Environmental control of sepalness and petalness in perianth organs of waterlilies: a new Mosaic Theory for the evolutionary origin of a differentiated perianth

Kate A. Warner1,2, Paula J. Rudall2 and Michael W. Frohlich2,*
1 Department of Botany, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
2 Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK

Received 5 February 2009; Revised 21 May 2009; Accepted 21 May 2009

Abstract

The conventional concept of an ‘undifferentiated perianth’, implying that all perianth organs of a flower are alike, obscures the fact that individual perianth organs are sometimes differentiated into sepaloid and petaloid regions, as in the early-divergent angiosperms Nuphar, Nymphaea, and Schisandra. In the waterlilies Nuphar and Nymphaea, sepaloid regions closely coincide with regions of the perianth that were exposed when the flower was in bud, whereas petaloid regions occur in covered regions, suggesting that their development is at least partly controlled by the environment of the developing tepal. Green and colourful areas differ from each other in trichome density and presence of papillae, features that often distinguish sepals and petals. Field experiments to test whether artificial exposure can induce sepalness in the inner tepals showed that development of sepaloid patches is initiated by exposure, at least in the waterlily species examined. Although light is an important environmental cue, other important factors include an absence of surface contact. Our interpretation contradicts the unspoken rule that ‘sepal’ and ‘petal’ must refer to whole organs. We propose a novel theory (the Mosaic theory), in which the distinction between sepalness and petalness evolved early in angiosperm history, but these features were not fixed to particular organs and were primarily environmentally controlled. At a later stage in angiosperm evolution, sepaloid and petaloid characteristics became fixed to whole organs in specific whorls, thus reducing or removing the need for environmental control in favour of fixed developmental control.

Key words: Environment, Nymphaea, Nuphar, organ identity, perianth evolution, petals, Schisandra, sepals, tepals.

Introduction

The perianth is an important element of most flowers. It consists of several sterile, dorsiventrally flattened organs (phyllomes) that surround the fertile floral organs. The perianth typically functions both to protect the immature reproductive organs, and to attract pollinators and direct them to the reproductive structures. In the majority of angiosperms, particularly the eudicots, the perianth is bipartite and differentiated into two (or more) distinct series or whorls that show different morphologies: an outer whorl of sepals (the calyx) and an inner whorl of petals (the corolla). By contrast, many early-divergent angiosperms, monocots, and some basal eudicots show little or no morphological distinction between the outer and inner perianth series. In these cases, the perianth members are collectively termed tepals, a term devised by AP de Candolle in 1827.

The term ‘tepal’ implies that the perianth organs from an individual flower are morphologically similar and not differentiated into sepals and petals. This condition occurs in many monocots, such as most Alliaceae, Amaryllidaceae, Hypoxidaceae, and Liliaceae, in which all the tepals are entirely petaloid. However, in other taxa, including some monocots, the tepals show a range of morphology within a single flower and/or are arranged spirally rather than in...
whorls, so differentiation between an outer and an inner perianth series is difficult to infer. For example, in many magnolilods there is a gradual transition from outer, green (i.e. sepaloid) tepals through to inner, coloured (i.e. petaloid) tepals (Dandy, 1927; Endress, 2001; Ronse De Craene et al., 2003).

Concepts for classifying perianth organs include an unstated assumption that the characteristics of sepalness (possessing sepal-like characters) and petalness (possessing petal-like characters) must be fixed to entire individual organs. However, our observations on the early-divergent angiosperms Nuphar L., Nymphaea L. (Nymphaeaceae), and Schisandra Michx. (Schisandraceae) indicate that sepaloid (green) and petaloid (colourful) patches can both occur on the same individual perianth organs, often delimited by sharp boundaries (Warner et al., 2008). In addition to the colour differences, these regions show morphological differences consistent with sepal-like and petal-like characters. Furthermore, it was observed that, in the waterlilies Nuphar and Nymphaea, the region of tepal that is exposed when the flower is in bud ultimately becomes sepaloid, whereas nearly all of the covered tissue becomes petaloid. This characteristic was also noted in Nymphaea by Conard (1905), who described the link between exposure and the presence of the sepal-like areas in this genus, although our observations on Nuphar appear to be novel. The positions of these sepal-like and petal-like patches on the tepals of Nuphar and Nymphaea led us to suspect that environmental factors such as light or physical contact could determine their developmental fates. In Schisandra, petaloid areas predominantly occur in the covered regions of the perianth, but they can also develop in exposed areas, so it is unclear whether environmental factors control the development of these areas.

To test our hypothesis of environmental control, a morphological study of normal flower buds in Nymphaea, Nuphar, and Schisandra was performed to document the characteristics, precise locations, and developmental trajectories of the sepal-like and petal-like patches. Developing buds in the field and greenhouse were also experimentally manipulated to determine whether changes in the environment of a tepal can affect the development of sepal-like and petal-like patches. Experiments were designed primarily to test whether exposure to light initiates sepaloid characteristics in Nuphar, Nymphaea, and Schisandra. Our observations on flower buds of Nuphar show that areas of the perianth that would normally be green are instead yellow if they are covered by natural debris. Most plants require light as a cue to initiate chlorophyll synthesis, and the presence of chlorophyll is an important sepal characteristic. In our study genera, light could control the development of chlorophyll synthesis in addition to any other morphological sepal-like characteristics.

Morphology of sepals and petals
The morphology of sepals and petals varies across the angiosperms, but if both are present, the green sepaloid organs enclose the colourful petaloid ones, rather than vice versa. Several characters are used collectively to distinguish between sepals and petals, although these are not always present, due to the variety of different forms of perianth organ (as discussed in Endress, 1994b, 2001, 2005, 2008). In fact, as Endress (1994b, page 26) stated, ‘If treated in isolation, there is no character combination which could stringently prove an organ’s nature as a petal or sepal’. Characters that distinguish petals from sepals include: organ colour (sepals green versus petals colourful), epidermal cell type (sepal cells flat versus petal cells conical), location in the flower (sepals outer versus petals inner), function (sepals protective versus petals attractive), vasculature (sepals three-traced versus petals one-traced), and width of insertion on the receptacle (sepals broad versus petals narrow) (Smith, 1928; Bierhorst, 1971; Endress, 1994b, 2001, 2005). More recently, another character can be added: gene expression (B-class genes not expressed in sepals versus B-class genes expressed in petals). Admittedly, gene expression has been studied in very few species, of which the majority are eudicots (Coen and Meyerowitz, 1991; Soltis et al., 2002, 2005; Jaramillo and Kramer, 2004; Drea et al., 2007). Furthermore, the actual specification of petal identity has been proved in only a handful of cases. However, B-gene expression is absent from sepals of a diverse range of taxa, including the magnolid Asimina, which has B expression in the petals but not the sepals (Kim et al., 2005). The clear correlation between petals and B-gene expression has been attributed to the independent recruitment of B-genes to specify petals (Kramer and Irish, 2000).

