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Evolution of Perianth and Stamen Characteristics with Respect to Floral Symmetry in Ranunculales

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• *Background and Aims* Floral symmetry presents two main states in angiosperms, namely polysymmetry and monosymmetry. Monosymmetry is thought to have evolved several times independently from polysymmetry, possibly in co-adaptation with specialized pollinators. Monosymmetry commonly refers to the perianth, even though associated androecium modifications have been reported. The evolution of perianth symmetry is examined with respect to traits of flower architecture in the Ranunculales, the sister group to all other eudicots, which present a large diversity of floral forms.

• *Methods* Characters considered were perianth merism, calyx, corolla and androecium symmetry, number of stamens and spurs. Character evolution was optimized on a composite phylogenetic tree of Ranunculales using maximum parsimony.

• *Key Results* The ancestral state for merism could not be inferred because the basalmost Eupteleaceae lack a perianth and have a variable number of stamens. The Papaveraceae are dimerous, and the five other families share a common trimerous ancestor. Shifts from trimery to dimery (or reverse) are observed. Pentamery evolved in Ranunculaceae. Ranunculales except Eupteleaceae, present a polysymmetric ancestral state. Monosymmetry evolved once within Papaveraceae, Ranunculaceae and Menispermaceae (female flowers only). Oligandry is the ancestral state for all Ranunculales, and polyandry evolved several times independently, in Papaveraceae, Menispermaceae, Berberidaceae and Ranunculaceae, with two reversions to oligandry in the latter. The ancestral state for androecium symmetry is ambiguous for the Ranunculales, while polysymmetry evolved immediately after the divergence of Eupteleaceae. A disymmetric androecium evolved in Papaveraceae. The ancestral state for spurs is none. Multiple spurs evolved in Papaveraceae, Berberidaceae and Ranunculaceae, and single spurs occur in Papaveraceae and Ranunculaceae.

• *Conclusions* The evolution of symmetry appears disconnected from changes in merism and stamen number, although monosymmetry never evolved in the context of an open ground plan. In bisexual species, monosymmetry evolved coincidently with single spurs, allowing us to propose an evolutionary scenario for Papaveraceae.

Key words: Floral symmetry, Ranunculales, perianth, androecium, stamen, spur, merism, evo-devo.

INTRODUCTION

Numbering at least 250 000 species, the angiosperms form a successful taxonomic group that makes up the majority of the extant land flora. The flower is the characteristic trait of this group and is probably a key factor in its evolutionary success. This complex structure associates, on a condensed axis, organs of both sexes surrounded by a perianth that often takes a showy aspect, possibly as a co-adaptation with pollinators. The floral ground plan is conserved throughout the angiosperms, with the sterile pieces outermost, then the stamens and finally the carpel, an innovative structure enclosing the ovule. The only notable exception is *Lacandonia schismatica* E. Martinez and Ramos (Triuridaceae) where stamens are central and surrounded by carpels (Ronse De Craene *et al.*, 2003, and references therein). This quite invariant organization provides the opportunity to develop the plant evo-devo paradigm, namely the ABC model of floral organ identity (Coen and Meyerowitz, 1991), and more recent developments as reviewed in Soltis *et al.* (2006). In contrast, there is a striking amount of diversity in flower architecture. Endress (2001) suggested a trend in floral trait evolution from an 'open' ground plan where variation occurs in the number and arrangement of floral organs, towards a 'closed' ground plan where the number and arrangement are fixed. In such fixed structures, further elaborations can take place through synorganization, which means fusion or close connection of parts (Endress, 1990). Open ground plans are common in basal angiosperms and early diverging eudicots, while closed ground plans are the rule in core eudicot and monocot taxa.

An architectural trait that can be superimposed onto a fixed or, more rarely, an open ground plan, is symmetry. Two main types of symmetry are recognized among angiosperms, actinomorphy or radial symmetry (or polysymmetry) and zygomorphy or bilateral symmetry (or monosymmetry). Symmetry is generally defined for the perianth, reflecting human eye perception. However, the androecium may also be concerned, due to the reduction (staminode) or abortion of one to several stamens, as happens in core eudicots and monocots. Very often, the gynoecium has its own merism, possibly resulting in still different internal symmetry. Monosymmetry can be extended to flowers with

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spiral phyllotaxy viewed in a plane perpendicular to the pedicel, although strictly speaking, these flowers are asymmetric. Monosymmetric flowers appear relatively late in the fossil record, as compared with the accepted period for angiosperm early diversification (Late Cretaceous *vs.* Early Cretaceous; Endress, 1999). Polysymmetry is generally considered as the ancestral state for angiosperms, with monosymmetry having evolved several times independently. As a derived architectural trait generally associated with lineage diversification, it constitutes a morphological innovation. Changes in floral symmetry may result in changes in efficient pollinator range, which in turn can set up sexual barriers leading to speciation. Accordingly, some of the most speciose taxa harbour monosymmetric flowers, in core eudicots (e.g. Fabaceae, Asteraceae) or monocots (e.g. Zingiberales, Orchidaceae). These taxa generally have closed floral ground plans, with more or less elaborate structures. In contrast, monosymmetry is almost absent from basal angiosperms, and is quite rare in early diverging eudicots (Ranunculales, Proteales: Endress, 1999; Ronse De Craene *et al.*, 2003) where open ground plans are the most frequent compared with the core eudicots.

