

Evolution / Évolution

Evolution of floral symmetry: a state of the art

Florian Jabbour^{a,b,*}, Sophie Nadot^b, Catherine Damerval^a

^a *Université Paris-Sud, unité mixte de recherche (UMR) génétique végétale, Institut national de la recherche agronomique (INRA), Centre national de la recherche scientifique (CNRS), AgroParisTech, Ferme du Moulon, 91190 Gif-sur-Yvette, France*

^b *Université Paris-Sud, Laboratoire écologie, systématique, évolution, unité mixte de recherche (UMR) 8079, Centre national de la recherche scientifique (CNRS), AgroParisTech, 91405 Orsay cedex, France*

Accepted after revision 21 July 2008

Available online 28 November 2008

Presented by Claude Combes

Abstract

The genetic determinants of the organisation and variation of the flower, a striking feature of the angiosperms, are only beginning to be deciphered. Floral symmetry has recurrently evolved among angiosperms, zygomorphy (monosymmetry) being a key innovation due to its role in the plant-pollination interaction. As such, it represents a case study for evo-devo. Phylogenetic comparative studies conducted in two eudicot clades, the Ranunculales and the Asteridae *sensu* APGII, have shown that the evolution of this trait is dependent upon the architectural context of the flower. Genetic and developmental bases of zygomorphy have been investigated in several unrelated model species. In all these species, zygomorphy appears to be controlled, at least partially, by genes belonging to the TCP gene family of transcription factors and named *CYC*-like genes. Exploring the molecular bases of zygomorphy in non-model species spanning the diversity of angiosperms, but also the developmental processes involved, are now essential to understand the evolution of floral symmetry. **To cite this article:** *F. Jabbour et al., C. R. Biologies 332 (2009).*

© 2008 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

Résumé

Évolution de la symétrie florale : une esquisse des connaissances actuelles. La symétrie de la fleur a subi des modifications au cours de l'évolution des angiospermes, et la zygomorphie (ou symétrie bilatérale) est considérée comme une innovation clé. Du fait de l'importance des processus de développement pour son élaboration et des questions soulevées par son homoplasie, la symétrie florale est devenue un modèle en evo-devo. Des analyses phylogénétiques comparatives menées dans deux clades d'eudicotylédones, les Ranunculales et les Asteridae *sensu* APGII, ont montré que l'évolution de ce trait était dépendante du plan d'organisation de la fleur. Chez différentes espèces modèles, le contrôle génétique de la zygomorphie ainsi que sa mise en place durant le développement sont en partie contrôlés par des gènes appartenant à la famille des gènes *CYC*-like, codant pour des facteurs de transcription. Décrypter les bases moléculaires de la zygomorphie chez des espèces non modèles, représentatives de la diversité des angiospermes, mais aussi les processus ontogéniques impliqués dans sa mise en place, sont deux aspects complémentaires essentiels pour la compréhension de l'évolution de la symétrie florale. **Pour citer cet article :** *F. Jabbour et al., C. R. Biologies 332 (2009).*

© 2008 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

* Corresponding author at: Université Paris-Sud, unité mixte de recherche (UMR) génétique végétale, Institut national de la recherche agronomique (INRA), Centre national de la recherche scientifique (CNRS), AgroParisTech, Ferme du Moulon, 91190 Gif-sur-Yvette, France.

E-mail address: florian.jabbour@u-psud.fr (F. Jabbour).

Keywords: Evo-devo; Floral symmetry; *CYC*-like genes; Development; Phylogeny; Zygomorphy

Mots-clés : Evo-devo ; Symétrie florale ; Gènes *CYC*-like ; Développement ; Phylogénie ; Zygomorphie

1. Introduction

The origin of phenotypic novelties is a central question for evolutionary biologists. For understanding how genomic changes can give rise to innovations at more integrative levels, it is mandatory to decipher the mechanisms underlying embryonic development. Evolutionary developmental biology or evo-devo addresses this question by using the knowledge about genes controlling development in model organisms, and comparing developmental processes and their molecular bases in different organisms in a phylogenetic framework [1]. Homology can be assessed and distinguished from homoplasy (parallel or convergent evolution) only by comparing characters in a phylogenetic context. The possibility to investigate the developmental and molecular bases of morphological structures led to redefine homology as a hierarchical concept dependent upon a phylogenetic framework [2], of which consistency at various levels (activator genes, target genes, regulatory networks, developmental processes, morphological structures) can be investigated. Consistency, or lack of consistency, of homology at different scales of complexity may help deciphering the relative part of selection and constraints in the evolution of morphological structures.

In animals, the concept of body plan has contributed to evolutionary analyses of morphological innovations like for instance, analyses of molecular evolution of *Hox* genes as related with segment formation in Arthropods [3]. It has pinpointed the role of the developmental genetic toolkit in determining the identity, number and pattern of body parts in animals. Most of the toolkit genes consist of transcription factor genes and genes involved in signalling pathways, *i.e.*, genes modifying the expression of other genes. Their functional consequences and further phenotypic impact are highly correlated with their spatial and temporal pattern of expression. In plants, the concept of body plan has been largely ignored until the late 1990s, even if the vascular plants have long been hypothesized to have evolved from a simple body plan that has diversified into the large array of architectures seen today. The study of the molecular and developmental bases of specific traits has long been restricted to a few model species (*e.g.* *Arabidopsis thaliana*, Brassicaceae, *Antirrhinum majus* or snapdragon, Plantaginaceae *sensu* [4], *Oryza sativa* or rice, Poaceae), most of which being derived angiosperms.

With the increasing influence of evo-devo as an integrative discipline, there is now a trend towards extending the analyses to a broader taxonomic sampling and larger array of traits [5,6].

Seminal evo-devo studies in plants have been dedicated to one of the most remarkable innovation of angiosperms, the flower that may have played a major role in their evolutionary success (more than 235,000 extant species [7]). Considerable work has been devoted to unravelling determinants of the identity of floral pieces, namely sepals, petals, stamens and carpels. Based on mutant studies in the model species *An. majus* and *Ar. thaliana*, the ABC model was elaborated [8–10]. The A function determines sepal identity, A + B function, petal identity, B + C function, stamen identity and C function, carpel identity. This model was recently complexified by the discovery of the D function involved in ovule development, and the E function that interacts with the A, B and C functions [11]. Interestingly, all but one of the genes specifying the organ identity belong to a single family of transcription factors, the MADS box genes of MIKC type [12].

Although the genetic determinants of the basic floral ground-plan are now well established [13–15], the molecular bases underlying the large variation in flower architecture (shape, number, colour and synorganization of floral parts) (see Fig. 1) are far from being deciphered. Floral symmetry participates in the attractiveness and beauty of the flowers to the human eye. It is also an integrative architectural trait that account for the astonishing diversity of flower form. Two main types of symmetry are recognized, actinomorphy (polysymmetry, *i.e.*, radial symmetry) and zygomorphy (monosymmetry, *i.e.*, bilateral symmetry). Zygomorphic flowers appear relatively late in the fossil record (late Cretaceous, *ca.* 70 mya) compared to the accepted period for angiosperm origin (early Cretaceous, *ca.* 150 mya) [16–18]. Actinomorphy is considered as the ancestral state for angiosperms, and basal angiosperms mainly have actinomorphic flowers [19]. Zygomorphy evolved several times independently from actinomorphy throughout the angiosperms with multiple reversals towards actinomorphy [20,21]. Changes in floral symmetry may result in changes in efficient pollinator range, which in turn can set up sexual barriers leading to speciation [17]. Accordingly, as a derived architectural trait generally associated with lineage di-