Evolutionary origin of the differentiated (bipartite) perianth
There has long been discussion about the evolutionary origin of sepals and petals (Arber, 1937; Eames, 1961; Bierhorst, 1971; Meeuse, 1973; Takhtajan, 1991; Endress, 1994a, 2001, 2006). Based on comparative morphology, most authors agree that sepals are evolutionarily derived from bracts or leaves, but petals can be derived either from bracts or from stamens. The improved molecular phylogenetic context for flowering plants (Qiu et al., 2000, 2005; Angiosperm Phylogeny Group II, 2003; Borsch et al., 2003; Hilt et al., 2003) supports previous studies (Hiepko, 1965; Takhtajan, 1991; Kosuge, 1994) suggesting that a bipartite perianth has evolved multiple times within the angiosperms, and that petals are derived from different organs in different groups. In some groups, sepals and petals are homologous structures, since both are derived from bracts or leaves; in these cases the petals are termed bracteopetals. In other taxa, the petals are derived from stamens (termed andropetals) and hence are non-homologous with the sepals. Tepals can also be classed as andropetals or bracteopetals depending upon whether they have a stamen-like or leaf-like morphology (Hiepko, 1965; Takhtajan, 1991; Kosuge, 1994; Zanis et al., 2003).
Studies on early-divergent angiosperms allow the reconstruction of probable character states at the base of the extant angiosperms, and thus allow us to hypothesize evolutionary scenarios for the origin of the angiosperm flower (Endress, 2001; Soltis et al., 2002; Rudall et al., 2007, 2009; Albert et al., 2005; Bateman et al., 2006; Frohlich and Chase, 2007; Endress and Doyle, 2009). Recent molecular phylogenies (Qi et al., 2000, 2005; Angiosperm Phylogeny Group II, 2003; Borsch et al., 2003; Hilu et al., 2003) have demonstrated strong support for the placement of Amborellaceae and Nymphaeales (including Nuphar and Nymphaea) as the successive sisters to the remaining extant angiosperms (Soltis et al., 2005). A clade consisting of Austrobaileyaceae, Illiciaceae, Trimeniaceae, and Schisandraceae (including Schisandra) is sister to all other angiosperms except Amborella and Nymphaeales (Fig. 1). Environmentally controlled differentiation of sepaloid and petaloid regions within individual perianth organs observed in these early-divergent genera could, therefore, have been a trait of ancestral angiosperms.

Materials and methods

Collection and survey

Buds of Nymphaea caerulea Savigny, Nuphar advena Aiton, and Schisandra rubriflora Rehder and EH Wilson were obtained from the living collections at the Royal Botanic Gardens, Kew (see Supplementary Table S1 at JXB online for collection information). Buds of Nuphar lutea Sm. were also collected from field sites in Battle and Small Hythe (UK). Early-stage N. lutea buds (0.1–0.5 cm wide) were removed from between leaf bases. Very young buds (0.1–0.5 cm wide) were not available.

The external morphology of the perianths was recorded in the field by photography, after marking the boundaries of the exposed tepal regions with a series of pin pricks. The buds were photographed from all sides, then the tepals were folded down and removed in phyllotactic sequence, photographing the flower again at each step. Tepals were numbered from the outermost to the innermost. Visible differences between the exposed and unexposed areas were recorded. Orientation of the bud in photographs was tracked by a large-headed pin inserted through the pedicel. The material was fixed in Formalin Acetic Alcohol (FAA) for at least 48 h, and stored in 70% ethanol.

Experimental manipulation of flower buds

To test the effect of light, experiments were carried out on closed flower buds of Schisandra rubriflora, S. sphenanthera Rehder and EH Wilson, Nuphar (N. lutea and N. advena), and Nymphaea caerulea (see Supplementary Table S1 at JXB online for accession numbers). One or two areas of inner tepal were exposed (the Experimentally Exposed area/s, here designated ‘EE’) either by making a hole in the covering tepal using a razor blade, or by removing the outermost tepal. The buds were enclosed either (i) in a clear plastic bag so that the EE area was exposed to light (non-covered buds), (ii) in an opaque material (black plastic or aluminium foil) so that the EE area was not exposed to light (covered buds), or (iii) for buds with two regions of experimentally exposed inner tepal, the buds were covered so that one of the regions was exposed to light (through clear plastic) while the other was not (half-covered buds). Buds were left in situ for 3–18 d until they were about to open. At collection, the boundary of the EE areas on each experimentally exposed tepal was marked using pin pricks so that it would be apparent after fixation.

Sectioning and light microscopy

Two young normal flower buds of Nuphar lutea (1 cm in width) were prepared for serial sectioning. The buds were embedded in paraplast wax using standard methods and serial sections (14–18 μm thick) were cut using a Reichert Jung 2040 rotary microtome. Sections were stained with Alcian Blue and safranin, and mounted in DPX. Tepals from tested and untested buds of Nymphaea caerulea, Nuphar advena, and N. lutea were also hand-sectioned and mounted onto slides using water or glycerol. Slides were examined and photographed using a Leica Diaplan photomicroscope or a Leica microscope with attached Cannon Powershot G5 digital camera.

Scanning electron microscopy (SEM)

Individual tepals or entire buds were examined using a Hitachi S-4700 II coldfield emission SEM at the Jodrell Laboratory, Royal Botanic Gardens Kew. Material was dissected in 70% ethanol, dehydrated through an ethanol series and dried using a Baltec 030 Critical Point Dryer (CPD). Specimens were mounted on stubs and coated with platinum using an Emitech K550 sputter coater. Composite photographs were made of some buds and tepals using Adobe Photoshop.
Results

Nuphar lutea: differences between sepaloid and petaloid patches within individual tepals of non-experimental buds

Different regions of the abaxial tepal surface were distinguished by (i) whether they are covered by other tepals or naturally exposed when the flower is in bud, and (ii) their colour and appearance immediately prior to anthesis. Based on these two criteria, three types were identified in Nuphar and Nymphaea caerulea: (i) exposed green (EG) patches, (ii) covered green (CG) patches, and (iii) covered non-green (CNG) patches.

In all buds examined, the entire abaxial surface of the outermost tepal (tepal 1) was EG, whereas the inner four tepals (tepals 2–5) possessed EG, CG, and CNG patches (Fig. 2C). The position, shape, and size of the three patches varied between tepals of the same bud depending upon the arrangement of their overlying tepals (see Supplementary Fig. S2 at JXB online). In mature buds, the green (EG and CG) and non-green (CNG) areas were morphologically distinct. All buds >0.5 cm in width had EG patches with glandular trichomes (hairs), domed epidermal cells, and chlorophyll (Fig. 2H). CG patches also possessed trichomes and chlorophyll, but epidermal cells were slightly less domed (Figs 2A, L, M, 3A–D). By contrast, CNG patches lacked glandular trichomes and the epidermal cells were relatively flat (Fig. 2I). The EG and CG patches had sharp boundaries which seem to follow the impressions left by the perianth organ that covered the tepal when the flower was in bud (Fig. 3B–D). Transverse sections of N. lutea and N. advena tepals show that EG patches also had a relatively thicker upper cuticle than the CNG areas. Stomata were present in all three areas.

Morphological differences arose between the three areas as the flower bud matured. Glandular hairs developed from the base to the tip of the tepals until, by c. 0.25 cm in diameter, they were densely spread across the entire surfaces of the EG and CG areas (Fig. 2K–M). These trichomes were present on the tepals of buds that had not yet been exposed to light and were entirely pale yellow (Fig. 2D). At c. 0.5 cm in width, chlorophyll began to develop from the base to the tip of the EG and CG areas and the number of developing glands dramatically decreased (see Supplementary Fig. S1 at JXB online). By c. 2 cm in width, only mature glandular hairs were present and the EG and CG patches were entirely green. The CNG patch remained yellow-green until c. 2 cm in bud width, when it began to turn yellow, just before the bud had begun to open (Fig. 2A, C). After the buds opened, the green patches started to turn yellow from their tips downward until only a small patch of green was left at the base of the tepals in open flowers.

By contrast, the adaxial tepal surfaces were relatively uniform and entirely pale yellow throughout bud development. pavement cells at the tepal bases were circular in surface view; cells further towards the tip were more irregularly shaped (Fig. 2J). Stomata were occasionally present, but no epidermal protrusions were present.

