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MOLECULAR BASIS OF DEVELOPMENT IN PETALOID MONOCOT FLOWERS

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ABSTRACT

The molecular background of flower development has been intensively studied within core eudicots, and several studies have confirmed the extended ABC model as the molecular background of flower development in this plant group. The core eudicots are characterized as having one copy of each of the B-class genes and at least two copies of A-class genes: one is expressed in floral meristems, the other in inflorescence meristems. In monocots and non-core eudicots the validity of the ABC model is under discussion. Generally, more than one functional copy is found of at least one of the B-class genes. The A-class genes apparently are expressed in meristems of both flower and inflorescence. Morphologically petaloid stamens and styles are well known within the petaloid monocots, whereas the phenomenon is rare in core eudicots. A simple model based on the extra copies of B-class genes can explain the molecular background of petaloid stamens in the monocots; the only requirement is that two copies of the same gene have different expression patterns and are responsible for development of petals and stamens, respectively. The formation of petaloid styles can be explained in the same way, but this hypothesis requires that A- and C-class gene expression is not mutually exclusive in monocots. The difference in expression of the A-class genes outside the floral organs shows a fundamental difference between monocot and core eudicot flowers.

Key words: ABC model, A-class genes, B-class genes, homeotic mutants, MADS-box genes, monocot flower.

INTRODUCTION

The molecular background of flower development has been studied intensively, especially in core eudicots (for reviews see Theissen and Saedler 1999; Theissen et al. 2000; Johansen et al. 2002; Becker and Theissen 2003) and the ABC model has become generally accepted. Despite recent modifications (Colombo et al. 1995; Becker et al. 2000; Pelaz et al. 2001) the model still appears too simple; this is clearly indicated by an overview of published expression patterns (Johansen et al. 2002) and studies in non-core eudicots (Kramer and Irish 2000; Kramer et al. 2003). Information from non-core eudicots and monocots also indicates that the B-class genes at least show some unexpected expression patterns (Kramer and Irish 1999, 2000; Hsu and Yang 2002; Skipper 2002; Kramer et al. 2003). Nevertheless, in spite of sparse information, the ABC model generally has been accepted to explain floral development in monocots (e.g., Kang et al. 1998; Ambrose et al. 2000).

Within monocots, MADS-box genes have been predominantly studied in grasses (Chung et al. 1995; Kang et al. 1995; Mena et al. 1995; Theissen et al. 1995; Greco et al. 1997; Lopez-Dee et al. 1999; Moon et al. 1999; Ambrose et al. 2000; Heuer et al. 2000, 2001; Kyozuka et al. 2000; Prasad et al. 2001), but the homology of the different whorls of the very reduced grass flower are still under discussion. Thus, studies in petaloid monocots should give a better idea of the molecular evolution of monocot flowers, as the homologies of the different whorls are unquestionable, even in the highly specialized orchid flower. Unfortunately, MADS-box gene expression has been studied in very few petaloid monocots (Caporali et al. 2000; Kramer and Irish 2000; Tzeng and Yang 2001; Li et al. 2002; Kanno et al. 2003), including a few orchids (Lu et al. 1993; Yu and Goh 2000; Hsu and Yang 2002; Johansen and Frederiksen 2002; Yu et al. 2002). Orchids could be suitable objects for studying both general expression of the A-, B-, and C-class genes, and the *SEPALLATA* genes as well as for studying the influence of these genes on the development of specialized structures such as gynostemium, stigma, viscidium, and pollinium stalk (Johansen and Frederiksen 2002).

Petaloid monocots are characterized by having two whorls of tepals (sepals and petals) that are petal-like. However, the two whorls are often quite different from each other as in *Galanthus* L. or *Iris* L. In Ranunculaceae, where two petaloid whorls are common, expression of B-class genes is observed in the sepals, indicating that petaloid sepals could be the result of influence from the B-class genes (Kramer et al. 2003), and northern hybridization has shown B-class genes to be expressed in both sepals and petals in *Tulipa* L. (Kanno et al. 2003). If the molecular background for flower development in the petaloid monocots is similar to that found in Ranunculaceae we may assume that A- and B-class genes, as well as *SEPALLATA* genes, are expressed in both sepals and petals.

Core eudicots seem to be fixed in only having one copy of each B-class genes, *PISTILLATA* (*PI*) and *APETALA3* (*AP3*), whereas non-core eudicots such as Ranunculaceae, as well as magnoliids and monocots, have more copies of these genes (Kramer et al. 1998, 2003; Kramer and Irish 1999, 2000; Kanno et al. 2003). Some of these extra copies may merely be redundant genes, but in several cases these extra copies may have a function that could explain some of the variation in floral morphology observed in monocots, magnoliids, and non-core eudicots. Extra copies of B-class genes

may also be responsible for the variation observed in some core eudicots.

Furthermore, it has been shown that duplication in the A-class lineage has occurred in the core eudicots, leading to two different lines: the *AP1* lineage with an altered C-terminal (Johansen et al. 2002; Litt and Irish 2003) and the *FRUITFULL* lineage possessing a plesiomorphic C-terminal. The two lineages appear to have different transcription patterns in the core eudicots; *AP1* genes are transcribed in floral meristems, whereas *FRUITFULL* genes are mainly transcribed in inflorescence meristems (Johansen et al. 2002; Litt and Irish 2003).

The key question is: do the flowers in monocots, magnoliids, and non-core eudicots develop according to the ABC model?

To answer this question four different approaches may be used: observations of naturally occurring homeotic mutants, phylogenetic-based comparisons among sequences of MADS-box genes from different species throughout the angiosperms, studies of MADS-box genes transcription patterns, and studies of transgenic mutants. The last approach is not addressed here, as it is difficult to produce transgenic monocots, and as the generation time in our main study group, the orchids, is often long.

