Diversity and Evolution of CYCLOIDEA-Like TCP Genes in Relation to Flower Development in Papaveraceae

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Monosymmetry evolved several times independently during flower evolution. In snapdragon (Antirrhinum majus), a key gene for monosymmetry is CYCLOIDEA (CYC), which belongs to the class II TCP gene family encoding transcriptional activators. We address the questions of the evolutionary history of this gene family and of possible recruitment of genes homologous to CYC in floral development and symmetry in the Papaveraceae. Two to three members of the class II TCP family were found in each species analyzed, two of which were CYC-like genes, on the basis of the presence of both the TCP and R conserved domains. The duplication that gave rise to these two paralogous lineages (named PAPACYL1 and PAPACYL2) probably predates the divergence of the two main clades within the Papaveraceae. Phylogenetic relationships among angiosperm class II TCP genes indicated that (1) PAPACYL genes were closest to Arabidopsis (Arabidopsis thaliana) AtTCP18, and a duplication at the base of the core eudicot would have given rise to two supplementary CYC-like lineages; and (2) at least three class II TCP genes were present in the ancestor of monocots and eudicots. Semiquantitative reverse transcription-polymerase chain reaction and in situ hybridization approaches in three species with different floral symmetry indicated that both PAPACYL paralogs were expressed during floral development. A pattern common to all three species was observed at organ junctions in inflorescences and flowers. Expression in the outer petals was specifically observed in the two species with nonactinomorphic flowers. Hypotheses concerning the ancestral pattern of expression and function of CYC-like genes and their possible role in floral development of Papaveraceae species leading to bisymmetric buds are discussed.

Organismal evolution has been punctuated by key innovations that constitute major driving forces for species diversification and colonization of new environmental niches. A key innovation in angiosperms is the flower, which brings together sterile and fertile organs in a condensed structure. Considerable work has been devoted to the identification of determinants of the identity of floral organs, leading to the ABCDE model (Coen and Meyerowitz, 1991; Colombo et al., 1995; Pelaz et al., 2000). The molecular bases underlying the diversity of flower shape are far less understood (Weiss et al., 2005). Floral symmetry is presently the most investigated character (Cubas, 2004). Two main types of symmetry are described, actinomorphic or radial symmetry (or polysymmetry) and zygomorphic or bilateral symmetry (or monosymmetry). Zygomorphic flowers appear in late Cretaceous fossil records, which is relatively late comparatively to the accepted period for angiosperm origin (early Cretaceous [Endress, 1999]). Actinomorphic is considered as the ancestral state for angiosperms and zygomorphy has evolved several times independently, possibly in coevolution with specialized pollinators (Crepet, 1996).

Changes in floral morphology and associated pollinators can set up reproductive isolation that may contribute to speciation. Indeed, some of the most-species-rich taxa harbor zygomorphic flowers (Fabaceae, Orchidaceae, Asteraceae; Sargent, 2004).

Remarkable progress in understanding the mechanisms underlying floral symmetry has been achieved in the model species snapdragon (Antirrhinum majus). Snapdragon has pentameric flowers, with five petals fused in a tube ending in two lips, four fertile stamens, and a dorsal staminode. The petals are of three types, dorsal, lateral, and ventral, building a monosymmetric flower. Four genes have been characterized, whose interaction accounts for dorsoventral asymmetry...
(Corley et al., 2005). Mutation in the CYCOIDEA (CYC) gene has the strongest phenotypic effect, with a loss of a full dorsal identity and a ventralization of the lateral petals. Together with its paralog DICHTOMA (DICH), CYC is expressed in the dorsal domain of the early floral meristem, resulting in retarded growth of petals and stamen. At later stages, CYC expression persists throughout the dorsal domain, where it promotes petal lobe growth while it represses stamen development (Luo et al., 1995). DICH expression is restricted to the dorsal part of the dorsal petals, participating in their internal asymmetry (Luo et al., 1999). The dorsal effect of CYC and DICH appears largely mediated by another gene, RADIALIS (RAD). Indeed, RAD has been shown to be activated by CYC in the dorsal domain of floral meristems (Costa et al., 2005), where it acts antagonistically with DIVARICATA (DIV) that is expressed all over the meristem (Corley et al., 2005). DIV is responsible for the ventral identity of petals, which can be observed in actinomorphic flowers of the *cyc-dich* double mutant (Galego and Almeida, 2002; Almeida and Galego, 2005).

Both CYC and DICH belong to the TCP gene family that encodes transcriptional factors characterized by a conserved basic helix-loop-helix domain (TCP) unique to plants (Cubas et al., 1999a). Twenty-four TCP genes were found in the completely sequenced genomes of Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*; Riechmann et al., 2000; Cubas, 2002; Damerval and Manuel, 2003; Xiong et al., 2005). Based on the characteristics of the TCP domain, two classes, I and II, were defined (Kosugi and Ohashi, 2002). CYC and DICH belong to class II, together with the *TB1* gene that plays a role in branching and floral development in maize (*Zea mays*; Doebley et al., 1997). CYC, DICH, and TB1 share a conserved domain particularly rich in polar residues, the R domain, which is not encountered in all class II members (Cubas et al., 1999a).

That genes homologous to CYC may play a role in the evolution of floral symmetry in diverse taxa is an intriguing possibility that prompted the analysis of molecular evolution of class II TCP genes, at first in Asterids and more recently in other core eudicot taxa. In all, the evolution of the family has appeared quite complex, with taxon-specific duplications and gene losses (Citerne et al., 2000, 2003; Reeves and Olmstead, 2003; Howarth and Donoghue, 2005, 2006). Phylogenetic analyses do not support the presence of orthologs of CYC and DICH in non-Antirrhineae species (Damerval and Manuel, 2003; Gübitz et al., 2003). The duplication giving rise to these two paralogs would have occurred within the Veronicaceae before the divergence of the Antirrhineae (Gübitz et al., 2003) and the two paralogs would then have evolved through subfunctionalization (Hileman and Baum, 2003). Examples of involvement of CYC-like genes in floral symmetry are described among Antirrhineae species (Cubas et al., 1999b; Hileman et al., 2003).