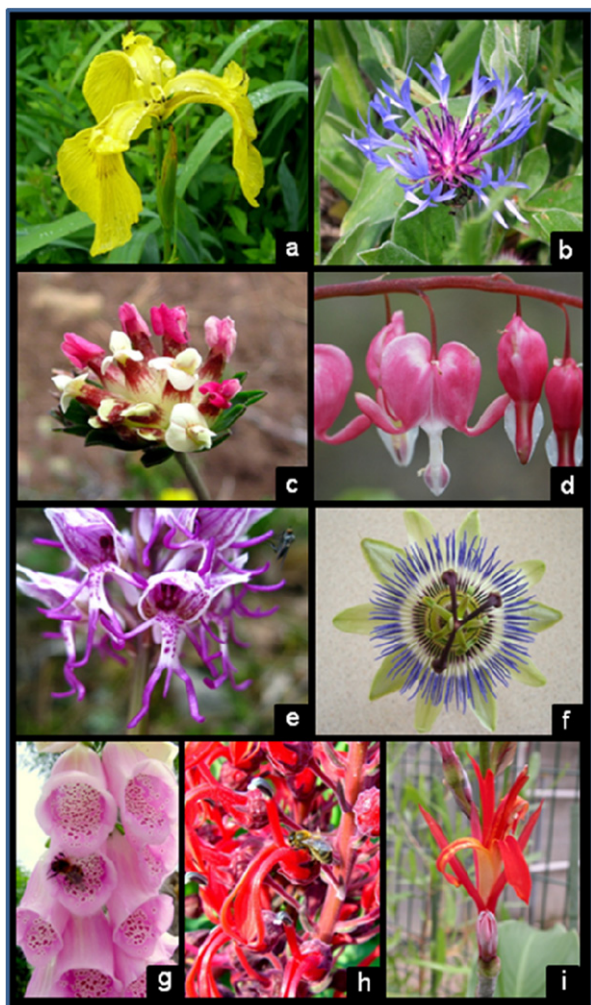


Fig. 1. A glimpse at the diversity of floral forms. a: *Iris pseudacorus* (Iridaceae). b: *Centaurea cyanus* (Asteraceae). c: *Anthyllis montana* (Fabaceae). d: *Lamprocapnos spectabilis* (Fumariaceae). e: *Orchis simia* (Orchidaceae). f: *Passiflora coerulea* (Passifloraceae). g: *Adonis autumnalis* (Plantaginaceae). h: *Lobelia tupa* (Campanulaceae). i: *Canna occidentalis* (Cannaceae). Polysymmetric flowers: a, inner flowers of b, f. Monosymmetric flowers: outer flowers of b, c, e, g, h. Disymmetric flower: d. Asymmetric flower: i. Photographs g and h represent flowers and one of their associated visitors. Photographs: Florian Jabbour (a, b, d, f, g, h), Sophie Nadot (c, e), Bruno Lascaux (i).

versification, zygomorphy constitutes a morphological innovation. In fact, some of the most speciose taxa possess zygomorphic flowers [22], such as Fabaceae and Asteraceae in the core eudicots, Orchidaceae in the monocots (see Fig. 1). As a component of the pollination syndrome [23], floral symmetry has probably an adaptive value, and may have coevolved with other traits of the flower. Until now, very few studies have been devoted to this aspect of coevolution of floral traits,

although it might help to appraise the relative contributions of adaptation and constraints in the emergence of zygomorphy.

Floral symmetry is thus worth being considered as a case study for evo-devo. It is a multiform and integrative morphological trait, genetically determined, set up at various stages during flower development. Moreover, mutants for floral symmetry exist in nature or can be obtained in the lab, enabling molecular genetics analyses. In this review, we will give a quick overview of the diversity of floral symmetry in relation with development, before addressing the question of the correlated evolution between symmetry and other floral traits. Then the focus will be put on the molecular bases of zygomorphy in model species that have allowed candidate genes for studies in non-model species to be pinpointed. Accordingly, a large part of this review will be dedicated to a state of the art about the diversity and evolutionary history of these candidate genes and what can be anticipated about their role in floral symmetry. Finally, some of the major questions that have to be addressed in the future to get a better knowledge of the origins and evolution of floral symmetry will be raised.

2. Diversity of floral symmetry

2.1. Diversity of symmetry types in angiosperm flowers

In its current acception, symmetry mainly characterizes the perianth aspect, even though the most elaborate forms also include modifications at the androecium level (see below). Angiosperm flowers are predominantly symmetric or very rarely asymmetric [17] (see Fig. 1i). It was shown that some pollinators (bees, for instance) are only attracted by symmetric flowers [24–26]. A symmetric flower can be polysymmetric, disymmetric (with two perpendicular symmetry planes) or monosymmetric (Fig. 1). Zygomorphic and disymmetric flowers are commonly found in clades characterized by a closed ground-plan (*i.e.*, where floral organ number and arrangement are fixed within and between individuals) as in core eudicots (Lamiales, Fabaceae, Asterales) and monocots (Orchidaceae and Zingiberales). In contrast, actinomorphic flowers are widespread in open ground-plan clades, like basal angiosperms and early diverging eudicots [17,19]. Zygomorphy involves more or less pronounced shape differentiation between organs within a single whorl (or along spirally inserted organs in rare cases). A strong morphological differentiation at the perianth level is often associated with alterations at the androecium level, including stamen reduction (staminodes) or even abor-

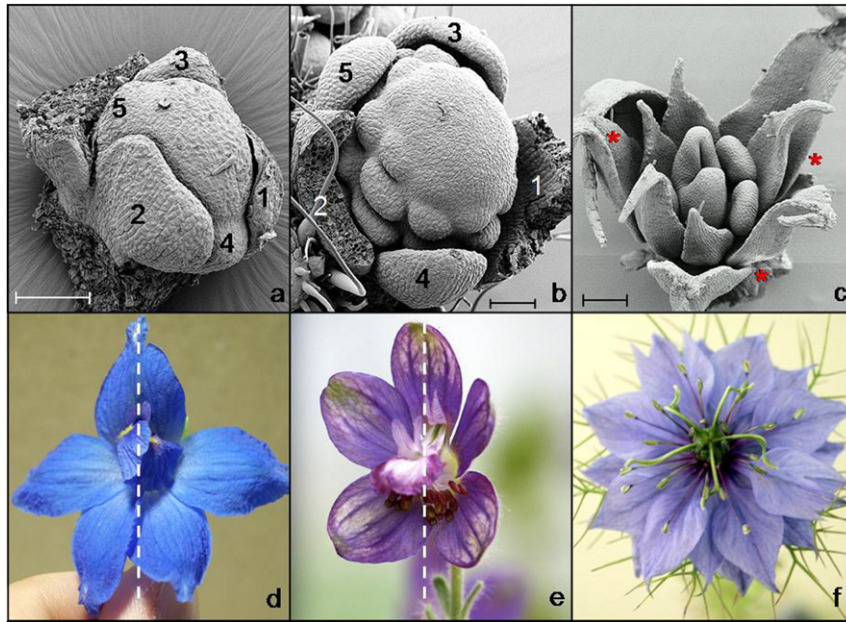


Fig. 2. Three examples of buds with spiral phyllotaxis developing into approximately symmetric flowers. (a) and (b) Young buds of *Delphinium grandiflorum* and *Delphinium staphisagria* showing the spiral initiation of sepals (scale bars: 100 μ m). (c) Late bud of *Nigella damascena* showing the spiral aestivation of sepals (red stars). (d) and (e) Approximately monosymmetric flowers of *D. grandiflorum* and *D. staphisagria*. Dotted lines indicate axes of mirror symmetry. (f) Approximately polysymmetric flower of *N. damascena*. SEM pictures: Florian Jabbour and Louis Ronse de Craene. Photographs: Florian Jabbour.

tion [27]. It must be noted that floral symmetry is never perfect and small deviations always exist. The minimum rate of deviation is observed in flowers with a high degree of synorganization (e.g. Orchidaceae and Apocynaceae which are respectively zygomorphic and generally actinomorphic) [17].

Asymmetry can be generated by enantiostyly (the style is deviated to the right or the left side of the flower) [28] or reduction, such as in Cannaceae (Fig 1i) or Valerianaceae. In these cases, asymmetry is not irregularity, but rather a highly complicated and ordered phenotype. Unordered simple asymmetric flowers occur in a few basal angiosperms, with chaotic organization where the innermost perianth organs and the stamens are irregularly arranged [17].