MATERIALS AND METHODS

Apart from MADS-box genes observed in *Cleisostoma racemiferum* (Lindl.) Garay, MADS-box genes referred to are all published in GenBank. A phylogeny of MIKC genes and a phylogeny-based transcription analysis were published by Johansen et al. (2002) and a more detailed phylogenetic analysis of A-class, including *FRUITFULL,* by Litt and Irish (2003). In situ RT-PCR on *CracOM1* was performed according to the methods of Johansen (1997) and Skipper (2002).

RESULTS

Homeotic Mutants

Although the petals in many angiosperm groups are thought to have evolved from stamens (Albert et al. 1998), spontaneous homeotic mutants showing petaloid functional stamens (pollen producing) are uncommon among dicots; the phenomenon is more frequently found in Ranunculaceae, Rosaceae, and Caryophyllales. The molecular background of homeosis has only been studied in Ranunculaceae, where the boundary between petals and anthers seems rather blurred (Kramer et al. 1998, 2003; Kramer and Irish 1999, 2000; Skipper 2002).

In monocots functional petaloid stamens are common among cultivated ''plena-like'' varieties and are found in Amaryllidaceae, Colchicaceae, Hemerocallidaceae, Hyacinthaceae, and Liliaceae (Fig. 1, 3). Some families are even characterized by having such functional petaloid stamens (Cannaceae, Marantaceae), and in Alliaceae, Hyacinthaceae, and Stemonaceae, many species have leaf-like filaments and/or connectives.

Transformation of styles to more or less petal-like organs is, to our knowledge, unknown, at least in core eudicots, whereas it is common in the monocots. Petaloid styles occur in Cannaceae, Iridaceae, and Marantaceae (Fig. 2), but they are also known from several cultivated varieties of, for example, *Tulipa* and *Hippeastrum* Herb. At the apex of these petaloid styles normal stigmas can be identified (Fig. 3).

In the zygomorphic flowers of orchids the sepals and petals are often morphologically identical but differ from the labellum. However, in some orchids sepals and petals differ strongly (e.g., Bulbophyllinae, Cypripedioideae, Pleurothallidinae).

In orchids spontaneous homeotic mutants (termed peloria or pseudopeloria) are well known, showing transformation of different parts of the flower. See Bateman and Rudall (2006) for a complete overview of different types of peloria. Perhaps the best known of these peloric orchids is *Cattleya intermedia* Hook. f. var. *aquinii* Barb. Rodr., a naturally occurring homeotic mutant where the two lateral petals are transformed into labellum-like organs (Type A of Bateman and Rudall 2002), but peloric orchids are also known from Cypripedioideae (e.g., *Paphiopedilum* Pfitzer*),* Orchidoideae (e.g., *Ophrys* L.*, Orchis* L.*, Platanthera* Rich.), and apart from *Cattleya* Lindl. several other genera in Epidendroideae (e.g., *Cymbidium* Sw.*, Oncidium* Sw.*, Phalaenopsis* Blume*, Zygopetalum* Hook.). Instead of having the petals transformed into labellum-like organs some of these peloric individuals have the labellum transformed into a petal (Type B or C in Bateman and Rudall 2002). This is a common homeotic mutation in *Arundina graminifolia* Hochr. in Malaysia (Johansen 2001; pers. obs.). In *Ophrys* (Fig. 4) the sepals are large and often green, the two petals are usually much smaller and often colored, while the labellum is especially conspicuous. Homeotic mutants have shown that petals and labellum can be transformed into sepals or petals can be transformed into labellum-like organs (Rudall and Bateman 2002, 2003). Complete transformation of sepals into labellum-like organs has not been reported (Rudall and Bateman 2002; Bateman and Rudall 2006). Thus, spontaneous mutants involve petals in preference to sepals.

Comparison of MADS-Box Genes

MADS-box genes code for DNA-binding proteins that regulate transcription of other genes. The MADS-box genes of interest here belong to the so-called MIKC genes, which include four different more or less conserved regions (Alvarez-Buylla et al. 2000). The DNA-binding region, the MADS-box, is strongly conserved, the I-region is only partly conserved and is supposed to take part in the dimerization, as is the rather conserved K-box. In contrast, the last part, the C-terminal, is quite variable except for a few highly conserved parts that are believed to be important in connection with formation of multicomponents (Riechmann et al. 1996*a*, *b*; Kramer et al. 1998, 2003; Egea-Cortines et al. 1999; Johansen et al. 2002; Becker and Theissen 2003; Messenguy and Dubois 2003). Phylogenetic analyses of MIKC genes indicate that A-, B-, and C-class MADS-box genes constitute monophyletic groups (Fig. 5) and the conserved amino acid sequences of the C-terminal are identical or nearly identical within each group (Johansen et al. 2002; Kramer et al. 2003).

Here we will focus on the A- and B-class genes, which according to the ABC model are responsible for flower mer-