The lack of a CYC ortholog in other taxa does not dismiss the hypothesis that similar genes may be repeatedly recruited for producing a similar trait. Actually, this was recently demonstrated in Papilionoideae, a Rosid taxon where zygomorphy is thought to have evolved independently from zygomorphy in the Asterids. In Fabaceae, the CYC closest lineages, LEGC1 and LEGC2, occurred from a duplication probably anterior to the evolution of the Papilionoideae, and the LEGC1 lineage underwent further duplication (LEGC1A and 1B; Citerne et al., 2003; Fukuda et al., 2003). It was recently demonstrated that a LEGC1B gene, *LjCYC2*, has dorsalizing activity during floral development of *Lotus japonicus*, similar to CYC (Feng et al., 2006). Similarly, molecular evolution results suggest that positive selection has been operating in LEGC1B lineage in relation to a shift in floral morphology among Lupinus species (Ree et al., 2004).

Outside the core eudicots, large clades with zygomorphic flowers exist among monocots (e.g. Orchidaceae). In contrast, zygomorphy is absent in basal angiosperms and quite rare in early diverging eudicots (Ranunculales, Proteales [Endress, 1999; Ronse De Craene et al., 2003]). Characterization of CYC-like genes in an early diverging eudicot taxon exhibiting diverse floral types would shed light on the evolutionary history of the TCP class II family gene and the recruitment of genes of this family in relation to floral symmetry in eudicots. Among the Ranunculales that are considered as the sister group to all other eudicots, the Papaveraceae sensu lato constitute a morphologically diverse monophyletic group (Hoot et al., 1999; Kim et al., 2004). Fumariaceae, including the basal genus Hypocoum, on the one hand, and Papaveraceae sensu stricto, on the other hand, are sister groups, with Pteridophyllum Sieb. and Zucc. being basal to both (Kadereit et al., 1995; Gleissberg and Kadereit, 1999; Fig. 1). The dimeric ground plan of the flower and especially the perianth with two sepals and two whorls of opposite decussate petals probably offers a favorable context for development of bisymmetric adult flowers through differentiation of the morphology of the two petal whorls. This is achieved in Fumariaceae, whereas Papaveraceae sensu stricto are characterized by actinomorphic flowers and the full enclosure of flower buds by the sepals (Kadereit et al., 1995). In addition, in Fumarioideae, zygomorphy occurs in monospur flowers.

In this article, we characterize class II TCP genes in the Papaveraceae sensu lato with a specific focus toward genes having both TCP and R domains, called CYC-like genes. Papaveraceae genes are then included in an extensive phylogeny of known class II TCP genes in an attempt to decipher the ancestral state of complexity of this gene family among angiosperms. Finally, using gene expression approaches in developing inflorescences and flowers, we address the question of ancestral expression and function for CYC-like genes.
and possible role in floral development and symmetry in the Papaveraceae sensu lato.

RESULTS

TCP Domain Characterization and Sequence Elongation in Papaveraceae Sensu Lato

To characterize class II TCP genes in the Papaveraceae, three combinations of degenerate primers arranged in nested PCRs were initially tested on Chelidonium majus and Lamprocapnos spectabilis, and about 20 clones were sequenced from each experiment (Fig. 2). It appeared that combination I was less powerful than the other two for retrieving different sequence types and thus only combinations II and III were used on all seven species for extensive clone sequencing (Table I). For every species, two to three sequence types could be defined, which differed by at least 19 among 85 nucleotides, except for CvA and CvC, which differed by 7 nucleotides. Some types were quite rare (e.g. type C in Hypecoum procumbens and Cysticapnos vesicarius) and would have been missed if the sequencing effort was lower. Each sequence type encoded a different amino acid sequence, which strongly suggested that they correspond to fragments of paralogs rather than alleles. Preliminary analysis of relationships of the 17 TCP types using the neighbor-joining (NJ) method indicated at least three well-supported groups. In particular, PrB and HpC appeared close to each other and very divergent from all other sequence types (Supplemental Fig. S1).

A seminested PCR strategy enabled recovery of longer genomic sequences for all 17 types, except CmA, HpB and HpC, and PrA and PrB (Table I). Most of the R domain was sequenced (36 of 54 nucleotide positions). An inverse PCR strategy was successful on other sequence types, except HpC. The R domain was found about 200 to 250 nucleotides downstream of the TCP domain for CmA, HpB, and PrA. In the case of PrB, no R domain was found up to 850 nucleotides downstream of the TCP domain. A BLAST search showed similarity between this Pr3 elongated sequence and the CIN gene of snapdragon, a class II TCP gene devoid of an R domain (Nath et al., 2003). Because we were mostly interested in CYC-like genes (TCP genes with an R domain), we did not investigate further the HpC type that appeared very close to PrB from the preliminary phylogenetic analysis (Supplemental Fig. S1).

CYC-Like Sequences in Papaveraceae Sensu Lato

A first objective was to assess whether the 15 characterized CYC-like Papaveraceae genes could be
orthologs of other known core eudicot genes. Evolutionary relationships were analyzed with the five Arabidopsis class II TCP genes with an R domain (Fig. 3A). Because it was not possible to confidently assess primary homology in the intermediary domain comprised between the TCP and R domains, analysis was conducted on the two most conserved domains only (174-nucleotide alignment matrix, comprising 78% and 67% of the complete TCP and R domains, respectively). NJ, maximum likelihood (ML), and Bayesian reconstruction methods were congruent, supporting a monophyletic group for all Papaveraceae sequences, split in two groups, each of which included one (two for C. vesicarius) sequence of each species. These two groups, however, were not highly supported in all three analyses (Fig. 3A).

To investigate in more detail the relationships among Papaveraceae CYC-like sequences, an alignment spanning the TCP, R, and intermediary domains was considered. Within the latter domain, a region where primary homology was difficult to assess was excluded, which resulted in a 381-position matrix, including gaps inserted to optimize alignment. Both NJ and Bayesian methods revealed two well-supported clades, including one (two in C. vesicarius) sequence of each species. These two groups, however, were not highly supported in all three analyses (Fig. 3A).