The increasing resolution of the phylogenetic relationships among angiosperms has opened the way for reconstructing character evolution by plotting traits onto phylogenetic trees, using parsimony or maximum likelihood methods. Several studies examined the evolution of various floral traits in basal angiosperms and eudicots, revealing that the patterns of perianth and stamen phyllotaxy are often closely associated (Doyle and Endress, 2000; Ronse De Craene *et al.*, 2003; Endress and Doyle, 2007). The present study focuses on the Ranunculales, to examine the architectural features allowing emergence of monosymmetry in a phylogenetic context. Ranunculales are sister to all other eudicots (Stevens, 2001 onwards) and include seven families. The most recent molecular phylogenetic studies indicate that the monogeneric Eupteleaceae are the sister group of the other six families,

although with a moderate bootstrap support (Ro *et al.*, 1997; Kim *et al.*, 2004), in contrast to earlier studies that placed the Papaveraceae *sensu lato* as the basalmost family. The order as a whole presents a huge range of floral forms, including diversity in merism, phyllotaxy and symmetry. Species with monosymmetric flowers occur in the Papaveraceae *s.l.* that are considered to have a closed ground plan in subfamily Fumarioideae, and in the Ranunculaceae that have an open ground plan (Endress, 1999; Ronse De Craene *et al.*, 2003). In this paper, the evolution of selected floral traits that are considered to be potentially linked to the evolution of symmetry are analysed. The present study includes a broad taxonomic sampling and takes into account the most recent improvement in family phylogenetic relationships. Evolutionary scenarios are discussed in relation with current knowledge in flower ontogeny and genetic determinants of flower architecture in eudicots.

MATERIALS AND METHODS

Composite tree

A detailed phylogenetic tree of the Ranunculales was obtained by assembling published trees of the different families of the Ranunculales into one. Composite trees include more taxa than individual trees and therefore provide better resolution for character optimization (e.g. Sillen-Tulberg, 1993; Weiblen *et al.*, 2000). The backbone of the composite tree (i.e. relationships among families) was obtained from the APG website (Stevens, 2001 onwards). Most trees that were used to generate the composite tree were based on molecular data, either from a single region or from the combined analysis of several regions (Table 1). The phylogeny used for the Menispermaceae results from a recent detailed cladistic analysis of morphological and anatomical characters (Jacques, 2006). Taxa within families were selected from the molecular phylogenies as to represent the floral morphological diversity of the family. All genera were represented by only one species, except *Epimedium* L. (Berberidaceae) and *Aquilegia* L. (Ranunculaceae) in which intra-generic variation in spurs was recorded. Since the composite tree resulted from an assemblage of trees obtained from heterogeneous datasets, branch lengths were not available. All branches were attributed a default length of 1.

Character optimization

The composite tree was used to examine the evolution of the following set of floral characters: merism, calyx symmetry, corolla symmetry, androecium symmetry, number of stamens and number of spurs. Table 2 gives the character states defined for each character considered. Symmetry was recorded independently from the organ phyllotaxy. When the perianth was not differentiated into a calyx and a corolla, which often happens in Ranunculales, we chose to code the symmetry under calyx symmetry, the corolla being considered as missing. The evolution of androecium

TABLE 1. *Phylogenetic trees used to generate the composite tree of Ranunculales*

Clade	Reference	Data type
Ranunculales	Stevens (2001 onwards)	*atpB*, *rbcL*, 18S nrDNA
Berberidaceae	[1] Kim *et al.* (2004)	*ndhF*
Lardizabalaceae	[2] Hoot *et al.* (1995)	*atpB*, *rbcL*, 18S nrDNA
Papaveraceae *s.l.* (incl. Fumariaceae)	[3] Hoot *et al.* (1997)	*atpB*, *rbcL*, *trnK* restriction sites, *rps16* intron, morphology
	[4] Liden *et al.* (1997)	
Menispermaceae	[5] Jacques (2006)	Morphology
Ranunculaceae	[6] Hoot (1995), [7] Ro *et al.* (1997)	*atpB*, *rbcL*, 18S and 26S nrDNA

The numbers before the references refer to the individual family trees that were grafted onto the backbone of the Ranunculales (see Fig. 1).

TABLE 2. *List of characters and character states examined in this paper*

Character	State 0	State 1	State 2	State 3
1. Merism	Variable	Trimerous	Dimerous	Pentamerous
2. Calyx symmetry	Polysymmetric	Monosymmetric	Disymmetric	
3. Corolla symmetry	Polysymmetric	Monosymmetric	Disymmetric	
4. Androecium symmetry	Polysymmetric	Monosymmetric	Disymmetric	
5. Stamen number	Over 10	10 or fewer		
6. Spurs	Absence	Single	Equal to merism	

Merism was considered with respect to the perianth only. For the dioecious Menispermaceae, calyx and corolla symmetry were coded as polymorphic (0/1) when appropriate since monosymmetry occurs only in female flowers.

symmetry was examined in the context of oligandry *vs.* polyandry, not considering whether the number of stamens is related or not to the perianth merism. The number of stamens is an extremely variable character in the Ranunculales, and we deliberately chose to use a binary coding: one state corresponds to a number equal or inferior to ten (oligandry), the other corresponds to a number superior to ten (polyandry). This coding is meant to reflect the common view that when the number of stamens exceeds ten, stamens are considered as 'numerous'. Character states for the species represented in the phylogeny were compiled mainly from Cronquist (1981) and from various electronic databases: eFloras (www.efloras. org), Angiosperm Phylogeny Website (Stevens, 2001 onwards) and Delta (Watson and Dallwitz, 1992 onwards). The characters were optimized on the composite tree with the maximum parsimony (MP) method implemented in the software Mesquite (Maddison and Maddison, 2006). The ML method is also implemented in this software but was not used for two reasons: (1) this method is optimal when branch lengths are known, which is not the case here; and (2) this method does not deal with polymorphisms, which occur twice in our matrix of characters. Phylogenetic trees were represented as rectangular cladograms, with species names coloured by groups according to the family, and branches coloured according to the ancestral state inferred from the MP reconstruction. Unresolved ancestral character states are indicated by yellow branches and can result either from missing character states in the present taxa or from ambiguities (more than one ancestral character state is found in the reconstruction).

