DIVISION OF SPECIATION MECHANISMS II

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All living organisms evolved from a common ancestor more than 35 billion years ago, and accumulated mutations on their genomes caused the present biodiversity. The traces of evolutionary processes are found in the genomes of extant organisms. By comparing the genomes of different organisms, we can infer (1) the phylogenetic relationships of extant organisms and (2) the genetic changes having caused the evolution of morphology and development. The inferred phylogenetic relationships give important insights on problems in various fields of evolutionary biology, and our group focuses on biogeography, evolution of morphological traits, and systematics in wide range of taxa. On the evolution of morphology and development, we aim to explore genetic changes led the evolution of plant body plan. We selected *Arabidopsis* (angiosperm), *Gnetum* (gymnosperm), *Ginkgo* (gymnosperm), *Ceratopteris* (pteridophyte), *Physcomitrella* (bryophyte), and some green algae as models to compare the gene functions involved in development of the reproductive organs and shoot apical meristem of land plants.

I. Evolution of reproductive organs in land plants

A flower is the most complex reproductive organ in land plants and composed of sepals, petals, stamens, and gynoecium. Female haploid reproductive cells are covered with a sporangium (nucellus) and two integuments, and further enclosed in a gynoecium. Male haploid reproductive cells (pollens) are covered with a sporangium (pollen sack). On the other hand, gymnosperms and ferns have simpler reproductive organs than angiosperms and lack sepals and petals. Female sporangia (nucellus) of gymnosperms are covered with only one integument. Sporangia of ferns have no integuments and are naked on the abaxial side of a leaf.

The development of floral organs is mainly regulated by A-, B-, C-function genes, which are members of the MADS-box gene family. These genes are transcription factors containing the conserved MADS and K domains. MADS-box genes of angiosperms are divided into more than 10 groups based on the gene tree. The *LEAFY* gene is the positive regulator of the MADS-box genes in flower primordia.

What kind of changes of the MADS-box genes caused the evolution of the complex reproductive organs in the flowering plant lineage ? Comparisons of MADS-box and *LFY* genes in vascular plants suggest that the following sequential changes occurred in the evolution of reproductive organs. (1) Plant-type MADS-box genes with both MADS and K domains were established. (2) The number of MADS-box genes increased, and the three ancestral MADS-box genes that later generate A-, B-, C-functions genes were likely originated before the divergence of ferns and seed plants. (3) Specifically expressed MADS-box genes in reproductive organs evolved from generally expressed ones in the seed plant lineage. (4) The ancestral gene of the AG group of MADS-box genes acquired the C-function before the divergence of extant gymnosperms and angiosperms. (5) The gene duplication that formed the AP3 and PI groups in MADS-box genes occurred before the diversification of extant gymnosperms and angiosperms. (6) The ancestral gene of angiosperm A-function gene was lost in extant gymnosperm lineage. (7) *LFY* gene becomes positively regulate MADS-box genes before extant gymnosperms and angiosperm diverged. (8) Spatial and temporal patterns of A-, B-, C-function gene expression were established in the angiosperm lineage.

Homeobox genes play indispensable roles for development in metazoa, instead of MADS-box genes. This difference is likely caused by the fact that metazoa and land plants established multicellular organs independently after their last common ancestor, which was presumably a unicellular organism or a multicellular organism without multicellular organs. Of note, in both land plants and metazoa, an increase in the number of specific transcription factors (MADS-box genes in land plants and homeobox genes in metazoa) and the subsequent diversification of their expression patterns and regulation of downstream genes are the principal mechanisms for the evolution of body plans.