In both PAPACYL1 and PAPACYL2, the TCP and R domains were highly conserved. At the amino acid level, the percentage of identity among pairs of sequences for the TCP domain ranged from 72% to 98% in PAPACYL1 and 63% to 100% in PAPACYL2. The R domain, values lay between 66 and 100% in PAPACYL1 and 58% to 92% in PAPACYL2. PAPACYL2 sequences were generally longer than PAPACYL1 (435 ± 33 nucleotides versus 388 ± 22 nucleotides), due to variable length of the intermediary domain. In Papaveraceae

### Table 1. Number of clones sequenced over all PCR primer combinations, number and relative frequencies of validated sequence types for the TCP domain in each species, and name of genes obtained following sequence elongation

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Clones Sequenced (No. of Sequence Type)</th>
<th>Type Code</th>
<th>Relative Frequency of Types</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. majus</td>
<td>133 (2)</td>
<td>CmA²,³</td>
<td>0.77</td>
<td>CmCYL1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CmB²,³</td>
<td>0.23</td>
<td>CmCYL2</td>
</tr>
<tr>
<td>P. rhoeas</td>
<td>59 (3)</td>
<td>Pr²</td>
<td>0.39</td>
<td>PrCYL2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pr³</td>
<td>0.12</td>
<td>Pr³</td>
</tr>
<tr>
<td>H. procumbens</td>
<td>78 (3)</td>
<td>PC²,³</td>
<td>0.49</td>
<td>PrCYL1</td>
</tr>
<tr>
<td>L. spectabilis</td>
<td>153 (2)</td>
<td>Ls²,³</td>
<td>0.66</td>
<td>LsCYL1</td>
</tr>
<tr>
<td>C. sempervirens</td>
<td>130 (2)</td>
<td>Ca²,³</td>
<td>0.88</td>
<td>CaCYL2</td>
</tr>
<tr>
<td>C. vesicarius</td>
<td>90 (3)</td>
<td>Cv²,³</td>
<td>0.54</td>
<td>CvCYL2A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cv²</td>
<td>0.41</td>
<td>CvCYL1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cb²,³</td>
<td>0.12</td>
<td>CvCYL2B</td>
</tr>
<tr>
<td>D. torulosa</td>
<td>88 (2)</td>
<td>Da²,³</td>
<td>0.5</td>
<td>DcCYL2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Db²,³</td>
<td>0.5</td>
<td>DcCYL1</td>
</tr>
</tbody>
</table>

*Superscripts 1, 2, and 3 refer to the PCR primer combinations (respectively, I, II, and III; see Fig. 2), allowing retrieval of the TCP sequence types.
sensu stricto, this difference was quite limited, ranging from 3 to 27 nucleotides in *Papaver rhoeas* and *C. majus*, respectively. In Fumarioideae, the difference was much higher, ranging from 66 to 93 nucleotides in *Capnoides sempervirens* and *C. vesicarius*, respectively. Conversely, in *H. procumbens*, *PAPACYL1* length exceeded *PAPACYL2* by 24 nucleotides. This was congruent with the large divergence of *H. procumbens* sequences from their Fumarioideae orthologs in the phylogenetic analysis.

**Relationships of Papaveraceae Sequences with Other Class II TCP Genes**

We investigated relationships of Papaveraceae sensu lato genes, as represented by sequences of *P. rhoeas*, *H. procumbens*, and *L. spectabilis*, with 34 other known class II TCP genes, including the full repertoires of Arabidopsis and rice (see “Materials and Methods”). Phylogenetic analyses were performed on an alignment including the 3’ 138 nucleotides of the TCP domain and on the conceptual translation of this domain. Parsimony analysis of the 104 informative nucleotide characters produced 55 equally parsimonious trees of 902 steps with a consistency index of 0.273 and a retention index of 0.554, indicating fairly high homoplasy. The 50% majority rule consensus tree had poor resolution (data not shown). ML and Bayesian analyses resulted in very similar topologies. Both analyses set apart two main groups (posterior probability $P = 1.0$ in the Bayesian analysis; Fig. 4A). The first one (called the ECE clade, following Howarth and Donoghue [2006]) included 23 sequences, among which are three Arabidopsis and three rice genes. Within this group, resolution was low, except for a *TB1*-like clade comprising the two Poaceae *TB1* genes and two related rice sequences ($P = 1.0$). The closest nonmonocot sequences to this subgroup appeared to be an Asterid sequence, *LoTCP2*, and *AtTCP12*. The Papaveraceae sequences were grouped with the Aquilegia *CYC*-like gene, *Populus balsamifera* *TB1*-like, *AtTCP18*, and the Lotus sequence *LjCYC5*, in a weakly supported cluster ($P = 0.57$). The second main group included most rice and Arabidopsis sequences (eight of 11 for both). A subclade included a *CIN* and *P. rhoeas* *CIN*-like sequence (*Pr3*) together with one rice and three Arabidopsis genes ($P = 0.89$). Well-supported subgroups included one rice and three Arabidopsis genes (*AtTCP13*-like; $P = 1.0$), and a set of six rice genes ($P = 1.0$). Most of these groups were also observed in the phylogenetic reconstruction by ML on amino acid-translated sequences (Fig. 4B).

**Expression of PAPACYL1 and PAPACYL2 in Vegetative and Floral Organs**

Expression analysis was undertaken in three species representative of each of the three types of floral symmetry encountered in the Papaveraceae sensu lato family: *C. majus* for actinomorphic, *L. spectabilis* for bisymmetric, and *C. sempervirens* for zygomorphic flowers (Fig. 1). Because zygomorphy was reported to begin to settle at the time of ovule development in Fumarioideae (Ronse De Craene and Smets, 1992), relatively late-stage floral buds (with well-differentiated stamens) were analyzed.

