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All living organisms evolved from a common ancestor that lived more than 3.5 billion years ago, and the accumulation of mutations in their genomes has resulted in the present biodiversity. Traces of the evolutionary process are found in the genomes of extant organisms. By comparing the gene networks (and their functions) of different organisms, we hope to infer the genetic changes that caused the evolution of cellular and developmental processes.

I. Phylogeny and biogeography of land plants and insects

Phylogenetic trees with reliable, statistical confidences are the basis for evolutionary biology. We infer phylogenetic relationships of major lineages of land plants and insects.

The subfamily Apaturinae consists of 20 genera and shows disjunct distributions and unique host-plant associations. Most genera of this subfamily are distributed in Eurasia, South-East Asia and Africa, whereas the genera *Doxocopa* and *Asterocampa* are distributed mainly in South America and North America, respectively. Although Apaturinae larvae mainly feed on Cannabaceae, those of the genus *Apatura* are associated with *Salix* and *Populus* (Salicaceae), which are distantly related to Cannabaceae. Here, we infer the phylogeny of Apaturinae and reconstruct the history of host shifting and of colonization in the New World. We analyzed 9761 bp of nuclear and mitochondrial DNA sequence data, including the genes encoding *EF1a*, *Wg*, *ArgK*, *CAD*, *GAPDH*, *IDH*, *MDH*, *RpS5*, *COI*, *COII*, *ATPase8*, *ATPase6*, *COIII*, *ND3*, and *ND5* for 12 apaturine genera. We also inferred the phylogeny with six additional genera using mitochondrial sequence data alone. Within Apaturinae, two major clades are recovered in all the datasets. These clades separate the New World genera, *Doxocopa* and *Asterocampa*, indicating that dispersal to the New World occurred at least twice. According to our divergence time estimates, these genera originated during the Early Oligocene to the Early Miocene, implying that they migrated across the Bering Land Bridge rather than the Atlantic Land Bridge. The temporal estimates also show that host shifting to *Salix* or *Populus* in *Apatura* occurred more than 15 million years after the divergence of their host plants. Our phylogenetic results are inconsistent with the previously accepted apaturine genus groups and indicate that their higher classification should be reconsidered.

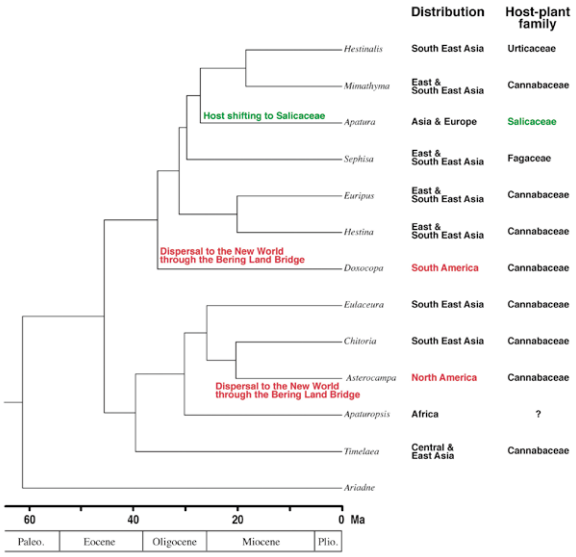

Figure 1. Evolution of Apaturinae

II. Evolution from cells to tissues based on molecular mechanisms of cytokinesis

The cells of land plants and their sister group, charophycean green algae, divide by the insertion of cell plates at cytokinesis. This is in contrast to other green algae, in which the invagination of the plasma membrane separates daughter cells at cytokinesis. The cell plate appears in the middle of daughter nuclei, expands centrifugally towards the cell periphery, and finally fuses to the parental cell wall. Cell wall materials are transported to the expanding cell plate with a phragmoplast, which is mainly composed of microtubules. Centrifugal expansion of the phragmoplast is a driving force for that of the cell plate, although elucidating the molecular mechanism for the expansion was a challenge. We have found that γ-tubulin complexes on existing phragmoplast microtubules nucleate new microtubules as branches. Although elongation of the branched microtubules is likely a driving force of the phragmoplast expansion, the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

mechanism by which phragmoplast microtubules redistribute towards the cell periphery is unclear. Because an inhibitor of microtubule depolymerization inhibits phragmoplast expansion, analyses of microtubule depolymerization might be a key for understanding the mechanism. We developed a method for quantifying the rate of microtubule depolymerization in the phragmoplast, and found that the rate of microtubule depolymerization gradually increases from the outer surface to the inside of the phragmoplast. Based on the results, we propose a hypothesis that random branching of microtubules coupled with biased depolymerization lead directional redistribution of microtubules, which drives centrifugal expansion of the phragmoplast. Takashi Murata was this study's main researcher.

