Evolution of Primitive Land Plants: A Review

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Abstract   Our understanding of the evolution of primitive land plants is overviewed. Molecular phylogenetic data suggest that the suggested ancestor of the land plants is Charophyceae, although other ancestry is suggested based on different lines of evidence. Invasion to land environment during the mid-Ordovician or earlier should have been made possible by chemical compounds, ozone, flavonoid, cutin, sporopollenin, and lignin, against UV irradiation, drought and gravity. Recent molecular analyses with large data sets and fossil data provide robust evidence on land plant evolution and phylogeny, but the deep relationship of land plants is still unclear. It is likely that the earliest land plants are liverwort-like, and pteridophytes are biphyletic while seed plants are monophyletic. Evolutionary innovations, such as the appearance of different heteromorphic alternation of generations, sporophyte apical meristem, polysporangiate sporophyte, vascular tissue, leaf (microphyll and megaphyll), and seed, have been increasingly understood not only by paleobotanical, comparative morphological, biochemical, and developmental studies, but also by recent molecular genetic analyses.

Key words: alternation of generations, leaf, molecular phylogeny, polysporangiate plants, seed, sporophyte, vascular tissue.

Ancestor of Land Plants

The earth, born 4.6 billion years ago, was long inorganic but experienced a chemical evolution until the appearance of the first prokaryotic organisms at least 3.5 billion years ago. Subsequently eukaryotes appeared 1.5–2.7 billion years ago, and three major lineages of life, i.e., animals, fungi and plants, were diverged and much later (1 billion years later) multicellularized (Futuyma, 2005). All of those organisms lived aquatic first and invaded the land 450–500 million years ago. The first terrestrial organisms were plants (which may have been preceeded by microorganisms) and followed by animals. Invasion to land was a process of conquering virgin environments characterized by drought and gravity.

The land plants evolved from diversified green algae sensu lato, among which charophytes are the most closely related to the land plants (Graham, 1993). Close relationships to other green algae or secondarily symbiotic brown algae are not considered to be true, although they had ever been postulated. The hypothesis for the land plants-charophytes relationship is based on correspondence in several microstructures and biochemical characters, e.g., chrolophylls *a* and *b*, starch as stored carbohydrates, cellulose as a major component of cell walls, phragmoplasts appearing at cytokinesis and a cell plate dividing daughter cells, multilayered structure (MLS) at the base of the flagellar apparatus, and presence of glycolate oxidase. Molecular phylogenetic analyses also support the monophyly of land plants and charophytes, which hence are together called streptophytes (Fig. 1; Lewis and McCourt, 2004; references cited therein). From recent molecular phylogenetic studies it is shown that the land plants are sister to Charophyceae of charo-
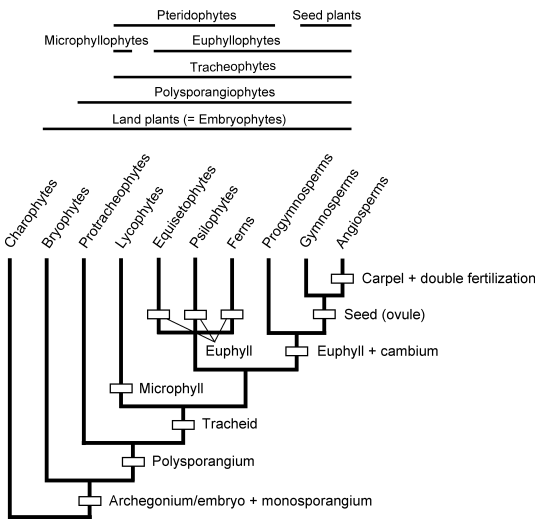
Fig. 1. Innovations mapped on simplified phylogenetic tree of streptophytes (Adapted from Kato, 2000).

phytes and together sister to *Coleochaete*. If it is correct, the land plants evolved from the common algal ancestor to Charophyceae some 1 billion years ago or earlier (Sanderson *et al*., 2004). However, land plant ancestry remains unsettled (e.g., Turmel *et al*., 2007).