Nuphar lutea and N. advena: tepal regions experimentally exposed to light develop sepaloid characteristics

In Nuphar, only non-covered experimental buds formed sepaloid characteristics in their experimentally-exposed areas. In the four half-covered buds that showed a reaction to exposure, only the EE area subjected to light turned green, while the covered EE area was yellow and/or did not differ from the remaining area of tepal (see Supplementary Table S4 at JXB online). Twelve of the 26 non-covered buds developed green patches in their EE areas and differed from their the surrounding covered area of tepal (Table 1). SEM examination on three non-covered buds (N3 bud 4, NA1 and NA2; see Supplementary Table S2 at JXB online) revealed that the EE areas also had domed pavement cells similar to the green areas (EG and CG patches) of untested buds. By contrast, the area of tepal surrounding the EE areas had flat cells and resembled CNG patches (Fig. 2N). In bud NA 2, the EE area was dark green and closely resembled EG areas of the outer tepals (Fig. 2E, F; see Supplementary Table S2 at JXB online). In this bud, the completely covered tepal underneath the tepal with the EE patch had a slightly greener region directly below the EE patch (Fig. 2G). The 21 covered buds did not develop any macroscopically visible sepal-like characteristics in their EE areas nor did EE areas of the three buds examined under SEM (NL/B12, 13, and 14) show microscopic sepal-like characters. The EE areas in covered buds resembled the surrounding CNG area of tepal both in colour and epidermal cell morphology, which typically had relatively flat epidermal cells without trichomes.

Nymphaea caerulea: differences between sepaloid and petaloid patches within the perianth of non-experimental buds

In addition to EG, CG, and CNG patches, several further regions were identified on the abaxial tepal surfaces of Nymphaea caerulea: (iv) exposed green-striped (EGS) patches, (v) covered green-striped (CGS) patches, (vi) covered purple (CP) patches, and (vii) covered white (CW) patches. In the CP patches the colour is diffuse, in contrast to striped patches which show sharply defined dark purple stripes.

In buds longer than 3.5 cm, EGS patches of tepals 1–4 were dark green with dark purple irregular stripes or small blotches (Fig. 4D). EGS patches bore a striking resemblance to the leaf abaxial surface in this species (Fig. 5H). In contrast to many other Nymphaea species and cultivars (Fig. 4A) the CGS patches from tepals 2–4 of N. caerulea were light green and striped rather than petaloid (Fig. 4D). In young buds, the (future) EGS patches were orange/brown at the base with dark green tips. As the bud matured, chlorophyll development spread from the tip downwards,
and dark purple stripes developed from the base upwards, until they both occurred over the entire EGS patches (see Supplementary Fig. S3 at JXB online).

In more mature buds (>3.5 cm) the remaining tepals (tepals 5–c. 20) had a CP patch that covered from one-half to three-quarters of the length of the tepal, grading into
a white (CW) area covering the rest of the tepal (Fig. 4D). Green (CG and CGS) and colourful (CP and CW) regions co-occurred on the larger second-whorl petaloid tepals (tepals 5–8). The position and extent to which the covered green area covered these tepals varied from bud to bud and even tepal to tepal in the same whorl, but in the majority of cases covered the very base and the midrib of the tepal, forming green lines on tepals 5, 6 and, occasionally, on tepals 7 and 8. Dissection of flower buds showed that the green lines usually followed the margin of the covering tepal (Fig. 4C). In some buds the tips of tepals 5 and 6 were exposed; these areas were dark green. In a few open flowers examined, tepals 5 and 6 possessed a large sepaloid patch (Fig. 4E).

Epidermal cells of the petaloid tepals (tepals 5–c. 20) were long, striated, and papillate (Fig. 5A, B); the purple pigment was spread diffusely within the upper epidermal cells and the cells directly below the epidermis, and the mesophyll was spongy. Epidermal cells were relatively shorter in EGS areas than in CP areas; EGS cells were non-papillate and did not bulge outward, so cell boundaries were poorly defined in surface view (Fig. 5C), unlike the petaloid tepal where epidermal cells are noticeably domed. In EGS patches chloroplasts were abundant in abaxial epidermal cells and in the upper mesophyll. The dark purple pigment that forms the stripes was present in the vacuole of upper epidermal cells and sometimes in the subepidermal cells. There were no obvious morphological differences between CP and CG patches from the same tepal, although, in some tepals, epidermal cells of the CG areas were flatter than in CP areas.

In contrast, the adaxial surfaces of the outer tepals did not show morphological differences corresponding to the

---

**Table 1. Summary of experimental results on Nuphar advena and N. lutea**

| Experiments       | Total number of buds | EE green and visibly differs from covered area |
|-------------------|----------------------|---------------------------------------------|
|                   |                      | Yes | No   |
| Non-covered buds  | 26                   | 12  | 14   |
| Covered buds      | 21                   | 0   | 21   |
| Half-covered buds | 18                   | 4   | 14   |
| Total             | 65                   | 16  | 49   |

---

**Fig. 3.** Composite SEM image of tepals 1 and 3 from a 1.5 cm wide bud. Glands in EG patches are dotted red, glands in CG area dotted white (A) The outermost tepal (tepal 1) is totally exposed and glandular hairs cover the entire abaxial surface. (B, C) Tepal 3. Holes mark the exposed area of the tepal. The EG and CG patches have sharp boundaries. (D) The boundary of the CG patches follows the impression (I) left by the covering tepal.
EGS and CP patches on their abaxial surfaces. The adaxial surfaces of tepals 1–3 were uniformly white and glossy. Tepal 4 resembled tepals 1–3 except that the adaxial surface of the CGS patch was tinged purple. In tepals 5 onwards, the adaxial surface was light purple (see Supplementary Fig. S3H at *JXB* online). Adaxial epidermal cells on all tepals were striated, papillate, and similar to CP regions on the abaxial surface (Fig. 5D). Short-stalked glandular trichomes and stomata were present over the entire abaxial and adaxial tepal surfaces.
Nymphaea caerulea: experimentally exposed tepal regions develop sepaloid characteristics

In *Nymphaea caerulea* both covered and non-covered buds developed sepaloid characteristics in their EE areas. Twenty (of 23) non-covered buds, two (of four) half-covered buds and 15 (of 31) covered buds responded to experimental exposure in EE areas. Most non-covered buds developed sepaloid characteristics in their EE areas (Fig. 4E–G). Most of the covered buds with positive results developed stripes in EE areas but on a petaloid (diffuse purple) background (nine out of 15 buds; Table 2; Fig. 4I, J). None of the covered buds developed chlorophyll in their EE areas, but some young buds (0.4 cm–1.5 cm) did not develop pigment in EE areas and remained pale yellow (six buds) (see Supplementary Fig. S4 at *JXB* online). Stripes and chlorophyll formed on EE areas only 3 d after exposure (during at least a week of exposure). It was found that these experimentally induced changes were confined to the EE areas of the experimental tepal while the rest of the tepal remained petaloid (Fig. 4H, J; see Supplementary Fig. S4 at *JXB* online).

Experimentally exposed tepals from NyCA bud 5 (non-covered) and NyCA buds 25, 35, 36, 40, and 44 (covered-buds) were examined using SEM (see Supplementary Tables S5–7 at *JXB* online). All except bud 40 responded to exposure in their EE area. In bud 5, EE epidermal cells were slightly less papillate than in the remaining covered area (Fig. 5E–G). This result also occurred in one (of three) EE tepals from buds 36 and 25; the other two tepals showed...
no obvious differences between their EE and unexposed tepal areas. Epidermal cells in both EE tepals from bud 50 were relatively less papillate and striate in their EE area. Buds 40 and 44 had no obvious differences between EE and covered areas.

**Schisandra: colourful and green patches do co-occur on individual tepals**

In very young buds of *Schisandra rubriflora* the tepals were all entirely green (Fig. 4L). In more mature buds, the edges of the outer and inner tepals had turned red while the rest of the tepal remained green. These petaloid edges occurred around the entire tepal, in the exposed and covered areas (Fig. 4M). At c. 0.3 cm in width, the rest of the tepal had begun to turn red, beginning in the covered areas of the perianth, i.e. all the adaxial surfaces, the abaxial surfaces of the inner covered tepals, and the abaxial areas of the tepals that were underneath overlying tepals. The exposed green areas of the tepals were the last to turn red. At this stage of bud development, the exposed patches were green and the covered regions were petaloid (Fig. 4K), similar to *Nuphar*.