RESULTS

Evolution of perianth merism

Merism is highly variable in the whole clade of Ranunculales, as shown by the number of states (four) that had to be defined, and is also a highly labile character (Fig. 1). The ancestral condition of this character for the whole clade could not be inferred, since the monogeneric Eupteleaceae that are sister to all other Ranunculales, have no perianth and display variation in the number of stamens. The ancestral condition for the rest of Ranunculales is equivocal, due to the splitting into two large clades each with a different ancestral condition: one is dimerous (Papaveraceae *s.l.*) and the other is trimerous. Within the dimerous clade,

trimery evolved at least once, in *Platystemon* Benth. Instability in the number of perianth parts (coded as variable) evolved twice, in *Arctomecon* Torr. & Frem. and *Sanguinaria* L. All these changes are nested within the clade that corresponds to the subfamily Papaveroideae. Trimery appears unambiguously as the ancestral condition for Lardizabalaceae, Menispermaceae (in which dimery evolved once, in *Cissampelos* L.) and Berberidaceae. In this latter family, dimery evolved once and characterizes the genus *Epimedium* and the perianth is absent in *Achlys* DC. The ancestral condition for the Ranunculaceae remains unresolved, due to the trichotomy of the dimerous *Glaucidium* Siebold & Zucc. and the trimerous *Hydrastis* Ellis ex L. together with the rest of Ranunculaceae that are ancestrally pentamerous. Dimery evolved at least twice in this pentamerous clade, in *Thalictrum* L. and *Clematis* L., and instability in the number of perianth parts evolved at least four times, in *Anemonella* Spach, *Trautvetteria caroliniensis* Fisch. & C.A.Mey., *Adonis* L. and *Cimicifuga* Wernisch. Some species of *Ranunculus* L. that were not included in the composite tree also show instability in the number of tepals, such as for instance *Ranunculus ficaria* L. (Salisbury, 1919).

Evolution of perianth symmetry

Due to the lack of a perianth in Eupteleaceae, the ancestral type of symmetry for the whole order could not be inferred (Fig. 2A). For the rest of Ranunculales, two types of monosymmetry evolved from a clearly polysymmetric condition of the corolla: monosymmetric corollas evolved once in the Ranunculaceae (in the ancestor of the sister genera *Aconitum* L. and *Delphinium* L.) and once in the Papaveraceae, within Fumarioideae (subfamily marked with a dot in Fig. 1). In this latter clade, our reconstruction shows that the transition from polysymmetry to monosymmetry involves an intermediate disymmetric state, followed by a reversion from monosymmetry to disymmetry in *Dactylicapnos macrocapnos* Hutchinson. Monosymmetry also evolved once in the dioecious Menispermaceae, in the female flowers of the sister genera *Cissampelos* and *Sarcopetalum* F. Muell. The male flowers of both genera are actinomorphic, which is why this character was coded as polymorphic for these taxa (Fig. 2A). In many genera of the Ranunculales, the perianth consists of a single whorl of tepals that were coded as sepals for practical reasons, without making any assumption concerning their homology with sepals or petals. The lack of a corolla in

these taxa accounts for the numerous yellow branches found. Concerning calyx symmetry, two transitions from polysymmetry to monosymmetry (indicated by the black bars on Fig. 2A) were found: one in the Menispermaceae, corresponding to the transition in corolla symmetry, and one in the Ranunculaceae, corresponding to the transition in corolla symmetry in the *Aconitum + Delphinium* clade. The calyx in Papaveraceae *s.l.* consists of two identical sepals and we coded it as actinomorphic, although strictly speaking, it should be qualified as disymmetric.
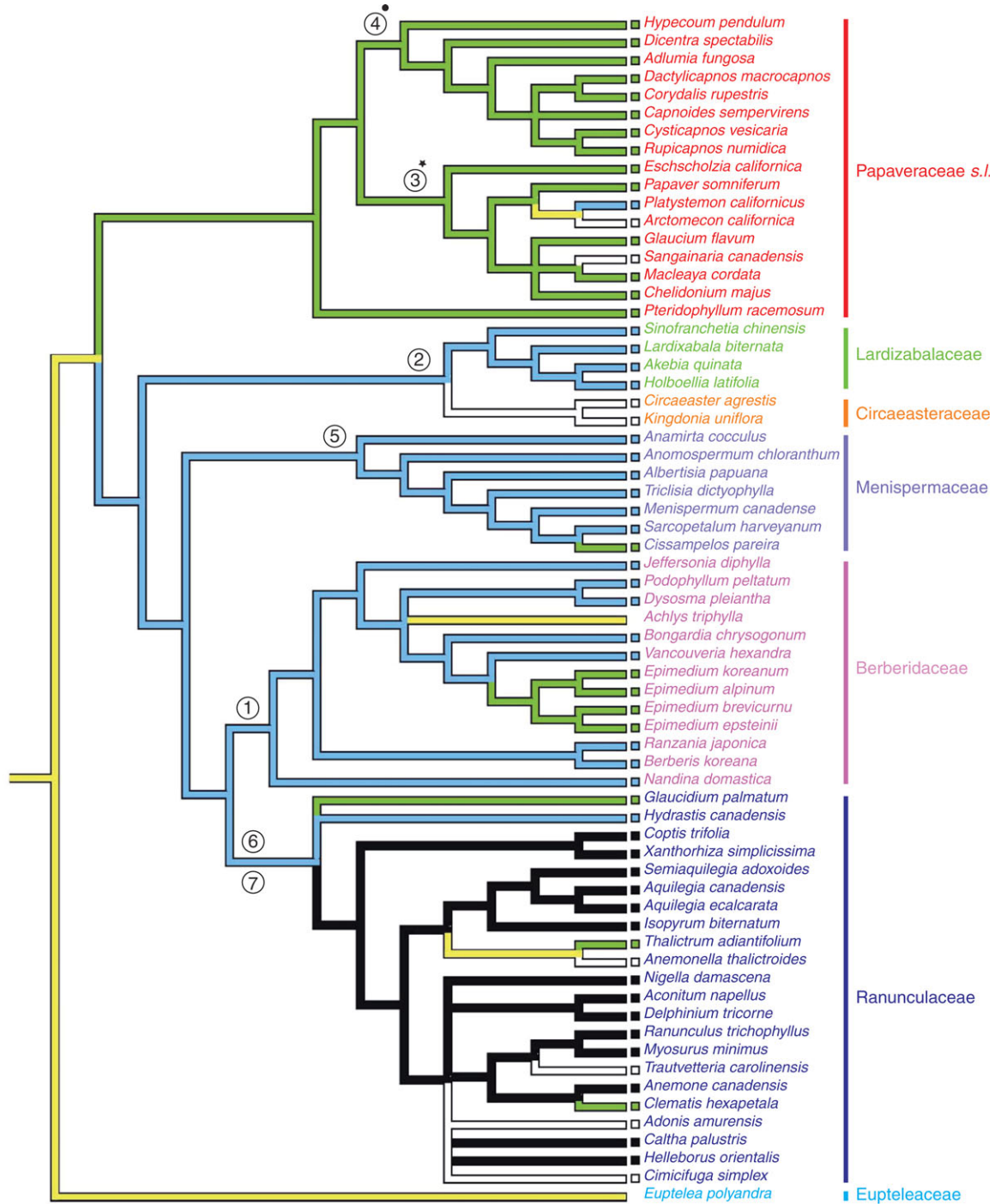