Fossil data show that organisms expanded their habitats from the water (perhaps fresh or brackish) to the land several hundred million years ago. It is likely that, although ancestral multicellular algae may have attempted to invade the land before the success, the land may not have provided habitats suitable for terrestrial life. The then terrestrial environments are likely to have differed from the present ones in which the ozone layer in the atmosphere (stratosphere) reduces harmful UV to reach the earth surface. In the early phase of the evolution of living organisms, cyanobacteria (blue green algae), then together with eukaryotic algae, produced O_2, which increased in the air, as a byproduct of photosynthesis. Accompanied with O_2 increase in the air, part of it converted to O_3 in the atmosphere zone, resulting in the development of the ozone layer. Several hundred million years ago, the ozone layer became as thick as for organisms to be able to live terrestrial. Thus, aquatic photosynthetic algae have changed the global environment to the one that allowed terrestrial life.

Biochemical Compounds That Supported Invasion

The ozone layer may be called the outer shield because it was an important environmental element for the terrestrial life. In the earlier environments in which the ozone layer was not so sufficiently thick and effective, plants protected themselves from UV by means of secondary metabolites such as flavonoids (Cooper-Driver, 2001). Most bryophytes and other land plants can biosynthesize flavonoids. Ancestral land plants are likely to have protected their cells, in particular DNA by absorbing UV with these intracellular flavonoids. Therefore such chemical compounds may be called the inner shield. The cuticle covering the plant epidermis may play a similar role of the inner shield. These inner shields have protected plant bodies since the land invasion through the present. Recently, the appearance and development of the ozone holes has been a global environmental issue and, in response to such an environmental change, the Antarctic bryophytes (mosses) were reported to have increased biosynthesis of flavonoids. A report that the algal charophytes also produce flavonoids is evaluated with caution.

The terrestrial organisms, in contrast to the aquatic organisms, are exposed to the stress of drought and gravity. Invasion from water to land may not have been abrupt, but perhaps passed via unstable waterfronts that were apt to be dried temporarily. Land plants became adapted to the terrestrial environment with some innovations. The cuticular layer covering the surface of the body of land plants protects it from drought. This structure is also useful to protect from microbe infection and reflect UV. The cuticular layer contains cutin that is synthesized from both fatty acids and phenolics, but the evolutionary origin of its synthetic pathway is uncertain. Interestingly, the body of *Coleochaete*, a member of the charophytes, like bryophytes (Cook and Graham,

1998) is covered by a cuticle-like envelope (Graham, 1993).

Spores (and also pollen) are disseminules and/or propagules indispensable to the terrestrial life. Spores and pollen are covered by thick and resistant spore and pollen walls, which protect them from stresses of drought, low temperature, and UV, and allow air-borne dispersal. Spore and pollen walls are primarily composed of polymeric sporopollenin. This chemical compound is present not only in spores and pollen of land plants but also in the cell wall of the Charophyceae zygote, suggesting that its origin is old. Sporopollenin, along with cutin and lignin (see below), is a member of a family of biopolymers that are biosynthetically related. The biosynthesis and its timing of sporopollenin, which is likely to be delayed from zygote formation seen in Charophyceae to sporogenesis in land plants, are an interesting issue in order to understand the origin of land plants.

Lignin is also an important chemical compound for further evolution of land plants. Lignin is a major substance of the secondary wall of conductive and supporting cells in vascular plants. In contrast to aquatic algae living in the buoyant environment, land plants are obliged to live under a strong stress of gravity. Vascular plants and mosses develop supporting tissues. Bryophytes do not have vascular tissues, due to absence of lignin biosynthesis, but vascular plants have such biosynthetic passways. In the early evolution of land plants, a group with vascular tissue-like tissues (called provascular plants, see below) appeared from bryophytes and then evolved into vascular plants. Another role of the vascular tissue is conduction of water and nutrients. Tracheids and vessel elements of the vascular tissue are dead cells with thick secondary walls pitted or perforated on the lateral sides and terminal ends. Vascular tissues conduct water absorbed from the soil, and carbohydrates synthesized in leaves, to various other parts of the plant body. Thus lignin is a leading biochemical compound contributing to both support and conduction in vascular land plants.