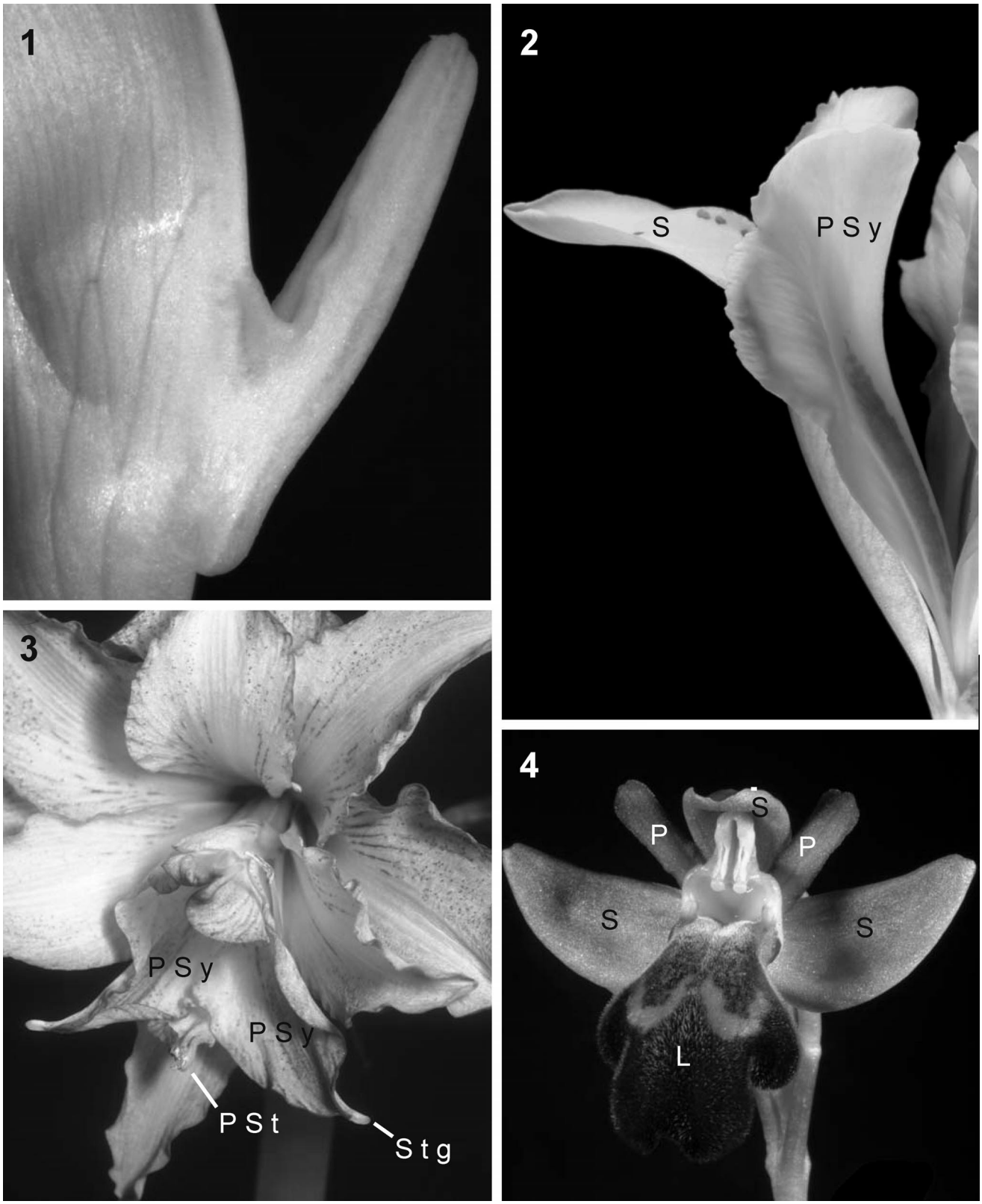


Fig. 1–4.—Details of different parts of flowers of selected petaloid monocots.—1. Part of petaloid, functional stamen from a cultivated ''plena-like'' *Tulipa*.—2. Petaloid style and one of the sepals from a ''normal'' *Iris*.—3. Flower of cultivated ''plena-like'' *Hippeastrum* with stamen and styles transformed to petaloid organs.—4. ''Normal'' flower of *Ophrys* demonstrating differences between sepals and petals. (L = labellum; P = petal; PSt = petaloid stamen; PSy = petaloid style; S = sepal; Stg = stigma.)

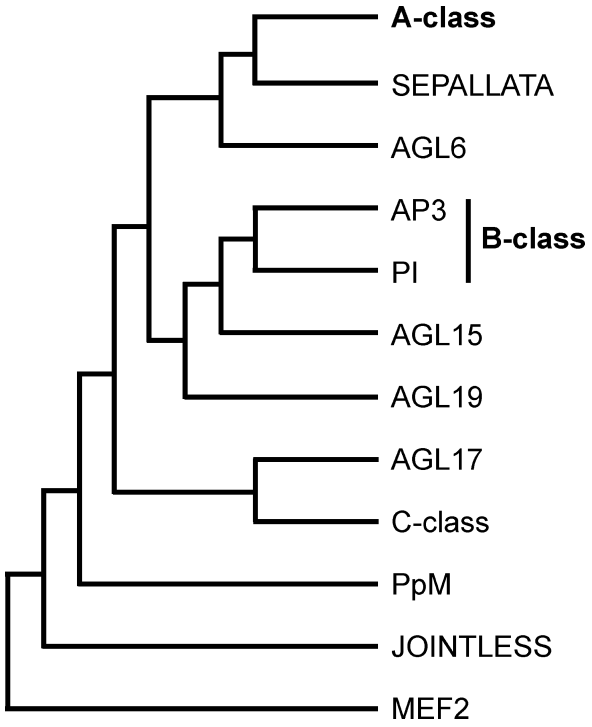


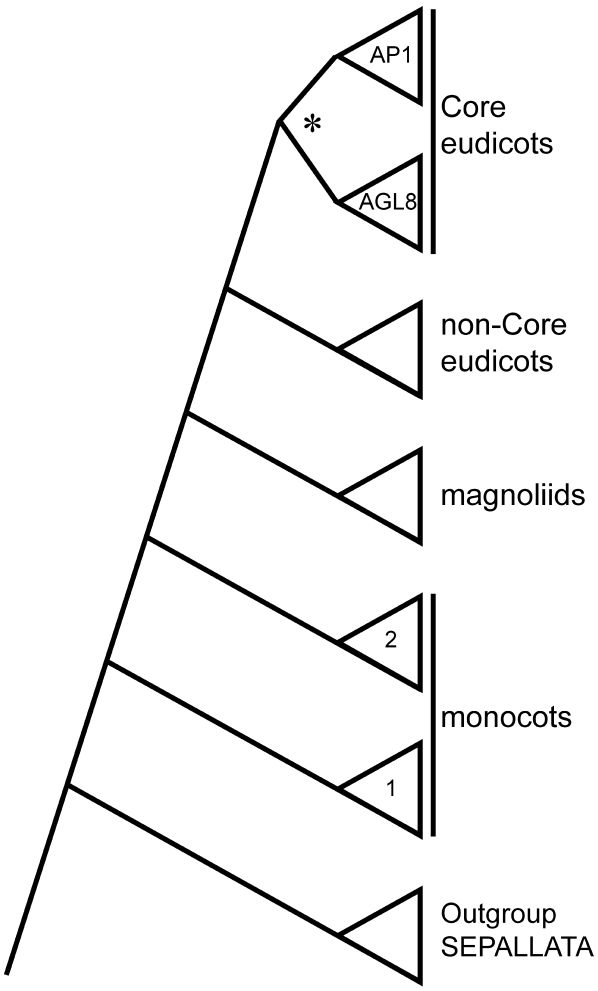
Fig. 5.—Summary of the different MIKC genes. Gene families of interest here are in boldface. After Johansen et al. 2002.