2.2. Changes in symmetry during development

An interesting observation deduced from the growing body of studies of flower development is that the symmetry of a mature flower can be different from symmetry at early developmental stages, and that symmetry of the floral primordium may change during development. Accordingly, an actinomorphic flower can present transitory zygomorphic or even asymmetric de-

velopmental stages [29]. Zygomorphy is expressed either from organ initiation with no change during the subsequent ontogeny, or later, as a result of differential growth or heterochrony [30], or formation of superstructures due to synorganisations [17]. Both situations occur in the Asteridae *sensu* [4]. For example, zygomorphy is the product of organ initiation in the subfamily Rhinanthioideae (now included in Orobanchaceae *sensu* [4]), but it is preceded by an actinomorphic stage during development in the Antirrhinoideae (now included in Plantaginaceae *sensu* [4]), Bignoniaceae and Lecythidaceae [30].

Symmetry of the floral primordium can also be influenced by the position of the flower in inflorescences, especially during early stages [17]. In contrast, the symmetry of mature flowers can be independent from phyllotaxis, and flowers with either whorled or spiral phyllotaxis can be actinomorphic or zygomorphic (Fig. 2).

2.3. Comparison between the evolution of floral symmetry and the evolution of other floral traits

As previously said, floral symmetry mainly refers to perianth aspect. An interesting question is then whether other architectural traits of the flower, and even fur-

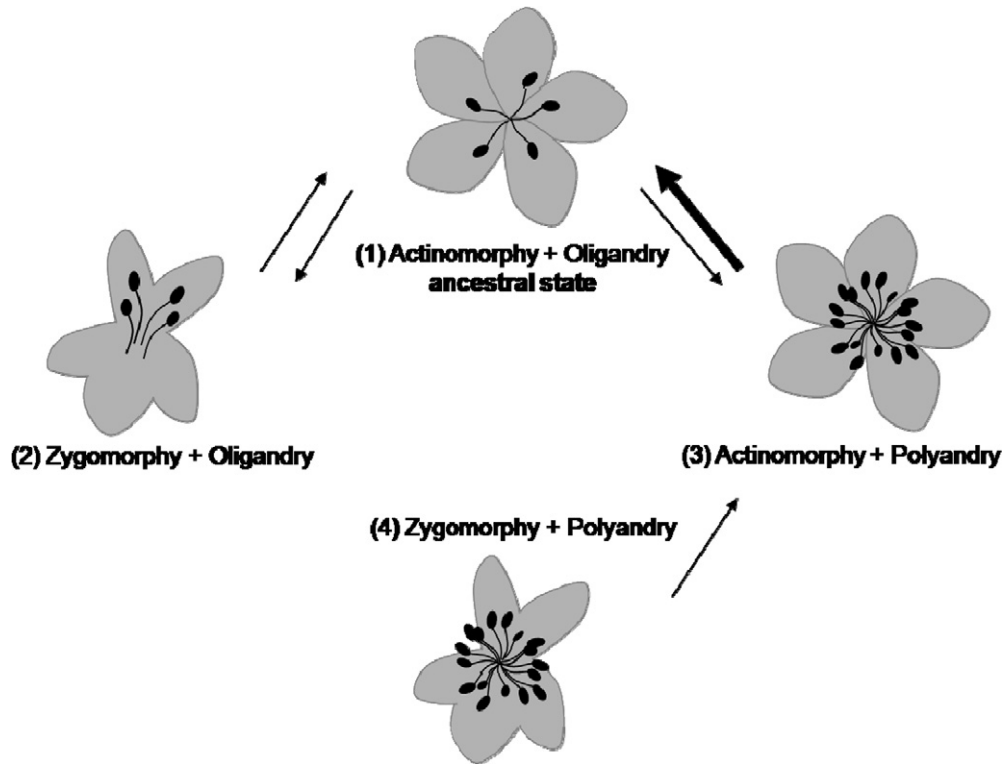


Fig. 3. Evolutionary scenario for correlated evolution between perianth symmetry and androecium size in Asteridae (adapted from [32]). Arrows indicate the possible transitions between the associations of traits during the evolutionary history of Asteridae. The bold arrow supports a transition rate significantly superior to the three others. A zygomorphic perianth associated with polyandry was observed very few times in Asteridae.

ther of the inflorescence, can exert a role in the evolution of perianth morphology, either as an initial trigger of adaptive evolution or as steric constraints during development. One possibility to address this question is to examine simultaneously the evolution of several traits of the flower, trying to elucidate the chronology of evolutionary transitions. First results using such an approach were obtained on Ranunculales [31] and on Asteridae [32]. In Ranunculales, zygomorphy appeared three times independently: in the Papaveraceae and the Ranunculaceae, it is associated with the presence of a single spur, while in the Menispermaceae it is limited to the female flowers of some dioecious species. In Asteridae, a phylogenetic comparative analysis revealed that the evolution of perianth symmetry is conditioned by both androecium size and fixed perianth organ number, and that zygomorphy is a prerequisite to the emergence of nectar spurs. The correlation between perianth zygomorphy and oligandry suggests that androecium size might be a developmental or spatial constraint exerted on floral symmetry (Fig. 3), as if a high number of stamens prevented the spatially or temporally heterogeneous development of organs, which characterizes zy-

gomorphic flowers. The results of [31] and [32] revealed different architectural contexts for the evolution of perianth zygomorphy between Ranunculales and Asteridae. In particular, the diversity of evolutionary pathways appears larger in the Ranunculales than in the Asteridae, which may suggest a higher canalization of development in the latter, and possibly different genetic bases in the two clades.

3. Molecular bases of zygomorphy in model species

3.1. Interplay between four genes in *Antirrhinum*

Genetic and developmental bases of zygomorphy have been investigated in depth in *An. majus*, where numerous mutants of flower architecture are available. This species has pentamerous flowers, with five petals fused into a tube ending in two lips, and a dorsal stamen reduced to a staminode. Zygomorphy takes place through dorsoventral asymmetry of the corolla and androecium. The petals are of three types, dorsal, lateral and ventral. Petal type and abortion of the dorsal stamen were found to be controlled by the interplay among

four genes belonging to two transcription factor gene families [33,34]. Mutation in the *CYCLOIDEA* (*CYC*) gene has the strongest phenotypic effect, with a loss of the dorsal identity and a ventralization of the lateral petals [34]. Together with its paralog *DICHOTOMA* (*DICH*), *CYC* is expressed in the dorsal domain of the young 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 [34]. *DICH* expression is restricted to the dorsalmost part of the dorsal petals, participating in their internal asymmetry [35]. 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 [36], where it acts antagonistically with *DIVARICATA* (*DIV*) that is expressed all over the meristem [33]. *DIV* is responsible for the ventral identity of petals, which can be observed in the actinomorphic flowers of the *cyc-dich* double mutant [37,38].

Both *DIV* and *RAD* belong to the MYB family. *DIV* has two MYB-like domains and belongs to so-called R2R3-MYB subfamily [38]. This subfamily appears as the largest in plants, encompassing 97 genes in *Arabidopsis* for example [39]. *RAD* has one MYB domain with high similarity with the N-terminal domain of *DIV*, suggesting that *RAD* may have evolved from *DIV*-like genes by C-terminal deletion [33]. It belongs to a recently described subfamily of SANT/MYB proteins [40]. *RAD*-like genes constitute a small family in both *An. majus* and *Ar. thaliana* [40].

CYC and *DICH* belong to the TCP gene family characterized by a conserved basic helix-loop-helix domain unique to plants [41–44]. TCP proteins were defined by the family founding members, namely *TEOSINTE BRANCHED 1* (*TBI*) in maize, *CYCLOIDEA* in snapdragon and *PCF* (Proliferating Cell Factor) in rice [41]. More than 20 TCP genes were found in the completely sequenced genomes of *Ar. thaliana* and *O. sativa* [44–48]. Based on the characteristics of the TCP domain, two main classes, I and II, were defined. The resulting proteins were shown to recognize slightly different DNA consensus sequences [49]. Both class I and class II proteins are involved in growth processes. Class I proteins studied up to now function as positive regulators [49–51] whereas class II proteins rather act as negative regulators, even though this role may vary according to the organ or the developmental stage [34,35]. *CYC* and *DICH* belong to class II, and share a short conserved domain particularly rich in polar residues, the R domain, which is however not present in all class II

genes [41]. In the following, such genes with both domains (TCP and R) will be referred to as *CYC*-like genes.