Appearance of Sporophyte Generation

Land plants clearly differ from algae in having multicellular gametangia, i.e., antheridia and archegonia, so they are called archegoniophytes. Land plants are also designated as embryophytes, because the embryo grows within the archegonium. Both antheridia and archegonia are multicellular reproductive organs, compared to unicellular antheridia and oogonia of charophytes and other green algae. The gametangia of Charophyceae are exceptionally multicellular, but differ from those of land plants in morphological and functional aspects. The zygote or fertilized egg develops into an embryo within the multicellular archegonium in the land plants, whereas the zygote develops alone after fertilization in algae. The embryo is heterotrophic, absorbing nutrients and water from the mother gametophytic plant through the foot at the base of the embryo. Thus the archegonium is a multifunctional organ that not only produces an egg cell but also brings up the zygote or embryo. Certainly, such an archegonium and embryonic foot were associated with the appearance of embryophytes or land plants, but the evolutionary origin of these organs remains uncertain.

Land plants show alternation of generations (Fig. 2). The sporophyte is an asexual generation to propagate by spores, while the gametophyte is a sexual generation that yields male and female gametes (sperm and egg cell). The zygote or fertilized egg develops to the next sporophyte, which produces spores through meiosis. It is noted that there is no alternation of generations in charophytes, although they are considered to be close to the algal ancestor of land plants. The visible multicellular plant is a gametophyte generation in which fertilization occurs. The yielded zygote immediately undergoes meiosis to produce reduced zygospores, which later grow to individual plants (gametophytes). Thus the sporophytic generation is absent from charophytes. If the life cycle of the algal ancestor to land plants is similar to that of living charophytes, it is most likely that the sporophytic gen-
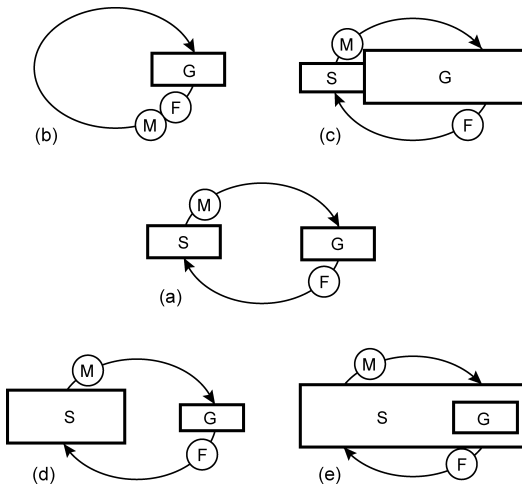
Fig. 2.   Life cycles. *a*. Alternation of isomorphic generations. *b*. Life cycle with gametophyte generation (e.g. charophytes). *c*. Bryophyte-type alternation of heteromorphic generations. Sporophyte is parasitic to gametophyte. *d*. Pteridophyte-type alternation of heteromorphic generations. Both generations are free-living. *e*. Seed plant-type alternation of heteromorphic generations. Gametophyte is endosporic and parasitic in sporophyte. *F*, fertilization; *G*, gametophyte; *M*, meiosis; *S*, sporophyte.

eration was interpolated to the life cycle with the gametophyte generation, resulting in alternation of generations in land plants. This is called the interpolation (antithetic) hypothesis (Graham, 1993). The first cell divisions of the zygote were altered from meiotic to mitotic and meiotic divisions were delayed to sporogenesis. How the mode of the first cell divisions in the zygote changed to interpolate the sporophytic generation is a significant issue to be solved.

The patterns of alternation of generations differ between bryophytes and vascular plants in the plant size and trophism. In bryophytes, the gametophyte is larger than the sporophyte, autotrophic, and is a thallus or a complex shoot with leaves, while the sporophyte is smaller, short-lived in the life cycle, and heterotrophic or semi-heterotrophic (the sporophyte of hornworts and some liverworts and mosses photosynthesize in certain degrees). The gametophyte of bryophytes

is a plant of limited size, on which sperms can swim to egg cells to fertilize (the largest is about 50 cm tall in *Dawsonia*). By contrast, the gametophyte of vascular plants is small and simple (thalloid, corm, or axial), whereas the sporophyte is large and differentiated into various organs. Thus the generations of land plants are heteromorphic but differ between bryophytes and vascular plants in the dominant generation.