First, semiquantitative reverse transcription (RT)-PCR was used to compare *PAPACYL* expression in leaves, early inflorescences, and flower buds of increasing size, with *actin* as a standard (Fig. 5). For a given species, both paralogs were generally shown to have low expression, with similar patterns. In *C. majus*, no expression was detected in leaves, whereas expression in the inflorescence decreased as development proceeded (Fig. 5A). In *L. spectabilis* and *C. sempervirens*, both paralogs were preferentially expressed in young leaves and in the first stage of developing inflorescence, where *PAPACYL1* paralogs appeared...
more expressed than those of PAPACYL2 (Fig. 5, B and C).

Tissue-specific expression was further investigated by in situ hybridization on inflorescences and buds displaying PAPACYL expression in RT-PCR experiments (2-mm buds). For each studied species, expression of the two PAPACYL paralogs was compared to actin expression. As expected, actin was found ubiquitously expressed. In contrast, PAPACYL1 and PAPACYL2 paralogs displayed restricted, tissue-specific expression (Fig. 6; Supplemental Fig. S2). In all the species, expression of the two PAPACYL paralogs appeared similar, even though in L. spectabilis mRNA accumulation seemed stronger for PAPACYL1 than PAPACYL2 (data not shown). Interestingly, an asymmetric repartition of PAPACYL transcripts in the main part of the outer petals was often observed in the C. sempervirens monosymmetric species (Fig. 6I). Supplementary patterns were specific to L. spectabilis. LsCYL1 expression was observed at the basis of the dorsal ridge on the inner petals (Fig. 6C; Supplemental Fig. S2). Moreover, very clear expression of LsCYL1 was apparent in the developing anther connective, sometimes surrounding the pollen sacs, whereas LsCYL2 expression was much fainter in the connective, but occurred in the first layer of the tapetum (Fig. 6, E–G). These results

Figure 4. Reconstruction of evolutionary history of class II TCP genes. The Institute for Genomic Research (TIGR) locus names for rice genes are OsTCP1, Os01g11550; OsTCP2, Os03g57190; OsTCP3, Os07g05720; OsTCP4, Os01g55750; OsTCP6, Os07g04510; OsTCP7, Os12g02090; OsTCP8, Os02g51310; OsTCP9, Os12g4190; OsTCP10, Os09g24480; and OsTCP11, Os08g33530. The Arabidopsis Information Resource (TAIR) locus names for Arabidopsis genes are AtTCP1, At1g67260; AtTCP2, At4g18390; AtTCP3, At1g51230; AtTCP4, At3g15030; AtTCP5, At5g60970; AtTCP10, At2g1070; AtTCP12, At1g68800; AtTCP13, At3g02150; AtTCP17, At5g80870; AtTCP18, At3g18550; and AtTCP24, At1g30210. References of other CYC-like genes are in the text. Gray boxes are for genes displaying an R domain. A, Majority rule consensus tree obtained by Bayesian inference analysis of the 138-nucleotide alignment matrix including 41 sequences (posterior probabilities of nodes above 0.6 x 100 indicated). B, Phylogenetic reconstruction based on ML analysis of the 46-amino acid alignment matrix (bootstrap values above 60% are indicated; 500 bootstraps were performed).
DISCUSSION

The Papaveraceae sensu lato constitutes an early diverging eudicot taxon encompassing about 45 genera. Large morphological diversity exists between species, most specifically concerning leaf dissection patterns (Gleissberg, 2004) and floral features (Kadereit et al., 1995). Species of this family recently emerged as a possible early diverging eudicot model for evolutionary developmental genetics (Floral Genome Project; http://fgp.bio.psu.edu/fgp; Becker et al., 2005).

In this study, TCP transcription factor genes were characterized in seven Papaveraceae sensu lato species belonging to Fumariaceae, including Hypecoum, and Papaveraceae sensu stricto. We focused our attention on class II TCP genes, whose founding members CYC and TB1 were shown to play a role in growth processes underlying aerial architectural traits (Hubbard et al., 2002; Costa et al., 2005). The chosen strategy relies on the initial extensive characterization of TCP domains closely related to CYC, AtTCP1, and TB1, then sequence elongation. Two to three TCP sequences were recovered in each of the seven study species, which represent a minimal estimate of the number of class II TCP genes in the concerned species. One sequence in P. rhoeas appeared devoid of the R domain (CIN-like sequence), which is most probably the case for the homologous H. procumbens sequence. Fifteen other sequences exhibited an R domain downstream of the TCP domain (CYC-like sequences). Phylogenetic analyses based on the TCP and R domains using the Arabidopsis genes as outgroup supported the hypothesis that these genes formed two paralogous lineages that we called PAPACYL1 and PAPACYL2. Using a different gene characterization strategy, Kölsch and Gleissberg (2006) recently obtained two Papaveraceae sequences closely related to the Arabidopsis genes AtTCP2 and AtTCP24. The specific structure of the TCP domain 3’ end in these genes may explain that we did not recover such paralogus. Thus, in all, it appears that at least four class II TCP genes are present in the Papaveraceae sensu lato, three of which have both a TCP and an R domain. Among the latter, only the PAPACYL lineages belong to the ECE clade.

Duplication giving rise to PAPACYL1 and PAPACYL2 most probably predates the divergence between Papaveraceae sensu stricto and Fumariaceae. Genome duplication has recently been detected in Eschscholzia californica, independent from other duplications in monocot and core eudicot lineages (Cui et al., 2006). Consistently, two CYC-like genes were recently found in E. californica (Kölsch and Gleissberg, 2006); indeed, we found that these two paralogs belong to the PAPACYL1 and PAPACYL2 lineages. This duplication may concern the common ancestor of all the Papaveraceae and account for the two PAPACYL lineages. Indeed, each of our study species displayed only one copy of each paralog, except C. vesicarius, which exhibited specific duplication of PAPACYL2. This is probably due to the tetraploid structure of the latter species (C. Damerval and S. Siljac-Yakovlev, unpublished data). Within both paralogous lineages, the topology reflected the species phylogeny (Fig. 1). C. majus and P. rhoeas orthologs were closer to each other than to any others, consistent with their placement in two subfamilies of Papaveraceae sensu stricto (Kadereit et al., 1995). A large divergence of H. procumbens sequences from their Fumarioideae orthologs was observed in the phylogenetic reconstruction, consistent with characteristics of their intermediary domain. Within Fumarioideae, the L. spectabilis orthologs appeared the most divergent from the group including C. sempervirens, Dactyliscapnos torulosa, and C. vesicarius sequences.

