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Floral development of *Lavatera trimestris* and *Malva hispanica* reveals the nature of the epicalyx in the *Malva* generic alliance

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The epicalyx is a structure below the calyx that is often integrated in floral display. In Malvales, the epicalyx is interpreted to be formed by bracts derived from inflorescence reduction. In this study, we compare the epicalyx and flower development of *Lavatera trimestris* and *Malva hispanica*, which are close relatives but show contrasting morphologies. Both species exhibit cymose branching, stipulate subtending leaves, a short plastochron between the appearance of the alternating epicalyx and calyx, a centrifugally developing androecium and a multicarpellar gynoecium. The predominantly trimerous structure and leafy morphology of the epicalyx suggest its origin from a former subtending leaf with leaf-like stipules. The bilobed epicalyx in *M. hispanica* represents a loss of the adaxial epicalyx lobe rather than modified bracts. In Malvoideae, the bracts and bracteoles in the flowering branches can be completely absent and are variable in position and number when present. Individual bracts and bracteoles could correspond to further reductions of former subtending leaves instead of precursors of the epicalyx. Although the centrifugal androecium behaves as a branched-like structure, it is a dynamic complex floral whorl with extended growth capacity. The umbrella in *L. trimestris* is a swollen part of the style without a well-understood role in floral or fruit morphology. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **181**, 84–98

ADDITIONAL KEYWORDS: bracts – flower – homology – inflorescence – leaf – Malvaceae – monadelphous androecium – umbrella.

INTRODUCTION

The evolutionary success of angiosperms is attributed in large part to the evolution of flowers (Burger, 1981; Stebbins, 1981; Dilcher, 2000; Endress, 2006), and the whorled organization of flowers can be considered an innovation allowing extraordinary diversification (Endress, 2001). The structures positioned between the vegetative and floral meristems are an intriguingly varied but not fully understood part of this morphological diversity. In the core eudicots, the presence of phyllomes just below the flower results in the formation of an epicalyx, as found in genera such as *Agrimonia* L. (Rosaceae), *Coris* Tourn. ex L. (Primulaceae), *Cuphea* P.Browne (Lythraceae), *Dianthus* L. (Caryophyllaceae), *Dipelta* Maxim. (Caprifoliaceae), *Dirachma* Schweinf. ex Balf.f. (Dirachmaceae), *Knautia* L. (Caprifoliaceae), *Morina* L. (Caprifoliaceae), *Neurada* B.Juss. (Neuradaceae), *Olax* L. (Olacaceae), *Olinia* Thunb. (Penaeaceae) and *Triplostegia* Wall. ex DC. (Caprifoliaceae) (Hofmann & Göttmann, 1990; Mayer & Svoma, 1998; Bayer & Kubitzki, 2003; Donoghue, Bell & Winkworth, 2003; Schönenberger & Conti, 2003; Ronse De Craene & Miller, 2004; Wanntorp & Ronse De Craene, 2009; Ronse De Craene, 2010).

The epicalyx can exhibit a large range of morphological variation and is thought to play roles in pollinator attraction, ovary protection, germination and seed dispersal (Mayer & Svoma, 1998; Bayer & Kubitzki, 2003; Donoghue *et al.*, 2003; von Balthazar *et al.*, 2004; Carlson, Mayer & Donoghue, 2009). This phenotypic diversity has led to different interpretations of the nature of the epicalyx, for example as a whorl of stipules (Payer, 1857) or bracteoles (Schumann, 1895) in Malvaceae, as congenitally fused

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*Corresponding author: E-mail: mabello2@rjb.csic.es sepals in *Lythrum* and *Cuphea* (Lythraceae: Cheung & Sattler, 1967; Mayr, 1969) and as sepal-like appendages (Heywood *et al.*, 2007), stipules of adjacent sepals (Bell, 1991) or bracts in several families of angiosperms (Weberling, 1989; for a detailed review see Bayer, 1994).

In Malvaceae, an epicalyx is present in six out of nine subfamilies: Bombacoideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicteroideae and Malvoideae (Bayer & Kubitzki, 2003). In the last of these, the epicalyx is present in members of subtribe Malvinae and absent among members of subtribe Abutilinae (clades A and B, respectively, of Tate *et al.*, 2005). Bayer (1999) performed a comparative study of the inflorescence structure in Malvales and introduced the 'bicolour unit' hypothesis. The inflorescences consist of determinate units, each of which bears three bracts: one sterile and the others subtending lateral cymes or single flowers. According to Bayer (1999), the epicalyx in Malvales thus consists of these three bracts after the loss of the lateral cymes and pedicels. Despite the importance and broad impact of this hypothesis in Malvales, few floral developmental studies have discussed it (e.g. Naghiloo, Esmaillou & Dadpour, 2014).

The *Malva* L. generic alliance, hereafter called the MGA clade (Bates, 1968; Ray, 1995; Tate *et al.*, 2005; Escobar García *et al.*, 2009), displays a broad epicalyx morphology (Fig. 1A). It includes *Althea* Crantz, *Lavatera* L., *Malope* L., *Malva*, *Malvalthaea* Iljin and *Navaea* Webb & Berthel. (Escobar García *et al.*, 2009). Traditionally, the number and degree of fusion of the epicalyx lobes have been traits used to differentiate *Althaea*, *Lavatera* and *Malva* (Linnaeus, 1753). However, molecular-based phylogenetic analyses suggest that these characters are homoplasious in the MGA clade and that the trimerous epicalyx with free lobes is ancestral in this group (Ray, 1995; Escobar García *et al.*, 2009; Fig. 1A). To interpret epicalyx morphology and development in the MGA clade, we compared the two closely related Mediterranean species *Lavatera trimestris* L. and *Malva hispanica* Loefl. ex L. from the southern Iberian Peninsula and north-western Africa. According to the phylogenetic analysis of Escobar García *et al.* (2009), *L. trimestris* and *M. hispanica* form a subclade with *L. punctata* All (Fig. 1A). Although they exhibit a high degree of similarity in several floral characteristics, their epicalyx morphology is highly contrasting, with two free and narrow lobes in *M. hispanica* vs. three fused and wide lobes in *L. trimestris*. Given this differential epicalyx morphology and their close phylogenetic relationship, we studied the epicalyx origin, development and integration into the flowers in these species and compared it further with other members of the MGA clade, contrasting our evidence with the available interpretations of the epicalyx nature. Additionally, the branch-like aspect of the androecium and the differential gynoecial morphology of *L. trimestris* and *M. hispanica* are discussed.

MATERIAL AND METHODS

Seeds of *M. hispanica* from Campanario (Badajoz, Spain, *J. Fuertes 1033 & A. González*, MA 883608) and *L. trimestris* from Tarifa, (Cádiz, Spain, *J. Fuertes 1037 & A. González*, MA 883609) were collected from natural populations. Seeds were sown in the greenhouse of the Real Jardín Botánico, Madrid (CSIC), and grown under constant conditions (16 h light, 24 °C). Young flowers were dissected under a stereo-microscope and fixed in formalin, acetic acid and ethanol for a minimum of 24 h, then stored in 70% ethanol for scanning electron microscopy (SEM) and light microscopy (LM). For SEM, buds from 15 individuals were dissected under a Nikon SMZ 1000 stereomicroscope and dehydrated under an increasing ethanol series. Samples were dried with CO_2 using the Polaron CPD7501 critical-point dryer and mounted on aluminium stubs. After being covered with gold using a Balzers SDC-004 sputter coater at 45 mA, the floral buds were observed and photographed using a Hitachi S-3000N scanning electron microscope. For *L. trimestris* and *M. hispanica*, 484 and 591 images were recorded, respectively. Digital images were treated using Adobe Photoshop CS 4 v.1.0.2. For LM, pre-anthetic buds of *M. hispanica* (four individuals) and *L. trimestris* (five individuals) were dehydrated through an ethanol series to 100% and then taken through an ethanol–Histoclear series to 100% Histoclear. Buds were embedded in MacCormick Paraplast Plus paraffin, sectioned into 12-μm slices using a Jung AG microtome and stained using safranin and Alcian blue. Slides were mounted with DePeX mounting medium (Serva) and observed and photographed using a Nikon Eclipse 80i bright-field microscope. A similar procedure was followed for anatomical study of the subtending leaves and stipules. The floral formulae of *L. trimestris* and *M. hispanica* were constructed following Prenner, Bateman & Rudall (2010).

GLOSSARY

Androclad: lateral and bracteate subordinate branch bearing only homosporangiate (androecial) organs (Meeuse, 1966).

Androecial unit: stalked monothecate bilocular structures in the androecium of Malvoideae (von Balthazar *et al.*, 2004).