istem identity and development of sepals, petals, and stamens.

Phylogenetic analyses of A-class genes (including *FRUITFULL*) show several more or less well-defined clades (Fig. 6; Johansen et al. 2002; Litt and Irish 2003). One of the main duplication events has occurred in the core eudicots where one of the copies—the *euSQUAMOSA* genes (= *euAP1* or *AP1*)—shows a unique conserved C-terminal (Johansen et al. 2002; Litt and Irish 2003). The remaining A-class genes, including all monocot genes, possess the plesiomorphic *SQUAMOSA* motif (the *AGL8* or *FUL* motif), this resembles the motif found in *SEPALLATA* genes, the sister group to the A-class genes (Fig. 6). Although few monocot genes are included in the analysis, it appears that two monocot clades exist (Fig. 6; Johansen et al. 2002; Litt and Irish 2003), indicating that all monocots most probably possess at least two different copies.

Within the B-class genes it has been demonstrated that a duplication event in the angiosperm ancestor resulted in two paralog lineages of B-class genes, the *APETALA3* (*DEFICIENS*) lineage and the *PISTILLATA* (*GLOBOSA*) lineage (Kramer et al. 1998; Sundstrom et al. 1999; Kramer and Irish 2000). Core eudicots generally have only one copy of each of these genes, but in *Malus* Mill. (Rosaceae) at least two copies of each are found in GenBank. Intensive studies in the non-core eudicot family Ranunculaceae have shown more than one copy of B-class genes to exist, and experiments indicate that all copies play an important role, at least in the development of ''normal'' petals (Kramer et al. 2003). Recently, several copies of *PI* and/or *AP3* have been found in several members of Laurales, Magnoliales, and Piperales (Stellari et al. 2004).

More than one copy of one or both B-class genes is known from several monocots, including *Lilium* L., *Tulipa, Hyacinthus* L., orchids, and grasses. In *Lilium* two copies of *PI* (*LRGLOA, LRGLOB*), but only one of *AP3* (*LRDEF*) are known (Tzeng et al. 2001). In *Tulipa* one *PI* (*TGGLO*) and



euSQUAMOSA motif:

AP1 motif	**..VYNCNLGCFAA**

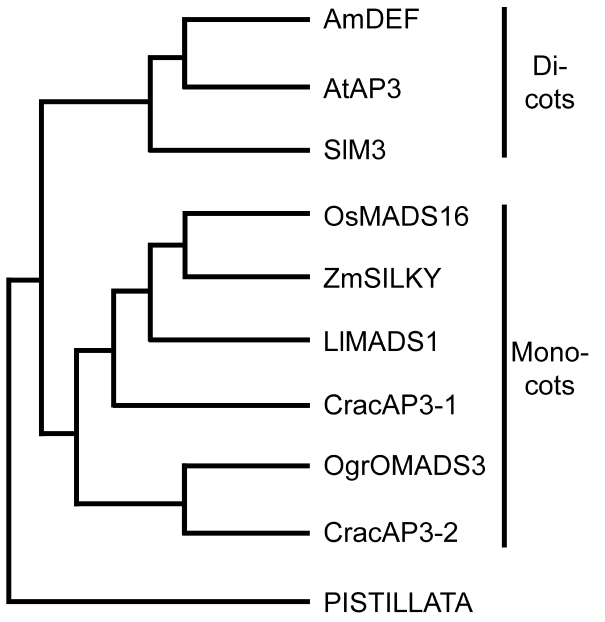
plesiomorphic SQUAMOSA motif:

AGL8 motif	**..LPAWMLRTPTTNE**
Non-core eudicot	**..MPPWMLSHLR**
Magnoliids motif	**..MPPWMLRHVNE**
Monocots 2 motif	**..LPPWMLSHING**
Monocots 1 motif	**..LPPWMLRTSHT**

Outgroup motif

SEPALLATA motif	**..MPPWLP**

Fig. 6.—Summary of a phylogenetic analysis of A-class genes. Different C-terminal motifs are shown below (* = a duplication event within the core eudicots). After Litt and Irish (2003).



eu AP3 motif:

AmDEF	**..DLTTFALLE**
AtAP3	**..DIITFHLLE**
SlM3	**..CVTTYALL**

paleo AP3 motif:

OsMADS16	**..NHDLRLG**
ZmSILKY	**..FHDLRLG**
LIMADS1	**..SHDLRLA**
CracAP3-1	**..PHDLRLA**

Orchid derived AP3 motif

CracAP3-2	**..SFIAEDLSGIYDSSISMANQR..**
OgrOMADS3	**..SFIAEDLSGVYNSAISMANQR**

Fig. 7.—Summary of a phylogenetic analysis of the B-class gene *AP3.* Different C-terminal motifs are shown below. After Johansen et al. 2002.

two *AP3* (*TGDEFA, TGDEFB*) genes are known (Kanno et al. 2003), but additional as yet unknown *PI* and *AP3* genes may be present. In *Hyacinthus* two *PI* (*HPI1, HPI2*) copies have been isolated. In orchids we have isolated *PI* from 40 species, covering all five subfamilies, and in all species we have found only one copy of *PI.* However, exceptions exist in subfamily Epidendroideae. In *Dendrobium* Sw. we have found three different copies of *AP3* and in *Cleisostoma* Blume at least four different *AP3* paralogs exists (Johansen, Skipper, and Frederiksen unpubl. data). One of the *AP3* genes in *Cleisostoma* is very similar to the *OMADS3* gene (*OgrOMADS3*) isolated from an *Oncidium* hybrid (Hsu and Yang 2002; Fig. 7). As in the *OMADS3* gene the typical paleo *AP3* C-terminal motif (Kramer et al. 1998; Kramer and Irish 2000) is lacking in the ortholog from *Cleisostoma,* but both these genes share another unique 21 amino acid conserved motif in the C-terminal (SFIAEDLSGVYNSAISMANQR).