3.2. Molecular and genetic studies in *Arabidopsis* and *Lotus*

In *Arabidopsis thaliana*, only five of the 11 class II genes identified, (*AtTCP1*, *AtTCP2*, *AtTCP12*, *AtTCP18* and *AtTCP24*) exhibit an R domain. Phylogenetic analyses [41,42,47] indicate that *AtTCP1* could be the closest paralog to *CYC*. *Arabidopsis* has an almost actinomorphic flower displaying the tetramerous ground-plan typical of the Brassicaceae (four sepals, four petals, six stamens and two fused carpels). Expression of *AtTCP1* was observed only transiently during early stages of flower development. It is expressed asymmetrically, in the dorsal region of early primordia, possibly delaying the emergence of the dorsal sepal primordium [54]. Six *RAD*-like genes have been characterized in *Ar. thaliana*. None of them is expressed in the dorsal domain of the flower, in contrast with what was found in *An. majus* [40]. Transformation experiments indicate that an interaction between *CYC* and *RAD* can be obtained in *Arabidopsis*. However, *CYC* cannot control the transcription of any of the *Arabidopsis* *RAD*-like genes, which suggests that this interaction may have specifically evolved in the *Anthirrhinum* lineage [36,40].

The genetic bases of zygomorphy have also been investigated more recently into details in the legume species *Lotus corniculatus* var. *japonicus* (abbreviated to *L. japonicus*). Like other Fabaceae species, *L. japonicus* has strongly zygomorphic flowers with a pentamerous ground-plan. Contrasting with *An. majus*, the flower of *L. japonicus* comprises five free petals. The petals are of three morphological types: a large standard formed by the dorsal petal, two lateral wings, and a keel formed by the two joined ventral petals. The androecium is bent, with the dorsal stamens shorter than the ventral ones joined into a tube. It surrounds the pistil and both are enclosed in the keel. The mutant *sql* has flowers bearing an abnormal squared standard, while mutant *kew1* has abnormal wings, resembling keel petals [55]. In *L. japonicus*, four *CYC*-like genes have been identified. It was shown that the *sql* mutant is affected in the *LjCYC2* gene, a close paralog of *AtTCP1* and *CYC*. Further analyses and transformation experiments indicate that *LjCYC2* plays a dorsaling role during flower development comparable to *CYC*. The double mutant *sqlkew1* exhibits fully ventralized flowers, indicating

epistatic interactions of *LjCYC2* and *kew1*. The molecular identity of *kew1* is unknown at present [55].

4. *CYC*-like genes as candidates to investigate the origin of floral zygomorphy in eudicots

Analyses in species phylogenetically related to the three previously described model species suggest that *CYC*-like genes play a general role in the establishment of zygomorphy, at least in core eudicots. For example, hypermethylation of an ortholog of *CYC* resulting in gene silencing was found to be responsible for the peloric mutant of *Linaria vulgaris* initially described by Linnaeus [56]. In *Mohavea confertiflora*, a close relative of *An. majus* with almost actinomorphic flowers, a change in the territory of expression of the orthologs of *CYC* and *DICH* was observed, in a pattern that is consistent with the hypothesis that these genes play a role in the shift in floral symmetry [57]. In *Cadia purpurea*, a legume species with actinomorphic flowers, an extension of the expression domain of the *CYC*-like gene *LEGCYC1* was observed compared to its zygomorphic close relative *Lupinus nanus*, consistent with dorsalization of the whole flower [58]. *Iberis*, one of the few Brassicaceae genera with zygomorphic flowers, displays two ventral petals three to four times larger than the dorsal ones [59]. The ortholog of *TCPI* in *I. amara* (*IaTCPI*) was found to be expressed symmetrically in young floral meristems, but as development proceeds, the expression becomes stronger in dorsal than in ventral petals, the difference reaching a peak of ~90-fold after anthesis. Analysis of a peloric mutant with dorsal petals as large as ventral ones indicates a reduced expression of *IaTCPI* as compared to wild-type in the corolla, consistent with the hypothesis that *IaTCPI* may act to restrain dorsal petal growth in the wild type [59].

CYC-like genes were chosen as candidates to investigate the origin of zygomorphy in the Ranunculales that are considered as the sister group to all other eudicots. Studies have been focused so far on the Papaveraceae [60,61], one of the basalmost families in Ranunculales, which constitutes a morphologically diverse clade in which the genus *Pteridophyllum* is sister to two subfamilies, Fumarioideae and Papaveroideae. The dimeric ground plan of the flower and especially the perianth with two sepals and two whorls of opposite decussate petals probably offered a favourable context for the development of disymmetric adult flowers through morphological differentiation of the two petal whorls. This was achieved in Fumarioideae, whereas Papaveroideae all have actinomorphic flowers. In addition,

in Fumarioideae, disymmetry further evolved into monosymmetry by reduction of one spur. Two paralogous lineages of *CYC*-like genes were identified in Papaveraceae, named *PAPACYL1* and *PAPACYL2*. They originated through a duplication probably predating the divergence between Papaveroideae and Fumarioideae. Gene expression studies of *PAPACYL1* and *PAPACYL2* in vegetative and floral organs showed that these two paralogs are expressed during inflorescence and flower development, mainly at organ junctions, and suggested a correlation between *PAPACYL* expression patterns and flower symmetry [61].

5. Evolutionary history and functional diversification of *CYC*-like genes

Elucidating the phylogenetic relationships of the *CYC*-like genes characterized in the eudicots in the context of the evolutionary history of the TCP gene family may help to shed light on the evolutionary forces shaping their diversity and evolution, and to put forward hypotheses about their ancestral function(s) and pattern(s) of expression in relation with floral symmetry.

5.1. Evolutionary history of TCP genes: a story of duplications

Among land plants, sequences belonging to TCP genes were found in mosses (*Physcomitrella patens*), ferns, gymnosperms [43] and angiosperms. Interestingly, homologous sequences could be amplified with degenerate oligonucleotides in *Chara* and *Cosmidium*, two green algae, indicating that the duplication that gave rise to class I and class II genes most probably occurred within Zygnematophyta, before the emergence of land plants [43]. Series of specific duplications appear in land plants, suggesting that this gene family may belong to the toolkit that enabled colonisation of the terrestrial environment [62].

Concerning the class II genes, three main clades comprising monocot and eudicot sequences were tentatively identified (*CYC*-like or 'ECE', *CIN*-like typified by the *CINCINNATA* gene of *An. majus* [63] and a third poorly supported clade), suggesting that at least three class II TCP genes were present in the common ancestor of monocots and eudicots [61]. A complex history of taxon specific duplications and gene losses further took place in each clade [43,48,61,64]. The R domain is present in most sequences of the *CYC*-like clade, while completely absent from the *CIN*-like clade. It is very likely homoplastic, since wherever the root of the phy-