FIG. 1. Optimization of floral merism on the composite phylogenetic tree of Ranunculales, with maximum parsimony. The systematic position of the species (family) *sensu* APG is indicated on the right-hand side of the tree and species names are coloured according to the family. Circled numbers refer to the individual family trees that were grafted onto the backbone of Ranunculales. For the Papaveraceae *s.l.*, the two subfamilies are labelled with a dot (Fumarioideae) and a star (Papaveroideae). External nodes are coloured according to the character state observed, internal nodes are coloured according to the ancestral state inferred: white = variable (i.e. intra-specific variability); blue = trimerous; green = dimerous; black = pentamerous. Yellow branches correspond to unresolved ancestral character states.
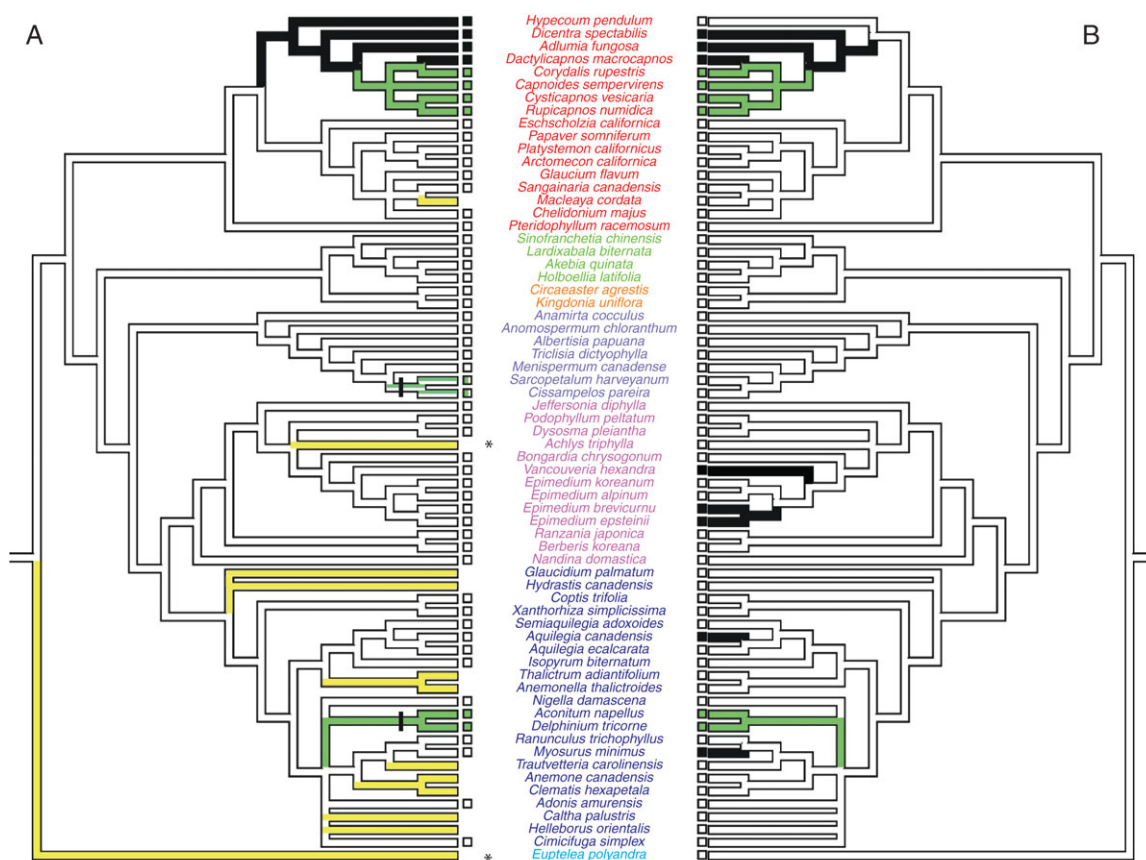
FIG. 2. Mirror trees showing the optimization of corolla symmetry (tree A) and spurs (tree B) on the composite phylogenetic tree of Ranunculales, with maximum parsimony. Switches in calyx symmetry are indicated by black bars on tree A and correspond to transitions from polysymmetry to monosymmetry. Species names are coloured according to the family to which they belong, as in Fig. 1. External nodes are coloured according to the character state observed, internal nodes are coloured according to the ancestral state inferred. Tree A (corolla symmetry): white = actinomorphic; green = monosymmetric; black = disymmetric. Species with a calyx and no corolla are always actinomorphic; species lacking a perianth altogether are labelled with a star. Tree B (spurs): white = absence; green = single; black = equal to merism. Yellow branches correspond to unresolved ancestral character states.