Three possibilities have been put forward on the passway in which the generations of land plants diverged in bryophytes and vascular plants. First, the generations of ancestral plants were isomorphic and diverged to the gametophyte-dominant generations of bryophytes (Fig. 2, *a* to *c*) and to the sporophyte-dominant generations of vascular plants (*a* to *d*). This interpretation is called the homologous (transformation) theory. Second, the small sporophyte of bryophytes was reduced from the large one of vascular plants (*d* to *c*). This reduction is linked with the phylogenetic hypothesis in which bryophytes were derived from among vascular plants. Both possibilities are not supported by recent molecular phylogenies in which bryophytes and vascular plants form a monophyletic group that is sister to charophytes.

Third, the earliest land plants had gametophyte-dominant alternation of generations similar to that of living bryophytes, and the sporophyte was increasingly magnified to become dominant (*b* to *c*, then to *d*). This, most supported possibility is consistent with the interpolation hypothesis on the origin of alternation of generations. Fossil data suggest that earliest land plants (e.g., the sporophytic *Aglaophyton* and the gametophytic *Lyonophyton*) had semi-isomorphic alternation of generations and that the sporophyte-dominant alternation of generations of vascular plants was derived from the gametophyte-dominant one of bryophytes via isomorphic alternation. This change of the dominant generation is considered to be an adaptation for terrestrial life under stresses of drought and gravity.

The Earliest Land Plant

Fossils can provide direct evidence on events that occurred in the past. Land plant fossils can be identified in having cuticles, meiotic spores with resistant spore walls, and/or stomata, which all are confined to land plants. The oldest spore fossils discovered in the Early Ordovician (475 million years ago) suggest that land plants invaded at or before this time (Graham and Gray, 2001). Although the plants that produced cryptospores in this period are uncertain, dyads or tetrads, which were sometimes massive, suggest that they are products in bryophyte-like, multicellular sporangia. Spore-containing plant fragments are recorded from the Ordovician of Oman and liverwort affinity is suggested (Wellman *et al*., 2003). Spores from the Early Silurian are very similar to those of extant liverworts (in particular Sphaerocarpales) in the microstructure of spore wall. From these microfossils and later plant megafossils, it was inferred that bryophytes, in particular liverworts, were the earliest land plants and anteceded vascular plants, the earliest of which was discovered 426 million years ago. Molecular data estimated the time of origin of land plants to be various, i.e. 480–490, 593, 700, or even 1,000 million years ago. Discrepancy in the suggested time of origin among molecular and fossil data requires further re-estimation (Wellman, 2004; Sanderson *et al*., 2004; Hedges and Kumar, 2009).

Phylogeny of Primitive Land Plants

The appearance of bryophyte-like land plants was followed by diversification leading to extant groups, bryophytes, pteridophytes (ca. 430 million years ago) and gymnosperms (370 million years ago). In relation to this early evolution of land plants, molecular data suggest that extant land plants are monophyletic or of single origin, and bryophytes are the most basal in phylogenetic trees. These results are in consistency with the fossil evidence for the earliest bryophyte-like land plants.

There are alternative hypotheses on the phylogeny of extant bryophytes: one, bryophytes are monophyletic and derived from a common ancestor, from which vascular plants are diverged prior to diversification of living members of bryophytes. Second, vascular plants were branched from one of the three groups of bryophytes. Third, bryophytes were reduced from a group of vascular plants, but this hypothesis is not supported by recent analyses. Recent multigene and chloroplast genome analyses have proposed different phylogenetic relationships of bryophytes, i.e., liverworts are branched first and mosses are sister to vascular plants; a clade of hornworts and mosses is sister to vascular plants; hornworts are the most basal and a clade of liverworts and mosses is sister to vascular plants; and bryophytes are monophyletic (Goffinet, 2000; Shaw and Renzaglia, 2004). At present there is no convincing hypothesis on the bryophyte phylogeny. Close examination is necessary for the phylogenetic relationships among bryophytes and between bryophytes and vascular plants, in order to understand better the early evolution of land plants.

