Bull. Univ. of Tokyo For, 129, 15 − 24 (2013)

Original Article

# Phylogenetic analysis of MADS-box genes in gymnosperms

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## Introduction

The floral organs of higher dicotyledonous plants are arranged in 4 different whorls, namely, sepals, petals, stamens, and carpels. Organ identity within each floral whorl is determined by certain combinations of gene expression, according to the now-renowned ABC model [1, 2]. Within this framework, only the A-function gene specifies sepal formation. The combination of A- and B-function genes specify the formation of petals, while B- and C-function genes specify stamen formation, and the C-function gene alone determines carpel formation. Recently, the model was extended to include D-function genes, which specify the identity of the ovules that develop within carpels [3, 4, 5]. Even more complex models (i.e., the ABCDE and quartet models [6]) have been proposed, which incorporate other functions involved in the specification of petals, stamens, and carpels into the interaction with canonical A-, B-, and C-function genes [7]. Researchers have demonstrated that in the *Arabidopsis* genus (part of the Brassicaceae family), both *APETALA1* and *APETALA2* contribute to the A-function, *APETALA3* and *PISTILLATA* contribute to the B-function, and *AGAMOUS* contributes to the C-function[2]. In addition, most A-, B-, and C-function genes encode a common DNA-binding domain in the respective transcription factors, which is called MADS-box [8].

MADS-box is a conserved sequence motif that is found in genes belonging to the MADS-box genes family, and is associated with sexual reproduction in plants. MADS-box genes are found in a wide range of eukaryotes, including metazoans, fungi, slime molds, and green plants, [3] and have been classified into several groups [9]. In vascular plants, MIKC-type MADS domain proteins are known for their conserved structure, including MADS, intervening (I), keratin-like (K), and C-terminal (C) domains [8, 10, 11]. The MADS domain is a major determinant of DNA binding, but it also performs dimerization and accessory factor-binding functions [8]. The K-domain, which has not been found in any animal or fungal MADS domain proteins so far [3, 8], is characterized by the conserved regular spacing of hydrophobic residues. These residues have been proposed to facilitate the formation of an amphipathic helix involved in protein dimerization [10, 12].

It is important to investigate MADS-box genes because they are key factors in the evolution of

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reproductive organs. The flowers of angiosperms are the most complex reproductive organs of land plants, with ancestral plants having simpler reproductive organs [3, 6, 13]. Some MADS-box genes serve as homeotic selector genes for floral organ development in angiosperms. For instance, the floral meristem identity gene, *LEAFY*, is the positive regulator of MADS-box genes in primordial flowers [14]. The number of MADS-box genes is assumed to increase via gene duplication before the divergence of ferns and seed plants [15]. In gymnosperms, some MADS-box genes are specifically expressed in reproductive organs, whereas most MADS-box genes are expressed similarly in both the vegetative and reproductive organs of ferns [3, 15]. This difference indicates that an increase in the number of MADS-box genes, and the subsequent recruitment of some MADS-box genes as homeotic selector genes, was important for the evolution of complex reproductive organs [13].

Therefore, in the current study, we attempted to isolate novel partial nucleotide sequences of MADS-box genes in ferns, Pterophyta, and 4 gymnosperm divisions (namely, Coniferophyta, Cycadophyta, Gnetophyta, and Ginkgophyta). In addition, we analyzed the phylogenetic relationships of these groups to evaluate the evolution of floral organs.