PAPACYL2 sequences were generally longer than PAPACYL1 due to the variable length of the intermediary

Figure 5. Semiquantitative RT-PCR profiles obtained for the two PAPACYL paralogs in young and mature leaf and in inflorescences and floral buds of increasing size in C. majus (A), L. spectabilis (B), and C. sempervirens (C). The actin gene was used in the three species as a control of equivalent cDNA quantity in the reaction mix. 0, inflorescences with buds ≤2 mm; 1, 3- to 5-mm buds; 2, 7- to 10-mm buds; 3 (for L. spectabilis and C. sempervirens only), 11- to 20-mm buds.

show that the two PAPACYL paralogs are expressed during inflorescence and flower development of the three Papaveraceae sensu lato studied species. Furthermore, they suggest a correlation between PAPACYL-specific patterns and flower symmetry.


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The intermediary domain appeared quite long (217 nucleotides for \textit{PAPACYL1} and 264 nucleotides for \textit{PAPACYL2}) compared to other characterized sequences, for example, in Arabidopsis (110–250 nucleotides) or snapdragon \textit{CYC} and \textit{DICH} (about 170 nucleotides). Differences in length were also observed among the three paralogous lineages found in Dipsacales (Howarth and Donoghue, 2005). Such variation may have some functional consequences through the positioning of the R domain relative to the TCP domain that is involved in DNA binding and dimerization. As observed in other botanical families, the intermediary domain was also highly variable in amino acid sequence, suggesting relaxed selective constraints (Hileman and Baum, 2003; Ree et al., 2004).

**Figure 6.** In situ hybridization analysis of \textit{PAPACYL} gene expression in inflorescences and ≈2-mm flower buds of \textit{C. majus} (actinomorphic flower), \textit{L. spectabilis} (bisymmetric flower), and \textit{C. sempervirens} (monosymmetric flower). A, Longitudinal section of a \textit{C. majus} flower receptacle hybridized with a \textit{CmCYL2} antisense probe; arrows point out \textit{CmCYL2} expression in the receptacle at the base of floral organs. B, Longitudinal section of a \textit{C. majus} flower hybridized with a \textit{CmCYL1} antisense probe; note \textit{CmCYL1} expression at the junction between pedicels and bracts. C, Longitudinal-oblique section of a \textit{L. spectabilis} flower bud hybridized with an \textit{LsCYL1} antisense probe; arrows point out expression of \textit{LsCYL1} in the receptacle at the base of floral organs, in sepalis, in the outer petals, and at the base of the ridge developing on the inner petals. D, Schematic drawing of a \textit{L. spectabilis} flower bud showing the approximate level of the section displayed in C. E to G, Transversal sections of a \textit{L. spectabilis} flower bud hybridized with an \textit{LsCYL1} (E), \textit{LsCYL2} (F), or \textit{actin} (G) antisense probe; the arrows point out specific expression of \textit{LsCYL1} in the anther connective (E) and of \textit{LsCYL2} in the tapetum (F). H, Transversal oblique section of a \textit{C. sempervirens} flower bud hybridized with a \textit{CsCYL2} antisense probe; arrows point out expression of \textit{CsCYL2} in the sepals and in the outer petal. I, Transversal section of a \textit{C. sempervirens} flower bud hybridized with a \textit{CsCYL1} antisense probe; arrows point out asymmetric expression of \textit{CsCYL1} in the two outer petals. J, Schematic drawing of a 2-mm \textit{C. sempervirens} flower bud indicating the approximate levels of the transverse sections shown in H and I. br, Bract; dst, dithecal stamen; ip, inner petal; mst, monotheical stamen; op, outer petal; pe, pedicel; pi, pistil; pib, pistil basis, being embedded in receptacle; re, receptacle; rip, ridge of inner petal; s, sepal; st, stamen. Scale bars = 200 μm.

**Taxon-Specific Duplication Has Played a Major Role in TCP Class II Gene Evolution**

The full repertoire of TCP transcription factor genes has been obtained in the completely sequenced genomes of Arabidopsis and rice. Both species have a similar number of members (Riechmann et al., 2000; Cubas, 2002; Damerval and Manuel, 2003; Xiong et al., 2005), approximately equally distributed among the two classes that were defined from specific structural features of the TCP domain (Cubas et al., 1999a).
Phylogenetic analyses showed that the two classes constitute two distinct clades (Cubas et al., 1999a; Cubas, 2002; Citerne et al., 2003; Damerval and Manuel, 2003). Accurate reconstruction of the evolutionary history of class II genes is complicated by the high rate of substitution and insertion/deletion outside the TCP domain, which makes primary homology assessment all the more difficult when a large angiosperm taxon sampling is intended. Therefore, we considered only the TCP domain for phylogenetic reconstruction. Genes available from representatives of major angiosperm clades (Rosids: *P. balsamifera* TB1-like and *L. japonicus* CYC-like sequences; Asterids: snapdragon *CIN*, *CYC*, and *DICH*, *Scrophularia alpina* TCP2, and *Ligustrum* ovalifolium TCP2; early diverging eudicots: *Papaveraceae* paralogs and the Ranunculaceae paralogs) were included in the analysis, together with the full repertoire of rice (monocot) and Arabidopsis (Rosid). Even though the resolution was quite low, several trends appeared consistent in both nucleotide- and amino acid-based analyses (Fig. 4). First, homoplasy of the TCP domain for phylogenetic reconstruction. Genes were used in semiquantitative RT-PCRs. 