SEM examination of the two outermost tepals of *S. rubriflora* suggests that there could be further differences between these regions, as the epidermal cells along the edges of the tepals are relatively domed compared with the rest of the tepal. However, cell structure did not vary across the third outer tepal, which was also green with red margins.

**Schisandra: sepaloid patches rarely develop in experimentally exposed tepal regions**

Two buds of *Schisandra* reacted to exposure: bud 9 of *S. sphenanthera* and bud 10 of *S. rubriflora* (Table 3; see Supplementary Tables S8 and S9 at JXB online). Bud 9 was exposed to light and, at the end of the experiment, the EE area was darker green than the covered area of that tepal. Bud 10 was not exposed to light and one of the two EE areas was partly green; the rest of the tepal was red (Fig. 4N), and the other EE area was entirely red. The remaining buds exhibited no visible response to exposure in their EE areas.

**Discussion**

**Morphological differences between green and non-green areas**

Results from this study contradict the supposition that sepalness and petalness must be attributes of whole organs. It is shown that, in *Nuphar lutea, N. advena*, and *Nymphaea caerulea*, sepal–petal differentiation can occur within the same organ, creating distinct sepaloid and petaloid regions within individual perianth members. Their distinction as ‘sepaloid’ and ‘petaloid’ patches is supported by numerous morphological differences, some of which are typical petal or sepal characteristics, such as colour (green sepals versus colourful petals) and epidermal cell patterning (papillate petal cells versus flat sepal cells) (Warner et al., 2008; this study). In *Nuphar*, abaxial surfaces of green (EG and CG) patches differ from non-green (CNG) patches. Specifically, domed pavement cells with trichomes occur in green regions, and relatively flat pavement cells without trichomes occur in non-green regions (Fig. 2H–J). These differences were present in all buds that measured over 0.25 cm in width, including early buds in which the tepals were still pale yellow (Fig. 2L, M), demonstrating that morphological differences between the green and non-green patches develop prior to any colour differences. By contrast, adaxial tepal surfaces are entirely pale yellow throughout development and resemble abaxial non-green areas (Fig. 2J).

In some *Nymphaea caerulea* buds studied under the SEM there were differences between the CP and CG patches; the epidermal cells in the CG patches are flatter than the remaining CP area of the tepal. There are also many differences between green areas of the outermost tepals and colourful areas of the inner tepals (Fig. 4D, E; 5A–C).
Furthermore, in *N. caerulea*, CP patches have typical petal attributes (papillate striate pavement cells and diffuse colourful pigmentation) while EGS patches have typical sepal attributes (flat pavement cells and chloroplasts). The differences between EGS patches of first-whorl organs and CP patches of second-whorl organs could indicate that these are different organ types (i.e. sepal and petal). However, our experimental results show that EGS-like patches can be induced on inner tepals, indicating that EGS/sepaloid patch identity is not organ-specific, as in most eudicots, as it can occur on both the inner and outer tepals.

It is interesting to note that the epidermal morphology of the exposed green patches in *Nuphar* and *Nymphaea caerulea* is similar to the abaxial surfaces of their leaves, not only in colour but also in the dark purple stripes and blotches of *Nymphaea* (Fig. 5H) and also in the presence of trichomes in *Nuphar*. A leaflike quality is a typical attribute of sepal in many higher dicots (Eames, 1931; Endress, 1994), thus supporting our interpretation of the green patches on *Nuphar* and *Nymphaea* tepals as sepaloid (see Warner et al., 2008, for further discussion).

**Complex environmental control of development of sepaloid areas in Nuphar and Nymphaea**

Many organisms use environmental cues to trigger developmental processes. Such environmentally controlled plasticity is particularly important in sessile organisms such as plants. Use of environmental cues such as light, temperature, and nutrient availability to trigger morphological or physiological changes means that plants can predict future favourable conditions for the next developmental stage (e.g. seed germination, flowering) and/or adopt a phenotype that maximizes their fitness under the prevailing environmental conditions (e.g. shade-avoidance syndrome, morphological differences between sun and shade leaves) (Smith, 1982; Clough et al., 1983; Casal and Smith, 1989; Dudley and Schmitt, 1995; Schmitt et al., 1995; Weinig, 2000). In plants, the best-documented environmental signal is light. Extensive research on photomorphogenesis has revealed that light is used to initiate a suite of changes through a plant’s life, including seed germination, de-etiolation, cotyledon expansion, chlorophyll synthesis, suppression of hypocotyl elongation, flowering, and the shade avoidance syndrome (i.e. stem elongation, suppression of branching) (recent reviews on photomorphogenesis include Kendrick and Kronenberg, 1994; Chory et al., 1996; Ballaré et al., 1997; Briggs and Christie, 2002; Bechtold et al., 2005; Spalding and Folta, 2005; Roberts and Paul, 2006; Lee et al., 2008; King et al., 2008). These developmental changes often depend on the colour, intensity, direction, or the photoperiod of the light source. A variety of photoreceptors gather this information from the light and use it to control genes ultimately involved in photomorphogenesis at the transcriptional or post-transcriptional level. Expression of over one-third of all genes in *Arabidopsis* is influenced by light signals (Nagatani et al., 1993; Huq et al., 2000; Ma et al., 2001; Tepperman et al., 2001; reviewed by Quail, 2007).

Our experimental results from *Nuphar* and *Nymphaea caerulea* show a strong correlation between the development of green, sepaloid regions and environmental exposure. This link is supported by two factors. (i) There are strong similarities between sepal-like experimentally-exposed areas in experimental buds, and sepaloid patches from non-experimental buds in both *Nuphar* and *Nymphaea caerulea*. (ii) In the majority of experimental buds that displayed visible changes in their EE areas, the induced sepaloid patch was restricted to the experimentally-exposed area of the tepal while the rest of the tepal was normal/petaloid (Figs 2E, F, 4F–J).

In *Nuphar*, only experimental buds that were exposed to light (non-covered buds and the light-exposed region in half-covered buds) formed sepaloid characteristics in their experimentally-exposed areas (16 out of 44 buds; Table 1). These buds formed chlorophyll in their EE area; non-covered buds examined under SEM showed domed pavement cells similar to the green areas (EG and CG patches) of untested buds. By contrast, the areas of tepal surrounding the light-exposed EE areas had flat cells and resembled the CNG patches (Fig. 2N). The EE areas from buds not exposed to light (covered buds) did not develop chlorophyll (Table 1) and there was no difference in colour or epidermal cell morphology between the EE area and the surrounding CNG area of tepal, which had relatively flat epidermal cells without trichomes. These results support our hypothesis that light controls the differentiation of sepal- and petal-like regions in the perianth of *Nuphar*. The lack of morphological changes in the EE areas of some covered buds could be due to bud age or the durations of the experiments, at least in those viewed under the SEM (as discussed later in this section). The failure of many buds to respond to exposure could also be due to their differing circumstances in the field; buds that became covered by floating leaves or that were oriented on their sides would have received much less light than those held erect without floating leaves above them. Weather conditions, whether cloudy or sunny, during the experimental interval could also have influenced the outcome.

In *N. caerulea*, both covered and non-covered experimental buds developed sepal-like features in their experimentally-exposed areas, demonstrating that light is not required for the development of all sepaloid characteristics in this species (Table 2). Experimentally-exposed areas of many tepals from covered and non-covered buds were morphologically similar
to EGS patches of untested buds except that the covered regions were not green. A large number of the experimental buds (26 of 54 covered and non-covered buds) formed dark purple stripes in their experimentally-exposed (EE) areas; their pavement cells were relatively flat compared with the rest of the tepal and less papillate, making them similar to EGS patches in untested buds (Fig. 5E–G). As in Nuphar, these sepal-like areas were generally confined to the experimentally-exposed region, while the remaining covered area of tepal showed characteristics of CP regions from untested buds (Fig. 5A, G).

