵⁸

Acknowledgements

I thank Professor Frantisek Baluska for his encouragement to publish this paper.

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