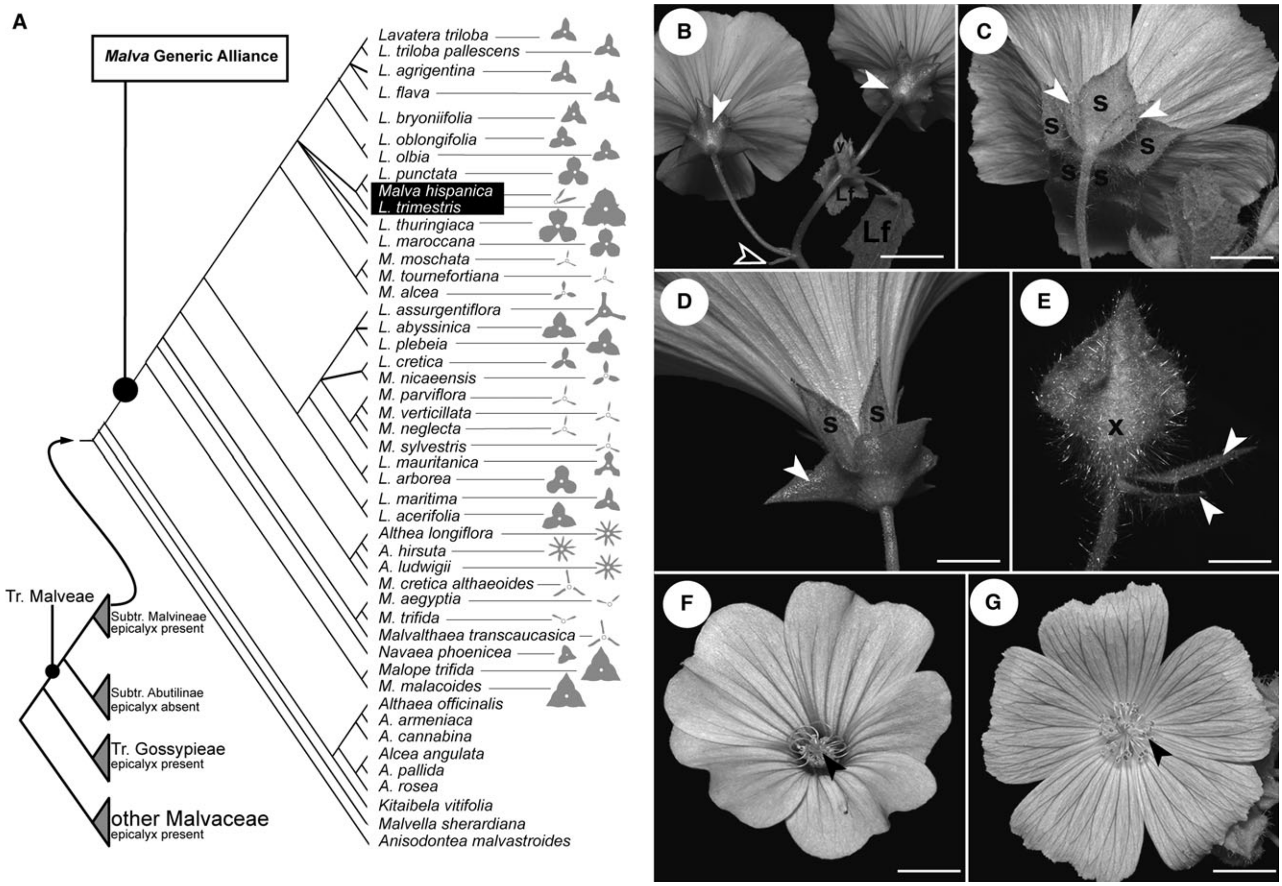

Figure 1. Schematic phylogeny and epicalyx diversity of the Malva generic alliance, flowers of *Lavatera trimestris* (B, D, F) and *Malva hispanica* (C, E, G). A, diagram of the main clades of Malvaceae with detailed members of the *Malva* generic alliance (modified from Escobar García *et al.*, 2009). B, bottom view of a flowering branch and epicalyx (white arrowheads). Older flower with bracteoles at the base (black arrowhead). C, detail of the epicalyx (white arrowheads) and the calyx. D, lateral view of the perianth and the epicalyx (white arrowhead). E, pre-anthetic floral bud with extended epicalyx (white arrowheads). F, G, anthesis flowers with exposed stamens and styles (black arrowheads). Lf, subtending leaf; s, sepal; x, calyx tube; y, young floral branch. Scale bars: B, 2 cm; C, 0.75 cm; D, 1 cm; E, 0.5 cm; F, G, 1 cm.

Bracteoles: in non-monocots, the two first reduced leaves of the floral axis associated with the flower (Endress, 1994; Ronse De Craene, 2010).

Bract: differentiated scale-like reduced leaf associated with inflorescences or flowers, sometimes with photosynthetic capacity (Weberling, 1989; Endress, 1994; Ronse De Craene, 2010).

Carpophore: extension of the receptacle to which the carpels are attached (Ronse De Craene, 2010).

Congenital fusion: process of fusion of two organ meristems occurring from the beginning of development, in which the epidermis is not involved (Endress, 2015).

Cymose: branching pattern of the inflorescences, in which the first-order axis never has more than two-second-order axes or more than two extrafloral leaves (Endress, 2010).

Double flower: bud with additional petal or petaloid structures and fewer stamens than single wild flowers (Reynolds & Tampion, 1983; Roeder & Yanofsky, 2001).

Epicalyx: whorl of structures inserted at the base of the calyx (Ronse De Craene, 2010).

Isomerous: number of parts of a whorl being the same as the other whorls in the flower (Ronse De Craene, 2010).

Monochasial: individual axes with only one lateral branch in a cymose complex (Endress, 2010).

Obdiplostemony: arrangement of the stamens in two whorls, the outer opposite to the petals and the inner opposite to the sepals (Ronse De Craene, 2010).

Plastochron: time between the initiation of two successive organs (Endress & Doyle, 2007).

Phyllome: organ categories of plants representing leaves and their homologous variations (Sachs, 1874).

Stipule: reduced leaf-like appendage or outgrowth, single or in pairs, associated with the base of the petiole of a leaf from early stages of development (Bell, 1991; Ronse De Craene, 2010).

Secondary polyandry: polyandry is the presence of numerous stamens, more than double the number of petals. Secondary polyandry refers to the division of the primary (common) primordia into several stamens (Ronse De Craene, 2010).

Unifacial style: upper part of the carpel that forms the style; the product of a carpel-restricted closure in which the primary margin does not encompass the entire length of the carpel above the ascidiate zone (Endress, 2015).

Whorled phyllotaxis: organs appear in pulses with unequal plastochrons and unequal divergence angles. Within a whorl, the organs are initiated either simultaneously or in a rapid spiral sequence (Endress & Doyle, 2007).

RESULTS

Morphology of the mature flowers

Lavatera trimestris

The partial inflorescences consist of a subtending leaf with a well-developed green lamina and two long lanceolate stipules with an axillary shoot (Figs 1B, 2A). Along the proximal part of the shoot, there are older individual flowers, also subtended by leaves (5–7 in Fig. 2A). There is no evidence of additional bracteate prophylls associated with the distal flowers, but bracteoles were observed in proximal older flowers (Fig. 1B). In the distal part of the partial inflorescence, there is a monochasial branching (4–7 in Fig. 2A). Accessory buds were not observed. The epicalyx lobes are located below the sepal whorl, separated from it by *c.* 1–3 mm, surrounding the flower base (Fig. 1B, D). They are fused to two-thirds of their length and are angulate–ovate, coriaceous and tomentose with stellate trichomes on the apex and the abaxial surface (Fig. 1B, D). The median lobe of the epicalyx is adaxial (Fig. 2C). The epicalyx is persistent in the fruit. The calyx is valvate, pentamerous and basally fused (Fig. 2C). The sepals are lanceolate with stellate and glandular hairs on the abaxial side (Figs 1D, 4F). They remain united by coiled trichomes in their distal portion (Fig. 3H). In the proximal adaxial side of each sepal, there is a patch of uni-/biserial glandular trichomes that form nectaries (Figs 4G, 7A, B). The corolla alternates with the calyx and consists of five pink–lilac, shortly connate petals with contorted aestivation and contrasting darker veins (Figs 1D, F, 2C). At the edges of the proximal adaxial side of each petal, there are two lateral rows of long hairs extending to the connate areas of the corolla (not shown). The androecium is formed by *c.* 70–80 connate androecial units in five groups, with a long staminal tube proximally adnate to the corolla. The stamen groups are opposite the petals (Fig. 2C). The anthers are dorsifixed, disporangiate and extrorse with longitudinal dehiscence (Figs 4H, 7C). The multicarpellate and superior gynoecium consists of 11–13 unilocular carpels forming a whorl, each with a single ovule (Figs 2C, 4E, 7D, E). The style branches, one per carpel, are fused proximally (Fig. 4A, B) and free distally, with the styles forming compressed rows (Figs 4C, 7F). The stigmas have a papillose dry surface (Fig. 4D). Placentation is axile and the ovules are anatropous (Fig. 7D, E). As the carpel matures, the style bases expand to form the umbrella, a discoid-like structure (Figs 4B, E, 7G, H). There is a central cylindrical structure that sclerifies and persists beyond the dehiscence of the mericarps at the fruiting stage, known as the columella (Fig. 7F).