## Materials and Methods

### Plant materials and genomic DNA extraction

We sampled the fresh leaves of 10 species (Table 1). Genomic DNA was extracted using Nucleon PhytoPure (Amersham Biosciences, UK). About 0.1 g leaf tissue was ground to fine powder in liquid nitrogen, and mixed with 600 μl of Nucleon PhytoPure Reagent I and 200 μl of Nucleon PhytoPure Reagent II in a 1.5-ml Eppendorf tube. The mixture was incubated at 65℃ for 10 min, and then placed on ice for 20 min. The sample was removed from ice, and 500 μl of chloroform and 100 μl of PhytoPure Resin were added. The mixture was agitated on a tilt shaker for 10 min. Then, the sample was centrifuged at 8,000 rpm for 10 min. Subsequently, 680 μl of

Table 1. The 10 gymnosperm species used for the isolation of MADS-box genes.

| Tree species | Division | Group | Sampling site |
|---|---|---|---|
| *Thelypteris torresiana* | Pterophyta | fern | KBG |
| *Metasequoia glyptostroboides* | Coniferophyta | gymnosperm | KBG |
| *Cryptomeria japonica* | Coniferophyta | gymnosperm | KBG |
| *Taxus cuspidata* | Coniferophyta | gymnosperm | KBG |
| *Picea abies* | Coniferophyta | gymnosperm | HFUT |
| *Picea jezoensis* | Coniferophyta | gymnosperm | HFUT |
| *Cycas revoluta* | Cycadophyta | gymnosperm | KBG |
| *Ephedra sinica* | Gnetophyta | gymnosperm | KBG |
| *Gnetum gnemon* | Gnetophyta | gymnosperm | IBG |
| *Ginkgo biloba* | Ginkgophyta | gymnosperm | YUT |

*KBG: Koishikawa Botanical Gardens
HFUT: Hokkaido Forest, University of Tokyo
IBG: Itabashi Botanical Gardens
YUT: Yayoi campus, University of Tokyo

the upper phase containing DNA, which was located above the brown resin layer, was transferred into a fresh tube with a pipette. A volume of 680-μl isopropanol was added to the solution, and the tube was gently inverted until the DNA precipitated. Then the tube was centrifuged at 8,000 rpm for 5 min, and the supernatant was discarded. After the DNA pellet was dried in a centrifugal thickener for about 10 min, the DNA was resuspended in Tris EDTA buffer or water, as required.

**Primer design**

We used nucleotide sequences of known gymnosperm MADS-box genes for the design of degenerate primers, as follows: *GbMADS1-11* (AB029463-AB029473) and *GBM5* (AY114304) of *Ginkgo biloba; GGM1-19* (AJ132207-AJ132217) of *Gnetum gnemon*; *DAL11-13* (AY269276-AY269278) of *Picea abies*; and a MADS-box sequence of *Cryptomeria japonica* (AB124812). The 20 primers were designed using conserved regions as degenerate primers (Table 2). Figure 1 shows the position of the primers based on the *APETALA3* amino acid sequence, which is one of

Table 2. Sequence primers used for the amplification of MADS-box genes. Forward primers are Nos. 1–9; reverse primers are Nos. 10–20.

| Primer | Sequence |
|---|---|
| No. 1: | 5′-AAGATYGARATAAARA-3′ |
| No. 2: | 5′-ATGGGRCGDGGRAAGAT-3′ |
| No. 3: | 5′-GAAGTBGCYCTCATCGT-3′ |
| No. 4: | 5′-GCKCTMATMATTTTCTC-3′ |
| No. 5: | 5′-ATGGGRCGDGGKMGRGT-3′ |
| No. 6: | 5′-ATGGGSMGDGGRAAGATYG-3′ |
| No. 7: | 5′-AGATMAAGAGGATMGAGAAY-3′ |
| No. 8: | 5′-KGSNMGDGGRAARATHSARAT-3′ |
| No. 9: | 5′-AAGATYGARATAAARAKGATYGAGA-3’ |
| No. 10: | 5′-AGRTYRACRAC-3′ |
| No. 11: | 5′- RAGRTTRACRAC-3′ |
| No. 12: | 5′-GRTTYAGRAGAGG-3′ |
| No. 13: | 5′-GRTYTAGRAGWGG-3′ |
| No. 14: | 5′-GRTYCAGRAGSGG-3′ |
| No. 15: | 5′-TGDAVHCGTAGTGTBTYBTG-3′ |
| No. 16: | 5′-TTGAGYATYTCRAAVGGAGMC-3′ |
| No. 17: | 5′-CTYTTYTGCTACTCYCGBTGAAG-3′ |
| No. 18: | 5′-CTCTTTTAMTAMYCKCGBTGRAG-3′ |
| No. 19: | 5′-CGTAGTGTBTYTTGNCSYTYGAG-3′ |
| No. 20: | 5′-GTTCAGAAGAAYATRGAWAGYTARC-3′ |