The presence of covered green patches in non-experimental buds of Nuphar and Nymphaea caerulea, and the experimental results from N. caerulea both imply that sepaloid characteristics can develop in the absence of light. However, light clearly plays a critical role in perianth organ differentiation in Nuphar and in N. caerulea. The presence of chlorophyll is a typical sepal characteristic; synthesis of this pigment in all plant organs is stimulated by light. Consequently, untested buds of N. lutea and N. caerulea formed chlorophyll only after exposure to light (Fig. 2D). In both species, chlorophyll was absent from experimentally-exposed areas of all covered buds, which, in many cases, also showed signs of etiolation (Fig. 4I, J). In untested buds, chlorophyll also developed in regions of the perianth that were covered when the flower was in bud (CG patches and CGS patches). CG patches of Nuphar and CGS patches of N. caerulea are only covered by a single tepal layer (Fig. 2B, 4D). Since light is required for chlorophyll formation, it is possible that CG patches represent areas where incident light was especially strong or the overlying tepal is relatively thin, so that sufficient light passed through it to the tepal beneath. The result from bud 2 of Nuphar advena supports this explanation, as the area of tepal directly below the experimentally exposed (EE) area also turned green (Fig. 2G). If this is the case, then in N. caerulea light must be able to pass through the (thinner) edges of the two covering tepals to form the CG areas of tepals 5 and 6 (Fig. 4C).

In N. caerulea, light also seems to affect tepal response to other environmental factors. Experimental results show that the percentage of buds that developed stripes in their experimentally-exposed areas (as stripes can develop in both covered and non-covered buds) was greater in buds exposed to light (87%) than in buds not exposed to light (48%). Light appears to be an important stimulus in N. caerulea, perhaps even increasing the responsiveness of the tepal to other environmental factors that are involved in the initiation of sepaloid characters.

Some characteristics of the green patches in untested buds, such as stripes in N. caerulea and domed pavement cells and trichomes in N. lutea, do not appear to require exposure to light in order to form (Figs 2K, M, 4I, J). This suggests that there are probably other environmental factors, in addition to light, that control the development of sepaloid areas. One additional environmental cue is the absence of physical contact with another tepal. In both species, CG patches have well-defined boundaries, parts of which tend to follow impressions made by the overlying tepal (Figs 3B–D, 4C). The CG and EG regions could therefore be areas in which contact with the covering layer is absent or reduced. Whether this is mediated by the mechanical attribute of contact, or by some other signal that moves from one tepal to the other is unknown. Attempts to mimic contact by placing gold leaf or other materials between tepals were unsuccessful; once dislodged, tepals would not press against the inner parts of the bud.

It is crucial to understand that the sepaloid and petaloid patches also differ by morphological features unrelated to chlorophyll presence, including such features as papillate and striate epidermal cells, which are a typical characteristic of petals. Some of these differences can develop in the absence of light. Hence, the differences seen between sepaloid and petaloid patches cannot be explained as an automatic response to premature exposure to light. Instead, two or more signals related to environmental exposure initiate complex developmental programmes resulting in morphologically distinct regions on these tepals.

**Bud age may be an important factor in the response to environmental signals**

Bud age seems to be a central factor in the response of tepals to environmental signals. In both waterlily species, tests commencing on young buds were more likely to form sepaloid characteristics in their experimentally-exposed areas than older buds. After the flowers had opened, the tepals no longer responded to exposure. In N. caerulea, the majority of buds that responded to exposure were 0.5–1.4 cm in length when experiments began (23 out of 35 covered and non-covered buds that reacted to exposure; Table 2). In Nuphar, young experimental buds also developed more sepaloid characteristics than older buds. Experiments on N. advena bud 2 commenced when the bud was about 1 cm in width (Fig. 2E–G). At the end of the test, the experimentally-exposed (EE) area closely resembled the EG patches. Interestingly, this was the only EE area examined under the SEM to have produced trichomes, though these were rare. Investigation of glandular trichome development on tepals of Nuphar revealed that the number of developing glands in the sepaloid areas decreased when the bud reached c. 0.25 cm in width (see Supplementary Fig. S1 at JXB online). Experiments on buds examined under the SEM commenced when the buds were slightly larger than 1 cm, after trichomes had largely ceased developing (see Supplementary Fig. S1 at JXB online). In order to induce trichomes in experimentally-exposed areas, it may be necessary to commence experiments on younger buds in which glandular trichomes are still forming; unfortunately, such young buds were not accessible for these studies.

The absence of trichomes and domed pavement cells in the EE areas of covered buds could be because the buds were at relatively late stages of development when the tests began (NL51, NL52) or/and the duration of the experiments was much shorter compared with the non-covered buds examined under the SEM (NL53) (see Supplementary
Table S2 and S3> at JXB online). Buds rarely survived experiments if they were covered at the early stages of development or were covered for an extended amount of time. As bud age affects the development of sepal-like characteristics in Nymphaeaceae, this difficulty in experimenting on very young buds would explain the lack of trichomes or domed cells in the EE areas of covered buds.

Developmental stage is a major aspect of an organism’s response to developmental cues, but it remains unclear why certain stages are more responsive to environmental cues than others (Larsen, 2003). Until rather late in development, perianth organ identity in *Nuphar* and *Nymphaea caerulea*, i.e. sepaloid versus petaloid, is not yet fixed as their features are still forming; consequently, organ identity is still plastic and responsive to environmental signals. This would mean that perianth organ identity in *Nuphar* and *Nymphaea caerulea* is determined relatively late compared with that of eudicots. Most studies of developmental genetics in angiosperms have focused on the earliest stages of flower development, corresponding with the stages of *Arabidopsis* and *Antirrhinum* when the developmental fates of the sepal and petals are specified in wild-type plants. Late-acting genetic lesions can destabilize normal petal development in *Antirrhinum*, for example, by excision of a transposon inactivating a B-gene, but such events are not normal possibilities in wild-type plants. Our demonstration that developmental fate can be reversed late in flower development solely by environmental cues underlines the need to study the late as well as the early stages of flower development in order to understand organ specification, at least in these basal angiosperms. This late specification of developmental fate (and perhaps even the reversal of fate by environmental cues) is reminiscent of the switch from floral to vegetative development of potential floral apices reported in *Impatiens* (Tooke *et al.*, 2005).

The early and developmentally fixed organ specification of model organisms such as *Arabidopsis* and *Antirrhinum* could facilitate the very rapid development of their flowers from newly specified floral apices, as in many eudicots. Sepal, petal, stamen, and carpel primordia arise in very quick succession, and not always in acropetal (centripetal) sequence. In *Nuphar* and *Nymphaea*, primordia are initiated over a long period of time; the outer tepals in *Nymphaea odorata* can be nearly 1 cm long before all the inner tepals have appeared (M Frohlich, personal observation).

**Petal-sepal identity in Schisandra could be partly controlled by the environment**

In *Schisandra*, green and colourful patches both occur on the outer three tepals, but it is unclear whether the environment controls their positions in the perianth. In untested mature flower buds of *S. rubriflora*, the petaloid (red) regions are restricted to the covered areas of the perianth, as in *Nuphar*. However, in young buds, the outer two tepals are green with petaloid margins that surround the entire tepal and forms in the exposed and covered areas (Fig. 4M). During the experiments, *Schisandra* buds showed a low response to exposure (Table 3; Fig. 4N), but this could be due to practical difficulties in working with this species. The results from *N. lutea* and *N. caerulea* demonstrate that the experimentally-exposed areas are more likely to develop sepaloid characteristics if exposed at a very early stage of bud development. *Schisandra* buds are difficult to manipulate at the early stages, as the buds are very small, often only 0.5 cm wide at maturity. As a consequence, the majority of buds that survived the experiments were at relatively late stages of development; 53% of the experimental buds (31 out of 59 buds) died. The results of the experimental and morphological study suggest that the environment may only partially control sepalness in *Schisandra*. To determine the extent of environmental influence on this type of differentiation requires further study.