Malva hispanica

In this species, there is a monochasial branching pattern with the proximal second-order branches bearing individual flowers. In the distal part or the partial inflorescences, there are up to five branching orders, including accessory buds (Fig. 2B). There are no bract-like additional prophylls. The epicalyx has two abaxial lanceolate free-lobes (Figs 1C, E, 2D). There is no adaxial epicalyx lobe. The epicalyx lobes are covered with stellate, forked or trifid trichomes, which are particularly abundant on the abaxial surface (Fig. 6P). The calyx and corolla are pentamerous and alternate with each other (Fig. 2D). The sepals are valvate and connate in their proximal portion (Figs 1E, 2D). Abaxially, they are covered by multiseriate glandular hairs and stellate trichomes (Fig. 6P). In the proximal adaxial side of each sepal, there is a patch of uni-/biserial nectarial trichomes that form nectaries (Fig. 6M–O). The contorted corolla displays darker veins on its adaxial surface (Figs 1G, 2D). The petals are connate in their proximal part, forming a short tube with many long hairs (Fig. 6Q). The connate androecium is formed by 50–60 monothecal androecial units in five groups opposite the petals (Figs 2D, 6R). The gynoecium is single-whorled, usually with 12–14 carpels facing the fused bases of the styles (Fig. 6I–K). Placentation is axile and the ovules are anatropous (not shown). The styles are free in their distal part, and the stigmas are papillose and dry (Fig. 6K, L).

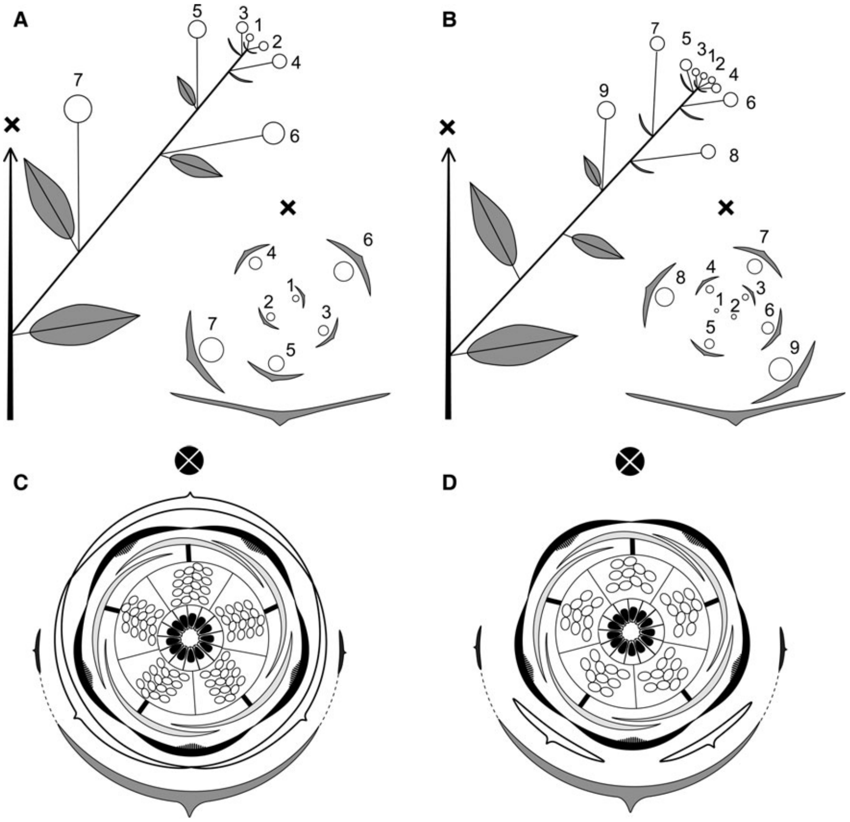

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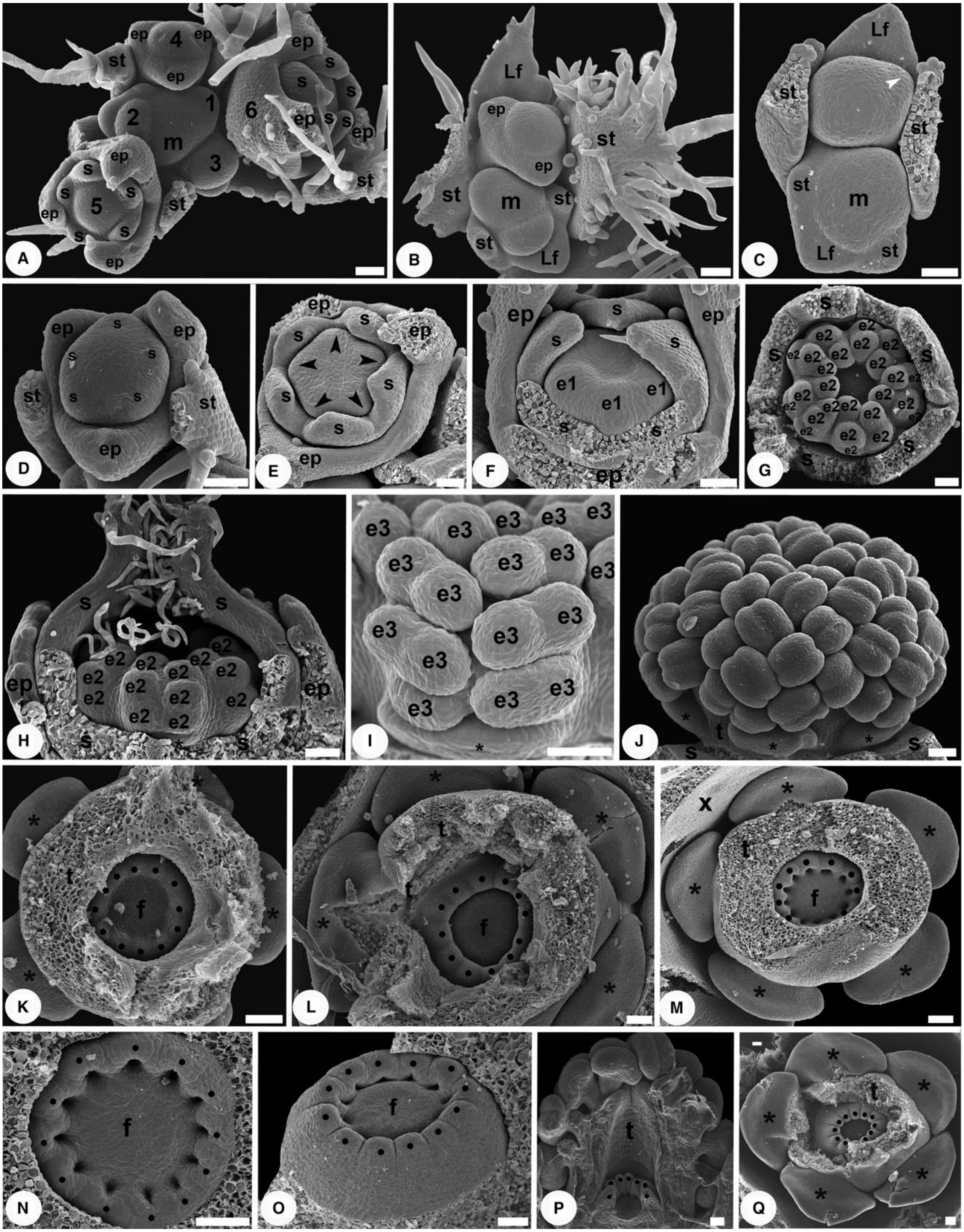
Figure 2. Diagrams of partial inflorescences and individual flowers of *Lavatera trimestris* (A, C) and *Malva hispanica* (B, D). A, B, lateral and top views of partial inflorescences and their order of succession (numbers). All floral buds are subtended by a leaf except in *M. hispanica* (B), in which there are two accessory flowers (1 and 2). Vegetative axis represented by a cross. C, D, floral diagrams of *L. trimestris* (C) and *M. hispanica* (D). Floral axis at the top (crossed black dot). From outer to inner whorls: subtending leaf (grey) united by dashed lines to the stipules in C and D, epicalyx lobes (white shapes), valvate sepals with hairy nectaries (black crescents with filiform adaxial structures), contorted petals (light grey) adnate to the androecial tube, androecium (central ring divided into five sectors, ovals represent androecial units) and central polymerous gynoecium with axile ovules (black ovals). Floral formulae abbreviations: A, androecium; B, epicalyx; C, corolla; G, gynoecium; K, calyx; Vx, axile placentation. Numbers indicate the number of organs per whorl. The double arrow indicates the opposite positions of the petals and the androecium sections. The symmetry of each whorl is indicated by the downward pointing arrow (zygomorphy) and the asterisk (actinomorphy).