III. Evolution of molecular mechanisms in plant development

Seed plants form shoot and root apical meristems containing multiple pluripotent stem cells in the sporophyte (diploid) generation, but do not form pluripotent stem cells in the gametophyte (haploid) generation. On the other hand, mosses, one of the basal groups of land plants, form stem cells in both gametophyte and sporophyte generations. No genes responsible for the initiation and maintenance of pluripotent stem cells in the moss gametophyte generation have been reported, and the common mechanisms and evolution of pluripotent stem cell formation in land plants are still unrevealed. We showed that *AINTEGUMENTA/PLETHORA/BABY BOOM (APB)* orthologs *PpAPB*s (*PpAPB1*, *2*, *3*, and *4*) regulate the formation of stem cells in the haploid generation in the moss *Physcomitrella patens*. Quadruple disruption mutants did not form any gametophores, indicating that *APB*s regulate gametophytic pluripotent stem cell formation. These *APB*s are transcriptionally regulated by auxin, and synergistically function with cytokinin signaling. The primary researchers for this study were Tsuyoshi Aoyama and Yuji Hiwatashi.

Flowers are the most complex reproductive organs in land plants, whose development is regulated by MADS-box transcription factors. To understand the origin of a genetic network of floral homeotic genes, we analyzed six MIKC classic type MADS-box genes in *P. patens*. Investigation of the effects of all six gene deletions is currently being undertaken by Yuji Hiwatashi. He also participated in collaborative works with Drs. Kenichiro Hayashi (Okayama Science University), Kazuhiko Nishitani (Tohoku University), and Koji Mikami (Hokkaido University) on the characterization and evolutionary significance of *ent*-kaurene synthases, xyloglucan endotransglucosylase/hydrolases, and a phosphatidylinositol phosphate kinase in *P. patens*, respectively.

Evolution of a branched system is a conspicuous novelty in land plant evolution, although the origin and evolution of its gene network is not known because of the lack of study in the basal land plants. We found that a deletion mutant of a polycomb repressive complex 2 gene *PpCLF* forms a branched sporophyte-like organ in *P. patens*. Analyses of auxin distribution, expression patterns of class 1 KNOX genes suggest that the active site of auxin signaling is localized to the initiation site of the branch. This work was mainly done by Yuji Hiwatashi.

V. Molecular mechanisms of reprogramming of gametophore leaf cells to pluripotent stem cells in the moss *Physcomitrella patens*

Differentiated cells can be reprogrammed to become undifferentiated pluripotent stem cells with abilities to both self-renew and give rise to most cell types in the organism. An induction of reprogramming is more easily manipulated in plants than in animals, although the genetic and molecular bases of the difference are mostly unknown. This is likely because the callus usually used in reprogramming studies in seed plants is a cell mixture composed of reprogrammed and unreprogrammed cells. We noticed that *P. patens* should overcome this problem by its rapid reprogramming ability from a single cell (see http://www.nibb.ac.jp/evodevo/ERATO/movie/MacMovie.mp4). Cells in a dissected leaf of *P. patens* are reprogrammed to become chloronema apical cells with pluripotency within 24 hours. We can continuously observe the reprogramming process of a specific cell under a microscope.

One of the key factors of reprogramming is the change in the epigenomic profile. In the differentiated cells, a gene expression profile that fits to the cell function is stably maintained. This stable maintenance is performed through chromatin modifications such as trimethylation of histone H3 at lysine 27 (H3K27me3) for gene repression and H3K4me3 for gene activation. In contrast, in the pluripotent stem cells, most genes are ready to be activated. In animal pluripotent stem cells, many genes with H3K27me3 also have H3K4me3, and this bivalent state is presumed to keep genes poised for transcription. Thus, in the process of reprogramming, the epigenomic profile of differentiated cells should be changed into a pluripotent stem cell-specific epigenomic profile. However, the mechanisms of the establishment of such epigenomic profiles are almost unknown. We are currently attempting to reveal these mechanisms using *P. patens*. We found that in young protonemata, which contain many pluripotent stem cells, more than 6,000 genes are in the bivalent state, and there are only a small portion of genes that have H3K27me3 but not H3K4me3. This epigenomic profile is almost identical to that in animal pluripotent stem cells, and we thus revealed that the epigenomic profile of pluripotent stem cells is similar in plants and animals. This study is mainly conducted by Takaaki Ishikawa and Yosuke Tamada.