Evolution of spurs

From an ancestral condition with no spurs, spurs evolved four times in the order in two different ways (Fig. 2B). Single spurs evolved once in Ranunculaceae (*Delphinium* and *Aconitum*) and once within the subfamily Fumarioideae (Papaveraceae). Multiple spurs, with a number equal to the merism, evolved twice in Berberidaceae (*Vancouveria* C.Morren & Decne. and the ancestor of *Epimedium epsteinii* Stearn. and *E. brevicornu* Maxim.), twice in Ranunculaceae (*Myosurus* L. and *Aquilegia*) and once at the base of the Fumarioideae, with a reversal from single to double spurs in *Dactylicapnos macrocapnos* that corresponds to the reversal from monosymmetry to disymmetry in the corolla (Fig. 2A).

Evolution of the androecium

For stamen number, the ancestral state of Ranunculales, as inferred from the MP reconstruction, was resolved as ten or less (Fig. 3A). This suggests that the polymorphism in stamen number that occurs in Eupteleaceae is derived. The ancestral state for all other Ranunculales is unambiguously less than ten stamens. From this ancestral condition, numerous stamens (over ten) evolved at least seven times in Ranunculales: at least three times independently in Menispermaceae, once in Circaeasteraceae, in Berberidaceae, and once also in the Papaveroideae (subfamily marked with a star in Fig. 1) and in Ranunculaceae, numerous stamens being synapomorphic for both groups. In the latter family, two reversals to the ancestral state (oligandry) can be noted: one in *Myosurus* (ten stamens) and another one in *Xanthorhiza* Marshall (five stamens). The androecium has a radial symmetry in most Ranunculales with one transition towards disymmetry at the base of Fumarioideae (Fig. 3B). Eupteleaceae are described as having an androecium with two symmetry planes (Endress, 1986, 1999). Their sister group relationship with the other Ranunculales results in an ambiguous ancestral condition at the level of the order.

DISCUSSION

Ancestral floral states in the Ranunculales

Eupteleaceae, with their key position at the base of Ranunculales, were reasonably expected to provide information concerning the floral features of the ancestral
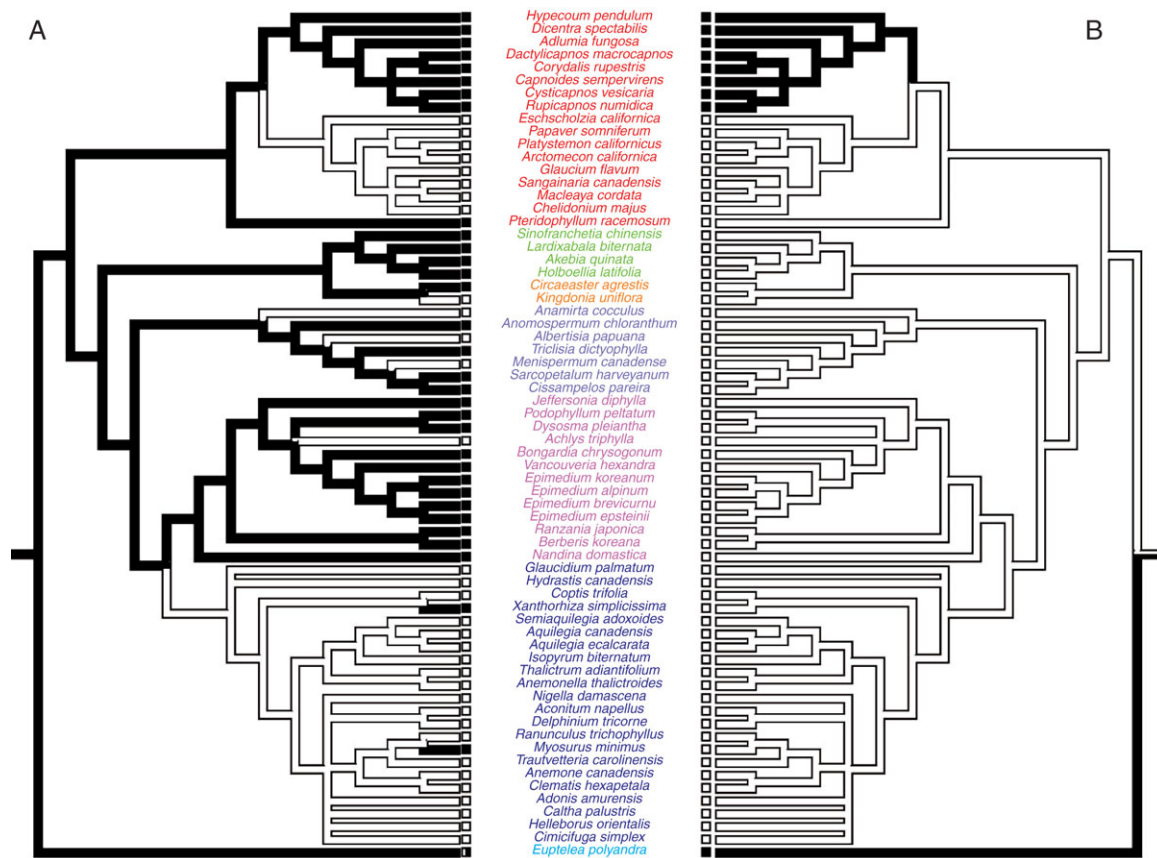
FIG. 3. Mirror trees showing the optimization of stamen number (tree A) and androecium symmetry (tree B) on the composite phylogenetic tree of Ranunculales, with maximum parsimony. Species names are coloured according to the family to which they belong, as in Fig. 1. External nodes are coloured according to the character state observed, internal nodes are coloured according to the ancestral state inferred. Tree A (stamen number): white = polyandry; black = oligandry. Tree B (androecium symmetry): white = actinomorphic; black = disymmetric.