II. Evolution of vegetative organs in land plants

The ancestor of land plants was primarily haploid. The only diploid cell was the zygote, which immediately underwent meiosis. It is believed that early during land plant evolution, zygotic meiosis was delayed and a multicellular diploid sporophytic generation became interpolated into the life cycle. In the early stages of land plant evolution, sporophytes are epiphytic to gametophytes, as observed in extant bryophytes. During the course of evolution, both generations started to grow independently at the stage of pteridophytes. Finally gametophytes became much reduced and epi-

phytic to sporophytes in seed plant lineage. Molecular mechanisms of development in a diploid generation have been well studied in some model angiosperms, but we have scarce information on those in a gametophyte generation. For example, mosses have leaf- and stem-like organs in their haploid generation, but it is completely unknown whether similar genes involved in angiosperm leaf and stem development are used in the gametophytic generation of mosses or not. To understand the evolution of body plans in diploid and haploid generation at the molecular level, we focus on the comparison of molecular mechanisms governing shoot development between Arabidopsis and the moss *Physcomitrella patens*. *P. patens* is known by its high rate of homologous recombination and suitable for analyze gene functions using the gene targeting. The moss homologs of *SHOOTMERISTEMLESS*, *ZWILLE*, and HD-Zip genes, which are involved in *Arabidopsis* shoot development, have been cloned and their characterization is in progress. We also established enhancer and gene trap lines and tagged mutant libraries of *P. patens* to clone genes involved in the leafy shoot development (Nishiyama et al. 2000).

III. Biogeography of *Coriaria*

Coriaria is, which has been mentioned to be the most conspicuous disjunct distribution in flowering plants distributed in four separate areas in the world. The phylogenetic relationships of 12 *Coriaria* species collected from the representative disjunct areas were inferred by comparing 2416 base pairs of the combined data set of *rbcL* (a large subunit of ribulose 1,5-