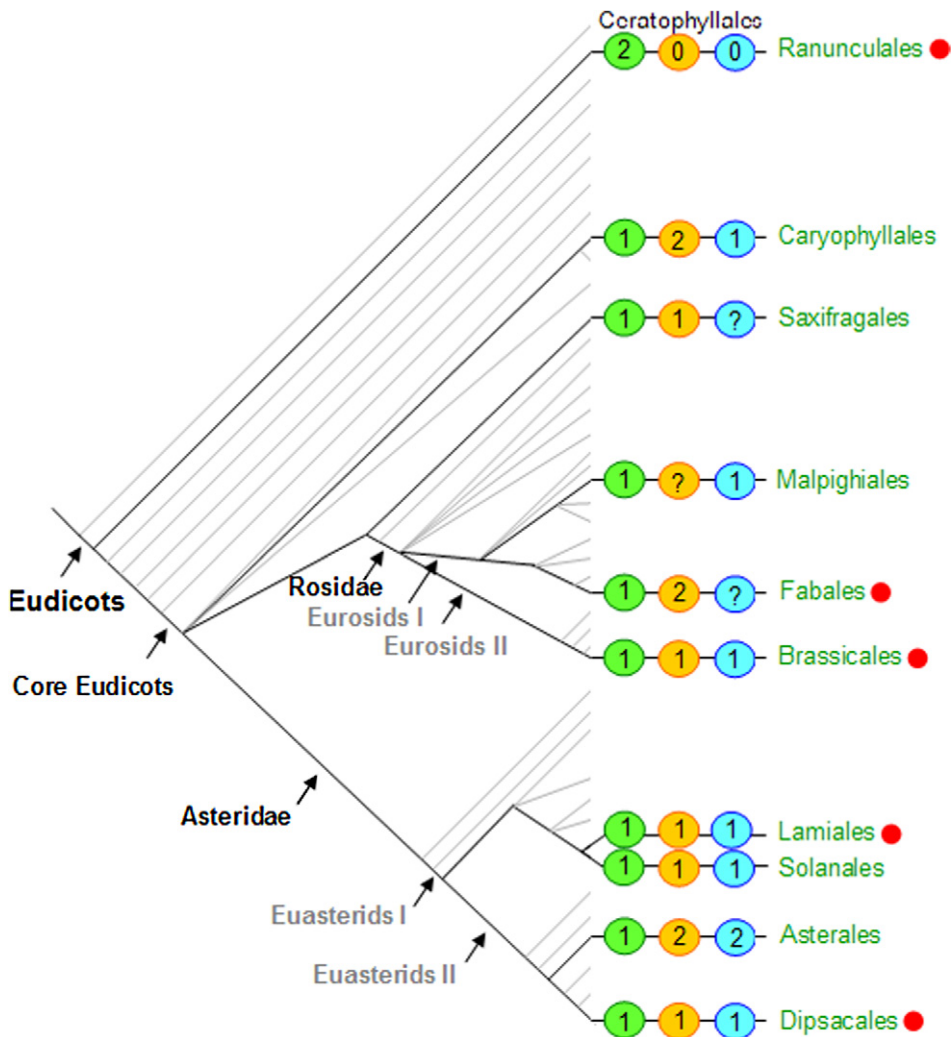


Fig. 4. Eudicot tree adapted from the Angiosperm Phylogeny Website [76]. Orders in green include the species in which members of the ECE clade, as described in [65], were found. Orders marked with a red dot are those in which the genetic bases of floral symmetry have been investigated mainly using expression studies (see Table 1). The numbers in the green, orange and blue circles respectively refer to the minimum number of *CYC* paralogs identified in each of the three lineages *CYC1*, *CYC2* and *CYC3*, for each species in the order considered.

logenic tree is placed, four events of acquisition/loss are required to account for its distribution.

Focusing on *CYC*-like sequences in core eudicots, Howarth and Donoghue [65] found three major gene lineages *CYC1*, *CYC2* and *CYC3*. All sequences were characterized by a short motif of amino-acids in the intermediary domain between the TCP and R domains, leading the authors to propose the denomination of ‘ECE’ clade for all these sequences. Phylogenetic analyses including several monocot sequences as well as basal eudicot sequences (*PAPACYL1* and *PAPACYL2*) are consistent with the hypothesis that at least two of these lineages (*CYC2* and *CYC3*) originated by a du-

plication at the base of the core eudicots. This duplication may have been genome-wide, since duplications just predating core eudicot emergence were also noticed in several gene families involved in floral development [65]. Enlarged taxonomic sampling is needed to elucidate the evolutionary relationships of core eudicot sequences from the lineage *CYC1* with early diverging eudicot and monocot sequences, in order to clarify the history of duplications in the ‘ECE’ clade. It is interesting to note that the *AtTCP18* gene that belongs to *CYC1* lineage is thought to be an ortholog of the monocot *TBI* gene, that played a major role in maize domestication [66]. Up to date in

Table 1

List of the main taxa studied to date for elucidating the genetic mechanisms underlying floral symmetry. Selected references are associated with each taxon.

Order	Studied taxon	Selected references
Ranunculales	Fumarioideae	(A. Kölsch and S. Gleissberg, 2006; C. Damerval, et al., 2007) [60,61]
Fabales	<i>Lotus japonicus</i>	(X. Feng, et al., 2006) [55]
	<i>Cadia purpurea</i>	(H.L. Citerne, et al., 2006) [58]
	<i>Lupinus nanus</i>	(R. Ree, et al., 2004) [72]
Brassicales	<i>Arabidopsis thaliana</i>	(P. Cubas, et al., 2001) [54]
	<i>Iberis amara</i>	(A. Busch and S. Zachgo, 2007) [59]
Lamiales	<i>Antirrhinum majus</i>	(D. Luo, et al., 1996; D. Luo, et al., 1999; J. Almeida and L. Galego, 2005; S.B. Corley, et al., 2005; M.M.R. Costa, et al., 2005; C.E.L. Baxter, et al., 2007) [33,34,36,37,40]
	<i>Linararia vulgaris</i>	(P. Cubas, et al., 1999) [42]
	<i>Mohavea confertiflora</i>	(L.C. Hileman, et al., 2003) [57]
	Gesneriaceae	(H.L. Citerne, et al., 2000) [75]
Dipsacales	21 genera	(D.G. Howarth and M.J. Donoghue, 2005) [67]

core eudicots, no duplication was recorded in clade *CYC1*, in contrast to clades *CYC2* (containing *CYC*, *DICH*, *AtTCP1* and *LjCyc2*) and *CYC3*, which exhibit numerous more or less recent taxon specific duplications [67] (Fig. 4). In the *CYC2* clade, which is hitherto the most widely studied, history of duplications has been elucidated in some cases. Accordingly, the duplication that gave rise to *CYC* and *DICH* took place in the ancestor of the tribe Antirrhineae within Plantaginaceae [68], enabling subsequent evolution of the two paralogs by subfunctionalisation [69]. In Legumes, three paralogous lineages (*LEGCYCIA* and *IB* – the latter one comprising the *LjCyc2* gene, *LEG-CYC2*) were observed, that probably originated through two successive duplications that took place at the base of the Faboideae, or even before [70,71]. Interestingly in the genus *Lupinus*, molecular evolution studies suggest that positive selection has been operating in *LEG-CYC1B* lineage in relation to a shift in floral morphology among species [72]. Several independent duplications also occurred within the Asteridae [73]. In Gesneriaceae, a duplication predating the divergence between the subfamilies Cyrtandroideae and Gesnerioideae was found (*GCYC1* and *GCYC2*), and a supplementary duplication took place in the Cyrtandroideae *Saintpaulia* + *Streptocarpus* clade [74,75]. In the Dipsacales, the *CYC2* and *CYC3* paralogs (*DipsCYC2* and *DipsCYC3*) were duplicated in the Caprifoliaceae that comprise mainly zygomorphic species, but not in the actinomorphic sister family Adoxaceae [65,67]. Fig. 4 shows the taxonomic sampling used for *CYC*-like gene characterisation among eudicots, and the minimum number of paralogs reported in each of the three lineages *CYC1*, *CYC2*, *CYC3*, and points to studies that have examined

gene expression in relation with floral symmetry (tree adapted from [76]).

5.2. Ancestral pattern of expression, function and recruitment for zygomorphy of *CYC*-like genes

In core eudicots, zygomorphy is considered to have evolved independently in Rosidae *sensu* [4] and Asteridae from an actinomorphic ancestral state. The results in *An. majus* and *L. japonicus* where two *CYC2* paralogs are responsible for dorsoventral asymmetry raised an interesting point. In both taxa, did the gene ancestral to the *CYC2* lineage have an ancestral function in flower asymmetry? Or were paralogs independently recruited for this trait? More generally, what can be the ancestral function of *CYC*-like genes, and how did it evolve following the successive duplications, in particular in the three lineages *CYC1*, *CYC2* and *CYC3*?