<table>
<thead>
<tr>
<th>Species</th>
<th>Floral Symmetry</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. majus</em></td>
<td>Actinomorphy</td>
<td>Ballainvilliers, France</td>
</tr>
<tr>
<td><em>P. rheas</em></td>
<td>Actinomorphy</td>
<td>Ballainvilliers, France</td>
</tr>
<tr>
<td><em>H. procumbens</em></td>
<td>Bisymmetry (no nectar spur)</td>
<td>Botanical Garden, Copenhagen (accession no. 1409)</td>
</tr>
<tr>
<td><em>L. spectabilis</em> (L.) Fukuhara</td>
<td>Bisymmetry (two nectar spur)</td>
<td>Horticultural</td>
</tr>
<tr>
<td><em>C. sempervirens</em> (L.) Borck</td>
<td>Monosymmetry (one nectar spur)</td>
<td>Botanical Garden, Copenhagen (accession no. 1395)</td>
</tr>
<tr>
<td><em>C. vesicarius</em> (L.) Fedde</td>
<td>Monosymmetry (one nectar spur)</td>
<td>Botanical Garden, Copenhagen (accession no. 51965)</td>
</tr>
<tr>
<td><em>D. torulosa</em> Hutchinson</td>
<td>Bisymmetry (two nectar spur)</td>
<td>Johannes Gutenberg-Mainz University</td>
</tr>
</tbody>
</table>

Table II. *Species of Papaveraceae sensu lato, floral symmetry, and origin of accessions used for molecular analyses*

(Xiong et al., 2005). This could indicate that TB1-like genes have been recruited for a function specific to the Poaceae lineage. If this is true, we might expect that TB1 orthologs would sustain similar function. Actually, the *OsTB1* gene was shown to regulate rice branching (Takeda et al., 2003), as was observed in maize (Doebely et al., 1997) and also in sorghum (Sorghum bicolor; Kebrom et al., 2006) and in pearl millet (*Pennisetum glaucum*; M.-S. Remigereau, personal communication). The closest eudicot gene to the TB1-like clade appeared to be an Asterid gene (LotTCP2); relationships with either AtTCP12 or AtTCP18 were not evenly supported in the nucleotide- and amino acid-based analyses. AtTCP1 has been reported as the CYC closest Arabidopsis paralog (Cubas et al., 2001). Our results were not inconsistent with this assumption, even though no strong relationship occurred between CYC, DICH, and AtTCP1. Additionally, they suggested homology between AtTCP1 and representatives of the Fabaceae LEGYC CYC genes (*LjCYC1–3*), and between the snapdragon sequences and a *S. californica* sequence, in agreement with the phylogeny of the concerned species.

As far as eudicot genes are concerned, the topology of the ECE clade is consistent with the phylogeny

Table III. *Primer sequences used to amplify PAPACYL sequences*

For *C. majus*, *L. spectabilis*, and *C. sempervirens*, the same primers were used in semiquantitative RT-PCRs. 

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CmCYL1-F</td>
<td>GAATAAAGACTTGTTCTTCGACAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>TGTTGTAGGGTTTGATTCTTCAAGG</td>
</tr>
<tr>
<td>CmCYL1-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>LsCYL1-F-R</td>
<td>CCAAGAGAATGAAGATGAGAT</td>
</tr>
<tr>
<td>-R</td>
<td>CTGTTGCTTCTTCGCCCTTC</td>
</tr>
<tr>
<td>LsCYL1-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>CsCYL1-F-R</td>
<td>CCAAGAGAATGAAGATGAGAT</td>
</tr>
<tr>
<td>-R</td>
<td>CTGTTGCTTCTTCGCCCTTC</td>
</tr>
<tr>
<td>CsCYL2-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
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<td>-R</td>
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<td>PrCYL1-F-R</td>
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<tr>
<td>-R</td>
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</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>Pr3-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>HpcCYL1-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
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<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
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<tr>
<td>HpcCYL2-F-R</td>
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</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>CVCYL1-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>CVCYL2A-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>CVCYL2B-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>DCYCL1-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
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</tr>
<tr>
<td>DCYCL2-F-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
<tr>
<td>-R</td>
<td>CGAGAGATCGGAAAGATGAGAC</td>
</tr>
</tbody>
</table>
recently obtained by Howarth and Donoghue (2006), focusing mainly on core eudicot CYC-like sequences. Three subclades were identified, each one including an Arabidopsis gene, AtTCP18 (CYC1 lineage), AtTCP1 (CYC2 lineage), and AtTCP12 (CYC3 lineage). CYC1 appeared as the basal-most clade and the sister group to both CYC2 and CYC3. In our analysis, CYC1, as represented by AtTCP18 and LjCYC5, was embedded in a loosely supported clade, including early diverging eudicot sequences (both PAPACYL1 and PAPACYL2 lineages and A. alpina CYC-like gene), supporting the hypothesis that CYC1 genes are the closest to the ancestor of the eudicot CYC-like genes. It has been hypothesized that successive duplications gave rise to CYC1 and the ancestor of CYC2 and CYC3, then to CYC2 and CYC3 (Howarth and Donoghue, 2006). Indeed, the CYC2 and CYC3 lineages appeared specific to core eudicot taxa. MADS-box gene lineages controlling the identity of floral organs have been demonstrated to undergo duplication events within the early diverging eudicots (Kramer and Hall, 2005). Large-scale studies of paralogous genes suggest genome-wide duplication at the base of the core eudicots (Cui et al., 2006) that might be responsible for the emergence of these duplicated lineages, and particularly CYC2 and CYC3, opening the way for novel functional recruitment. Whereas increasing expression data seem to indicate that the CYC2 genes in core eudicot taxa are expressed in the dorsal domain of the flowers, scarce expression data are still available for CYC3 genes (Feng et al., 2006; Citerne et al., 2006; Howarth and Donoghue, 2006), including AtTCP12. Anyway, the topology of the ECE clade suggests one ancestral gene common to monocots and eudicots, with a complex history of duplication in the various taxa.

In contrast, the number of putative ancestral genes accounting for the topology of the second main clade is not readily established. A CIN-like group, including genes without an R domain, namely, AmCIN, P. rheas CIN-like sequence, and one rice and three Arabidopsis genes, was supported by both amino acid- and nucleotide-based analyses, even though not very strongly. CIN has been shown to play a role in leaf curvature and epidermal cell differentiation and growth in petal lobe in snapdragon (Nath et al., 2003; Crawford et al., 2004). A possible orthologous relationship of a P. rheas sequence with CIN raises the interesting possibility that it plays a similar role in poppy leaf and petal development. Only one well-supported group (AtTCP13-like) included one rice gene and three Arabidopsis genes, suggesting a common ancestor. Thus, at least three TCP class II genes would have been present in the common ancestor of monocots and eudicots.