Figure 3. Floral development of *Lavatera trimestris*. Scanning electron micrographs. A, partial inflorescence with the order of initiation of the floral buds (numbers). B, floral bud with recently formed epicalyx (top bud) and early branch with differentiated young floral bud. C, top floral bud with epicalyx primordium (white arrowhead) and apex of growing inflorescence (bottom). D, sepal initiation. E, initiation of the primary androecial primordia (black arrowheads). F, lateral view of a floral bud recently enclosed by the epicalyx (front sepals and epicalyx lobes removed). G, top view of a floral bud during centrifugal initiation of the secondary androecial primordia. H, lateral view of a floral bud enclosed by the calyx (front sepals removed) with the petal primordium (asterisk). I, lateral view of tertiary androecial primordia in one of the five main sections of the androecium subtended by a petal (asterisk). J, androecium with differentiated anthers surrounded by petals (asterisks). K–M, congenitally fused carpels (dots) and locule differentiation during petal enlargement (asterisks). N, detail of a young gynoecium with thick flanking walls separating the fused carpels (dots). The floral apex is flat. O, gynoecium with the rising floral apex partially covering the locules and distally free carpels (dots). P, lateral view of a half floral bud with fused androecial tube, differentiated anthers and dorsally bulging carpels (dots). Q, top view of a floral bud displaying the relative size and stage of petals (asterisks) and carpels (dots). Anther tube removed. e1, primary androecial primordia; e2, secondary androecial primordia; e3, tertiary androecial primordia; ep, epicalyx; f, floral apex; Lf, lamina of the subtending leaf; m, floral meristem; s, sepal; st, stipule; t, androecial tube; x, calyx tube. Scale bars, 50 μm.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FLORAL ONTOGENY

Lavatera trimestris

The floral buds initiate sequentially within the inflorescence in the axils of the incipient subtending leaves (Fig. 3A). The stipules of the subtending leaf grow rapidly during the early stages of floral development, whereas the lamina extends more slowly (Fig. 3B, C). The epicalyx organs initiate as sequential independent primordia, beginning with the abaxial one and ending with one of the laterals (Fig. 3C, D). Once raised, they fuse proximally (Fig. 3A–D). The epicalyx lobes rapidly reach the same size. Then, the sepal primordia initiate simultaneously (Fig. 3D). The abaxial and adaxial sepal primordia alternate with the epicalyx lobes (Fig. 3D). By the time of initiation of the corolla/androecium ring wall, the sepals are connate at their base and display the dorso-ventrality of their lamina (Fig. 3E). The floral bud is perfectly pentagonal when the corolla/androecium ring wall is initiated. The alternisepalous corners of the pentagon correspond to five primary androecial primordia that initiate simultaneously (Fig. 3E, F). Each primary androecial primordium differentiates into two rows of alternating secondary primordia that develop centrifugally (Fig. 3G, H). The petals become apparent soon after differentiation of the androecial secondary primordia (Fig. 3H). They alternate with the sepals and are positioned opposite the primary androecial primordia (Fig. 3H, I). The petal primordia grow slowly during development compared with the androecial units. Each secondary androecial primordium begins to subdivide into two tertiary androecial primordia in a centrifugal direction (Fig. 3I). Subsequently, each tertiary androecial primordium differentiates into a two-locular theca and a filament. During development, these androecial unit primordia form a distinctive zigzag pattern and subsequently occupy most of the space available on the androecial tube (Fig. 3I, J). The carpel primordia emerge congenitally fused, forming a ring

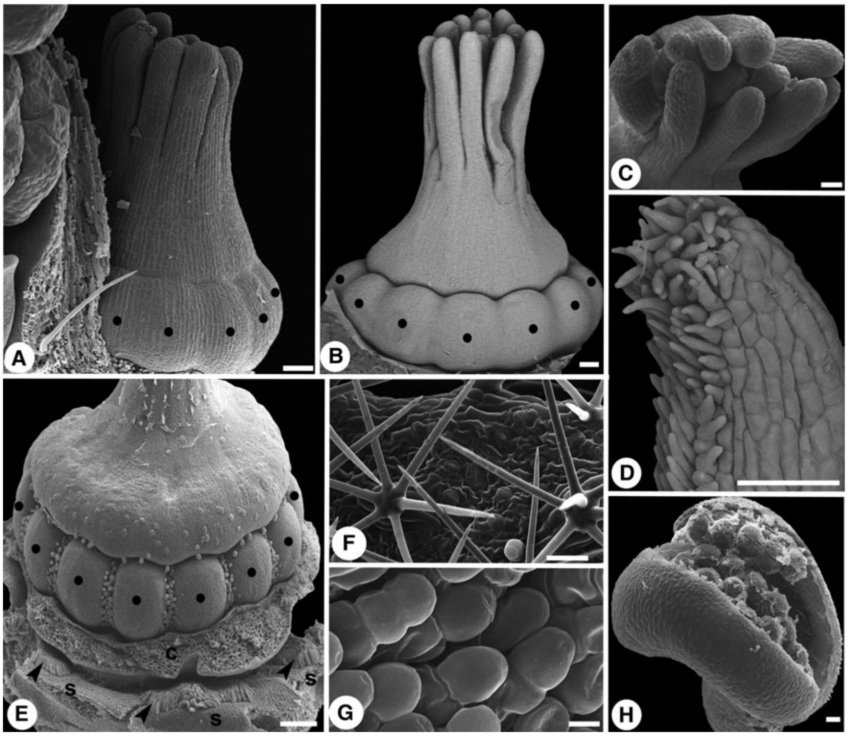

Figure 4. Late sepal, anther and gynoecium development in *Lavatera trimestris*. Scanning electron micrographs. A, bulging carpels (dots) and the fused proximal part of the styles forming a continuous structure. Distal part of the styles free. B, carpels (dots) flanked by conspicuous furrows and separated from the thick proximal part of the styles. C, top view of the distal and free part of the styles forming a flattened (compressed) closure. D, distal papillose stigmatic tissue differentiated along the ventral part of the styles. E, lateral view of mature carpels (dots) with multiple glandular trichomes along the furrows separating them. The proximal part of the styles is thick and wavy. Sepals and petals were partially removed, and remaining sepal nectaries are marked (arrowheads). F, detail of the abaxial side of a mature sepal with stellate and glandular trichomes. G, detail of the uniseriate glandular trichomes forming the sepal nectaries. H, dehiscent bilocular thecae. c, corolla; s, sepal. Scale bars: A–C, F–H, 50 μm; D, 25 μm; E, 0.2 mm.

inside the androecial tube after differentiation of the tertiary androecial primordia and when the petals are already flat, expanding organs (Fig. 3K–M). The carpels are basally connected at the ventral part of the flanks with their neighbours (Fig. 3L–N). The floral apex is initially flat, but soon elongates until it reaches the level of the tip of the carpels, leaving the locule beneath (Fig. 3N–P). When the carpels enlarge, they exhibit dorsal bulging, a free distal part and unifacial styles (Figs 3O–Q, 4A–C, E). The proximal part of the styles is congenitally fused (Fig. 4A, B, E), whereas the distal part is free, forming compressed rows. The stigmas are dry and papillose (Fig. 4C, D).

Malva hispanica

The floral buds initiate successively (Fig. 5A). The stipules and the lamina of the subtending leaf develop more slowly than in *L. trimestris* (compare Fig. 3B, C vs. . 5Fig. C, D). The stipules differentiate almost simultaneously and remain relatively small compared with the fast-growing epicalyx organs (Fig. 5B–D). Although the two epicalyx lobes initiate successively, they rapidly equalize in size and alternate with the lamina and the stipules of the subtending leaf (Fig. 5B–E). The five sepals initiate simultaneously when the epicalyx lobes have overtopped the floral bud, with the abaxial one occupying the median position (Fig. 5C). The sepals expand to reach a similar size to the epicalyx (Fig. 5E). The pentagonal corolla/androecium ring wall primordium initiates, alternating with the sepals (Fig. 5F). The petals initiate as the same time as the centrifugal differentiation of the secondary androecial primordia, opposite the five main androecial areas (Fig. 5G). The tertiary androecial primordia rows differentiate centrifugally and alternate with each other in a zigzag pattern (Fig. 5H). The androecial tube begins to enlarge, and the anthers differentiate (Fig. 5H, I). In abnormally developed floral buds, premature initiation of the petal primordia was observed, with the formation of more than five primary androecial primordia (Fig. 5J). With the androecial tube enlargement delayed, the secondary and tertiary androecial primordia are limited in number and do not form organized rows (Fig. 5K–M). Whereas a tetra- or pentamerous corolla expands in these abnormal flowers, the tertiary androecial units are not evenly arranged, and petaloid structures emerge on the internal side of the androecial tube (Fig. 6A). Gynoecial primordia were not observed in these abnormal flowers. In wild-type flowers, the gynoecium initiates, forming a congenitally fused ring inside the staminal tube (Fig. 6B), and all carpels grow and elongate simultaneously, leaving the distal part of the styles free (Fig. 6C–H). The styles become unifacial (not shown). As the expanding petals begin to be covered by short trichomes, the carpels enlarge, distinctive flanking zones form between them, and the fused proximal part of the styles becomes thicker and separated from the carpels (Fig. 6I, J). As the carpels develop conspicuous dorsal bulging, the proximal part of the styles sinks and forms grooves that differentiate individual styles (Fig. 6K). The distal portion of the styles remains smooth until preanthesis, when the dry and papillose stigmas differentiate (Fig. 6G, L).

DISCUSSION

Homology of the epicalyx with other phyllome structures

The bract and the bracteoles

In Malvales, the epicalyx is interpreted as homologous to the triad of bracts of a plesiomorphic modified inflorescence unit (Weberling, 1989; Bayer, 1999). However, Naghiloo *et al.* (2014) questioned the bracteate nature of the epicalyx proposed by Bayer (1999) in *Alcea rosea* L., in which the epicalyx is present independently from the bracts in the inflorescence. Bayer's (1999) hypothesis refers to bracts from a modified plesiomorphic repeating unit (a cymoid bicolour unit) rather than the bracts currently observed in the inflorescences of these particular species. In the particular case of *M. hispanica* and *L. trimestris*, the epicalyx is not satisfactorily associated with a bract-like origin. The epicalyx lobes do not subtend the shoots or flowers as such, but are grouped at the base of the flower with a whorled disposition. Moreover, although an important ancestral branching rearrangement in the inflorescences could promote the epicalyx origin in Malvaceae, as implied by Bayer (1999), it is difficult to associate the whorled epicalyx with individual and well-differentiated bracts in the bicolour unit of core Malvales.