The downstream networks of *CYC* and *LjCyc2* are at least partially different, since the *RAD* gene that belongs to this regulatory network in *An. majus*, is apparently not involved in the Rosidae examined so far [55]. This appears rather in favor of an independent recruitment of these two paralogs for zygomorphy. Nevertheless, a common pattern of asymmetric expression in lateral organs may exist for *CYC2* genes [54]. In *An. majus*, wild-type inflorescences lack a terminal flower and have zygomorphic axillary flowers. The *centroradialis* (*cen*) mutant produces an inflorescence terminated by an actinomorphic flower which resembles the axillary flowers of *cyc* mutant [77,78]. In this terminal flower, *CYC* is not active at early developmental stages, but a later transient asymmetric expression is observed in the dorsal regions of the sepals, like in the wild type axillary flowers [79]. In addition to

flowers, *CYC* is expressed asymmetrically in shoots. An asymmetric expression of *AtTCP1* was also observed in elongating shoots of *Ar. thaliana*. In flowers, an asymmetric expression is reported for most if not all *CYC2* genes studied (*CYC*, *DICH*, *Lonicera morrowii* *DipsCYC2B*, *LjCyc2*, *AtTCP1* – see above). An interesting exception appears to be the early expression of the *laTCP1* gene, which could be an apomorphy of *I. amara* [59].

Only a few data are available concerning the expression and function of genes in *CYC3*, the *CYC2* sister clade. In *Lonicera morrowii*, a paralog of *DipsCYC3* is expressed in flower buds [65]. In *Ar. thaliana*, *AtTCP12* (*BRC2* in [80]) prevents axillary bud outgrowth redundantly with *AtTCP18*, and is also expressed in flowers and siliques [80]. There is no report of asymmetric expression in any of these two cases.

Expression data of genes belonging to the *CYC1* lineage in core eudicots, or to related genes in Papaveraceae (*PAPACYL*) and Poaceae (*TB1*), indicate an expression in axillary positions and/or at junctions between organs. The *TB1* gene and the *Arabidopsis AtTCP18* gene (*BRC1* in [80]) play a major role in branching. Both genes are expressed in axillary meristems in an apparently radially symmetric pattern, resulting in inhibition of secondary branch growth [52,80]. The *TB1* gene in maize also plays a role in sex determination, and a correlation has been found between gene expression and organ (spikelet and especially stamen) abortion [52]. The *PAPACYL* genes are expressed at organ junctions during flower and inflorescence development. Their particular asymmetric expression observed in the transverse petals in *Capnoides semper-virens* during development [61] should be examined in other species in order to corroborate this apparent synapomorphy of zygomorphic Fumarioideae. Since the lineage *CYC1* and related genes are ancestral to both *CYC2* and *CYC3*, it is tempting to speculate that the asymmetric dorsoventral pattern of expression observed in *CYC2* has evolved as an apomorphy of this gene lineage after the *CYC2–CYC3* duplication. A proposed ancestral function is growth inhibition or retardation, based on data of several *CYC1* genes (see above) and other class II TCP genes [53,63]. Then, sustained asymmetric expression in flowers would be sufficient to promote zygomorphy.

Even though the increasing body of analyses dedicated to the genetic bases of floral symmetry using *CYC*-like genes as candidates has provided interesting results, our understanding about the origin of this trait during angiosperm evolution is still at its beginning. In particular, no genetic and molecular studies on mono-

cot species, where zygomorphy evolved independently in large clades such as Orchidaceae and Zingiberales, have yet been published. The complexity of class II TCP genes is largely unknown in these clades, the only data in monocots coming from the Poaceae [81]. Moreover, the dorsoventralisation of the flower in *An. majus* has been shown to rely on the interplay between four transcription factor genes, of which the conservation has not yet been evaluated among the Asteridae. The *LjCyc2* interacting partners in Rosidae are still unknown. Widening the sampled taxa and deciphering the gene networks involved during development of zygomorphic flowers are promising perspectives. Some peloric forms appear sporadically in basal eudicots, monocots and basal angiosperms and molecular techniques are now available such as VIGS (Virus Induced Gene Silencing) that enables down-regulation of developmental genes [82–84], which might give the opportunity to test for the role of candidate genes in shaping floral symmetry in various taxa. Other aspects of floral symmetry have to be taken into account in order to progress in the understanding of the evolutionary forces promoting the emergence of this trait among angiosperms. Studying the patterns of symmetry changes during flower development in a phylogenetic context would be of great interest to detect structural constraints and evaluate stability or canalization of development as related to more or less recent transitions towards a zygomorphic phenotype. It would enable to describe the various ways to switch from actinomorphy to zygomorphy, and pinpoint the steps leading to reversals towards ancestral phenotypes. The potential role of “superstructures” in the flower (synorganization of parts) or in the inflorescences should also be challenged. Analyses of relationships between floral symmetry, breeding systems and types of pollinators would help to gain a better view of the role of adaptation in the emergence of particular types of symmetry. Conducting integrative studies that combine molecular genetics, developmental analyses without passing over the ecological aspects is indubitably the key to success in grasping the evolutionary history of floral symmetry.

Acknowledgements

We are grateful to Louis Ronse de Craene for giving FJ the opportunity to conduct SEM studies at the Royal Botanic Garden, Edinburgh. We thank an anonymous reviewer for his constructive comments on the first version of the manuscript. A part of this research (the SEM studies) received support from the SYNTHESYS Project <http://www.synthesys.info/> which is financed

by European Community Research Infrastructure Action under the FP6 “Structuring the European Research Area” Programme. FJ was supported by a fellowship from the Ministère de l’Enseignement Supérieur et de la Recherche, France. We also acknowledge financial support from IFR 87 “La Plante et son Environnement”.