**Conclusion: a new perspective on the evolution of sepalis and petals**

Our observations demonstrate that sepal–petal differentiation occurs within individual perianth organs in Nymphaeaceae. This finding contradicts the widespread assumption that sepalness and petalness are always characteristics of whole organs. Our preliminary observations indicate that this feature also occurs in other Nymphaeaceae (*Euryale: Warner et al.*, 2008) and in a phylogenetically diverse range of other angiosperms, including some eudicots (e.g. *Berberis, Hypericum, Illicium*), some monocots (e.g. *Alstroemeria*) (see Supplementary Fig. S5 at JXB online), and probably also *Amborella* (*Buzgo et al.*, 2004), the putative sister to all other angiosperms. Even more surprisingly, our observations suggest that the environment has a role in determining this differentiation; i.e. the visible boundaries of sepalness and petalness on each perianth organ correspond closely with the areas that were exposed (the sepaloid regions) versus the areas that were covered (the petaloid regions) when in bud. Furthermore, according to a review by Endress (2008), several species display structural differences between the exposed and covered areas of their perianth. For example, in *Stellaria media* (Caryophyllaceae), *Chiranthodendron*, *Fremontodendron* (Malvaceae), *Rivea*, and *Convolvulus tricolor* (Convolvulaceae) hairs are predominantly restricted to the exposed areas of their perianths, as found in *Nuphar*. Also in *Ipomoea purpurea* (Convolvulaceae) the exposed areas are green and the covered areas are thin and hyaline. This suggests that, in many angiosperms, the environment plays an important role in differentiating between structurally different areas of the perianth organs.

We predict that additional studies will show that sepal–petal differentiation within individual organs occurs in multiple lineages within the angiosperm phylogeny and, therefore, it could have been present at the base of extant angiosperms and, perhaps, in ancestral (stem-group) angiosperms (with evidence potentially available from their flower buds, if stem-group angiosperms are identified).
These results lead us to propose a new Mosaic theory for the evolution of the dimorphic perianth, with two distinct steps. (i) Early in angiosperm history there was a distinction between sepalness and petalness, but these features were not fixed to particular organs and were primarily environmentally controlled. (ii) At later stage(s) in angiosperm evolution, sepalness and petalness became fixed to whole organs in specific whorls, forming distinct sepals and petals, thus reducing or removing the need for environmental control in favour of fixed developmental control. The occurrence of differentiation within individual organs of eudicots, as observed in Berberis (Berberidaceae) and Hypericum (Hypericaceae), would represent a reversal to the plesiomorphic condition.

In addition, it is noted that, in the observed taxa, including Nuphar and Nymphaea, the tepals are petaloid over their entire adaxial surfaces. Perhaps, at an even earlier stage of evolution, before environmental control of sepalness and petalness was established, these fates might have been controlled by abaxial versus adaxial determinants. If so, the appearance of a petaloid surface on the abaxial face of tepals could have been accompanied by the spread of some adaxial factors to the abaxial surface, which might be testable in living plants.

Morphological differences between the sepal-like and petal-like patches of the tepals of Nuphar and N. caerulea suggest that their underlying developmental programmes differ. In the higher eudicots, B-class genes are expressed in the petals but not in the sepals (Schwartz-Sommer et al., 1990; Coen and Meyerowitz, 1991; Hansen et al., 1993; van der Krol and Chua, 1993; Davies et al., 1996; Yu et al., 1999; Kim et al., 2005; Zang et al., 2008). Outside of the eudicots, B-class expression varies, but there appears to be a strong correlation between expression of B-class genes and the development of petaloid organs (Kramer and Irish, 2000; Tzeng and Yang, 2001; Kanno et al., 2003; Kramer et al., 2003, 2007; Tsai et al., 2004; Kim et al., 2005; Nakamura et al., 2005; for exceptions see Chung et al., 1995; Ambrose et al., 2000; Jaramillo and Kramer, 2004; Whipple et al., 2004). Therefore, it is possible that B-class gene expression is ‘on’ in the petaloid patches and ‘off’ in the sepaloid patches of Nuphar and Nymphaea caerulea. If so, B-gene expression could specify petalness in waterlilies. Studies by Kramer and her co-workers (Kramer and Irish, 1999, 2000; Jaramillo and Kramer, 2004) have found that, in several species, B-class gene expression is spatially restricted to specific areas of the perianth. For example, B-gene expression can be localized at the base or tip of the perianth organ, to a single cell layer (the adaxial epidermis) and even to the adaxial half of an organ. Furthermore, a study of B-class expression in Nuphar advena reported that expression of their B-class APETALA3 (AP3) and PISTILLATA (PI) homologues is more pronounced in the inner, covered, tepals (which have larger petaloid patches) and the tips of the outer tepals (which are often covered and petaloid) (Kim et al., 2005). In addition, a study by Kramer et al. (2007) of B-class gene function in the early-divergent eudicot Aquilegia found that B-class genes can confer petaloidy at late stages of organ development. In Aquilegia, B-class genes are expressed in the petaloid sepals but only during late stages of sepal development, possibly when the initially green sepals become colourful. Furthermore, down-regulation of the Aquilegia PI homologue corrupted the development of the petals, stamens, and staminodes but did not affect sepal identity (in strong mutant phenotypes sepal-like organs developed instead of petals). Therefore, it is plausible that B-class expression could also be restricted to the petaloid areas of an individual tepal of N. lutea and N. caerulea, i.e. the covered petaloid regions of the tepals and the adaxial surfaces of all the tepals. Perhaps the late expression of B genes is less extensive and/or post-transcriptional regulation limits B-gene function to petaloid regions. Furthermore, if B-class expression is absent from the sepaloid patches of the perianths, then B-class expression could also be ‘turned off’ in an area of tepal that is experimentally exposed and develops a sepaloid patch. B-gene function could have specified petalness (and stamens) even at the base of extant angiosperms (and possibly into the stem group). B-gene expression is found in petals but not in sepals in non-eudicots such as Asimina (Magnoliales) (Kim et al., 2005). Such expression patterns outside the eudicots have been explained as cases of parallel recruitment of these genes for the same function as in eudicots (Kramer and Irish, 2000). However, if B-genes specified petalness before distinct petal and sepals appeared, then no parallel change in B-gene function would be required for these cases. The only parallelism would involve the second step of the Mosaic theory.

The Mosaic theory is compatible with, but more detailed than the Fading Borders model (Buzgo et al., 2004; Soltis et al., 2007; Warner et al., 2008). However, the Fading Borders model implies that within the floral organ identity gradient each individual tepal is morphologically uniform, whereas we emphasize the presence of morphologically distinct sepal–petal regions on individual tepals as the first step towards the evolution of the bipartite perianth (Warner et al., 2008).

Future work on this project will focus on B-gene expression in the perianth of experimental and untested buds of Nuphar and Nymphaea to investigate whether there are differences in B-gene expression between green and colourful regions. We also plan to explore the control of sepalness and petalness in other angiosperms that display differentiation within individual perianth organs.

**Supplementary data**

Supplementary data are available at JXB online.

**Supplementary Fig. S1.** Average number of glands in an 810×570 m (×150 magnification) area at four chosen stages of gland development.

**Supplementary Fig. S2.** Tepals from buds of Nuphar lutea.

**Supplementary Fig. S3.** Nymphaea caerulea: tepals from buds at different developmental stages.
**Supplementary Fig. S4.** Experimental bud of *Nymphaea caerulea* that was not exposed to light (covered bud).

**Supplementary Fig. S5.** Several other angiosperms have sepaloid and petaloid patches on the abaxial surfaces of their tepals.