* Y: C/T, R: A/G, D: A/G/T, B: C/G/T, K: G/T, M: A/C, S: C/G, N: A/C/G/T, H: A/C/T, W: A/T, V: A/C/G

the MADS-box genes in *Arabidopsis thaliana*.

**Polymerase chain reaction (PCR)**

PCRs were carried out using *TaKaRa Ex Taq* ™ Hot Start Version kit (TAKARA BIO, Japan) according to the manufacturer's protocol. Each 10 μl of the mixture used for PCR contained 2.5 ng of genomic DNA, 1.0 μl of 10 × Ex Taq Buffer, 0.8 μl of dNTP mixture, 10 pmol of each designed primer, and 0.05 μl of TaKaRa Ex Taq ™ HS. DNA amplification reaction was performed in a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, USA), with the following cycling parameters: 1 cycle of 2 min at 94° C; 30 cycles of 30 s at 94° C, 30 s using an annealing temperature gradient of 46–54° C, and 110 s at 72° C; and 1 cycle of 3 min at 72° C. Ninety-one patterns were performed under the conditions specified here, for amplification with all conceivable combinations for Nos. 1–9 and Nos. 10–20, except for combinations of Nos. 3, and 4 and Nos. 15, 17–19, due to their locations (Fig. 1). The amplification products were electrophoresed in 0.75% agarose gel (Nacalai Tesque, Japan) with 40 mM Tris Acetate ETDA buffer and visualized by staining with ethidium bromide.

**Cloning and sequencing**

The amplified putative MADS-box specific bands were excised from the gel, eluted using the QIAquick Gel Extraction Kit (QIAGEN, Germany), cloned in the pGEM-T easy vector (Promega, USA), and then the transformed plasmids were inserted into Competent high DH5 α (TOYOBO, Japan). The inserts of the positive clones were amplified by PCR using the primers SP6 and T7, which flank the polylinker region of the plasmid vector. The plasmid DNAs were sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA) and SP6 or T7 primers, in addition to ABI PRISM ™ 310 with Genetic Analyzer (Applied Biosystems, USA). The obtained DNA sequences were compared with the MADS-box genes that had been registered in the database using a BLAST homology search in the NCBI (http://www.ncbi.nlm.nih.gov/). The percentage similarity was calculated for the region of the matched sequences in relation to the total sequence of the fragment.

**Homology search and phylogenetic analysis**

The 9 putative MADS-box sequences were aligned with the amino-acid sequences of *APETALA1*, *APETALA2*, *APETALA3*, *PISTILLATA*, *AGAMOUS* from *Arabidopsis thaliana* and

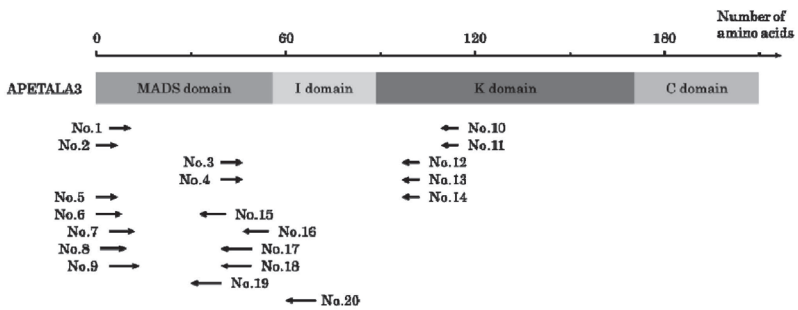


Figure 1. Positions of the primers based on the *APETALA3* amino acid sequence. Nine forward primers (Nos. 1-9) were designed in the MADS domain, and 11 reverse primers (Nos. 10-20) were designed in MADS, I, and K domains.