Transcription Studies

Transcription studies are performed in order to visualize where genes are active in the tissue. Transcription studies can be performed as Northern blotting, in situ hybridization, in situ RT-PCR, or real-time RT-PCR studies. Northern blotting and real-time RT-PCR does not offer the opportunity to localize a signal in specific cells. In situ hybridization is not very sensitive and it may be impossible to design probes that do not cross-hybridize to closely related genes. In situ RT-PCR is sensitive but difficult to perform. However, it is the only technique that is able to localize expression at the cell level and discriminate between closely related genes. Based on in situ hybridization it was found that *DOMADS1* (a *SEP3* ortholog) in *Dendrobium* was transcribed in the apical meristem of the inflorescence (Yu and Goh 2000). Our studies of the *SEP3* ortholog in *Cleisostoma racemiferum* (*CracOM1*) by in situ RT-PCR clearly demonstrated that *CracOM1* is not expressed in the apical meristematic tissue itself, but in the tissue immediately behind it (Fig. 8, 9). Thus, conclusions based on expression patterns cannot always be trusted. Furthermore, the presence of specific mRNA in a given tissue does not necessarily mean that the protein is present in the tissue, too.

A survey of known expression patterns of A-class genes shows that genes with the *AP1* motif (= *euSQUAMOSA* motif; Fig. 6) are expressed in the flower meristem, but not in the apical meristem of the inflorescence, whereas paralogs, such as *AGL8* (referred to as *euFUL* by Litt and Irish 2003) apparently are expressed in the apical meristem of the inflorescence. In non-core eudicots, magnoliids, and monocots, only genes possessing a plesiomorphic *SQUAMOSA* motif (Fig. 6) are known and they are expressed in flowers, inflorescences, and apical meristems (Johansen et al. 2002). However, in situ studies on A-class gene expression are almost lacking in the monocots, and we do not know whether genes belonging to the different clades of A-class genes in the monocots actually have contrasting expression patterns.

DISCUSSION AND CONCLUSIONS

The contrast in types of homeotic mutants found in monocots and core eudicots are most likely caused by expression of different (paralog) MADS-box genes or by different expression patterns of ortholog genes. Some of the differences are easily explained by the numerous copies of B-class genes found in monocots; flowers with similar sepals and petals develop if the same set of B-class genes is expressed in both whorls (Fig. 10). Differences between sepals and petals could emerge if expression of different paralogs from the same group of B-class genes occurs in the two whorls (Fig. 11). Furthermore, the apparently corresponding feature of producing petaloid, but functional stamens could be explained by the extra copies of B-class genes. If one of these copies is responsible for development of the petaloid organs and another copy is responsible for stamen development, a shift in expression from one copy to the other between the petal and staminal whorls results in normal flowers (Fig. 10, 11), whereas expression of both copies in the staminal

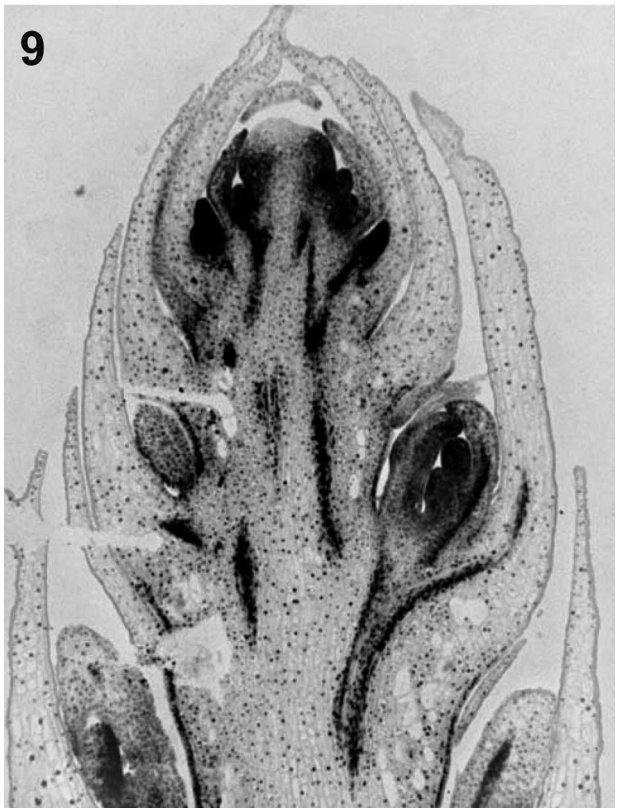
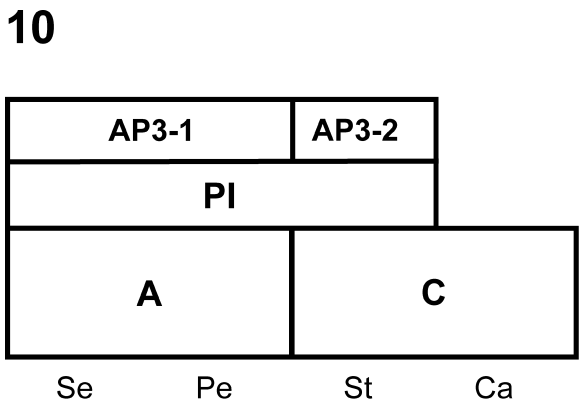
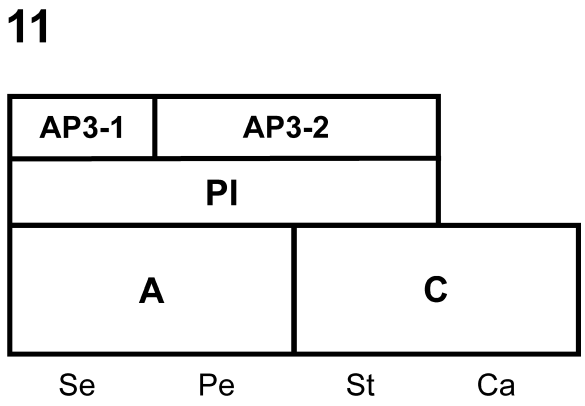