Ranunculales. However, the unusual floral morphology found in this monogeneric family, with a combination of traits that are traditionally considered as ancestral (intra-specific variability in the number of stamens) and traits that are considered as derived (absence of a perianth and disymmetric androecium), makes it difficult to resolve the character states for the ancestor of all Ranunculales. It is likely that the disymmetric androecium of *Euptelea* Siebold & Zucc. represents a specialized feature rather than an ancestral condition, disymmetry being exceptional at the level of all angiosperms. Similarly, although the lack of a perianth occurs in species distributed across several families of the Ranunculales (see Fig. 1), this character state appears as derived within the families. The perianthless condition in *Euptelea* could then result from a loss rather than an inheritance from the ancestor of all Ranunculales.

Overall, in spite of the good representation of floral diversity within Ranunculales, our reconstruction of merism evolution does not allow us to improve the resolution at the deepest nodes of the order, compared with previous studies (Drinnan *et al.*, 1994; Ronse De Craene *et al.*, 2003; Soltis *et al.*, 2003). However, this study provides a better picture of the diversity, especially concerning the frequency of the transitions to variable from a dimerous

(Papaveroideae), trimerous (Circeasteraceae) or pentamerous (Ranunculaceae) condition (Fig. 1). It has also led us to reappraise the character state for merism in a few species compared with previous studies. For example, *Circaeaster* Maxim. was coded as trimerous in Soltis *et al.* (2003) but due to the flexible number of floral parts within each whorl (Tian *et al.*, 2006), as in the related *Kingdonia* Balf.f. & W.W.Sm. (Ren *et al.*, 2004), we chose to code merism as variable in both species, resulting in an ancestral variable state for Circeasteraceae as a whole. *Hydrastis canadensis* was coded as dimerous in Drinnan *et al.* (1994) as well as in Ronse De Craene *et al.* (2003), although it is described as trimerous in different floras, hence our coding of this species as trimerous (Fig. 1). Considering that the base of Ranunculaceae is an unresolved trichotomy between the trimerous *Hydrastis*, the dimerous *Glaucidium* and the rest of the family that is mostly pentamerous, it is tempting to suggest that this pentamerous state might result from the superposition of dimery and trimery, as had been suggested by Drinnan *et al.* (1994).

Concerning the number of stamens, the reconstruction suggests that the ancestral condition for the deepest nodes of Ranunculales is a reduced number of stamens from which polyandry has evolved repeatedly throughout the

order (Fig. 3A). Here again, it is impossible to ascertain whether the variable number of stamens observed in *Euptelea* represents a plesiomorphic condition or a specialized feature.

Merism and flower symmetry

From our reconstructions, the evolution of perianth merism and symmetry, whether at the androecium or the perianth level, do not appear to be particularly linked (compare Fig. 1 with Figs 2A and 3B). However, whereas shifts in symmetry occur equally within dimerous (Papaveraceae *s.l.*), trimerous (Menispermaceae) or pentamerous (Ranunculaceae) clades, flowers with a variable merism are consistently regular. Following the view of Endress (2001) that floral organization in angiosperms has evolved from a relatively open ground plan to a more closed one, this suggests that the evolution of monosymmetry in Ranunculales was made possible only after fixation of the floral ground plan. Interestingly, variation in the number of floral parts in open ground plans is usually associated either with a spiral phyllotaxy or a variable number of whorls within each type of organ. It is noteworthy that in basal angiosperms (including basal eudicots), organ identity is not always completely fixed, with easy shifts between the different whorls, as for example the shift between petals and stamens observed in *Enemion* Raf. (Tucker and Hodges, 2005). Similarly, expression of the MADS-box genes responsible for the A, B and C functions, which, alone or in combination, specify the identity of floral organs in the core eudicots, appears much less organ specific in basal angiosperms and basal eudicots than in core eudicots (Kramer and Irish, 1999, 2000; Kramer *et al.*, 2003; Jaramillo and Kramer, 2004; Kim *et al.*, 2005).

Androecium and flower symmetry

One interesting thing revealed by the present results is that the shifts in androecium symmetry and stamen number are not necessarily associated with shifts in perianth symmetry (compare Fig. 2A and Fig. 3). The ancestral state for androecium symmetry is unresolved, while it is ten or less (oligandry) for stamen number (Fig. 3). In the Papaveraceae, androecium disymmetry evolved in the Fumarioideae from an actinomorphic ancestral state observed in *Pteridophyllum* Siebold & Zucc., where the androecium consists of four diagonally placed dithecal stamens. In *Hypecoum* L. the androecium consists of two whorls of two dithecal stamens opposite the two whorls of petals, with the larger stamens opposite the inner petals. In the remaining Fumarioideae species (Corydaleae and Fumarieae tribes), six stamens are associated in two sets of three in a transverse position (opposite the outer petals). Each set is composed of a central dithecal stamen and two monothecal stamens, whose filaments may be partially fused (Ronse De Craene and Smets, 1992). Polyandry evolved as a synapomorphy of the Papaveroideae (Fig. 3A). However, different ontogeneses account for adult multistaminate androecia in various genera, involving a ring primordium (*Papaver* L.) or