An alternative interpretation suggests that the epicalyx consists of a whorl of bracteoles, as in *Dirachma socotrana* Schweinf. ex Balf (Dirachmaceae; Bell, 1991). The persistence of three lobes in the epicalyx in the MGA clade (or multiples of three, such as the nine-lobed epicalyx in *Althaea*, Fig. 1A) makes it difficult to associate the origin of the epicalyx with paired bracteoles. In *M. hispanica*, there are two free epicalyx lobes. These epicalyx lobes are located abaxially, alternating with the lamina of the subtending leaf, as also observed in the epicalyx of *L. trimestris* (Fig. 2C, D). Then, in the context of the MGA clade (Fig. 1A), we consider the bilobed epicalyx of *M. hispanica* to be derived from an original trimerous structure after loss of the adaxial lobe, rather than as an original paired bracteolar structure.

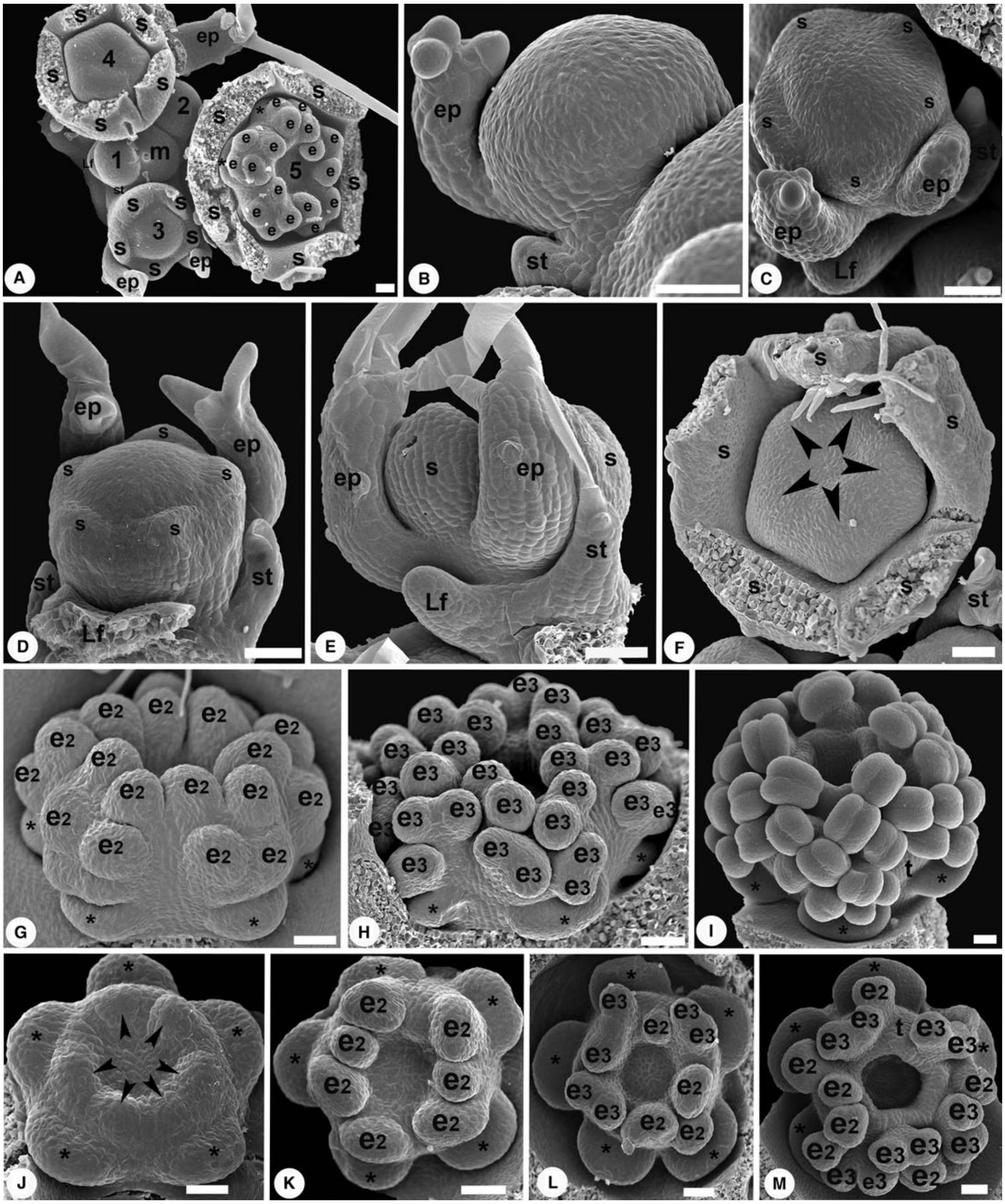

Figure 5. Floral development of *Malva hispanica*. Scanning electron micrographs. A, partial inflorescence. Numbers indicate the order of initiation of the buds. B, epicalyx lobe differentiation. C, successive growth of the epicalyx lobes and sepal initiation. D, floral bud with sepal primordia surrounded by the epicalyx lobes and stipules of the subtending leaf. E, lateral view of the subtending leaf, sepals and epicalyx. F, primary stamen primordia initiation (arrowheads). Sepals are partially removed. G, centrifugal differentiation of the secondary stamen primordia with petals subtending different androecial areas (asterisks). H, centrifugal differentiation of the tertiary stamen primordia. I, anther differentiation and rise of androecial tube. Petals (asterisks) remain separated. J–M, abnormal young floral buds. J, initiation of six primary androecial primordia (arrowheads) after precociously formed petals (asterisks). K, differentiation of uneven secondary androecial primordia and enlarged petals (asterisks). L, irregular differentiation of tertiary androecial primordia alternating with undifferentiated secondary androecial primordia. M, enlargement of the androecial tube bearing androecial primordia at different stages. e1, primary stamen primordia; e2, secondary stamen primordia; e3, tertiary stamen primordia; ep, epicalyx; f, floral apex; Lf, lamina of the subtending leaf; m, floral meristem; s, sepal; st, stipule; t, androecial tube. Scale bars: 50 μm.

Stipules and subtending leaves

Evidence for the homology between epicalyx and sepals or stipules of the adjacent sepals has been found in, for example, *Cuphea* (Mayr, 1969), *Lythrum salicaria* L. (Cheung & Sattler, 1967) and *Potentilla* L. (Rosaceae; Ronse De Craene, 2010). As far as we know, there is no evidence of a calyx-related origin of the epicalyx in Malvaceae. However, this possibility could not be ruled out entirely for *Anisodontea anomala* (Link & Otto) D.M.Bates (Malvaceae, *Anisodontea* C.Presl alliance), in which the epicalyx and the calyx are adnate (see fig. 14B, C in Bates, 1969).

The origin of the epicalyx from a stipulate subtending leaf has been considered previously (Payer, 1857; Eichler, 1878; Bates, 1968; Trimbacher, 1989). The interpretation of leaf-like structures surrounding flowers as modified subtending leaves is found in Tofieldiaceae (Remizowa & Sokoloff, 2003; Remizowa *et al.*, 2010). The homology of subtending leaves of the flower with inflorescence phyllomes has been suggested in Malvaceae (Helictereae and Hermannieae), in which a reduction of the prophyll lamina with persistent stipules was proposed to explain the origin of the bracts surrounding their two-flowered basic structure (Bayer, 1999). In *Malachra* L. (Hibisceae), there is no epicalyx (except in *Malachra radiata* L.) and the subtending leaf of the partial florescence forms a conspicuous involucrum (fig. 19A in Schumann, 1895; Bayer, 1998). In *Nototriche* Turcz. (Malvinae), a high-Andean genus with *c.* 75 species without an epicalyx due to an independent secondary loss (Tate *et al.*, 2005), the pedicel of the flower is inserted at the level or below the point of the stipules of a whorled-like subtending leaf (Hill, 1909; Chanco & Ulloa Ulloa, 2004).

Similarly, the mostly trilobed structure of the epicalyx is consistent with the idea of its homology with the lamina and the two stipules of a floral subtending leaf. In *Lavatera oblongifolia* Boiss, *L. valdesii* Molero & J.M.Monts. and *L. bryoniifolia* Mill. (Escobar García *et al.*, 2009), the lobes of the trimerous epicalyx are often unequal, with the middle one wider than the lateral ones (Molero Briones & Montserrat Martí, 2007), resembling a typical leaf structure with a lamina and its stipules. Remarkably, in cases where there are more than three lobes of the epicalyx in subtribe Malvinae, the number of lobes is usually a multiple of three (six or nine), suggesting an equal division of the whole initial trimerous structure. Multi-lobed epicalyces in Malvinae (with six or more segments) appear in *Alcea* L., *Althaea* and *Kitaibela* Willd. (Escobar García *et al.*, 2009, 2012; Naghiloo *et al.*, 2014).