In a long-accepted, phenetic classification, pteridophytes are classified into four groups, lycophytes, psilophytes, equisetophytes, and ferns (Gifford and Foster, 1989). Among the four, ferns are considered to be the most closely related to seed plants. Fossil records also suggest that progymnosperms, the presumed ancestor of gymnosperms, have a common ancestry with ferns, and equisetophytes are also closely related to ferns. By contrast, lycophytes are considered to be very remotely related to seed plants, if any. These classification systems treated pteridophytes as paraphyletic. This treatment is generally consistent with a molecular analysis of a gene order in the chloroplast genome, with a result that pteridophytes are classified into two, i.e., lycophytes and the remaining three groups. The results of recent molecular researches differ from previous classifications, except for monophyly of lycophytes (Pryer *et al*., 2004). They show that psilophytes (*Psilotum*, *Tmesipteris*) are sister to
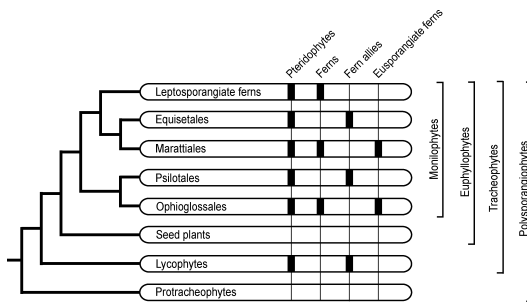
Fig. 3. Major groups of pteridophytes on simpli-
fied phylogenetic tree, showing monophyletic
groups on the right and paraphyletic groups on
the top (Adapted from Kato, 2005).

Ophioglossales, a member of ferns, and equiseto-
phytes (*Equisetum*) are sister to Marattiales,
another member of ferns, and do not support the
traditional classifications to classify pterido-
phytes into four groups (Fig. 3). Different from
these classifications, a vascular plant clade that
remains after separation of lycophytes, is di-
verged into seed plants and a group of ferns,
psilophytes and equisetophytes.

Extant gymnosperms are diverse morphologi-
cally and are traditionally classified into four
groups, conifers, cycads, gnetophytes (*Ephedra*,
Gnetum, *Welwitschia*) and *Ginkgo*. Many classifi-
cations regarded gnetophytes as sister to an-
giosperms. However, recent molecular phyloge-
netic analyses show monophyly of the extant
gymnosperms, suggesting that angiosperms were
derived from an extinct gymnosperm (Soltis *et
al*., 2005). Molecular analyses also suggested
monophyly of angiosperms, which is congruent
with an angiosperm-monophyly hypothesis based
on apomorphic characters such as double fertil-
ization and angiospermy (carpel).

Paleobotanical analyses that examined changes
in the number of species in the past 400 million
years recognized four stages in the evolution of
vascular plants (Stewart and Rothwell, 1993).
The first stage is characterized by morphological-
ly relatively simple, primitive plants, e.g., rhynio-
phytes and trimerophytes that thrived in the Early
and Middle Devonian; the second stage, by pteri-
dophytes in the Late Devonian and Carbonifer-

ous; the third stage, by gymnosperms in the Tri-
assic and Jurassic; and the fourth stage, by an-
giosperms from the Late Cretaceous and Tertiary
through the present. There is a common pattern
in the change of biodiversity in the four stages.
Appearance of a certain group of plants was fol-
lowed by rapid speciation and then by culmina-
tion of species number. As another group of
plants likewise appeared and then undertook
rapid speciation and increasing of species num-
ber, the anteceding group decreased and was re-
placed by the later appearing group. In extreme
cases it got extinct, and a typical example of it is
gymnosperms.