Fig. 8–9.—Expression studies of *CracOM1,* a *SEP3* ortholog, on a longitudinal section of a young inflorescence of *Cleisostoma racemiferum,* made by in situ PCR.—8. Negative control.—9. Positive. *CracOM1* is not expressed in the apical meristem of the inflorescence.





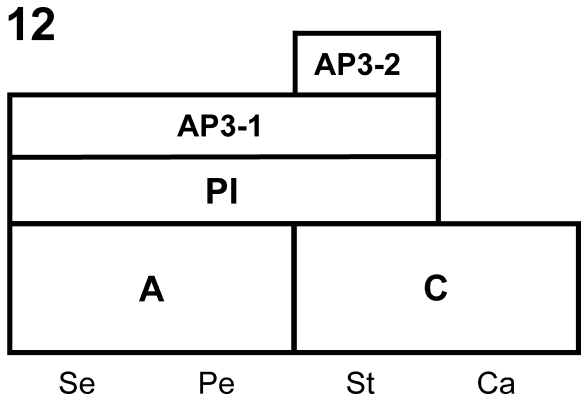


Fig. 10–12.—Hypothetical ABC models for gene expression within plants possessing extra copies of *AP3. AP3–1* and *AP3–2* are believed to be responsible for development of different organs in combination with *PI,* A-, and C-class genes.—10. Monocot with sepals and petals alike, stamens normal.—11. Monocot with sepals and petals different, stamens normal.—12. Monocot with sepals and petals alike, stamens petaloid. (Se = sepals; Pe = petals; St = stamens; Ca = carpels.)

whorls results in petaloid anthers (Fig. 12). This model requires that two copies of either *PI* or *AP3* are present and that the copies show different expression patterns. Apparently this is not the case in *Tulipa gesneriana* L., where the two copies of *AP3* are expressed in all three whorls (Kanno

et al. 2003). However, as the probes used clearly cross-hybridized between the two *AP3* genes this study does not contradict the model; one of the genes may actually be expressed in both sepals and petals and the other gene only in the stamens. The hypothesis is further strengthened by the observations of Lamb and Irish (2003) that *AP3* from *Dicentra eximia* (Ker Gawl.) Torr. was unable to support petal development in transgenic *Arabidopsis* Heynh., whereas stamen development was supported, showing that a given *AP3* copy may only function within a single whorl. This hypothesis could also explain how the reversed order of stamens and carpels observed in *Lacandonia* E. Martínez S. & Ramos (Triuridales; Martínez and Ramos 1989) could emerge. The classical ABC-model must assume that B-class genes are turned on in the first two whorls, then off in the carpels and on again in the stamens. In the model presented here delayed expression of one ore more stamen-specific B-class genes could explain the altered order of stamens and carpels.

At present the ABC-model is unable to adequately explain the molecular background of the formation of petaloid styles within the monocots. The presence of petaloid styles and homeotic mutants in several families indicates that the character probably is governed by one or very few mutations. One of the basic features of the ABC model is the cadasteral activity of A- and C-class genes—if one is expressed the other is not (Mizukami and Ma 1992; Irish and Kramer 1998). If petaloid styles are produced as a result of A- and B-class gene expression, expression of A-, B-, and C-class genes may be expected in the carpel whorl, as the petaloid styles are the apical part of otherwise normal carpels. However, the cadasteral activity of A- and C-class genes has only been demonstrated between e*uSQUAMOSA* (*AP1*) genes and C-class (*AG*) genes (Mizukami and Ma 1992; Irish and Kramer 1998). Apparently, the plesiomorphic type of A-class genes found in non-core eudicots, magnoliids, and monocots can be expressed simultaneously with C-class genes.

It is interesting that in monocot flowers with different sepals and petals, such as *Ophrys* and *Iris,* the sepals are never transformed into petals whereas petals may be transformed into sepals (Rudall and Bateman 2002; Bateman and Rudall 2006); thus, a molecular ''developmental limit'' is hypothesized to exist between sepals and petals. In contrast, both types of mutants are known in *Arabidopsis,* where simple ectopic expression of *AP3*/*PI* in the first whorl leads to petals, whereas knockout of B-class genes results in two whorls of sepals (Krizek and Meyerowitz 1996).

The duplication in the A-class gene of the core eudicots (Johansen et al. 2002; Litt and Irish 2003) resulted in functional specialization of the two paralogs, one gene (from the *AP1* clade) controlling the flower meristem and another (from the *AGL8* clade) controlling the inflorescence meristem. A-class gene transcription patterns have not been studied in detail among petaloid monocots, but as two distinct clades occur (Fig. 6), it seems likely that one gene is responsible for inflorescence meristem identity and the other for flower meristem identity, as in the core eudicots. If so, all organs that are under the influence of the A-class genes are non-homologous in monocots and core eudicots, and hence the monocot flower as a whole is non-homologous with the core eudicot flowers on which the ABC model was based.