A comparison of the presence and position of subtending leaves and bracts/bracteoles on the inflorescences of Malvoideae (Bayer, 1994; M.A. Bello, A. Martínez-Asperilla & J. Fuertes-Aguilar, unpubl. data) suggests that the latter are related to particular inflorescence structures rather than to the presence, absence or disposition of the epicalyx. Although the bract and bracteole disposition forms a trimerous structure around individual flowers when present, these structures are independent organs and do not originate from the same meristem as the subtending leaf and its stipules. The presence and position of bracts/bracteoles is variable between and within genera with or without the epicalyx. In epicalyx bearers such as *Althaea*, individual flowers can be either surrounded by bracts/bracteoles, displaying an almost symmetrical structure (e.g. in *A. armeniaca* Ten. and *A. officinalis* L.; figs 74, 76 in Bayer, 1994), or selectively deprived of bracts/bracteoles (*A. cannabinna* L., fig. 75 in Bayer, 1994). In genera without an epicalyx, such as *Abutilon* Mill., bracts/bracteoles can flank individual flowers or can be absent from the partial inflorescence (fig. 61 in Bayer, 1994). Species with an epicalyx [*Anoda cristata* (L.) Schldl., *Lavatera arborea* L., *L. bryoniifolia*, *L. cretica* L., *L. triloba* L., *L. trimestris*, *Malachra capitata* (L.) L., *Sphaeralcea miniata* (Cav.) Spach and several *Malva* species (this study; figs 60–89 in Bayer, 1994)] and without an epicalyx [e.g. *Abutilon indicum* (L.) Sweet, *A. megapotamicum* (Spreng.) A.St.-Hil. & Naudin, *A. sonneratianum* Sweet and *A. striatum* Dickson, fig. 61 in Bayer, 1994; fig. 10.20 in Ronse De Craene, 2010] do not have bracts/bracteoles in their partial inflorescences. It is possible that the bracts are a product of the further modification of former subtending leaves. In *Navaea phoenicea* Webb & Berthel., one of the first divergent species of the MGA clade (Fig. 1A), there are three free bracts and two bracteoles alternating with the epicalyx lobes (our pers. observ.). Each of the three bracts seems to be the product of the congenital fusion of two or three organs, which could correspond to modified individual subtending leaves. Therefore, the epicalyx and the bracts could share the same original structure, i.e. a subtending leaf, but represent a parallel morphological differentiation.

Floral morphology and development

Does the androecium call to mind a branched structure?

The flowers of *L. trimestris* and *L. hispanica* display features of Malvoideae such as pentamerous structure, nectarial trichomatous patches on the adaxial side of the sepals, valvate sepals, contorted petals, an androecial tube with uniformly inserted monothecate units and anatropous ovules with axile placentation (Vogel, 2000; Bayer & Kubitzki, 2003; von

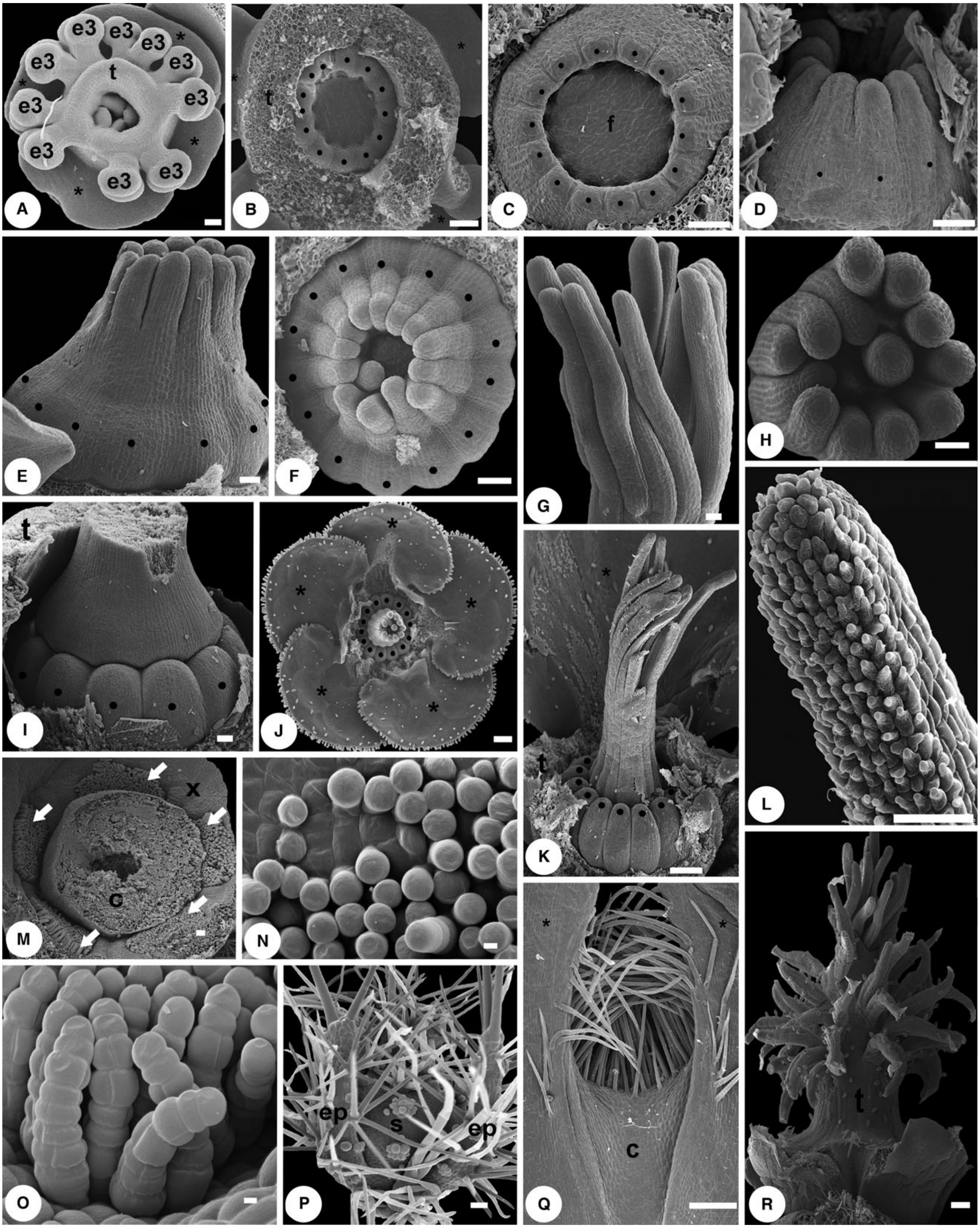
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Figure 6. Middle and late stages of flower development of *Malva hispanica*. Scanning electron micrographs. A, abnormally developed floral bud with four flattened petals (asterisks) with an androecial tube bearing unevenly distributed stamens outside and petal-like structures inside. B, top view of recently initiated gynoecium (dots) and surrounding flattened petals (asterisk). Androecial tube removed. C, congenitally fused carpels (dots) with differentiated locule. D, lateral view of a gynoecium before closure with elongated styles and individual carpels undifferentiated in their proximal part (dots). E, carpels (dots) and fused proximal part of the styles. F, gynoecium with further differentiation of individual carpels (dots) with converging styles. G, distal part of young styles. H, styles converging in different rows. I, carpels (dots) covered by the solid structure formed by the proximal part of the styles. J, top view of mature petals (asterisks) surrounding an enlarged gynoecium. K, detail of a gynoecium with the base of the styles sunken on the top of the carpels (dots). Mature petal at the back (asterisk). L, dry and papillose stigma. M–P, nectarial patches on the sepals. M, distribution of the five nectarial patches at the bottom of the calyx tube (white arrows). N, young glandular trichomes in early stages of nectar development. O, developed glandular uni-/biserial trichomes. P, epicalyx lobes flanking a sepal, all covered by stellate and multicellular glandular trichomes. Q, proximal part of a developed corolla at the area between two petals (asterisks), showing the corolla tube and abundant filiform hairs. R, androecial tube (anthers removed) displaying branching stamens. e2, secondary stamen primordia; c, corolla; e3, tertiary stamen primordia; ep, epicalyx; s, sepal; t, androecial tube; x, calyx tube. Scale bars: A–I, L, M, P, Q, 50 μm; J, K, R, 0.2 mm; N, O, 5 μm.

Balthazar *et al.*, 2004). Some developmental traits of Malvales, such as initiation of the calyx and corolla, connation of the proximal part of the sepals, centrifugal development of the androecium and closure of carpels with unifacial styles (van Heel, 1966; Ronse De Craene & Smets, 1995; von Balthazar *et al.*, 2004; Schönenberger & von Balthazar, 2006; Endress, 2015), were found in these species (Figs 3–6). One of the important features in Malvales is androecium development. This whorl has a transitional state in terms of merism, manifesting the pentamerous framework of the perianth whorls in early development and the increased merism of the gynoecium. In Malvales, the stamen fascicles are a case of secondary and complex polyandry inserted in an oligomerous framework (Ronse De Craene & Smets, 1992; Nandi, 1998; von Balthazar *et al.*, 2006; Schönenberger & von Balthazar, 2006).