successive centripetal whorls [*Macleaya* R.Br., *Eschscholzia* Cham. (Ronse De Craene and Smets, 1990; Becker *et al.*, 2005)]. Polyandry appears as the ancestral state for the whole Ranunculaceae (Fig. 3A). All taxa exhibiting polyandry are reported with an actinomorphic androecium, whether the phyllotaxy is whorled [Lardizabalaceae, Menispermaceae, Berberidaceae except *Achlys* (Feng and Lu, 1998), Papaveroideae, some Ranunculaceae], spiral [*Kingdonia* (Ren *et al.*, 2004), Ranunculaceae] or chaotic [*Achlys* (Endress, 1989; Ronse De Craene and Smets, 1993)].

Monosymmetry at the perianth level evolved in female flowers only in the *Sarcopetalum* + *Cissampelos* clade (Menispermaceae) and in the context of oligandry in the Fumarioideae, and of polyandry in the *Aconitum* + *Delphinium* clade (Ranunculaceae) (Fig. 2A and Fig. 3A). This latter point is in contrast with reports in monocots and core eudicots, where the evolution of monosymmetry is described in the context of oligandry, usually associated with changes including stamen reduction to staminodes or even abortion (Endress, 1997, 1999; Rudall and Bateman, 2004).

In core eudicots, remarkable progress has been achieved in understanding the genetic mechanisms underlying floral symmetry, through the initial work on the model species *Antirrhinum majus* (Asterids) and the most recent work on *Lotus japonicus* (Rosids). Monosymmetry is thought to have evolved independently in these two species, affecting both corolla and androecium in different respects. *Antirrhinum majus* has a sympetalous pentamerous flower, with a dorso-ventral differentiation of the petals into two lips generating monosymmetry. The androecium consists of four fully developed stamens and one dorsal staminode. In this species, the interplay of four genes belonging to two families of transcription factors appears to be responsible for the full achievement of monosymmetry in corolla and androecium (Corley *et al.*, 2005). CYCLOIDEA (*CYC*) appears as the master gene (Luo *et al.*, 1995), retarding growth in the dorsal domain of the floral meristem, and inhibiting the development of the dorsal stamen; its paralogue DICHOTOMA (*DICH*) has a partially redundant function (Luo *et al.*, 1999). *Lotus japonicus* has a typical papilionoid flower, with three distinctive types of free petals, a dorsal one (standard), two lateral (wings) and two lower ones (keel petals). The androecium is diadelphous, the dorsal stamen being free from the tube joining the nine other ones. *LjCyc2*, a *CYC* paralogue, has recently been shown to be expressed in the dorsal domain of floral primordia and to play a role in the dorsal identity of the petals, similarly to *CYC* (Feng *et al.*, 2006). In a few other species, a role of *CYC*-like genes in floral monosymmetry has been demonstrated or suspected through shifts in gene expression associated to shifts in symmetry. A naturally occurring mutant of floral symmetry has been known for a long time in *Linaria vulgaris*, a close relative of *Antirrhinum*. It was shown that a defect in the *LCYC* gene, consisting in an extensive methylation of the gene associated with transcriptional silencing, is involved in the shift from monosymmetry to polysymmetry (Cubas *et al.*, 1999). As in *A. majus*, the *LCYC* gene appears to be involved in the

dorsal identity of the petals and in the repression of the dorsal stamen development. *Mohavea confertiflora*, also a close relative of *A. majus*, has a superficially actinomorphic corolla and only two functional stamens, the dorsal and lateral ones being aborted. An extension of the expression domain of orthologues of *Cyc* and *Dich* was observed, which could account for the changes in flower morphology, provided that the genes have equivalent function in the two species (Hileman *et al.*, 2003). Similarly in *Cadia purpurea*, an actinomorphic papilionoid species, an extension of the expression domain of *LegCyc* genes was observed, as compared with the dorsal expression observed in the monosymmetric close relative *Lupinus nanus*. In addition, while in *L. nanus* the three dorsal stamens are shorter than the lateral and ventral ones resulting in monosymmetry at the androecium level, all stamens have equal length in *C. purpurea* (Citerne *et al.*, 2006). In the *L. vulgaris* mutant, *M. confertiflora* and *C. purpurea*, the shift in symmetry can be interpreted as a result of homeotic transformations, leading to the expansion of the ventral identity within the flower in the former, and of the dorsal identity in the two latter (Hileman *et al.*, 2003; Citerne *et al.*, 2006).

A possible role of *Cyc*-like genes in the evolution of flower symmetry in angiosperms has been a tempting hypothesis that prompted molecular evolution studies, first in various asterid and rosid lineages (Citerne *et al.*, 2000, 2003; Reeves and Olmstead, 2003; Howarth and Donoghue, 2005), and more recently in Ranunculales, Caryophyllales, Saxifragales and also Poales in the monocot clade (Howarth and Donoghue, 2006; Damerval *et al.*, 2007). Phylogenetic analyses of the gene family support at least three major clades in the eudicots, associated with supplementary taxon-specific duplications and gene losses. Two of the major clades (one of which includes *Cyc*, *Dich* and *LjCyc2*) would have originated through a duplication that might have taken place at the base of core eudicots (Howarth and Donoghue, 2006; Damerval *et al.*, 2007). In the Papaveraceae, a duplication of *Cyc*-like genes independent from the one observed in the core eudicot gave rise to two paralogous lineages, *PapaCyL1* and *PapaCyL2*. Gene expression has been investigated in three species illustrating the three types of symmetry in the family. A specific expression in the outer petals was observed in both non-actinomorphic species and seems to be correlated with corolla symmetry.