VI. Molecular mechanisms of mimicry

Mimicry is an intriguing phenomenon in which an organism closely resembles another, phylogenetically distant species. An excellent example is the flower-mimicry of the orchid mantis *Hymenopus coronatus*, in which pink and white coloration and petal-like structures on its walking legs enable this insect to blend perfectly into flowers. To elucidate the evolutionary mechanism of this complex mimicry at the molecular level, we first focused on the mechanism of body coloration in the orchid mantis. HPLC and mass

spectrometric analyses indicated that xanthommatin, a common red pigment of the ommochrome family, contributes to the pink body coloration of the orchid mantis. Integuments of the mantis have an absorption peak at 534 nm, which is different from that of oxidized (440 nm) and reduced forms (495 nm) of xanthommatin solubilized in a neutral buffer. On the other hand, it well agrees with an absorption peak of a reduced and precipitated form of xanthommatin (533 nm). These results suggest that the coloration of the orchid mantis is formed by the change of specific chemical states of a component common to other mantis. We also found that integuments of the orchid mantis contain a large amount of uric acid, which serves for white coloration in other insects such as the silkworm. These results indicate that it is possible this unique coloration of the orchid mantis is formed by the combination of usual pigments. This work was mainly done by Hiroaki Mano.

VII. Molecular mechanisms of host shifting

Adaptation to a novel environment often requires evolution of multiple traits. In phytophagous insects a precise combination of performance and preference traits for particular host plants is crucial for host shifting because a new host plant can be incorporated into an insect's diet if adults accept it for oviposition and if the larvae are able to complete their development on it. However, very little is known about the genetic bases of the performance and preference, which are fundamental to infer the process and evolutionary consequence of host shifting. To address the molecular mechanism of host shifting, we use two host races of a tiny moth, *Acrocercops transecta*, as a model system. A QTL analysis revealed that only a restricted region of a single autosome was responsible for the larval performance. This indicates that host shifting from *Juglans* to *Lyonia* in *A. transecta* involved changes in few genes with large effect, suggesting that a small number of genetic changes in larval performance allowed the successful host shifting. To test whether preference genes are physically linked with performance genes or not, a mapping analysis of preference genes is in progress. This study was conducted mainly by Issei Ohshima.

VIII. Molecular mechanisms of Plant Movement using *Mimosa pudica*

The molecular mechanisms and evolutionary significance of plant movement, including seismonastic and nyctinastic movements, are enigmatic. We are working to establish chemically mutagenized lines that lack movement to compare fitness to wild types. We are also attempting to set up a method for transformation to characterize the genes involved in movement. To achieve this goal, we use a cotyledonary node explant, which can regenerate multiple shoots in the presence of 6-benzylaminopurine (BAP), as a target of Agrobacterium-mediated gene transfer. Although the node explant is highly recalcitrant to Agrobacterium infection, we successfully obtained several lines of transformed calluses that were capable of developing new shoots. We are now trying to regenerate whole plants from these transformed shoots in addition to further improvement of transformation efficiency. This study was conducted mainly by Hiroaki Mano.

IX. Evolution of pitcher leaves in carnivorous plants

Development and evolution of the unique morphology of pitcher-shaped leaves of the carnivorous plant family Sarraceniaceae remains problematic. Since 1870's, the pitcher leaves have been hypothesized to have a similar developmental program to that of peltate leaves. However, this hypothesis could not explain the formation of the keel, a structure specific to pitcher leaves. To understand the development and evolution of pitcher leaves, we analyzed expression patterns of leaf developmental gene orthologs in *Sarracenia purpurea*. Unexpectedly, the results suggested that adaxial-abaxial patterning of pitcher leaves was different from those of peltate leaves and have enabled us to hypothesize the evolutionary process of pitcher leaves. This study was conducted mainly by Kenji Fukushima.

Publication List

〔Original papers〕

- Hayashi, K., Horie, K., Hiwatashi, Y., Kawaide, H., Yamaguchi, S., Hanada, A., Nakashima, T., Nakajima, M., Mander, L.N., Yamane, H., Hasebe, M., and Nozaki, H. (2010). Endogenous diterpenes derived from ent-kaurene, a common gibberellin precursor, regulate protonema differentiation of the moss *Physcomitrella patens*. Plant Physiol. *153*, 1085-1097.
- Mikami, K., Saavedra, L., Hiwatashi, Y., Uji, T., Hasebe, M., and Sommarin, M. (2010). A dibasic amino acid pair conserved in the activation loop directs plasma membrane localization and is necessary for activity of plant type I/II phosphatidylinositol phosphate kinase. Plant Physiol. *153*, 1004-1015.
- Ohshima, I., Tanikawa-Dodo, Y., Saigusa, T., Nishiyama, T., Kitani, M., Hasebe, M., and Mohri, H. (2010). Phylogeny, biogeography, and host-plant association in the subfamily Apaturinae (Insecta: Lepidoptera: Nymphalidae) inferred from eight nuclear and seven mitochondrial genes. Mol. Phylogenet. Evol. *57*, 1026-1036.
- Yokoyama, R., Uwagaki, Y., Sasaki, H., Harada, T., Hiwatashi, Y., Hasebe, M., and Nishitani, K. (2010). Biological implications of the occurrence of 32 members of the XTH (xyloglucan endotransglucosylase/hydrolase) family of proteins in the bryophyte *Physcomitrella patens*. Plant J. *64*, 645-656.