References

- [1] B.K. Hall, Guest Editorial: Evo-devo or devo-evo – does it matter? *Evo. Dev.* 2 (4) (2000) 177–178.
- [2] E. Abouheif, Developmental genetics and homology: a hierarchical approach, *Trends Ecol. Evol.* 12 (10) (1997) 405–408.
- [3] S.B. Carroll, Homeotic genes and the evolution of arthropods and chordates, *Nature* 376 (1995) 479–485.
- [4] APG, An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGII, *Bot. J. Linn. Soc.* 141 (4) (2003) 399–436.
- [5] C.P. Scutt, M. Vinauger-Douard, C. Fourquin, C. Finet, C. Dumas, An evolutionary perspective on the regulation of carpel development, *J. Exp. Bot.* 57 (10) (2006) 2143–2152.
- [6] S.K. Floyd, J.L. Bowman, Distinct developmental mechanisms reflect the independent origins of leaves in vascular plants, *Curr. Biol.* 16 (2006) 1911–1917.
- [7] G. Lecointre, H. Le Guyader, *Classification phylogénétique du vivant*, Editions Belin, France, 2001.
- [8] E. Coen, E. Meyerowitz, The war of the whorls: genetic interactions controlling flower development, *Nature* 353 (1991) 34–37.
- [9] T. Jack, Molecular and genetic mechanisms of floral control, *Plant Cell* 16 (2004) S1–S17.
- [10] E.M. Krizek, J.C. Fletcher, Molecular mechanisms of flower development: an armchair guide, *Nature Rev. Gen.* 6 (2005) 688–698.
- [11] S. Pelaz, G.S. Ditta, E. Baumann, E. Wisman, M.F. Yanofsky, B and C floral organ identity functions require SEPALLATA MADS-box genes, *Nature* 405 (2000) 200–203.
- [12] G. Theissen, A. Becker, A. Di Rosa, A. Kanno, J.T. Kim, T. Münster, K.W. Winter, H. Saedler, A short history of MADS-box genes in plants, *Plant Mol. Biol.* 42 (2000) 115–149.
- [13] J. Nam, K. Kaufmann, G. Theissen, M. Nei, A simple method for predicting the functional differentiation of duplicate genes and its application to MADS-box genes, *Nucleic Acid Res.* 33 (2) (2005) e12.
- [14] E.M. Kramer, J.C. Hall, Evolutionary dynamics of genes controlling floral development, *Curr. Opin. Plant Biol.* 8 (1) (2005) 13–18.
- [15] L.M. Zahn, H.Z. King, J.H. Leebens-Mack, S. Kim, P.S. Soltis, L.L. Landherr, D.E. Soltis, C.W. de Pamphilis, H. Ma, The evolution of the SEPALLATA subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history, *Genetics* 169 (4) (2005) 2209–2223.
- [16] W. Crepet, Timing in the evolution of derived floral characters: Upper Cretaceous (Turonian) taxa with tricolpate and tricolpate-derived pollen, *Rev. Palaeobot. Palynol.* 90 (3) (1996) 339–359.
- [17] P.K. Endress, Symmetry in flowers: diversity and evolution, *Int. J. Plant Sci.* 160 (6 Suppl.) (1999) S3–S23.
- [18] W. Crepet, K.C. Nixon, M.A. Gandolfo, Fossil evidence and phylogeny: the age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits, *Am. J. Bot.* 91 (10) (2004) 1666–1682.
- [19] L.P. Ronse De Craene, P.S. Soltis, D.E. Soltis, Evolution of floral structures in basal angiosperms, *Int. J. Plant Sci.* 164 (5 Suppl.) (2003) S329–S363.
- [20] M.J. Donoghue, R.H. Ree, D.A. Baum, Phylogeny and the evolution of flower symmetry in the Asteridae, *Trends Plant Sci.* 3 (8) (1998) 311–317.
- [21] G.L. Stebbins, *Flowering Plants, Evolution Above the Species Level*, Harvard University Press, Massachusetts, 1974.
- [22] R.D. Sargent, Floral symmetry affects speciation rates in angiosperms, *Proc. R. Soc. B* 271 (2003) 603–608.
- [23] C.B. Fenster, W.S. Armbruster, P. Wilson, M.R. Dudash, J.D. Thomson, Pollination syndromes and floral specialization, *Annu. Rev. Ecol. Syst.* 35 (2004) 375–403.
- [24] M. Giurfa, B. Eichmann, R. Menzel, Symmetry perception in an insect, *Nature* 382 (6590) (1996) 458–461.
- [25] E.L. West, T.M. Lavery, Effect of floral symmetry on flower choice and foraging behaviour of bumble bees, *Can. J. Zool.* 76 (1998) 730–739.
- [26] M. Giurfa, A. Dafni, P.R. Neal, Floral symmetry and its role in plant-pollinator systems, *Int. J. Plant Sci.* 160 (1999) S41–S50.
- [27] P.J. Rudall, R.M. Bateman, Evolution of zygomorphy in monocot flowers: iterative patterns and developmental constraints, *New Phytol.* 162 (2004) 25–44.
- [28] P.K. Endress, Evolution of floral symmetry, *Curr. Opin. Plant Biol.* 4 (2001) 86–91.
- [29] P.K. Endress, *Diversity and Evolutionary Biology of Tropical Flowers*, Cambridge University Press, Cambridge, 1994.
- [30] S.C. Tucker, Evolutionary liability of symmetry in early floral development, *Int. J. Plant Sci.* 160 (6 Suppl.) (1999) S25–S39.
- [31] C. Damerval, S. Nadot, Evolution of perianth and stamen characteristics with respect to floral symmetry in Ranunculales, *Ann. Bot. (Lond.)* 100 (2007) 631–640.
- [32] F. Jabbour, C. Damerval, S. Nadot, Architectural constraints involved in the evolution of floral symmetry: a comparative analysis in Asteridae, *Ann. Bot. (Lond.)* 102 (2) (2008) 153–165.
- [33] S.B. Corley, R. Carpenter, L. Copsey, E. Coen, Floral asymmetry involves an interplay between TCP and MYB transcription factors in *Antirrhinum*, *Proc. Nat. Acad. Sci.* 102 (14) (2005) 5068–5073.
- [34] D. Luo, R. Carpenter, C. Vincent, L. Copsey, E. Coen, Origin of floral asymmetry in *Antirrhinum*, *Nature* 383 (1996) 794–799.
- [35] D. Luo, R. Carpenter, L. Copsey, C. Vincent, J. Clark, E. Coen, Control of organ asymmetry in flowers of *Antirrhinum*, *Cell* 99 (1999) 367–376.
- [36] M.M.R. Costa, S. Fox, A.I. Hanna, C. Baxter, E. Coen, Evolution of regulatory interactions controlling floral asymmetry, *Development* 132 (2005) 5093–5101.
- [37] J. Almeida, L. Galego, Flower symmetry and shape in *Antirrhinum*, *Int. J. Dev. Biol.* 49 (2005) 527–537.
- [38] L. Galego, J. Almeida, Role of DIVARICATA in the control of dorsoventral asymmetry in *Antirrhinum* flowers, *Gene Dev.* 16 (2002) 880–891.
- [39] R. Stracke, M. Werber, B. Weisshaar, The R2R3-MYB gene family in *Arabidopsis thaliana*, *Curr. Opin. Plant Biol.* 4 (5) (2001) 447–456.
- [40] C.E.L. Baxter, M.M.R. Costa, E.S. Coen, Diversification and co-option of RAD-like genes in the evolution of floral symmetry, *Plant J.* 52 (2007) 105–113.
- [41] P. Cubas, N. Lauter, J. Doebley, E. Coen, The TCP domain: A motif found in proteins regulating plant growth and development, *Plant J.* 18 (1999) 215–222.