their homologs from 5 gymnosperms (specfically, *DAL2*, *DAL11*, *DAL12*, and *DAL13* from *Picea abies*; *PrDGL* from *Pinus radiata*; *SAG1a* from *Picea mariana*; *CyAG* from *Cycas edentate*; and *GBM5* from *Ginkgo biloba*). Based on the results, a phylogenetic tree was constructed using the neighbor-joining (NJ) method by means of the program MEGA (version 5.1) [16]. These sequences were all obtained from the DDBJ database (http://www.ddbj.nig.ac.jp/). MADS-box genes of *PPM1*, *PPM2*, *PpMADS1*, *PpMADS2*, and *PpMADS3* from *Physcomitrella patens* were used as an out group to construct the phylogenetic tree. *APETALA2* from *A. thaliana*, which is not a MADS-box gene, was also used because it has an A-function.

## Results and Discussion

As a result of PCRs using combinations of 91 primer patterns derived from 10 species, only the combination of No. 9 and No. 17 was selected at an annealing temperature of 50°C to delineate putative MADS-box specific bands. Only 3 DNA species were amplified, from which 9 nucleotide sequences of 86–87-bp fragments were obtained. These 9 nucleotide sequences were named as: *Gb-1* and *Gb-2* from *Ginkgo biloba*; *Pj-1* to *Pj-4* from *Picea jezoensis*; and *Cr-1* to *Cr-3* from *Cycas revoluta*. All of the sequences with the highest homology were derived from MADS-box genes (Table 3). A homology search revealed that each of the 9 sequences is more

Table 3. The strongest homology obtained from 9 sequences of *Ginkgo biloba*, *Picea jezoensis*, and *Cycas revoluta*. Homologies for the 9 sequences were searched for by using the BLASTn program for targeting DNAs, and the BLASTx program for targeting proteins. The strongest homology of DNAs and proteins is indicated on gene names or sequence features, species, accession numbers, and identity percentage, which are ordered from top to bottom. The identity percentages were calculated using only matched sequences in relation to the total sequences of the fragments.

| | Accession number | DNA targeted | Protein targeted |
|---|---|---|---|
| Gb-1 | AB747316 | *GBM30*, *Ginkgo biloba*<br>(AF515027)<br>100% (66/66) | *GBM15*, *Ginkgo biloba*<br>(Q84LV2)<br>76% (19/25) |
| Gb-2 | AB747317 | *GBM21*, *Ginkgo biloba*<br>(AF515018)<br>100% (77/77) | *GBM21*, *Ginkgo biloba*<br>(Q84LU6)<br>100% (25/25) |
| Pj-1 | AB747318 | *CAG3*, *Chrysanthemum x morifolium*<br>(AB354251)<br>87% (67/77) | MADS-box protein, *Poa annua*<br>(Q9XGS1)<br>96% (27/28) |
| Pj-2 | AB747319 | *DAL11*, *Picea abies*<br>(AF158540)<br>100% (86/86) | *DAL11*, *Picea abies*<br>(Q9SEE3)<br>100% (28/28) |
| Pj-3 | AB747320 | *CAG3*, *Chrysanthemum x morifolium*<br>(AB354251)<br>100% (86/86) | MADS-box protein, *Poa annua*<br>(Q9XGS1)<br>96% (27/28) |
| Pj-4 | AB747321 | *CAG3*, *Chrysanthemum x morifolium*<br>(AB354251)<br>91% (57/62) | MADS-box protein, *Poa annua*<br>(Q9XGS1)<br>92% (25/27) |
| Cr-1 | AB747322 | MADS-box transcription factor, *Zea mays*<br>(EU962906)<br>91% (41/45) | MADS-box transcription factor, *Pseudotsuga macrocarpa*<br>(C6F724)<br>92% (25/27) |
| Cr-2 | AB747323 | MADS-box transcription factor, *Cycas edentata*<br>(AY295079)<br>97% (83/85) | *AOM2*, *Asparagus officinal*<br>(Q6TXR1)<br>100% (28/28) |
| Cr-3 | AB747324 | *MADS2*, *Elaeis guineensis*<br>(EF034155)<br>85% (66/77) | MADS-box transcription factor, *Pseudotsuga macrocarpa*<br>(C6F724)<br>96% (26/27) |