Ancestral Expression of CYC-Like Genes

Both class I and class II TCP proteins are involved in growth processes. Class I proteins studied to date appear as positive regulators (Kosugi and Ohashi, 1997; Tremousaygue et al., 2003; Li et al., 2005), whereas the function of class II genes appeared to vary according to the trait concerned. Indeed, CYC and DICH were shown to repress growth of dorsal organs at an early stage of floral development in snapdragon (Luo et al., 1999), whereas CYC promotes growth of dorsal petals at late developmental stages (Luo et al., 1995). In a similar way, overexpression of CYC in transgenic Arabidopsis plants results in contrasted effects in leaf and petals: whereas leaf growth is reduced, petals are enlarged as a consequence of cell enlargement (Costa et al., 2005). In maize, expression of TB1 was observed in inflorescences and axillary meristems, the latter being associated with reduced branching (Hubbard et al., 2002). In potato (Solanum tuberosum), the expression of a class II gene appeared correlated with the inactive state of lateral and apical meristems (Fairev-Rampant et al., 2004). CIN genes in snapdragon play a role in cell growth arrest during leaf development (Nath et al., 2003), but also promote growth of petal lobes (Crawford et al., 2004). These contrasted effects on growth patterns might take place through differential action of the transcription factor (e.g. activation of some target genes, such as the RAD gene, and repression of other target genes) or through a single molecular function, the different developmental consequences being in this case dependent on the function of different downstream target genes. Phylogenetic analysis of class II genes and gene products suggested primary structure divergence possibly associated with functional divergence. Analysis of this phylogenetic pattern may help to shed light on the ancestral function of class II genes and their taxon-specific functional recruitment.

Gene expression analysis in Papaveraceae species may help to get insight into this question. In C. majus and C. sempervirens, similar qualitative expression of both PAPACYL paralogs was observed in all tissues. Except in the anther tapetum, this was also the case in L. spectabilis, even though PAPACYL1 appeared highly expressed compared to PAPACYL2. Overlapping expression in spite of sequence divergence may suggest a conserved ancestral function and/or a cooperative action of the two gene products. Indeed, in rice, homo- and heterodimerization have been described, preferentially between members of the same class of TCP genes (Kosugi and Ohashi, 2002). Moreover, a pattern of expression common to both paralogs was observed in all three species, at organ junctions, between bracts and pedicels, or peduncles and pedicels, and on the receptacle, at the base of floral organs. It is worth noting that expression of TB1 was observed at the base of the pedicellate spikelet in the female inflorescence of teasl (Hubbard et al., 2002) and at the junction between sheath and lamina in rice (Takeda et al., 2003). In a similar way in L. japonicus, LjCYC5, a CYC-like gene close to AtTCP18, was reported to be expressed at the base of the secondary inflorescence (Feng et al., 2006). Moreover, the earliest expression of LjCYC2, a LEGCYC1 gene closely related
to AtTCP1, was observed at the boundary between the primary and secondary inflorescence meristems (Feng et al., 2006). We propose that this pattern of expression at organ boundaries could be associated with an ancestral function of CYC-like genes, possibly corresponding to repression of cell growth and division that would contribute to differentiated growth of lateral organs and/or relative positioning of organs.

**PAPACYL Expression in Relation to Floral Development**

In Papaveraceae sensu stricto, flowers are actinomorphic, with the two petals in the two whorls equally developed. In Fumariaceae, typical bisymmetry arises through the development of nectar spurs by outer petals (genus Dicentra sensu lato). Zygomorphy occurs in monospur flowers (e.g. Capnoides, Corydalis, and tribe Fumariaceae) and is initially transverse. A rotation of the pedicel brings the spurred petal upward in adult flowers. Development of the unique spur begins at the time of ovule development from a bisymmetric floral bud (Ronse De Craene and Smets, 1992). The phylogeny of Fumariaceae based on molecular and morphological data supports the hypothesis of bisymmetry being the ancestral state (Liden et al., 1997).

Three species representative of the main floral symmetries (actinomorphic, bisymmetry, and zygomorphy) were studied for PAPACYL gene expression in developing floral buds. PAPACYL mRNAs were detected specifically in the developing anther (connective and tapetum) of L. spectabilis. CYC and TB1 gene expression have been reported in stamens, associated with organ abortion or reduction (Luo et al., 1999; Hubbard et al., 2002). Expression of two other class II TCP genes, AtTCP2 and AtTCP3, was also reported in the stamens and developing anthers in Arabidopsis (Cubas et al., 1999). As in L. spectabilis, developmental consequences remain unknown, but are most probably not associated with reduced growth or abortion. As regards to perianth organs, PAPACYL was more or less expressed in sepalas of all three analyzed species. In contrast, expression in petals, preferentially in the ridge region of the outer ones, was observed in the two Fumarioideae species only, namely, the two non-actinomorphic species. It may be anticipated that PAPACYL expression in sepalas and petals has developmental consequences distinct from expression at organ boundaries. The specific expression in outer petals compared to inner ones might contribute to build early bisymmetric buds, a synapomorphy of the two Fumarioideae species. Moreover, the differential expression observed between the two outer petals in *C. sempervirens* is reminiscent of the zygomorphy of the adult flower. Further studies will be necessary to examine the generality of the expression patterns observed in this analysis and the possible role of PAPACYL genes in the evolution of floral development in Papaveraceae.

**MATERIALS AND METHODS**

**Study Species and Material Collection**

Seven species of Papaveraceae sensu lato were investigated (Fig. 1). They were either from natural populations, horticultural, or botanical garden sources (Table II). In the first case, plants were collected in the wild (Ballainvilliers, France); in the second and third cases, plants were grown in a greenhouse or outdoors (Gif-sur-Yvette, France). Organs were harvested and either processed immediately for genomic DNA extraction (mature leaves), frozen in liquid nitrogen for RNA extraction, or fixed in formaldehyde-acetic acid-ethanol fixative for in situ hybridization.

