

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 remained in the genomes of extant organisms, and we can infer (1) the phylogenetic relationships of organisms and (2) the genetic changes having caused the phenotypic evolution by comparing the genomes of different organisms. The inferred phylogenetic relationships give important insights on problems in various fields of evolutionary biology, and our group is now focusing on biogeography, evolution of morphological traits, and systematics in wide range of taxa. On the phenotypic evolution, we are especially interested in the morphological evolution and aim to explore genetic changes led the evolution of plant body plan. We selected *Arabidopsis* (angiosperm), *Gnetum* (gymnosperm), *Ginkgo* (gymnosperm), *Ceratopteris* (pteridophyte), and *Physcomitrella* (bryophyte) as models to compare the genetic cascades regulating morphogenesis especially in 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 (Hasebe and Ito 1999, Hasebe 1999). (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. New hypothesis on the homology of *Gnetum* reproductive organ

Gnetales, one of the extant gymnosperm orders has traditionally been recognized to be most closely related to flowering plants, because the reproductive organ of Gnetales has some morphological characters similar to flowering plants. Most recent molecular phylogenetic studies do not support the sister relationship of the Gnetales and flowering plants, but instead support a close relationship between Gnetales and other extant gymnosperms. The MADS-box genes are transcription factors, some of which are involved in reproductive organ development in flowering plants. To resolve the discrepancy in phylogenetic inferences, and to provide insights into the evolution of reproductive organs in seed plants, four MADS-box genes (*GpMADS1* - 4) were cloned from *Gnetum parvifolium* (Fig. 1A, B).

GpMADS2 is likely to be a pseudogene and the other three genes were characterized. A MADS-box gene tree based on partial amino acid sequences showed that *GpMADS3* is included in the AGL6 group, but the other two genes do not cluster with any previously reported MADS-box gene. The three *GpMADS* genes were expressed during the early stage of ovule development in the differentiating nucellus and three envelopes (Fig. 1C-F). A comparison of MADS-box gene expression among conifers, *Gnetum*, and flowering plants suggests that the comparable reproductive organs in *Gnetum* and flowering plants evolved in parallel, and is likely to support the homology between the ovule-ovuliferous scale complex of conifers and the *Gnetum* ovules including the three envelopes (Fig. 1G).

III. Evolution of gametophytic and sporophytic generations

Plants have the haploid gametophytic and diploid sporophytic generations, both of which are usually multicellular and morphologically diversified each other. Origin and evolution of the two generations have been discussed and some hypotheses are proposed, although the molecular basis of the both generations have not been studied. For example, it is not known whether different sets of genes contribute to the morphological differences of the two generations or similar genes are differently used. Ferns are good materials to study the evolution of gametophytic and sporophytic generations, because individuals of the both generations grow independently and it is much easier to analyze than other plants in which one of the two generations is enrolled in the tissue of another generation.

We have cloned 11 homeodomain - leucine zipper (HD-Zip) genes of the fern *Ceratopteris richardii* (*Crhb* genes) encoding a transcription factor and are characterized by the presence of a homeodomain closely linked to a leucine zipper motif. Most of *Crhb* genes belonging to the HD-ZIP I or II subfamilies are almost ubiquitously expressed in both sporophytes and gametophytes, while the amounts of expression are different among the tissues. The similarly ubiquitous expression patterns among different sporophytic tissues have been reported in some angiosperm HD-Zip genes, although the patterns of expression in gametophytic tissues in angiosperms are not known. Our study clearly showed that most of *Ceratopteris* HD-Zip genes are ubiquitously expressed even in gametophytes from which morphologically different tissues and organs of sporophytes develop. It may indicate that *Crhb* genes have unknown functions common in the both generations. On the contrary, *Crhb1* and *Crhb3* genes are expressed only in the gametophytes and it may suggest the existence of gametophyte specific regulatory systems which cause morphological diversity between the

two generations. Further studies on the regulatory elements of the *Crhb* genes and on more detailed spatial patterns of expression will give useful information to reveal the evolutionary aspects of differences between sporophytic and gametophytic generations.

IV. Establishment of tagged mutant library of the moss *Physcomitrella patens*.

Mosses have the different body plan from flowering plants. Leafy shoots of mosses are similar to the ones of angiosperms, but develop in the gametophytic generation instead of the sporophytic generation as angiosperms. Organs of mosses are much simpler than flowering plants. For example, the leaves are composed of one layer of cells. Therefore, the body plan of mosses may be regulated by different genes from angiosperms. In addition to analyses of the homologs of angiosperm genes governing morphogenesis, it is necessary to screen specific genes in the moss. We established enhancer and gene trap lines and tagged mutant libraries of *Physcomitrella patens* to clone genes related to leafy shoot development. *P. patens* is known by its high rate of homologous recombination and suitable for molecular biological analyses using the gene targeting. Our libraries should be also useful for other purposes.