LITERATURE CITED

Albert, V. A., M. H. G. Gustafsson, and L. Di Laurenzio. 1998. Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal, pp. 349–374. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, Vol. 2. Kluwer Academic Publishers, Boston, Massachusetts, USA.

Alvarez-Buylla, E. R., S. Pelaz, S. J. Liljegren, S. E. Gold, C. Burgeff, G. S. Ditta, D. P. Ribas, L. Martinez-Castilla, and M. F. Yanofsky. 2000. An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. *Proc. Natl. Acad. Sci. U.S.A.* **97**: 5328–5333.

Ambrose, B. A., D. R. Lerner, P. Ciceri, C. M. Padilla, M. F. Yanofsky, and R. J. Schmidt. 2000. Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. *Molec. Cell* **5**: 569–579.

Bateman, R. M., and P. J. Rudall. 2006. The good, the bad, and the ugly: using naturally occurring terata to distinguish the possible from the impossible in orchid floral evolution, pp. xx–xx. *In* J. T. Columbus, E. A. Friar, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], Monocots: comparative biology and evolution, 2 vols. Rancho Santa Ana Botanic Garden, Claremont, California, USA.

Becker, A., and G. Theissen. 2003. The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Molec. Phylogen. Evol.* **29**: 464–489.

———, K. U. Winter, B. Meter, H. Saedler, and G. Theissen. 2000. MADS-box gene diversity in seed plants 300 million years ago. *Molec. Biol. Evol.* **17**: 1425–1434.

Caporali, E., A. Spada, A. Losa, and G. Marziani. 2000. The MADS-box gene *AOM1* is expressed in reproductive meristems and flowers of the dioecious species *Asparagus officinalis. Sexual Pl. Reprod.* **13**: 151–156.

Chung, Y. Y., S. R. Kim, H. G. Kang, Y. S. Noh, M. C. Park, D. Finkel, and G. An. 1995. Characterization of two rice MADS-box genes homologous to *GLOBOSA. Pl. Sci. (Elsevier)* **109**: 45–56.

Colombo, L., J. Franken, E. Koetje, J. Van Went, H. J. Dons, G. C. Angenent, and A. J. Van Tunen. 1995. The petunia MADS-box gene *FBP11* determines ovule identity. *Pl. Cell* **7**: 1859–1868.

Egea-Cortines, M., H. Saedler, and H. Sommer. 1999. Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus. E. M. B. O. J.* **18**: 5370–5379.

Greco, R., L. Stagi, L. Colombo, G. C. Angenent, M. Sari-Gorla, and M. E. Pè. 1997. MADS-box genes expressed in developing inflorescences of rice and sorghum. *Molec. Gen. Genet.* **253**: 615–623.

Heuer, S., S. Hansen, J. Bantin, R. Brettschneider, E. Kranz, H. Lörz, and T. Dresselhaus. 2001. The maize MADS-box gene *ZmMADS*3 affects node number and spikelet development and is co-expressed with *ZmMADS*1 during flower development, in egg cells, and early embryogenesis. *Pl. Physiol. (Lancaster)* **127**: 33–45.

———, H. Lörz, and T. Dresselhaus. 2000. The MADS-box gene *ZmMADS2* is specifically expressed in maize pollen and during maize pollen tube growth. *Sexual Pl. Reprod.* **13**: 21–27.

Hsu, H. F., and C. H. Yang. 2002. An orchid (*Oncidium* Gower Ramsey) AP3-like MADS gene regulates floral formation and initiation. *Pl. Cell Physiol.* **43**: 1198–1209.

Irish, V. F., and E. M. Kramer. 1998. Genetic and molecular analysis of angiosperm flower development. *Advances Bot. Res.* **28**: 197–230.

Johansen, B. 1997. *In situ* PCR on plant material with sub-cellular resolution. *Ann. Bot. (Oxford)* **80**: 697–700.

———, and S. Frederiksen. 2002. Orchid flowers: evolution and

molecular development, pp. 206–219. *In* Q. C. B. Cronk, R. M. Bateman, and J. A. Hawkins [eds.], Developmental genetics and plant evolution. Taylor & Francis, London, UK.

———, L. B. Pedersen, M. Skipper, and S. Frederiksen. 2002. MADS-box gene evolution-structure and transcription patterns. *Molec. Phylogen. Evol.* **23**: 458–480.

Kang, H. G., J. S. Jeon, S. Lee, and G. An. 1998. Identification of class B and class C floral organ identity genes from rice plants. *Pl. Molec. Biol.* **38**: 1021–1029.

———, Y. S. Noh, Y. Y. Chung, M. A. Costa, K. An, and G. An. 1995. Phenotypic alterations of petal and sepal by ectopic expression of a rice MADS-box gene in tobacco. *Pl. Molec. Biol.* **29**: 1–10.

Kanno, A., H. Saeki, T. Kameya, H. Saedler, and G. Theissen. 2003. Heterotopic expression of class B floral homeotic genes supports a modified ABC model for tulip (*Tulipa gesneriana*). *Pl. Molec. Biol.* **52**: 831–841.

Kramer, E. M., R. L. Dorit, and V. F. Irish. 1998. Molecular evolution of genes controlling petal and stamen development: duplication and divergence within the *APETALA3* and *PISTILLATA* MADS-box gene lineages. *Genetics* **149**: 765–783.

———, and V. F. Irish. 1999. Evolution of genetic mechanisms controlling petal development. *Nature* **399**: 144–148.

———, and ———. 2000. Evolution of the petal and stamen developmental programs: evidence from comparative studies of the lower eudicots and basal angiosperms. *Int. J. Pl. Sci.* **161**: 29–40.

———, V. S. Di Stilio, and P. M. Schlüter. 2003. Complex patterns of gene duplication in the *APETALA3* and *PISTILLATA* lineages of the Ranunculaceae. *Int. J. Pl. Sci.* **164**: 1–11.

Krizek, B. A., and E. M. Meyerowitz. 1996. The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide B class organ identity function. *Development* **122**: 11–22.