The centrifugal development of androecial units in several members of Malvales, developing a tube around the ovary, is unique among angiosperms (von Balthazar *et al.*, 2004; Ronse De Craene, 2010). In other angiosperms with centrifugal androecia, such as Caryophyllales, there are several developmental patterns, including the centrifugal expansion of the androecial ring primordia [e.g. in *Monococcus equinophorus* F.Muell. (Petiveriaceae), *Phytolacca dodecandra* Sessé & Moc. (Phytolaccaceae) and *Corbichonia decumbens* (Forssk.) Exell (Lophiocarpaceae)], which differs from the developmental dynamics in Malvales (von Balthazar *et al.*, 2004, 2006; Ronse De Craene, 2013). In Malvaceae, centrifugal development occurs when the secondary androecial units and the androecial tube begin to grow and differentiate from top to bottom (Figs 3G, 5G). In *L. trimestris* and *M. hispanica*, the first differentiated secondary androecial units (at the top of the androecium) split to form the tertiary androecial units (Figs 3G, 5H). In abnormal flowers of *M. hispanica*, the distal and internal side of the androecial tube is occupied by petal-like structures, suggesting an incipient 'trial' of double flower formation (Figs 5J–M, 6A). A similar phenotype is observed in certain double flowers of *Hibiscus rosa-sinensis* L., in which petaloid structures replace either the androecial tube (fig. 37 in MacIntyre & Lacroix, 1996) or the gynoecium (van Heel, 1966).

Stamens developing centrifugally have been homologized to leaflet primordia (Leins, 1964) or as parts of androclads, i.e. lateral fertile branches (Meeuse, 1966). In *Euphorbia* L. *s.l.*, the centrifugal formation of male organs occurs within a single male partial inflorescence (Prenner & Rudall, 2007). Currently, there is no evidence to propose that the monadelphous androecium of Malvoideae is an inflorescence-like whorl. There are no subtending leaves of the androecial units; however, the opposite configuration of the petal–primary androecial meristem evokes a subtending leaf-branch structure (Figs 3I, 5G). In fact, obdiplostemony is likely to be plesiomorphic in Malvaceae (von Balthazar *et al.*, 2006). Notably, in the first type of double flowers documented in *Alcea rosea*, there is an abnormal second proliferation of androecial primordial regions on the axil of the petals at the bottom of the initially differentiated androecial units (fig. 5 in Naghiloo *et al.*, 2014). In this case, there is a centrifugal development of complexes of androecial primordia rather than only a centrifugal growth of individual androecial units. However, the evoked branch behaviour observed in the centrifugal androecium of Malvaceae is occasional and positionally irregular in the flower. Therefore, although the centrifugal androecium of Malvaceae can replicate itself in double flowers and is subtended by the petals, it is a dynamic flower whorl with an extended growth dynamic rather than

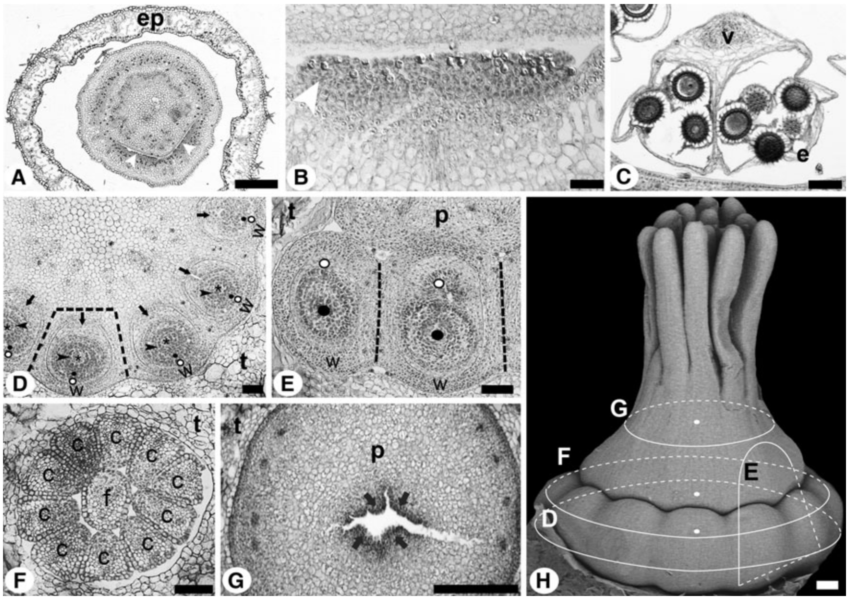

Figure 7. Transverse (A–D, F, G) and longitudinal (E) microtome floral sections of *Lavatera trimestris*. A, fused part of the epicalyx surrounding a floral base at the level of the calyx inception with the nectarial tissue of two sepals (white arrowheads). B, detail of a sepal nectary (white arrowhead). C, mature androecial unit with two locules bearing globose and equinate pollen grains. D, base of the carpels with one ovule each. Individual carpel delimited by black dashed lines. Ovules display a micropile (black arrow), outer (white circle) and inner (black circles) integuments, the nucellus (black arrowhead) and an undifferentiated embryo sac (asterisk). E, locules of two carpels with one ovule each, with differentiated outer (white circle) and inner integument (black circle). Individual carpel delimited (black dashed lines). F, section at the top of the carpels above the ovules. G, section at the fused part of the styles with an open centre surrounded by the pollen transmitting tissue (grey arrows). H, scanning electron micrograph. Gynoecium displaying the scheme of the gynoecium transverse and longitudinal sections shown in D–G. c, carpel; ep, epicalyx; f, floral apex; e, epidermis; p, proximal part of the styles; t, androecial tube; v, vascular bundle; w, carpel wall. Scale bars: A, 0.125 mm; B, 30 µm; C, 25 µm; D–F, H, 50 µm; G, 15 µm.

a regular branching structure embedded in the flower.

Umbrella in Lavatera trimestris

In *L. trimestris*, *M. hispanica* and the MGA clade in general, the one-whorled gynoecium displays concomitant traits of multicarpellate gynoecia (Endress, 2014), such as the compressed structure of parallel rows formed by the styles (Figs 4C, 6H) and the dorsal bulging of the carpels (Figs 3P, 6K). A distinctive pistil morphology of *L. trimestris* and *M. hispanica* is evident in which the proximal part of the styles in *L. trimestris* thickens and becomes wavy at the top of the carpels, forming a solid umbrella-like structure (Figs 4E, 7G, H). By contrast, in *M. hispanica*, the fused column formed by all styles sinks and does not extend to cover the carpels (Fig. 6K). Although within the MGA clade the fruits with fused mericarps that release seeds when ripe (lavateroid fruit) define the Lavateroid clade in which *L. trimestris* and *M. hispanica* are included (Escobar García *et al.*, 2009), the umbrella-like structure seems to grow differentially or be absent in different species. The umbrella-like structure covering the mericarps in *L. trimestris*, used as a diagnostic characteristic by De Candolle (1805) to describe the monotypic genus *Stegia* DC (nom. rejic.), is documented in several *Lavatera* species and is identified as a carpophore rather than as stylar tissue (Fernandes, 1993). With a fleshy initial aspect (Figs 4E, 7G), the umbrella differentiates from the distal part of the carpels and the extended floral apex, maintaining the surface of the transmitting pollen tissue as the communication tract with the carpels (Figs 4B, E, 7E–G). Given its structure and position at the top of the carpels, this floral outgrowth would be better identified as an umbrella with style origin rather than as a carpophore. Therefore, in addition to the *ad hoc* roles of the umbrella as a fruit/seed dispersal organ based on its former consideration as a carpophore and its persistence in the fruit, other functional possibilities are suggested by the proposal of a style origin, such as carpel protection.

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REFERENCES

von Balthazar M, Alverson WS, Schönenberger J, Baum DA. 2004. Comparative floral development and androecium structure in Malvoideae (Malvaceae *s.l.*). *International Journal of Plant Sciences* **165:** 445–473.

von Balthazar M, Schönenberger J, Alverson WS, Janka H, Bayer C, Baum DA. 2006. Structure and evolution of the androecium in the *Malvatheca* clade (Malvaceae *s.l.*) and implications for Malvaceae and Malvales. *Plant Systematics and Evolution* **260:** 171–197.

Bates DM. 1968. Generic relationships in the Malvaceae tribe Malveae. *Gentes Herbariorum* **10:** 117–135.

Bates DM. 1969. Systematics of the South African genus *Anisodontea* Presl (Malvaceae). *Gentes Herbariurum* **10:** 215–383.

Bayer C. 1994. *Zur Infloreszenzmorphologie der Malvales. Dissertationes Botanicae*, Vol. 212. Berlin: J. Cramer.

Bayer C. 1998. Synflorescences of Malvaceae. *Nordic Journal of Botany* **18:** 335–338.

Bayer C. 1999. The bicolor unit – homology and transformation of an inflorescence structure unique to core Malvales. *Plant Systematics and Evolution* **214:** 187–198.

Bayer C, Kubitzki K. 2003. Malvaceae. In: Bayer C, Kubitzki K, eds. *The families and genera of vascular plants, Vol. V*. Berlin: Springer, 225–304.

Bell AD. 1991. *Plant form, an illustrated guide to flowering plant morphology*. New York: Oxford University Press.