The extant land plants with ca. 270,000
species (vs. ca. 11,000 spp. of charophytes) are
usually classified into bryophytes (ca. 18,000
spp.), pteridophytes (ca. 12,000 spp.), gym-
nosperms (ca. 800 spp.), and angiosperms (ca.
235,000 spp.). These four groups are defined by,
e.g., the number of sporangia per sporophytes,
the heteromorphic alternation of generations, a
diversity of vegetative organs, the presence or
absence of vascular tissue and carpel, and dis-
seminule/propagule (spore, ovule or seed), all of
which are salient features for land plant evolution
(Fig. 1). The appearance of vascular tissue pro-
moted the evolution of upright plants standing
against gravity. The evolution of seeds (ovules)
allowed the evolution of seed plants from pteri-
dophytes, and then the further enclosure of
ovules within the carpel gave rise to the an-
giosperms. However, these synapomorphies do
not necessarily reflect monophyly of each of the
four groups. For example, recent phylogenetic
analyses show that bryophytes comprise horn-
worts, liverworts and mosses, and part of the
bryophytes evolved into vascular plants, although
the possibility that the bryophytes are mono-
phyletic is not entirely ruled out. It is noted that
the bryophytes were monophyletic when they
evolved from charophytes and before any deriva-
tive group was derived from them. If subsequent-
ly vascular plants would have arisen from moss-
es, the bryophytes became paraphyletic, because
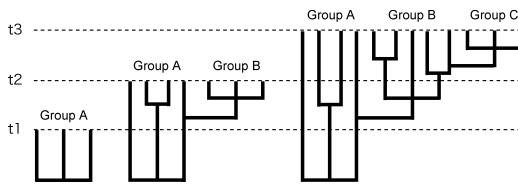they do not include the derived vascular plants.

Fig. 4. Temporal changes of phylogenetic relationship. Group *A* is monophyletic at time *t1* and becomes paraphyletic at *t2*. Similarly, group *B* is monophyletic at *t2* and becomes paraphyletic at *t3*.

As another example, dicotyledons of angiosperms became paraphyletic after monocotyledons arose from basal dicotyledons, which had been monophyletic. Thus, a group was initiated as a monophyletic group and diversified, and then part of the group evolved into another group, consequently leaving the original group as paraphyletic (Fig. 4).

Evolution of Vascular Plant Traits

Meristem and polysporangium

In a recently proposed systematic classification incorporating fossil plants, land plants are classified into two groups, i.e., polysporangiophytes and monosporangiophytes (Kenrick and Crane, 1997). In polysporangiophytes, the sporophyte is ramified with sporangia on branches, resulting in multiple sporangia per sporophyte and most likely raising fecundity per sporophyte. This sporophyte is more or less large in size (but less than 30 cm tall in earliest plants), develops a vascular system functioning as both conduct and support, and is likely autotrophic and independent of the gametophyte. The polysporangiophytes include all vascular plants and polysporangiate non-vascular plants (*Aglaophyton*, *Horneophyton*). The latter share polysporangium with vascular plants, and on the other hand, share the absence of true vascular tissue with bryophytes, so that they are called protracheophytes. The protracheophytes are not extant but were present as a middle evolutionary runner in the Late Silurian and the Early Devonian. Occurrence of past plants with such a combination of traits indicates that ramification or polysporangium anteceded vascularization. By contrast, bryophytes are monosporangiophytes with a single sporangium per sporophyte.

Extant polysporangiate tracheophytes have elaborate sporophytes. Both stem and root repeat ramifications, and leaves are borne on the stem in regular order. Therefore, this repetitive organization of the shoot is composed of units called modules, each of which comprises a node with (a) leaf (leaves) and an axillary branch (bud), and an internode below. Similarly, a root module comprises a lateral root and a portion of a mother root between the levels of lateral roots. This module structure is a product of development regulated primarily by the shoot apical meristem and the root apical meristem, which yield young cells, tissues or organs acropetally above old ones.

The shoot and root apical meristems are able to divide and cause ramification of the shoot and root. By contrast to the vascular plants, in the monosporangiate bryophytes the sporophyte is small and parasitic entirely or partly to the mother gametophyte. It does not ramify at all and comprises a file of a single sporangium, a stalk (seta), and a foot, which is embedded in the gametophyte and absorbs nutrients from it. The sporophyte does not possess an apical meristem comparable to the shoot apical meristem of vascular plants (Kato and Akiyama, 2005). There is an intercalary (basal) meristem at the base of the sporangium in hornworts, and in mosses an epibasal cell (apical cell) and a hypobasal cell occur on either end, but are ephemeral. Liverworts do not possess apical cells, and the sporophyte develops by non-localized cell divisions. Thus, the ramified sporophyte and the simple sporophyte, which are characteristic of vascular plants and bryophytes, respectively, are the results of the presence and absence of persistent and dividable apical meristems. The evolutionary origin of the apical meristem remains uncertain. It may be possible that the apical meristem appeared de novo, or was recruited from the apical meristem of the gametophyte in bryophytes.