Expression in stamens was variable according to the species, and apparently not related to androecium symmetry (Damerval *et al.*, 2007). Gene expression analyses are needed in a broader taxonomic sampling to examine whether this expression during floral development (and the possible role in floral symmetry) is an ancestral trait of *Cyc*-like genes, or an independently derived trait in various eudicot taxa.

Spurs and flower symmetry

We studied the evolution of spurs as an architectural trait of the perianth possibly linked to flower symmetry. Albeit of various ontogenetic origins, the spurs function as nectar collectors, in Ranunculales as in other angiosperms. The reconstructions presented show that spurs evolved in the context of a fixed merism (Fig. 2B), dimerous (Papaveraceae and *Epimedium*), trimerous (*Vancouveria*) or pentamerous (Ranunculaceae) (compare Fig. 1 and Fig. 2). Moreover, this evolution is not necessarily associated with monosymmetry in Ranunculales (Fig. 2), insofar as multiple spurs occur in actinomorphic corollas independently in three different families (Papaveraceae, Berberidaceae and Ranunculaceae), a phenomenon that is quite unusual among angiosperms (Endress, 1994). Nevertheless, single spurs are only found in monosymmetric flowers of bisexual species. Based on these patterns, a scenario for the evolution of flower symmetry can be proposed in the Papaveraceae (Fig. 4): the ancestral state is actinomorphy without spur (A: *Pteridophyllum racemosum* and Papaveroideae), then disymmetry is acquired at the base of the Fumarioideae in *Hypecoum* (B) through differentiation of the morphology of the two petal whorls; a next step is the evolution of two symmetric nectar spurs formed by the outer petals (C), meaning that these spurs are in transverse position; monosymmetry then evolves (D) – with a reversion in *Dactylicapnos* – by reduction
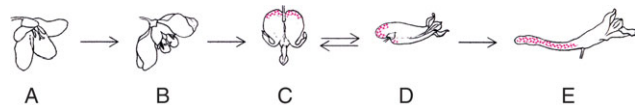

FIG. 4. Proposed scenario for the evolution of flower symmetry in the Papaveraceae. (A) Ancestral actinomorphic state: the petals assume the same shape in both whorls (*Pteridophyllum racemosum*). (B) Evolution of disymmetry through differentiation of the two petal whorls at the base of the Fumarioideae (*Hypecoum pendulum*). (C) Evolution of two nectar spurs in disymmetric flowers [*Lamprocapnos spectabilis* (L.) Fukuhara]. (D) Reduction of one of the spurs leading to monosymmetric flowers [*Capnoides sempervirens* (L.) Borckh]; possible reversion of this reduction is observed in *Dactylicapnos* sp. (E) Development of a single nectar spur in monosymmetric flowers (*Corydalis* sp.). Nectar spurs are indicated in colour.

FIG. 5. Flower of *Cysticapnos vesicarius* (L.) Fedde showing the dorsal nectary pouch (white arrow) and the bilabiate corolla. Scale bar = 5 mm (photography by Martine Le Guilloux).

of one of the spurs, associated with a rotation of the flower pedicel that brings the spur in an apparent dorsal position (Ronse De Craene and Smets, 1992) and a differential curvature of the outer petals resulting in two more or less differentiated lips. A large diversity of spur shape is encountered in the Corydaleae and Fumarieae (Liden, 1986), where the single spur can become extremely proeminent (E) while in other species such as *Cysticapnos vesicarius* (L.) Fedde, it can be reduced to a dorsal pouch (Fig. 5).

Within the Ranunculaceae, single and multiple spurs evolved independently and have various origins (Fig. 2B). Multiple spurs are found in some *Aquilegia* species and in the genus *Myosurus*. In the first case, the spurs are developed by the second petaloid whorl (Tucker and Hodges, 2005), whereas in the second, they are developed by the sepals. A single spur of complex structure is observed in the *Aconitum* + *Delphinium* clade, where the calyx is pentamerous and the upper sepal differentiates into a hood or a spur that encloses spurs developed by the two upper petals. Monosymmetry of the perianth in *Delphinium* and *Aconitum* is thus achieved through a dorso-ventralization of the flower mostly due to calyx spur or hood development, in sharp contrast with the Papaveraceae. Very few data are available concerning the genetic determinants of spurs. Mutations in two *KNOX* homeobox genes were reported in *A. majus*, that affect the corolla tube development, resulting in an invagination that resembles a spur in its morphology and position (Golz *et al.*, 2002). Whether homologues of these genes may be involved in spur development in other taxa is not known.

Thus in bisexual Ranunculales, perianth monosymmetry evolved in taxa of fixed but different merism, coincidently with a single nectar spur. It is noteworthy that in Ranunculaceae, where nectaries are borne on the petals, the evolution of perianth monosymmetry is not associated with shifts in androecium structure. In this family, the androecium generally consists of numerous stamens of indefinite number that are produced in a spiral phyllotaxy (or whorled in the case of *Aquilegia*), resulting in an open androecium ground plan that must have precluded the evolution of any symmetry plane. In contrast in the Papaveraceae, the presence of nectaries at the base of the stamen in an oligomerous disymmetric androecium may have offered a favourable context for the evolution of multiple nectar spurs, then reduction to a single one generating monosymmetry.

Monosymmetry was already considered as a highly homoplastic trait among the angiosperms. The present results indicate that this is also the case at the level of the Ranunculales and, moreover, that the associated evolutionary trends observed in this group are quite different from those recorded in monocots and core eudicots (Donoghue *et al.*, 1998; Rudall and Bateman, 2004). Such results should be kept in mind for evo-devo studies aiming at understanding the evolution of the genetic determinants of monosymmetry in the Ranunculales and beyond.

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