Burger WC. 1981. Why are there so many kinds of flowering plants? *BioScience* **31:** 577–581.

Carlson SE, Mayer V, Donoghue MJ. 2009. Phylogenetic relationships, taxonomy, and morphological evolution in Dipsacaceae (Dipsacales) inferred by DNA sequence data. *Taxon* **58:** 1075–1091.

Chanco M, Ulloa Ulloa C. 2004. Las especies de *Nototriche* (Malvaceae) de Ecuador. *Sida* **21:** 693–703.

Cheung M, Sattler R. 1967. Early floral development of *Lythrum salicaria*. *Canadian Journal of Botany* **45:** 1609–1618.

De Candolle AP. 1805. *Stegia*. In: Lamarck JB, ed. *Flore Française, Vol. (4) 2, 3rd edn*. Paris: H. Agasse, 835–836.

Dilcher DL. 2000. Toward a new synthesis: major evolutionary trends in the angiosperm fossil record. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 7030–7036.

Donoghue MJ, Bell CD, Winkworth RC. 2003. The evolution of reproductive characters in Dipsacales. *International Journal of Plant Sciences* **164:** S453–S464.

Eichler AW. 1878. *Blüthendiagramme*. Leipzig: Wilhelm Engelmann.

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

Endress PK. 2001. Origins of flower morphology. In: Wagner GP, ed. *The character concept in evolutionary biology*. San Diego: Academic Press, 495–508.

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

Endress PK. 2010. Disentangling confusions in inflorescence morphology: patterns and diversity of reproductive shoot ramification in angiosperms. *Journal of Systematics and Evolution* **48:** 225–239.

Endress PK. 2014. Multicarpellate gynoecia in angiosperms: occurrence, development, organization and architectural constraints. *Botanical Journal of the Linnean Society* **174:** 1–43.

Endress PK. 2015. Patterns of angiospermy development before carpel sealing across living angiosperms: diversity, and morphological and systematic aspects. *Botanical Journal of the Linnean Society* **178:** 556–591.

Endress PK, Doyle JA. 2007. Floral phyllotaxis in basal angiosperms – development and evolution. *Current Opinion in Plant Biology* **10:** 52–57.

Escobar García P, Pakravan M, Schönswetter P, Fuertes Aguilar J, Schneeweiss GM. 2012. Phylogenetic relationships in the species-rich Irano-Turanian genus *Alcea* (Malvaceae). *Taxon* **61:** 324–332.

Escobar García P, Schönswetter P, Fuertes Aguilar J, Nieto Feliner G, Schneeweiss GM. 2009. Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). *Molecular Phylogenetics and Evolution* **50:** 226–239.

Fernandes R. 1993. *Lavatera* L. In: Castroviejo S, Aedo C, Laínz M, Muñoz Garmendia F, Nieto Feliner G, Paiva J, Benedí C, eds. *Flora Ibérica 3*. Madrid: Real Jardín Botánico-CSIC: 232–243.

van Heel WA. 1966. Morphology of the androecium in Malvales. *Blumea* **13:** 177–394.

Heywood VH, Brummitt RK, Culham A, Seberg O. 2007. *Flowering plant families of the world*. Richmond Hill, Ontario: Firefly Books.

Hill AW. 1909. A revision of the genus *Nototriche* Turcz. *Transactions of the Linnean Society of London. 2nd Series: Botany* **7:** 201–266.

Hofmann U, Göttmann J. 1990. *Morina* L. und *Triplostegia* Wall. ex DC. im Vergleich mit Valerianaceae und Dipsacaceae. *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* **111:** 499–553.

Leins P. 1964. Das zentripetale und zentrifugale Androeceum. *Berichte der Deutschen Botanischen Gesellschaft* **77:** 22–26.

Linnaeus C. 1753. *Species plantarum*. London: Ray Society.

MacIntyre JP, Lacroix CR. 1996. Comparative development of perianth and androecial primordia of the single flower and the homeotic double-flowered mutant in *Hibiscus rosa-sinensis* (Malvaceae). *Canadian Journal of Botany* **74:** 1871–1882.

Mayer V, Svoma E. 1998. Development and function of the elaiosome in *Knautia* (Dipsacaceae). *Botanica Acta* **111:** 402–410.

Mayr B. 1969. Ontogenetische Studien an Myrtalen-Blüten. *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* **89:** 210–271.

Meeuse ADJ. 1966. *Fundamentals of phytomorphology*. New York: Ronald Press.

Molero Briones J, Montserrat Martí JM. 2007. A new species of *Lavatera* sect. *Olbia* (Medik.) DC. (Malvaceae) from north east Morocco. *Botanical Journal of the Linnean Society* **153:** 445–454.

Naghiloo S, Esmaillou Z, Dadpour MR. 2014. Comparative floral ontogeny of single-flowered and double-flowered phenotypes of *Alcea rosea* (Malvaceae). *Australian Journal of Botany* **62:** 217–228.

Nandi OI. 1998. Floral development and systematics of Cistaceae. *Plant Systematics and Evolution* **212:** 107–134.

Payer JB. 1857. *Traité d'organogénie comparée de la fleur*. Paris: Librairie de Victor Masson.

Prenner G, Bateman RM, Rudall PJ. 2010. Floral formulae updated for routine inclusion in formal taxonomic descriptions. *Taxon* **59:** 241–250.

Prenner G, Rudall PJ. 2007. Comparative ontogeny of the cyathium in *Euphorbia* (Euphorbiaceae) and its allies: exploring the organ–flower–inflorescence boundary. *American Journal of Botany* **94:** 1612–1629.

Ray MF. 1995. Systematics of *Lavatera* and *Malva* (Malvaceae, Malveae) – a new perspective. *Plant Systematics and Evolution* **198:** 29–53.

Remizowa M, Sokoloff D. 2003. Inflorescence and floral morphology in *Tofieldia* (Tofieldiaceae) compared with Araceae, Acoraceae and Alismatales *s.str*. *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* **124:** 255–271.

Remizowa MV, Sokoloff DD, Timonin AC, Rudall PJ. 2010. Floral vasculature in *Tofieldia* (Tofieldiaceae) is correlated with floral morphology and development. In: Barfod A, Davis JI, Petersen G, Seberg O, eds. *Diversity, phylogeny and evolution in the monocotyledons*. Århus: Aarhus University Press, 81–99.

Reynolds J, Tampion J. 1983. *Double flowers: a scientific study*. London: Pembridge Press.

Roeder AHK, Yanofsky MF. 2001. Unraveling the mystery of double flowers. *Developmental Cell* **1:** 4–6.

Ronse De Craene LP. 2010. *Floral diagrams: an aid to understanding flower morphology and evolution*. Cambridge: Cambridge University Press.

Ronse De Craene LP. 2013. Reevaluation of the perianth and androecium in Caryophyllales: implications for flower evolution. *Plant Systematics and Evolution* **299:** 1599–1636.

Ronse De Craene LP, Miller AG. 2004. Floral development and anatomy of *Dirachma socotrana* (Dirachmaceae): a controversial member of the Rosales. *Plant Systematics and Evolution* **249:** 111–127.

Ronse De Craene LP, Smets EF. 1992. Complex polyandry in the Magnoliatae: definition, distribution and systematic value. *Nordic Journal of Botany* **12:** 621–649.

Ronse De Craene LP, Smets EF. 1995. The distribution and systematic relevance of the androecial character oligomery. *Botanical Journal of the Linnean Society* **118:** 193–247.

Sachs J. 1874. *Traité de botanique conforme à l'état présent de la science*. Paris: Savy.

Schönenberger J, Conti E. 2003. Molecular phylogeny and floral evolution of Penaeaceae, Oliniaceae, Rhynchocalycaceae and Alzateaceae. *American Journal of Botany* **90:** 293–309.

Schönenberger J, von Balthazar M. 2006. Reproductive structures and phylogenetic framework of the rosids – progress and prospects. *Plant Systematics and Evolution* **260:** 87–106.

Schumann K. 1895. Malvaceae. In: Engler A, Prantl K, eds. *Die natürlichen Pflanzenfamilien, Vol. III*. Leipzig: Verlag von Wilhelm Engelmann, 30–53.

Stebbins GL. 1981. Why are there so many species of flowering plants? *BioScience* **31:** 573–577.

Tate JA, Fuertes-Aguilar J, Wagstaff SJ, La Duke JC, Bodo Slotta TA, Simpson BB. 2005. Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoideae) as inferred from ITS sequence data. *American Journal of Botany* **92:** 584–602.

Trimbacher C. 1989. Der Aussenkelch der Rosaceen. In: Weber A, Vitek E, Kiehn M, eds. *Symposium Morphologie, Anatomie und Systematik 9, Zusammenfassungen der Vorträge*, **66**. Vienna**:** Institute of Botany, University of Vienna.

Vogel S. 2000. The floral nectaries of Malvaceae *sensu lato* – a conspectus. *Kurtziana* **28:** 155–171.

Wanntorp L, Ronse De Craene LP. 2009. Perianth evolution in the sandalwood order Santalales. *American Journal of Botany* **96:** 1361–1371.

Weberling F. 1989. *Morphology of flowers and inflorescences*. Cambridge: Cambridge University Press.