than 85% and 76% similar to nucleotide and amino acid sequence identities with other plant MADS-box genes, respectively. These data indicate that all 9 sequences are homologous to MADS-box genes. One section of *Gb-1* and *Gb-2* shared 100% nucleotide sequence similarity with *GBM30* and *GBM 21*, indicating that they are identical to *GBM30* and *GBM21*, respectively. Our study provided more information about these 2 sequences, which have been previously reported[12]. It is also suggested that *Pj-2* is a *DAL11* homolog, because both sequences are 100% similar to their respective nucleotide and amino-acid sequences. Sequences identical to *Pj-1*, *Pj-2*, *Pj-3*, *Pj-4*, *Cr-1*, *Cr-2*, and *Cr-3* were not found in same species; therefore, these putative parts of the MADS domains were newly determined in the current study.

The alignment of the amino-acid sequences among the 9 putative MADS-box genes and previously determined MADS-box genes of *A. thaliana*, *Picea abies*, *Cycas edentate*, *Picea mariana*, *Pinus radiate*, and *Ginkgo biloba* are shown in Fig 2. The 9 sequences were aligned with the central 28 amino acids in the MADS domain.

A NJ phylogenetic tree was constructed using the 9 sequences, MADS-box genes and the *AP2* subfamily of gymnosperm and *A. thaliana*, using *Physcomitrella patens* as out group. In our study, only 86–87 bp sequences of the deduced MADS domains were determined; therefore, the topology of the phylogenetic tree is ambiguous, with low bootstrap confidence. *Cr-2* formed 1 clade with C-function genes, including *GBM5*, *AGAMOUS*, *CyAG*, *DAL2*, and *SAG1a*; therefore, *Cr-2* is suggested to have C-function. Only *Pj-2* and *Cr-2* from the 9 sequences of the deduced MADS domains formed the 2 clades containing genes of B- and C-functions, respectively;

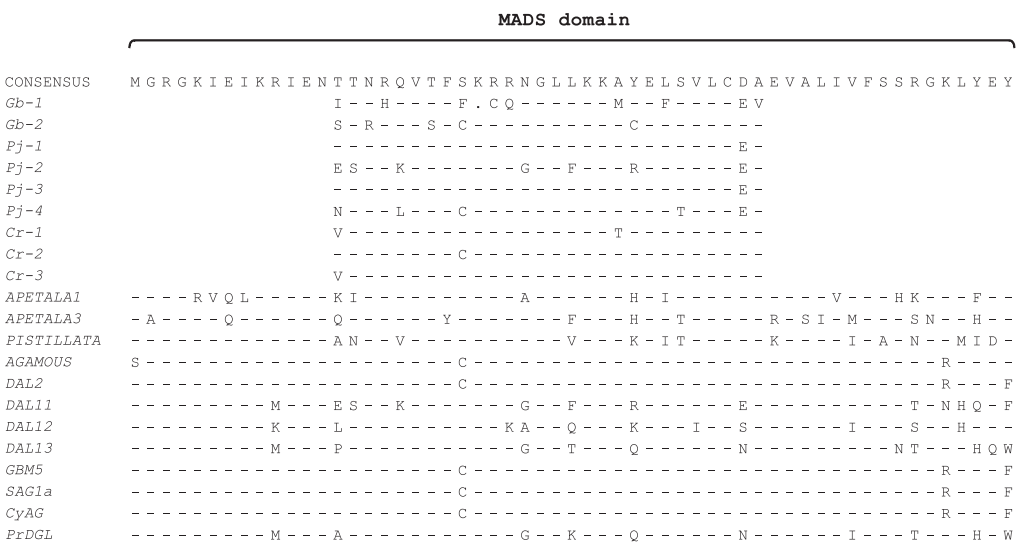


Figure 2. Comparison of amino acid sequences in the MADS-domain of the MADS-box genes, including putative amino acid sequences in gymnosperms and *Arabidopsis thaliana*. The multiple alignments were generated using the computer program MEGA (ver. 5.1). Identity with consensus is denoted by dashes, differences are indicated by the amino acid designation, and a stop-codon in the alignment is indicated by a period.