Kyozuka, J., T. Kobayashi, M. Morita, and K. Shimamoto. 2000. Spatially and temporally regulated expression of rice MADS-box genes with similarity to *Arabidopsis* class A, B and C genes. *Pl. Cell Physiol.* **41**: 710–718.

Lamb, R. S., and V. F. Irish. 2003. Functional divergence within the *APETALA3/PISTILLATA* floral homeotic gene lineages. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 6558–6563.

Li, Q. Z., X. G. Li, S. N. Bai, W. L. Lu, and X. S. Zhang. 2002. Isolation of *HAG1* and its regulation by plant hormones during in vitro floral organogenesis in *Hyacinthus orientalis* L. *Planta* **215**: 533–540.

Litt, A., and V. F. Irish. 2003. Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: implications for the evolution of floral development. *Genetics* **165**: 821–833.

Lopez-Dee, Z. P., P. Wittich, M. E. Pè, D. Rigola, I. Del Buono, M. Sari-Gorla, M. M. Kater, and L. Colombo. 1999. *OsMADS13,* a novel rice MADS-box gene expressed during ovule development. *Developmental Genet.* **25**: 237–244.

Lu, Z. X., M. Wu, C. S. Loh, C. Y. Yeong, and C. J. Goh. 1993. Nucleotide sequence of a flower-specific MADS-box cDNA clone from orchid. *Pl. Molec. Biol.* **23**: 901–904.

Martínez, E., and C. H. Ramos. 1989. Lacandoniaceae (Triuridales): una nueva familia de México. *Ann. Missouri Bot. Gard.* **76**: 128–135.

Mena, M., M. A. Mandel, D. R. Lerner, M. F. Yanofsky, and R. J. Schmidt. 1995. A characterization of the MADS-box gene family in maize. *Plant J.* **8**: 845–854.

Messenguy, F., and E. Dubois. Role of MADS-box proteins and their cofactors in combinatorial control of gene expression and cell development. *Gene* **316**: 1–21.

Mizukami, Y., and H. Ma. 1992. Ectopic expression of the floral homeotic gene *AGAMOUS* in transgenic *Arabidopsis* plants alters floral organ identity. *Cell (Cambridge)* **71**: 119–131.

Moon, Y. H., J. Y. Jung, H. G. Kang, and G. An. 1999. Identification of a rice *APETALA3* homologue by yeast two-hybrid screening. *Pl. Molec. Biol.* **40**: 167–177.

Pelaz, S., C. Gustafson-Brown, S. E. Kohalmi, W. L. Crosby, and M. F. Yanofsky. 2001. *APETALA1* and *SEPALLATA3* interact to promote flower development. *Plant J.* **26**: 385–394.

Prasad, K., P. Sriram, C. S. Kumar, K. Kushalappa, and U. Vijayraghavan. 2001. Ectopic expression of rice *OsMADS1* reveals a role in specifying the lemma and palea, grass floral organs analogous to sepals. *Developm. Genes Evol.* **211**: 281–290.

Riechmann, J. L., B. A. Krizek, and E. M. Meyerowitz. 1996*a*. Dimerization specificity of *Arabidopsis* MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 4793–4798.

———, M. Wang, and E. M. Meyerowitz. 1996*b*. DNA-binding properties of *Arabidopsis* MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA and AGAMOUS. *Nucl. Acids Res.* **24**: 3134–3141.

Rudall, P., and R. Bateman. 2002. Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium and labellum of orchids and other lilioid monocots. *Biol. Rev. (Cambridge)* **77**: 403–441.

———, and ———. 2003. Evolutionary change in flowers and inflorescences: evidence from naturally occurring terata. *Trends Pl. Sci.* **8**: 7–82.

Skipper, M. 2002. Genes from the *APETALA3* and *PISTILLATA* lineages are expressed in developing vascular bundles of the tuberous rhizome, flowering stem and flower primordia of *Eranthis hyemalis. Ann. Bot. (Oxford)* **89**: 83–88.

Stellari, G. M., M. A. Jaramillo, and E. M. Kramer. 2004. Evolution of the *APETALA3* and *PISTILLATA* lineages of MADS-box containing genes in the basal angiosperms. *Molec. Biol. Evol.* **21**: 506–519.

Sundstrom, J., A. Carlsbecker, M. E. Svensson, M. Svenson, U. Johanson, G. Theissen, and P. Engstrom. 1999. MADS-box genes active in developing pollen cones of Norway spruce (*Picea abies*) are homologous to the B-class floral homeotic genes in angiosperms. *Developmental Genet.* **25**: 253–266.

Theissen, G., A. Becker, A. Di Rosa, A. Kanno, J. T. Kim, T. Munster, K. U. Winter, and H. Saedler. 2000. A short history of MADS-box genes in plants. *Pl. Molec. Biol.* **42**: 115–149.

———, and H. Saedler. 1999. The golden decade of molecular floral development (1990–1999): a cheerful obituary. *Developmental Genet.* **25**: 181–193.

———, T. Strater, A. Fischer, and H. Saedler. 1995. Structural characterization, chromosomal localization and phylogenetic evaluation of two pairs of *AGAMOUS*-like MADS-box genes from maize. *Gene* **156**: 155–166.

Tzeng, T. Y., and C. H. Yang. 2001. A MADS box gene from lily (*Lilium longiflorum*) is sufficient to generate dominant negative mutation by interacting with *PISTILLATA* (*PI*) in *Arabidopsis thaliana. Pl. Cell Physiol.* **42**: 1156–1168.

Yu, H., and C. J. Goh. 2000. Identification and characterization of three orchid MADS-box genes of the AP1/AGL9 subfamily during floral transition. *Pl. Physiol. (Lancaster)* **123**: 1325–1336.

———, S. H. Yang, and C. J. Goh. 2002. Spatial and temporal expression of the orchid floral homeotic gene *DOMADS1* is mediated by its upstream regulatory regions. *Pl. Molec. Biol.* **49**: 225–237.