- [42] P. Cubas, C. Vincent, E. Coen, An epigenetic mutation responsible for natural variation in floral symmetry, *Nature* 401 (6749) (1999) 157–161.
- [43] O. Navaud, P. Dabos, E. Carnus, D. Tremousaygue, C. Hervé, TCP transcription factors predate the emergence of land plants, *J. Mol. Evol.* 65 (2007) 23–33.
- [44] J.L. Riechmann, J. Heard, G. Martin, L. Reuber, C.Z. Jiang, J. Keddie, L. Adam, O. Pineda, O.J. Ratcliffe, R.R. Samaha, R. Creelman, M. Pilgrim, P. Broun, J.Z. Zhang, D. Ghandehari, B.K. Sherman, C.L. Yu, Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes, *Science* 290 (2000) 2105–2110.
- [45] X. Yao, H. Ma, J. Wang, D.B. Zhang, Genome-wide comparative analysis and expression pattern of TCP gene families in *Arabidopsis thaliana* and *Oryza sativa*, *J. Integr. Plant Biol.* 49 (6) (2007) 885–897.
- [46] Y.Q. Xiong, T.Y. Liu, C.G. Tian, S.H. Sun, J.Y. Li, M.S. Chen, Transcription factors in rice: a genome-wide comparative analysis between monocots and eudicots, *Plant Mol. Biol.* 59 (4) (2005) 191–203.
- [47] P. Cubas, Role of TCP genes in the evolution of morphological characters in angiosperms, in: Q.C.B. Cronk, R.M. Bateman, J.A. Hawkins (Eds.), *Developmental Genetics and Plant Evolution*, in: Special Volume Series, vol. 65, The Systematics Association, London, 2002, pp. 247–266.
- [48] C. Damerval, M. Manuel, Independent evolution of Cycloidea-like sequences in several angiosperm taxa, *Comptes Rendus Palevol* 2 (2003) 241–250.
- [49] S. Kosugi, Y. Ohashi, DNA binding and dimerization specificity and potential targets for the TCP protein family, *Plant J.* 30 (2002) 337–348.
- [50] C.X. Li, T. Potusshak, A. Colon-Carmona, A. Gutierrez, P. Dörner, *Arabidopsis* TCP20 links regulation of growth and cell division control pathways, *Proc. Nat. Acad. Sci.* 102 (36) (2005) 12978–12983.
- [51] D. Tremousaygue, L. Garnier, C. Bardet, P. Dabos, C. Herve, B. Lescure, Internal telomeric repeats and ‘TCP domain’ protein-binding sites co-operate to regulate gene expression in *Arabidopsis thaliana* cycling cells, *Plant J.* 33 (6) (2003) 957–966.
- [52] L. Hubbard, P. McSteen, J. Doebley, S. Hake, Expression patterns and mutant phenotype of teosinte branched1 correlate with growth suppression in maize and teosinte, *Genetics* 162 (4) (2002) 1927–1935.
- [53] O. Favier-Rampant, G.J. Bryan, A.G. Roberts, D. Milbourne, R. Viola, M.A. Taylor, Regulated expression of a novel TCP domain transcription factor indicates an involvement in the control of meristem activation processes in *Solanum tuberosum*, *J. Exp. Bot.* 55 (398) (2004) 951–953.
- [54] P. Cubas, E. Coen, J.M.M. Zapater, Ancient asymmetries in the evolution of flowers, *Curr. Biol.* 11 (13) (2001) 1050–1052.
- [55] X. Feng, Z. Zhao, Z. Tian, S. Xu, Y. Luo, Z. Cai, Y. Wang, J. Yang, Z. Wang, L. Weng, J. Chen, L. Zheng, X. Guo, J. Luo, S. Sato, S. Tabata, W. Ma, X. Cao, X. Hu, C. Sun, D. Luo, Control of petal shape and floral zygomorphy in *Lotus japonicus*, *Proc. Nat. Acad. Sci.* 103 (13) (2006) 4970–4975.
- [56] P. Cubas, C. Vincent, E. Coen, An epigenetic mutation responsible for natural variation in floral symmetry, *Nature* 401 (6749) (1999) 157–161.
- [57] L.C. Hileman, E.M. Kramer, D.A. Baum, Differential regulation of symmetry genes and the evolution of floral morphologies, *Proc. Nat. Acad. Sci.* 100 (22) (2003) 12814–12819.
- [58] H.L. Citerne, R.T. Pennington, Q.C.B. Cronk, An apparent reversal in floral symmetry in the legume *Cadia* is a homeotic transformation, *Proc. Nat. Acad. Sci.* 103 (32) (2006) 12017–12020.
- [59] A. Busch, S. Zachgo, Control of corolla monosymmetry in the Brassicaceae *Iberis amara*, *Proc. Nat. Acad. Sci.* 104 (42) (2007) 16714–16719.
- [60] A. Kölsch, S. Gleissberg, Diversification of CYCLOIDEA-like TCP genes in the basal eudicot families Fumariaceae and Papaveraceae s.str., *Plant Biol.* 8 (2006) 680–687.
- [61] C. Damerval, M. Le Guilloux, M. Jager, C. Charon, Diversity and evolution of CYCLOIDEA-like TCP genes in relation to flower development in Papaveraceae, *Plant Physiol.* 143 (2) (2007) 759–772.
- [62] S.K. Floyd, J.L. Bowman, The ancestral developmental tool kit of land plants, *Int. J. Plant Sci.* 168 (1) (2007) 1–35.
- [63] U. Nath, B.C. Crawford, R. Carpenter, E. Coen, Genetic control of surface curvature, *Science* 299 (2003) 1404–1407.
- [64] P. Cubas, Floral zygomorphy, the recurring evolution of a successful trait, *BioEssays* 26 (2004) 1175–1184.
- [65] D.G. Howarth, M.J. Donoghue, Phylogenetic analyses of the ‘ECE’ (CYC/TB1) clade reveals duplications predating the core eudicots, *Proc. Nat. Acad. Sci.* 103 (24) (2006) 9101–9106.
- [66] J. Doebley, A. Stec, L. Hubbard, The evolution of apical dominance in maize, *Nature* 386 (6624) (1997) 485–488.
- [67] D.G. Howarth, M.J. Donoghue, Duplications in CYC-like genes from Dipsacales correlate with floral form, *Int. J. Plant Sci.* 166 (3) (2005) 357–370.
- [68] T. Gübitz, A. Caldwell, A. Hudson, Rapid molecular evolution of CYCLOIDEA-like genes in *Antirrhinum* and its relatives, *Mol. Biol. Evol.* 20 (9) (2003) 1537–1544.
- [69] L.C. Hileman, D.A. Baum, Why do paralogs persist? Molecular evolution of CYCLOIDEA and related floral symmetry genes in Antirrhineae (Veronicaceae), *Mol. Biol. Evol.* 20 (4) (2003) 591–600.
- [70] H.L. Citerne, D. Luo, R.T. Pennington, E. Coen, Q.C.B. Cronk, A phylogenomic investigation of CYCLOIDEA-like TCP genes in the Leguminosae, *Plant Physiol.* 131 (2003) 1042–1053.
- [71] T. Fukuda, J. Yokoyama, M. Maki, Molecular evolution of cycloidea-like genes in Fabaceae, *J. Mol. Evol.* 57 (2003) 588–597.
- [72] R. Ree, H.L. Citerne, M. Lavin, Q.C.B. Cronk, Heterogeneous selection on LEGCYC paralogs in relation to flower morphology and the phylogeny of *Lupinus* (Leguminosae), *Mol. Biol. Evol.* 21 (2) (2004) 321–331.
- [73] P.A. Reeves, R.G. Olmstead, Evolution of the TCP gene family in Asteridae: cladistic and network approaches to understanding regulatory gene family diversification and its impact on morphological evolution, *Mol. Biol. Evol.* 20 (12) (2003) 1997–2009.
- [74] J.F. Smith, L.C. Hileman, M.P. Powell, D.A. Baum, Evolution of GCYC, a Gesneriaceae homolog of CYCLOIDEA, within Gesnerioideae (Gesneriaceae), *Mol. Phylogenet. Evol.* 31 (2) (2004) 765–779.
- [75] H.L. Citerne, M. Möller, Q.C.B. Cronk, Diversity of cycloidea-like genes in Gesneriaceae in relation to floral symmetry, *Ann. Bot. (Lond.)* 86 (2000) 167–176.
- [76] P.F. Stevens, Angiosperm Phylogeny Website. Version 8, 2001; <http://www.mobot.org/MOBOT/research/APweb/>, onwards.
- [77] D. Bradley, R. Carpenter, L. Copey, S. Rothstein, E. Coen, Control of inflorescence architecture in *Antirrhinum*, *Nature* 379 (1996) 791–797.
- [78] F. Cremer, The delayed terminal flower phenotype is caused by a conditional mutation in the CENTRORADIALIS gene of snapdragon, *Plant Physiol.* 126 (2001) 1031–1041.

- [79] J.I. Clark, E. Coen, The cycloidea gene can respond to a common dorsoventral prepattern in *Antirrhinum*, *Plant J.* 30 (6) (2002) 639–648.
- [80] J.A. Aguilar-Martinez, C. Poza-Carrion, P. Cubas, *Arabidopsis* BRANCHED1 acts as an integrator of branching signals within axillary buds, *Plant Cell Online* (2007).
- [81] L. Lukens, J. Doebley, Molecular evolution of the teosinte branched gene among maize and related grasses, *Mol. Biol. Evol.* 18 (4) (2001) 627–638.
- [82] L.C. Hileman, S. Drea, G. de Martino, A. Litt, V.F. Irish, Virus-induced gene silencing is an effective tool for assaying gene function in the basal eudicot species *Papaver somniferum* (opium poppy), *Plant J.* 44 (2) (2005) 334–341.
- [83] S. Wege, A. Scholz, S. Gleissberg, A. Becker, Highly efficient virus-induced gene silencing (VIGS) in California poppy (*Escholzia californica*): An evaluation of VIGS as a strategy to obtain functional data from non-model plants, *Ann. Bot. (Lond.)* 100 (2007) 641–649.
- [84] B. Gould, E.M. Kramer, Virus-induced gene silencing as a tool for functional analyses in the emerging model plant *Aquilegia* (columbine, Ranunculaceae), *BMC Plant Methods* 3 (6) (2007).