however, other sequences of *Gb-1*, *Gb-2*, *Pj-1*, *Pj-3*, *Pj-4*, *Cr-1*, and *Cr-3* formed different clades. *Gb-1* includes a stop codon in its alignment, indicating that it is highly likely that *Gb-1* is a pseudo-gene. None of the 9 sequences formed a clade with A-function genes.

Exhaustive analysis of MADS-box genes indicates that only B- and C-functions existed in the common ancestor of angiosperms and gymnosperms [17, 18, 19]. None of the MADS-box genes of *Physcomitrella patens* (Fig. 3) formed any sister groups with MADS-box genes for which the functions are known. Therefore B- and C-function genes are estimated to have developed their functions when ferns or gymnosperms diverged, following the divergence of mosses. In addition, the A-function was first added in basal angiosperms, while the other functions remained unchanged [19, 20]. At this late phase, the homologs of *APETALA2*, which does not have a MADS domain but is known to have an A-function in *A. thaliana*, appeared in certain gymnosperms (namely, *Ginkgo biloba*, *Cycas revolute*, and *Gnetum parvifolium*) [21, 22]. These findings imply that the regulatory mechanisms of gene expression have been conserved over the three hundred

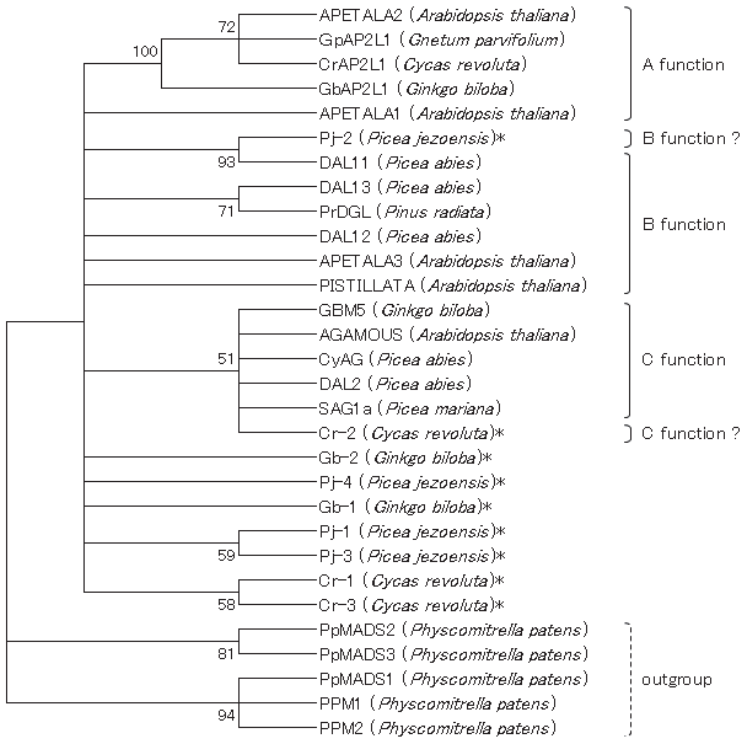


Figure 3. A phylogenetic tree showing the relationships of MADS-box genes and the *AP2* subfamily in gymnosperms, *Physcomitrella patens*, and *Arabidopsis thaliana*. The tree is a Neighbor-Joining (NJ) tree of amino acids. The sequences were rooted using *PPM1*, *PPM2*, and *PpMADS1-PpMADS3* from *Physcomitrella patens*. Bootstrap percentages on 1000 replicas are provided next to some nodes, and branches not supported by bootstrap percentages over 50% were collapsed. Genes and species names are shown to the left of each branch. Asterisks (*) show putative MADS domain sequences that were isolated in this study.