Publication List:

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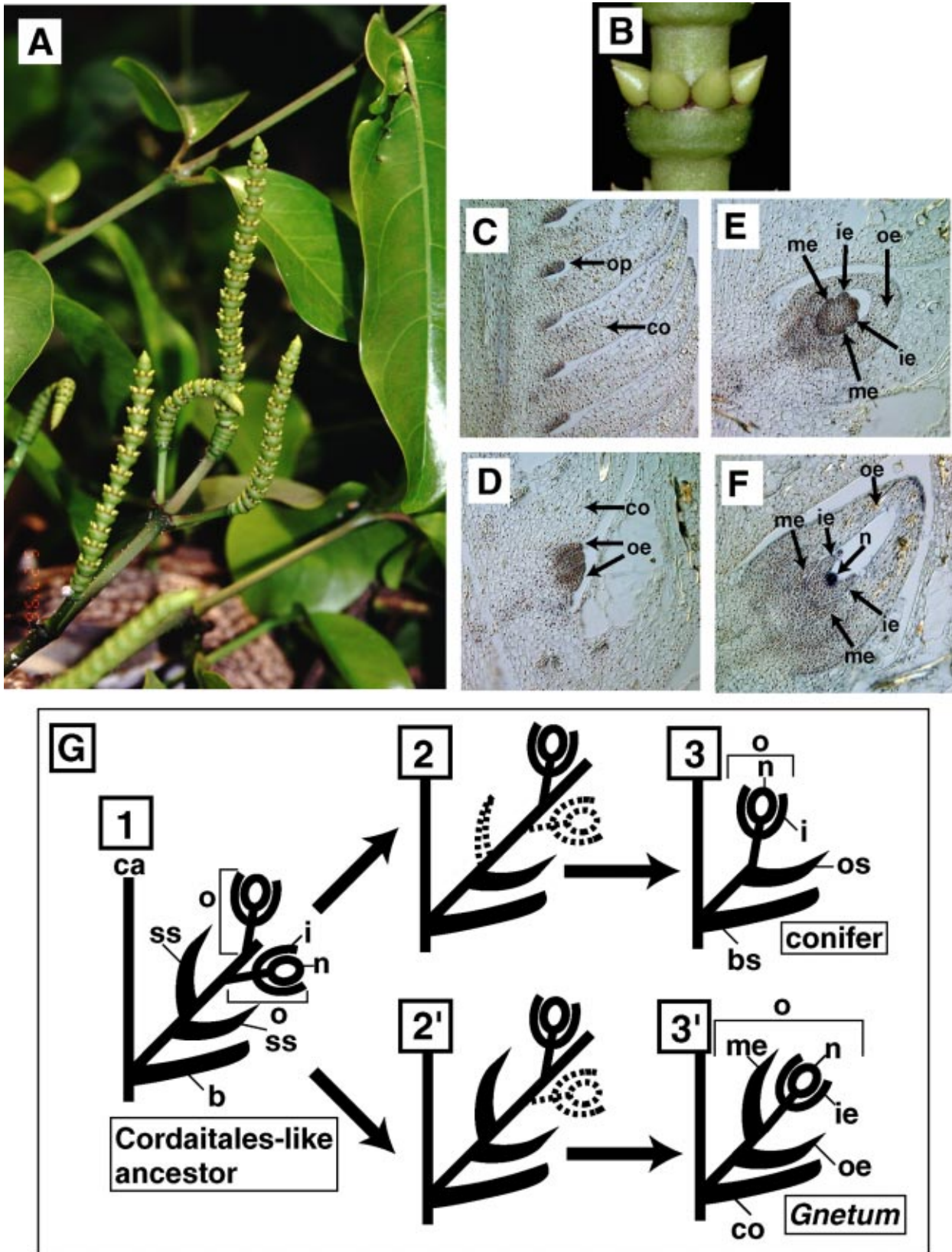


Fig. 1 New hypothesis on the homology between *Gnetum* and conifer reproductive organs. (A) *Gnetum parvifolium*. Female strobili and vegetative branches with leaves. (B) A whorl of nearly mature ovules subtended by annular collars. (C-F) *In situ* localization of *GpMADS3* mRNA in developing ovules of *Gnetum parvifolium*. (G) Scenario for the evolution of ovule-bearing structures in *Gnetum* and conifers from a Cordaitales-like prototype. b, bract; bs, bracteole scale; ca, cone axis; co, collar; i, inner integument; ie, inner envelope; me, middle envelope; n, nucellus; o, ovule; op, ovule primordium; oe, outer envelope; os, ovuliferous scale; ss, sterile scale.