**Genomic DNA Extraction and TCP Domain Characterization**

Genomic DNA was extracted from leaves ground to powder in liquid nitrogen, according to Doyle and Doyle (1987). Degenerate primers were designed in the TCP domain, based on alignment of CYC-like genes from databases. These primers were used in three combinations to perform nested PCRs, as described in Figure 2. According to species and 2C DNA amount (C. Damerval, unpublished data), 2 to 20 ng of genomic DNA were used in the first PCR; dilution of this first PCR (1/50–1/1,000 according to combination and species) was used as a matrix for the nested PCRs. PCRs were performed in 10-µl mix containing EurobioTaq buffer 1×, 2 or 3 mM MgCl₂ (for first or nested PCR, respectively), 200 µM dNTP, 1 µM each degenerate primer, 0.5 units EurobioTaq. Conditions consisted of 95°C for 5 min (initial denaturation), followed by 30 cycles of denaturation 95°C for 30 s, annealing 45°C or 48°C (for first or nested PCR, respectively, in combination I), 43°C or 49°C (for first or nested PCR, respectively in combination II), 42°C or 49°C (for first or nested PCR, respectively, in combination III) for 30 s, extension at 72°C for 1 min, followed by a final extension step at 72°C for 10 min. Bands of the expected size (110 to 130 bp according to the combination) were excised from agarose gel and purified using the QIAquick gel extraction kit (Qiagen). The purified products were used as a matrix for PCR with primers defined in the known TCP sequence.

**Sequence Elongation**

Two different strategies were used from genomic DNA. First, a seminested strategy, with the forward primer used in combinations II and III and a reverse degenerate primer designed in the R domain in the first PCR, and a specific forward primer and the same reverse primer in the nested PCR (Fig. 2, combination IV). The specific primers were designed on the basis of homology between previously recovered TCP sequence types. PCR conditions were the same as above, except for annealing temperature (47°C, 50°C, 52°C, or 55°C according to the specific primer used) and extension duration (2 min). Fragments from 200 to 600 bp were purified and either directly sequenced or cloned and sequenced as above. Second, inverse PCR was performed in *Chelidonium majus*, *Papaver rhoeas*, and *Hypecoum procumbens* for genes not obtained using the first strategy. Genomic DNA (500 ng) was digested by *Rsal* or *EcoRV* (without a cutting site within the known TCP sequence) in a final volume of 200 µl. Digestion was purified using the QIAquick PCR purification kit, then digested DNA was ligated using T4 DNA ligase (3 units, final volume 400 µl). Ligations were purified as above and the purified product used as a matrix for PCR with primers defined in the known TCP sequence. Here, again, nested PCRs were performed to increase specificity. Fragments obtained were either directly sequenced or cloned before sequencing, as above. Sequences obtained by both strategies were redone on new genomic DNA extracts using specific primers (Table III; sequences deposited in GenBank, accession nos. DQ659308–23).

**Expression Analysis**

Flower development was appraised through bud sizes. Adult flowers vary in size, from about 15 mm in *Capnoides sempervirens* to 20 mm in length in...
Actin PCR primers were: forward, 5'-CTCGTCAATAATAGATTAGGAGA-3'; and reverse, 5'-CCCTCAATCTCCAGACTGTA-3'. GeneAmplimer pAW109 PCR primers were: forward, 5'-AATCGGGTATATGATGAGAA-3'; and reverse, 5'-CCCTCAATCTCCAGACTGTA-3'. GeneAmplimer pAW109 PCR primers were: forward, 5'-CATTGACAAATTTGCTGCTCTGAC-3'; and reverse, 5'-TGGACCAACGACACATTCTTTGAC-3'. Primers allowing specific amplification of the two CYC-like paralogs in C. majus, L. spectabilis, and C. sempervirens are given in Table III. These primers were tested for specificity by sequencing PCR amplification products obtained from genomic DNA.

DNA Methods

Maximum parsimony (MP), ML, and NJ analyses were carried out using PAUP 4.0b7 (Swofford, 2003). For MP analysis, a heuristic search with 100 random addition replicates and tree bisection reconnection branch-swapping was performed with the multrees option selected. MODELEST Test Version 3.6 (Posada and Crandall, 1998) was used to estimate the best evolutionary model parameters according to Akaikes information criterion. ML analysis was then carried out with a heuristic search and 50 random additional replicates, tree bisection reconnection branch-swapping algorithm, and multrees option selected. The NJ reconstruction method was carried out on Kimura 2-parameter distances, proportion of invariable sites, and parameter for site heterogeneity obtained from MODELEST following Akaikes criterion. One thousand bootstrap replicates were done to calculate branch support value. Bayesian phylogenetic analyses were carried out using MrBayes Version 3.1.1 (Huelsenbeck and Ronquist, 2001), using a general time-reversible model with a proportion of invariable sites and a γ-distribution for site-specific rates partitioned by codons. For most analyses, four chains (three heated with temperature 0.2) were run twice for 2,000,000 generations, with a burn-in of 500,000 samples. For phylogenetic reconstruction within class II TCP genes, three chains were run twice with 5,000,000 generations and a burn-in of 12,500. In both cases, convergence was followed with potential scale reduction factor and average SD of split frequencies. A majority rule consensus tree with posterior probabilities of nodes was built.

Protein Methods

The online version of PHYLML (available at http://atgc.lirmm.fr/phyml; Guindon et al., 2005) was used to perform ML reconstruction of phylogenies based on amino acid translated sequences (Guindon and Gascuel, 2003). The evolutionary model was a Jones-Taylor-Thornton substitution model, with six substitution rate categories, γ-shape parameter, and proportion of invariable sites estimated from the data.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers DQ659308 to DQ659323.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. NJ tree resulting from analysis of 85 nucleotide alignments, including 17 TCP sequence types of seven Papaveraceae seu species.

Supplemental Figure S2. In situ hybridization analysis of PAPACYL gene expression in inflorescences and flowers of L. spectabilis (bistymetric) and C. sempervirens (monosymmetric).

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LITERATURE CITED