million years since the divergence of gymnosperms and flowering plant lineages [20], which generates controversy over the divergence of MADS-box genes. The evolution of flowers containing reproductive organs is suggested to be highly-diverse in gymnosperms and angiosperms, with new theories being currently in the process of construction [17, 18, 19]. Therefore, novel findings about MADS-domains are fundamental, such as the 3 sequences of *Cycas revoluta*, which belongs to the Cycadophyta division, and is thought to be an evolutionarily ancient in gymnosperm[19]. This is because such findings may contribute towards elucidating the mechanisms of organs evolution and sex determination in gymnosperms. Since the current study only focused on assessing a specific region, further sequencing and analysis are required.

**Acknowledgment**

We thank Arboricultural Research Institute, The University of Tokyo Hokkaido Forest, Koishikawa Botanical Gardens and Itabashi Botanical Gardens for providing the plant materials.

**Summary**

Angiosperms are known to have MADS-box genes, which regulate the formation of floral organs. We isolated sections of MADS-box genes, and conducted phylogenetic analyses to elucidate the evolution of reproductive organs in gymnosperms. Ten experimental gymnosperm and pteridophyta plant species were used (gymnosperms: *Ginkgo biloba*, *Gnetum gnemon*, *Ephedra sinica*, *Cycas revoluta*, *Picea jezoensis*, *Picea abies*, *Taxus cuspidata*, *Cryptomeria japonica*, and *Metasequoia glyptostroboides*; pteridophyta: *Thelypteris torresiana*). Degenerate primers were designed on the basis of conserved regions of MADS-box genes that were already known for gymnosperms (i.e., *Ginkgo biloba*, *Gnetum gnemon*, *Picea abies*, and *Cryptomeria japonica*). PCRs were performed using 91 primer combination patterns by using DNA extracted from the leaves of all 10 plants. Amplified DNA fragments were selected after cloning, and their nucleotide sequences were determined. As a result, 2, 3, and 4 sequences (86–87 bp long) were determined for 3 species (*Ginkgo biloba*, *Cycas revoluta*, and *Picea jezoensis*, respectively), and considered to be sections of MADS domains. While 2 types of sequences obtained from *Ginkgo biloba* have been reported previously (*GBM21* and *GBM30*), each extended sequence was newly determined. Moreover, 2 sequences from *Cycas revoluta* and *Picea jezoensis* were inferred to be homologs of known MADS-box genes. The newly determined putative MADS domain sequences of *Cycas revoluta* in this study are expected to be particularly important toward understanding the evolution of floral organs.