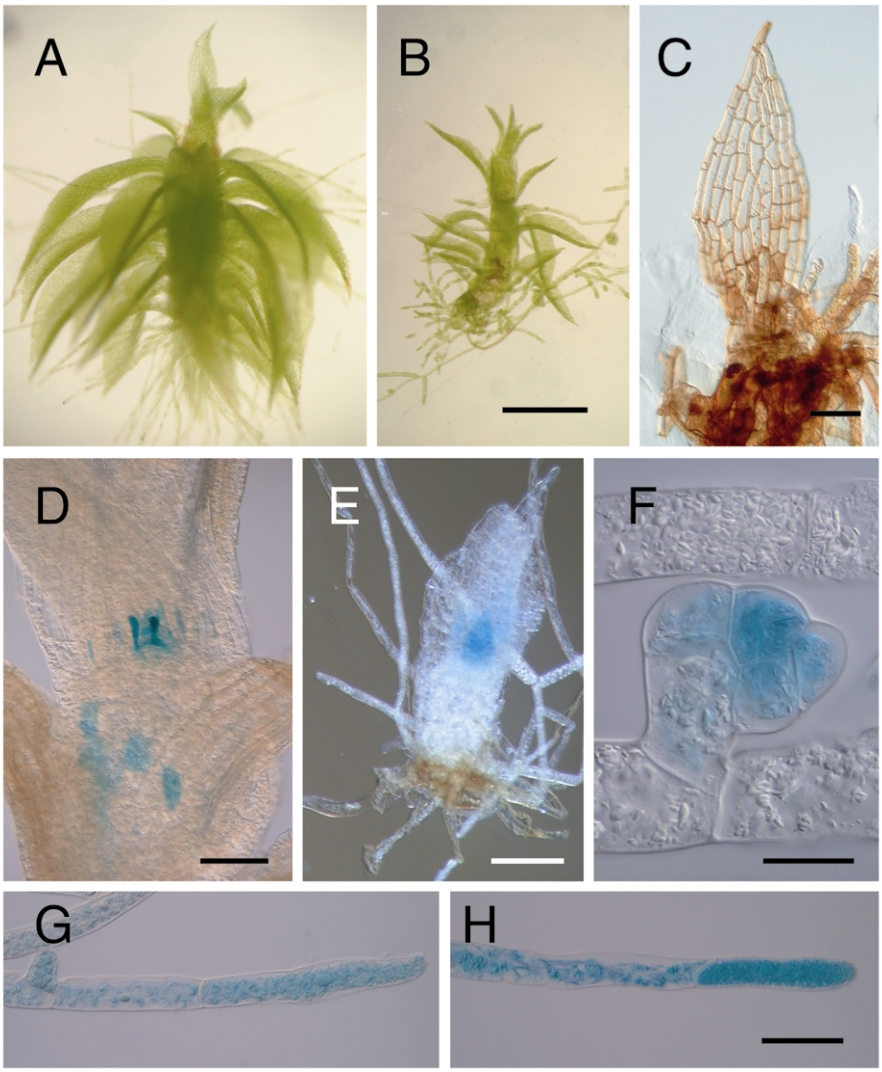

Figure 1. Example of mutant strains and gene-trap lines of *Physcomitrella patens.* A, transformant morphologically indistinguishable from the wild type (19980725084-3). B (TN1) and C (TN2), morphological mutants obtained by shuttle mutagenesis. D-H, histochemical staining of gene-trap lines. D, YH330, mucilage hairs are stained. E, YH78, young leaves are stained. F, YH229, a young bud is stained. G and H, YH206, the chloronema (G) and caulonema (H) are stained. Bar in B = 0.5 mm for A and B. Bars in C and D = 0.1 mm. Bar in E = 0.2 mm. Bar in F = 20 μm. Bar in H = 50 μm for G and H.

bisphosphate carboxylase / oxygenase) and *matK* (maturase K) genes (Yokoyama et al. 2000). The phylogenetic tree shows that the Chile - Papua New Guinea - New Zealand - Pacific islands species and the Central America -northern South America species form a sister group, and the Eurasian clade is more basal to them. The divergence time between the Eurasian group and the other species was estimated as 63 or 59 million years ago using *rbcL* and *matK* molecular clocks, respectively. These results do not support the previously proposed hypotheses to explain the disjunct distribution based on the continental drift, but suggest that the distribution pattern was formed by several geographical migrations and separations in the Cenozoic.

IV. Molecular phylogeny of athyrioid ferns

Nucleotide sequences of the chloroplast gene *rbcL* from 42 species of the fern tribe Physematieae (Dryopteridaceae) were analyzed to gain insights into the inter- and intrageneric relationships and the generic circumscriptions in the tribe. The phylogenetic relationships were inferred using the neighbor joining and maximum parsimony methods, and both methods produced largely congruent trees (Sano et al. 2000). These trees reveal that: 1) *Athyrium*, *Cornopteris*, *Pseudocystopteris*, and *Anisocampium* form a clade and *Athyrium* is polyphyletic; 2) *Deparia sensu lato* is monophyletic and *Dictyodroma formosana* is included in the *Deparia* clade; 3) *Diplaziopsis* forms a clade with *Homalosorus*, which is isolated from the other genera of the Physematieae; 4) *Monomelangium* is included in the monophyletic *Diplazium* clade; 5) *Rhachidosorus* is not closely related to either *Athyrium* or *Diplazium*.

It has been suggested that *Diplazium tomitaroanum* Masam. is a hybrid arising from *Diplazium subsinuatum* (Wall. ex Hook. et Grev.) Tagawa and *Deparia petersenii* (Kunze) M. Kato. *Di. subsinuatum*'s basic chromosome number differs from that of *Diplazium* but is consistent with that of *Deparia*, suggesting that *Di. subsinuatum* is closely related to *Deparia* but not to *Diplazium*. Traditional taxonomy based on morphological characteristics sometimes encounters difficulty in inferring phylogenetic relationships when dealing with taxa having few diagnostic morphological characteristics, such as *Di. subsinuatum* with its simple leaves. We obtained the *rbcL* nucleotide sequences from *Di. subsinuatum* and *Di. tomitaroanum* in order to infer their phylogenetic relationships to other *Deparia* and *Diplazium* species (Sano et al. 2000). We also examined the morphological characteristics of both the scales and leaf axes, which are regarded as diagnostic characteristics for *Deparia* but have not been described in detail for *Di. subsinuatum*. According to the *rbcL* nucleotide sequences, *Di. subsinuatum* and *Di. tomitaroanum* are included in the *Deparia* clade, not in the *Diplazium* clade. Furthermore, the articulate multicellular hairs on leaves, the shape of the rachis groove, the basic chromosome number and the spore morphology are all more similar to *Deparia* rather than to *Diplazium*, indicating that *Di. subsinuatum* should be classified as *Deparia*. We therefore propose a new taxonomic treatment of the two *Diplazium* species.

Publication List:

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