Recently, a molecular genetic analysis was performed for the model moss *Physcomitrella patens* (Okano *et al*., 2009). In deletion lines of the gene orthologous to the *Arabidopsis thaliana CURLY LEAF* (*PpCLF*), an ellipsoidal sporophyte-like body that is derived by apogamy has an apical cell and becomes branched, while it produces a sporangium-like organ when *PpCLF* is repressed in the lines. The results show that, in the gametophyte, *PpCLF* represses initiation of a sporophytic apical cell and, in the sporophyte, it represses its apical cell activity and induces sporangial development. The resulting sporophyte-like plant mimics the polysporangiate pteridophytic sporophyte. It is suggested that polycomb repressive complex 2 (PRC2) may be involved in evolutionary innovations in land plants. It may be likely that regulatory changes of a few keystone genes promoted sporophyte evolution.

Vascular tissue

The gametophyte of bryophytes, although much larger than the sporophyte, has not developed a vascular system and hence has not been greatly magnified, perhaps due to the necessity of sexual reproduction. A magnified gametophyte in relation to vasculature may have forced gametangia to be much apart from the ground and made fertilization using external water difficult. By contrast, the gametophyte of vascular plants usually is unvascularized and small. The vascularized, magnified sporophyte is able to produce sporangia at high levels, which are advantageous for spore dispersal.

There are conducting tissues similar to the vasculature, called central bundle, in some bryophytes, in particular the stem of large gametophytes and the seta of relatively large sporophyte of mosses. The central bundle comprises tracheid-like hydroids in the center, surrounded by sieve-cell-like leptoids. The hydroids, like tracheids, are fusiform, thick-walled, dead, and conduct water apoplastically, but differ from tracheids in the absence of the secondary cell wall composed of lignin.

The Early Devonian *Aglaophyton major* has long been regarded as an early vascular plant with a xylem lacking secondary thickening or the secondary wall disappearing during fossilization. A close reexamination showed that the plant had not a true vasculature but a bryophyte-like conducting tissue. It is the case with *Horneophyton*. As a result, both plants are polysporangiate protracheophytes leading to the clade of eutracheophytes.

The structure of the secondary wall of the early-plant tracheids has been made clear (Kenrick and Crane, 1997). Among three Devonian clades, the tracheid wall of rhyniophytes consists of a thin layer (2% of the entire thickness of the wall) of lignin enclosing a soft, spongy inner body. In early lycophytes the tracheid wall is two-layered with the inner layer prone to degradate and the thicker (30%) resistant. The tracheid wall of the earliest euphyllous *Trimerophyton* shows a similar, more complicate layered structure. The outer tracheid wall of all the three groups is thinner than that of modern taxa. A developmental anatomical study on *Huperzia lucidula*, an extant lycophyte, shows that secondary-wall thickening begins with a deposition of a template (inner) layer on the primary wall, followed by another deposition of the outer layer (50% of the entire thickness) (Cook and Friedman, 1998). It was speculated that in the evolution of vascular plants the tracheids evolved with a polarity of the degradation-resistant layer increasingly thickening, and eventually to the tracheids and vessel elements of angiosperms with the secondary wall entirely comprising resistant layers.

Leaf

One of the most prominent events in the evolution of land plant morphology is the evolution of the leaf in the sporophyte. The stem, which bears leaves, evolved in association with leaf evolution. The leaf does not exist in the bryophyte sporophyte (but present in the gametophyte), and did not exist in the protracheophytes and the simple early vascular plants (e.g., *Cooksonia*, *Psilophyton*). It is hypothesized that the euphyll (true leaf)

or megaphyll was derived from the ancestral axes named telomes (+mesomes) through planation and webbing (according to the telome theory), while the microphyll was derived from an enation borne on the ancestral axis (enation theory). It is suggested that the former was of multiple origins and the latter was of single origin (Fig. 1). The evolution of meristem in relation to the evolution of the leaf and stem is not known, an issue to be solved by comparing the morphogenetic behaviors of meristems involved in leaf and stem differentiation, and furthermore the underlying genetic regulations of the meristems (Friedman *et al*., 2004; Tomescu, 2009). It is noted that expression of *KNOX* genes in the shoot apical meristem and that of *ARP* genes in leaf primordia are shared by microphylls and megaphylls (Tomescu, 2009).