**Keywords**: gymnosperms, MADS-box genes, phylogenetic analysis, ABC model, reproductive organ evolution

## References


1) Coen, E.S., and Meyerowitz: E.M. (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353: 31-37.
2) Weigel, D., and Meyerowitz, E.M. (1994) The ABCs of floral homeotic genes. Cell 78: 203-209.
3) Theissen, G., *et al*. (2000) A short history of MADS-box genes in plants. Plant Mol. Biol. 42: 115-149.
4) Colombo, L., *et al*. (1995) The petunia MADS box gene *FBP11* determines ovule identity. Plant Cell 7: 1859-1868.
5) Angenent, G.C., and Colombo, L. (1996) Molecular control of ovule development. Trends Plant Sci. 1: 228-232.
6) Theissen, G. (2001) Development of floral organ identity: stories from the MADS house. Curr. Opin. Plant Biol. 4: 75-85.
7) Muriel, J., *et al*. (2003) MADS-box genes in *Ginkgo biloba* and the evolution of the *AGAMOUS* Family. Mol. Biol. Evol. 20: 842-854.
8) Theissen, G., *et al*. (1996) Classification and phylogeny of the MADS-box multigene family suggest defined roles of MADS-box gene subfamilies in the morphological evolution of eukaryotes. J. Mol. Evol. 43: 484-516.
9) De Bodt, S., *et al*. (2003) And then there were many: MADS goes genomic. Trends in Plant Sci. 8: 475-483.
10) Shore, P., and Sharrocks, A.D. (1995) The MADS-box family of transcription factors. Eur. J. Biochem. 229: 1-13.
11) Münster, T., *et al*. (1997) Floral homeotic genes were recruited from homologous MADS-box genes preexisting in the common ancestor of ferns and seed plants. Proc. Nat. Acad. Sci. USA 94: 2415-2420.
12) Winter, K.U., *et al*. (1999) MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. Proc. Nat. Acad. Sci. USA 96: 7342-7347.
13) Hasebe, M. (1999) Evolution of reproductive organs in land plants. J. Plant Res. 112: 463-474.
14) Himi, S., *et al*. (2001) Evolution of MADS-box gene induction by *FLO*/*LFY* genes. J. Mol. Evol. 53: 387-393.
15) Hasebe, M. (1998) Characterization of MADS homeotic genes in the fern *Ceratopteris richardii*. Proc. Nat. Acad. Sci. USA 95: 6222-6227.
16) Tamura, K., *et al*. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28(10): 2731-2739.
17) Alvarez-Buylla, E.R., *et al*. (2000a) MADS-box gene evolution beyond flowers: expression in pollen, endosperm, guard cells, roots and trichomes. Plant J. 24: 457-466.
18) Alvarez-Buylla, E.R., *et al*. (2000b) An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. Proc. Natl. Acad. Sci. USA 97: 5328-5333.
19) Hasebe, M., and Itou, M. (2000) Evolution of reproductive organs and MADS-box genes. Ser. Plant Cell Technol. 12: 188-191.
20) Zhang, P., *et al*. (2004) Conservation of class C function of floral organ development during 300 million years of evolution from gymnosperms to angiosperms. Plant J. 37: 566-577.
21) Shindo, S., *et al*. (1999) Characterization of MADS genes in the gymnosperm Gnetum parvifolium and its implication on the evolution of reproductive organs in seed plants. Evol. Dev. 3: 180-190.
22) Shigyo, M., *et al*. (2006) Molecular evolution of the AP2 subfamily. Gene 366: 256-265.


(Received Jul. 17, 2012)
(Accepted Jan. 23, 2013)

# 裸子植物における MADS-box 遺伝子群の系統解析

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**要　旨**

被子植物においては花器官形成に関わる MADS-box 遺伝子群が知られている。本研究は裸子植物における雌雄の器官形成の進化を知ることを目的とし，MADS-box 遺伝子の部分配列を決定し，系統解析を行った。対象植物は，裸子植物であるイチョウ，グネツム，マオウ，ソテツ，エゾマツ，ドイツトウヒ，イチイ，スギ，メタセコイア，およびシダ植物であるヒメワラビの計 10 種であった。実験手法は，裸子植物（イチョウ，グネツム，トウヒ，スギ）で既に知られている MADS-box 遺伝子の保存領域からデジェネレートプライマーを設計し，10 種の葉から抽出した DNA をテンプレートとして計 91 組のプライマー対で PCR を行った。増幅された DNA 断片はクローニング後選抜し，塩基配列を決定した。その結果，イチョウ，ソテツ，エゾマツの 3 種において，MADS-box 遺伝子の部分配列 86 ～ 87 塩基長がそれぞれ 2，3，4 種類得られた。このうちイチョウから得た 2 種類の配列は，既に報告されている配列（*GBM21*，*GBM30*）と一致したが，それぞれ新たに延長する配列を決定した。またソテツ，エゾマツからそれぞれ得た 2 種類の MADS-box 配列は，機能の知られる既存の MADS-box 遺伝子のホモログであることが推測された。特に今回新たに決定したソテツの MADS-box 配列は，花器官の進化を探る上で今後重要な情報になると考えられる。

**キーワード**：裸子植物，MADS-box 遺伝子，系統解析，ABC モデル，生殖器官進化