**Supplementary Table S1.** Accession numbers of specimens used in this paper from the Royal Botanic Gardens, Kew.

**Supplementary Table S2.** Results of experiments on *Nuphar* species: buds exposed to light.

**Supplementary Table S3.** Results of experiments on *Nuphar* species: buds not exposed to light.

**Supplementary Table S4.** Results of experiments on *Nuphar* species: buds half exposed to light.

**Supplementary Table S5.** Results of experiments on *Nymphaea caerulea*: buds exposed to light.

**Supplementary Table S6.** Results of experiments on *Nymphaea caerulea*: buds not exposed to light.

**Supplementary Table S7.** Results of experiments on *Nymphaea caerulea*: buds half exposed to light.

**Supplementary Table S8.** Experiments on *Schisandra sphenanthera* and *S. rubriflora*: buds exposed to light.

**Supplementary Table S9.** Experiments on *Schisandra sphenanthera* and *S. rubriflora*: buds not exposed to light.

**Acknowledgements**

We thank Chrissie Prychid for help in the Jodrell laboratory, John Sitch, Michelle Mathews, and Roselle Andrews for tending our specimens at the Royal Botanic Gardens, Kew, and the staff at Battle Abbey for allowing us access to their lake and *Nuphar lutea* plants. Merrie Dadd, Annie Warner, Shirley Warner, and Michael Warner provided assistance in collecting data and specimens during field trips. This project was funded by a Natural History Museum studentship to KAW, the Floral Genome Project (NSF Plant Genome Research Program project DBI-0115684), and also by NSF grant DEB-9974374 to MWF.

**References**

Albert VA, Soltis DE, Carlson JE, *et al.* 2005. Floral gene resources from basal angiosperms for comparative genomics research. *BMC Plant Biology* 5, 1–15.

Ambrose BA, Lemer DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RJ. 2000. Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. *Molecular Cell* 5, 569–579.

Angenent GC, Franken J, Busscher M, Colombo L, Vantunen AJ. 1993. Petal and stamen formation in *Petunia* is regulated by the homeotic gene *Fbp1*. *The Plant Journal* 4, 101–112.

Angiosperm Phylogeny Group. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141, 399–436.

Arber A. 1937. The interpretation of the flower. A study of some aspects of morphological thought. *Biological Reviews* 12, 157–184.

Ballaré CL, Scopec AL, Sánchez RA. 1997. Foraging for light: photosensory ecology and agricultural implications. *Plant, Cell and Environment* 20, 820–825.

Bateman RM, Hilton J, Rudall PJ. 2006. Morphological and molecular phylogenetic context of the angiosperms: contrasting the ‘top-down’ and ‘bottom-up’ approaches to inferring the likely characteristics of the first flowers. *Journal of Experimental Botany* 57, 3471–3503.

Bechtold U, Karpinski S, Mullineaux PM. 2005. The influence of the light environment and photosynthesis on oxidative signalling responses in plant-biotrophic pathogen interactions. *Plant, Cell and Environment* 28, 1046–1055.

Bierhorst DW. 1971. * Morphology of vascular plants*. New York: MacMillan.

Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W. 2003. Noncoding plastid *trnT-trnF* sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* 16, 558–576.

Briggs WR, Christie JM. 2002. Phototropins 1 and 2: versatile plant blue-light receptors. *Trends in Plant Science* 7, 204–210.

Buzgo M, Soltis PS, Kim S, Soltis DE. 2005. The making of the flower. *Biologist* 52, 149–154.

Buzgo M, Soltis PS, Soltis DE. 2004. Floral developmental morphology of *Amborella trichopoda* (*Amborellaceae*). *International Journal of Plant Sciences* 165, 929–947.

Casal JJ, Smith H. 1989. Effects of blue light pretreatments on internode extension growth in mustard seedlings after transition to darkness: analysis of the interaction with phytochrome. *Journal of Experimental Botany* 40, 893–899.

Chory J, Catterjee M, Cook RK, *et al.* 1996. From seed germination to flowering, light controls plant development via the pigment phytochrome. *Proceedings of the National Academy of Sciences, USA* 93, 12066–12071.

Chung YY, Kim SR, Kang HG, Noh YS, Park MC, Finkel D, An GH. 1995. Characterization of 2 rice MADS box genes homologous to *Globosa*. *Plant Science* 109, 45–56.

Clough JM, Terri JA, Tons SJ. 1983. Photosynthetic adaptation of *Solanum dulcamara* L. to sun and shade environments. IV. A comparison of North American and European genotypes. *Oecologia* 60, 348–352.

Coen ES, Meyerowitz EM. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature* 353, 31–37.

Conard HS. 1905. * The waterlilies: a monograph of the genus Nymphaea* (reprint 1991). Bury St. Edmunds: Lark Publications.

Dandy JE. 1927. The genera of Magnolieae. *Bulletin of Miscellaneous Information* 7, 257–264.

Davies B, Di Rosa A, Eneva T, Saedler H, Sommer H. 1996. Alteration of tobacco floral organ identity by expression of combinations of *Antirrhinum MADS-box* genes. *The Plant Journal* 10, 663–677.

De Candolle AP. 1827. *Organographie vegetale*. Paris: Deterville.

Ditta G, Pinyopich A, Robles P, Pelaz S, Yanofsky MF. 2004. The *SEP4* gene of Arabidopsis thaliana functions in floral organ and meristem identity. *Current Biology* 14, 1935–1940.
Drea S, Hileman LC, de Martino G, Irish VF. 2007. Functional analyses of genetic pathways controlling petal specification in poppy. Development 134, 4157–4166.

Dudley SA, Schmitt J. 1995. Testing the adaptive plasticity hypothesis: density dependent selection on manipulated stem length in Impatiens capensis. American Naturalist 147, 445–465.

Eames AJ. 1961. Morphology of the angiosperms. New York: McGraw-Hill.

Endress PK. 1994a. Floral structure and evolution of primitive angiosperms: recent advances. Plant Systematics and Evolution 192, 79–97.

Endress PK. 1994b. Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge University Press.

Endress PK. 2001. Origins of flower morphology. Journal of Experimental Zoology 291, 105–115.

Endress PK. 2005. Links between embryology and evolutionary floral morphology. Current Biology 89, 749–754.

Endress PK. 2006. Angiosperm floral evolution: morphological developmental framework. Advances in Botanical Research 44, 1–61.

Endress PK. 2008. The whole and the parts: relationships between floral architecture and floral organ shape, and their repercussions on the interpretation of fragmentary floral fossils. Annals of the Missouri Botanical Garden 95, 101–120.

Endress PK, Doyle JA. 2009. Reconstructing the ancestral flower and its initial specializations. American Journal of Botany 96, 22–66.

Frohlich MW, Chase MW. 2007. After a dozen years of progress the origin of angiosperms is still a great mystery. Nature 450, 1184–1189.

Hansen G, Estruch JJ, Sommer H, Spena A. 1993. Ntglo, a tobacco homolog of the GLOBOSA floral homeotic gene of Antirrhinum majus: cDNA sequence and expression pattern. Molecular and General Genetics 239, 310–312.

Hiepko P. 1965. Vergleichend-morphologische und entwicklungsgeschichtliche Untersuchungen über das Perianth bei den Polycarpicae. Botanische Jahrbücher für Systematik 84, 359–508.

Hilu KW, Borsch T, Muller K, et al. 2003. Angiosperm phylogeny based on matK sequence information. American Journal of Botany 89, 1758–1776.

Huq E, Tepperman JM, Quail PH. 2000. GIGANTEA is a nuclear protein involved in phytochrome signaling in Arabidopsis. Proceedings of the National Academy of Sciences, USA 97, 9789–9794.

Jaramillo MA, Kramer EM. 2004. APETALA3 and PISTILLATA homologs exhibit novel expression patterns in the unique perianth of Aristolochia (Aristolochiaceae). Evolution and Development 6, 449–458.

Kanno A, Saeki H, Kameya T, Saedler H, Theissen G. 2003. Heterotopic expression of class B floral homeotic genes supports a modified ABC model for tulip (Tulipa gesneriana). Plant Molecular Biology 52, 831–841.