Fossil data suggest that the microphyll evolved 40 million years earlier than the euphyll. This difference is explained to have been caused by the atmospheric change: the microphyll appeared in the Early Devonian when CO_2 concentration was very high, and subsequently the euphyll with a large lamina appeared, as the concentration was lowered in the Late Devonian (Beerling *et al*., 2001). The euphyll evolution is likely to have been linked with the increase in transpiration efficiency by the evolution of conducting tissues and stomata, which may have prevent leaves from temperature rising.

Seed

Most of the extant vascular plants (i.e., all seed plants and a few pteridophytes) are heterosporous with two spore size classes, while the remaining pteridophytes are homosporous with a single class. The evolutionary trend from homospory toward heterospory, which is a major trend in land plant evolution, is strongly connected with reproductive strategy of an escape from the consequence of reproduction exclusive to homosporous plants. In homosporous plants, the hermaphroditic gametophyte allows intragametophytic self-fertilization, which causes homozygosity at all loci and expression of deleterious or

lethal genes. Such intragametophytic selfing is excluded from heterosporous plants with gametophyte dioecy. The smaller spores (microspores) develop into male gametophytes, while the large spores (megaspores) develop into female gametophytes.

The vascular plants show an evolutionary polarity toward reduction of the gametophyte compared to the sporophyte (Fig. 2). The gametophyte is exosporic (i.e., extruded from the spore wall) and much larger than the spore in homosporous plants. By contrast, the gametophyte is endosporic (most part enclosed by the spore wall) and as small as or slightly larger than the spore in heterosporous plants. Furthermore, in seed plants, which are the most advanced heterosporous, both male and female gametophytes are reduced and grow within parent sporophytes, i.e., ovules or carpels (pollen can be free from the parent when dispersed). The angiosperm female gametophyte (embryo sac) is usually 7-celled and 8-nucleate, although 4-celled or 9-celled in basal angiosperms (Rudall, 2006), and the male gametophyte (pollen tube) is 3-celled.

The evolutionary reduction of the gametophyte is also strongly related to the mode of fertilization. In pteridophytes, like in bryophytes and aquatic algae, fertilization takes place by sperms swimming in external water under (on) the gametophyte, which is beyond the control of the gametophyte. In comparison, in seed plants, the pollen germinates within the ovule (in gymnosperms) or on the stigma (in angiosperms) and subsequently the male gametophyte (pollen tube) elongates toward the egg in the female gametophyte. Thus the gametophyte growth is influenced by the mother sporophyte (e.g., self compatibility), and fertilization occurs internally without using external water. This fertilization pattern may be a beneficial product of the gametophyte reduction.

Seed plants differ from bryophytes and pteridophytes in possessing ovules (which develop into seeds in post-fertilization) (Fig. 1). The earliest seed-fern fossils were discovered from the Lower Devonian (370 million years ago). Angiosperms evolved from among extinct gym-

nosperms. Pollen analyses suggest that angiosperms appeared in the Middle Triassic (230 million years ago). Different molecular evolutionary clock analyses proposed that angiosperms diverged from the extant gymnosperm lineage more than 200 million years ago, by 285 million years ago, or 330 million years ago; however, a most supported estimation is that angiosperms appeared in the Jurassic (208–145 million years ago) (Sanderson *et al.*, 2004). Angiosperms are most likely to have evolved at a certain time between the Carboniferous and the Jurassic when pteridophytes and then gymnosperms thrived.

Angiosperms achieved a remarkable diversification in the Late Cretaceous (100–65 million years ago) and the Tertiary (65–2 million years ago). The diversification, with a result that angiosperms account for about 90% of the present land plant flora, is owing mainly to intimate relationships between flowers and pollinators (e.g., insects) or, in other words, to strengthened relationships with different organisms. Since the flower, i.e., a reproductive organ complex unique to the angiosperms, has been analyzed by genetic research, the angiosperm diversification may be described in terms of gene evolution in future.

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