Kendrick RE, Kronenberg GHM, eds. 1994. Photomorphogenesis. Boston: Dordrecht.

Kim S, Koh J, Yoo MJ, Kong H, Hu Y, Ma H, Soltis PS, Soltis DE. 2005. Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators. The Plant Journal 43, 724–744.

King RW, Hisamatsu T, Goldschmidt EE, Blundell C. 2008. The nature of floral signals in Arabidopsis. I. Photosynthesis and a far-red photoreponse independently regulate flowering by increasing expression of FLOWERING LOCUS T (FT). Journal of Experimental Botany 59, 3811–3820.

Kosuge K. 1994. Petal evolution in Ranunculaceae. Plant Systematics and Evolution 8, 185–191.

Kramer EM, Di Stilio VS, Schluter PM. 2003. Complex patterns of gene duplication in the APETALA3 and PISTILLATA lineages of the Ranunculaceae. International Journal of Plant Sciences 164, 1–11.

Kramer EM, Holappa L, Gould B, Jaramillo MA, Setnikov D, Santiago PM. 2007. Elaboration of B gene function to include the identity of novel floral organs in the lower eudicot Aquilegia. The Plant Cell 19, 750–766.

Larsen EW. 2003. A view of phenotypic plasticity from molecules to morphogenesis. In: Hall BK, Pearson RD, Muller GB, eds. Environment, development and evolution: toward a synthesis. Cambridge: The MIT Press, 117–124.

Lee Y, Lee HS, Lee JS, Kim SK, Kim SH. 2008. Hormone- and light-regulated nucleocytoplasmic transport in plants: current status. Journal of Experimental Botany 59, 3229–3245.

Ma L, Li J, Qu L, Hager J, Chen Z, Zhao H, Deng XW. 2001. Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. The Plant Cell 13, 2589–2607.

Meeuse ADJ. 1973. The different origins of petaloid semaphylls. Phytomorphology 23, 88–99.

Nagatani A, Reed JW, Chory J. 1993. Isolation and initial characterisation of Arabidopsis mutants that are deficient in phytochrome A. Plant Physiology 102, 269–277.

Nakamura T, Fukuda T, Nakano M, Hasebe M, Kameya T, Kanno A. 2005. The modified ABC model explains the development of the petaloid perianth of Agapanthus praecox ssp. orientalis (Agapanthaceae) flowers. Plant Molecular Biology 58, 435–445.

Qiu YL, Dombrovska O, Lee J, et al. 2005. Phylogenetic analyses of basal angiosperms based on nine plastid, mitochondrial, and nuclear genes. International Journal of Plant Sciences 166, 815–842.

Quail PH, Lee J, Bernasconi-Quadrioni F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW. 2000. Phylogeny of basal angiosperms: analyses of five genes from three genomes. International Journal of Plant Sciences 161, Supplement, 3–27.

Quail PH. 2007. Phytochrome-regulated gene expression. Journal of Integrative Plant Biology 49, 11–20.
Roberts MR, Paul ND. 2006. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defense against pests and pathogens. New Phytologist 170, 677–699.

Ronse De Craene LP, Soltis PS, Soltis DE. 2003. Evolution of floral structures in basal angiosperms. International Journal of Plant Sciences 164, Supplement, 329–363.

Rudall PJ, Sokoloff DD, Remizowa MV, Conran JG, Davis JI, Macfarlane TD, Stevenson DW. 2007. Morphology of Hydatellaceae, an anomalous aquatic family recently recognized as an early-divergent angiosperm lineage. American Journal of Botany 94, 1073–1092.

Rudall PJ, Remizowa MV, Prenner G, Prychid CA, Tuckett R, Sokoloff DD. 2009. Non-flowers near the base of extant angiosperms? Spatiotemporal arrangement of organs in reproductive units of Hydatellaceae, and its bearing on the origin of the flower. American Journal of Botany 96, 67–82.

Saarela JM, Rai HS, Doyle JA, Endress PK, Mathews S, Marchant AD, Briggs BG, Graham SW. 2007. Hydatellaceae identified as a new branch near the base of the angiosperm phylogenetic tree. Nature 446, 312–315.

Schmitt J, McCormac AC, Smith H. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. American Naturalist 146, 937–953.

Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H. 1990. Genetic-control of flower development by homeotic genes in Antirrhinum majus. Science 250, 931–936.

Smith GH. 1928. Vascular anatomy of Ranalian flowers. II. Ranunculaceae (continued), Menispermaceae, Calycanthaceae, Annonaceae. Botanical Gazette 85, 152–177.

Smith H. 1982. Light quality, photoreception and plant strategy. Annual Review of Plant Physiology 33, 481–518.

Soltis DE, Chandler AS, Kim S, Buzgo M, Soltis PS. 2007. The ABC model and its applicability to basal angiosperms. Annals of Botany 100, 155–163.

Soltis DE, Soltis PS, Albert VA, Oppenheimer DG, dePamphilis CW, Ma H, Frohlich MW, Theissen G. 2002. Missing links: the genetic architecture of flower and floral diversification. Trends in Plant Science 7, 22–31.

Soltis DE, Soltis PS, Endress PK, Chase MW. 2005. Phylogeny and evolution of Angiosperms. Sunderland: Sinauer Associates.

Spalding EP, Folta KM. 2005. Illuminating topics in plant photobiology. Plant, Cell and Environment 28, 39–53.

Takhtajan A. 1991. Evolutionary trends in flowering plants. New York: Columbia University Press.

Tepperman JM, Zu T, Chang HS, Wang X, Quail PH. 2001. Multiple transcription-factor genes are early targets of phytochrome A signaling. Proceedings of the National Academy of Sciences, USA 98, 9437–9442.

Theissen G. 2001. Development of floral organ identity: stories from the MADS house. Current Opinion In Plant Biology 4, 75–85.

Tooke F, Ordidge M, Chiurugwi T, Battey N. 2005. Mechanisms and function of flower and inflorescence reversion. Journal of Experimental Botany 56, 2587–2599.

Tsai WC, Kuoh CS, Chuang MH, Chen WH, Chen HH. 2004. Four DEF-Like MADS box genes displayed distinct floral morphogenetic roles in Phalaenopsis orchid. Plant and Cell Physiology 45, 831–844.

Tzeng TY, Yang CH. 2001. A MADS box gene from lily (Lilium longiflorum) is sufficient to generate dominant negative mutation by interacting with PISTILLATA (PI) in Arabidopsis thaliana. Plant and Cell Physiology 42, 1156–1168.

Van der krol AR, Chua NH. 1993. Flower development in Petunia. The Plant Cell 5, 1195–1203.

Warner KA, Rudall PJ, Frohlich MW. 2008. Differentiation of perianth organs in Nymphaeales. Taxon 57, 1096–1109.

Weinig C. 2000. Limits to adaptive plasticity: temperature and photoperiod influence shade-avoidance responses. American Journal of Botany 87, 1660–1668.

Whipple CJ, Ciceri P, Padilla CM, Ambrose BA, Bandong SL, Schmidt RJ. 2004. Conservation of B-class floral homeotic gene function between maize and Arabidopsis. Development 131, 6083–6091.

Yu DY, Kotilainen M, Pollanen E, Mehto M, Elomaa P, Helariutta Y, Albert VA, Teeri TH. 1999. Organ identity genes and modified patterns of flower development in Gerbera hybrida (Asteraceae). The Plant Journal 17, 51–62.

Zanis MJ, Soltis PS, Qiu YL, Zimmer E, Soltis DE. 2003. Phylogenetic analyses and perianth evolution in basal angiosperms. Annals of the Missouri Botanical Garden 90, 129–150.

Zhang L, Xu Y, Ma RC. 2008. Molecular cloning, identification, and chromosomal localization of two MADS box genes in peach (Prunus persica). Journal of Genetics and Genomics 35, 365–372.