Special Invited Papers

Nonflowers near the base of extant angiosperms? Spatiotemporal arrangement of organs in reproductive units of Hydatellaceae and its bearing on the origin of the flower[1]

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ABSTRACT

Reproductive units (RUs) of *Trithuria*, the sole genus of the early-divergent angiosperm family Hydatellaceae, are compared with flowers of their close relatives in Cabombaceae (Nymphaeales). *Trithuria* RUs combine features of flowers and inflorescences. They differ from typical flowers in possessing an "inside-out" morphology, with carpels surrounding stamens; furthermore, carpels develop centrifugally, in contrast to centripetal or simultaneous development in typical flowers. *Trithuria* RUs could be interpreted as pseudanthia of two or more cymose partial inflorescences enclosed within an involucre, but the bractlike involucral phyllomes do not subtend partial inflorescences and hence collectively resemble a typical perianth. Teratological forms of *T. submersa* indicate a tendency to fasciation and demonstrate that the inside-out structure—the primary feature that separates RUs of Hydatellaceae from more orthodox angiosperm flowers—can be at least partially modified, thus producing a morphology that is closer to an orthodox flower. The *Trithuria* RU could be described as a "nonflower", i.e., a structure that contains typical angiosperm carpels and stamens but does not allow recognition of a typical angiosperm flower. The term nonflower could combine cases of secondary loss of flower identity and cases of a prefloral condition, similar to those that gave rise to the angiosperm flower. Nonhomology among some angiosperm flowers could be due to iterative shifts between nonfloral construction and flower/inflorescence organization of reproductive organs. Potential testing of these hypotheses using evolutionary-developmental genetics is explored using preliminary data from immunolocalization of the floral meristem identity gene *LEAFY* in *T. submersa*, which indicated protein expression at different hierarchical levels.

Key Words: angiosperm • development • evo-devo • flower • Hydatellaceae • immunolocalization • inflorescence • *LEAFY* • ontogeny • pseudanthium • *Trithuria*

The traditional paradigm of early angiosperm evolution presents a picture of an incremental accumulation of adaptive innovations leading to the major diversifications of monocots and eudicots. The angiosperm flower is often portrayed as a classic example of this paradigm; floral evolutionary innovations, especially the carpel, are considered too sophisticated and unique to be homoplastic. Since Charles Darwin in 1879 judged the evolutionary origin of the flowering plants to be an "abominable mystery" (cited in Darwin and Seward, 1903), the origin of the flower has remained one of the most popular discussion topics in evolutionary biology. Several factors lie at the root of the problem, including large numbers of ancient extinctions—both among the early angiosperm lineages and their stem-group—that weaken the value of both outgroup comparison and phylogeny reconstruction (both molecular and morphological) for interpreting the observed morphological variation (Bateman et al., 2006).

Another significant difficulty in determining the origin of the flower results from the high degree of diversity in reproductive morphology of early divergent extant angiosperms and their putative gymnosperm relatives, both extant and extinct. How do we resolve the considerable disparity among the minute perianthless flowers of *Chloranthus* with a single stamen and a single uniovulate carpel (Fig. 1A, B), the relatively cone-like unisexual flowers of plants such as *Kadsura* (Fig. 1C), and the bisexual flowers of plants such as *Nymphaea*, which possess numerous tepals, stamens, and carpels, each carpel containing two or more ovules? *Kadsura* (Schisandraceae) and *Nymphaea* (Nymphaeaceae) both belong among the earliest extant angiosperm lineages (the ANITA-grade [*Amborella*, Nymphaeales, Illiciales, Trimeniaceae, Austrobaileyaceae] or ANA-grade). Chloranthaceae and *Ceratophyllum* (Fig. 1D) are "wildcards" in molecular phylogenetic analyses of angiosperms; they are placed in isolated, though variable, positions among early-divergent extant angiosperms (Qiu et al., 2000, 2006; Soltis et al., 2000) and therefore further complicate phylogenetic optimization of critical morphological character states.

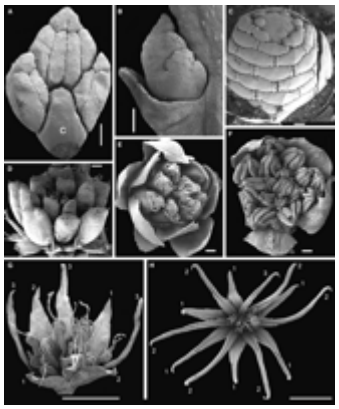

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Fig. 1. Flowers of early-divergent angiosperms, SEM. (A, B) *Chloranthus elatior* (Chloranthales) flower buds. (A) Flower removed from inflorescence and seen from adaxial side; a solitary carpel (c) in center; a single trilobed stamen, central lobe with four microsporangia and each lateral lobe with two microsporangia. (B) Flower on inflorescence axis in the axil of its subtending bract; only the three-lobed stamen is visible (the carpel is hidden by the stamen). (C) *Kadsura* sp. (Schisandraceae, Austrobaileyales), developing male flower with tepals removed, showing laterally elongated anthers of spirally arranged stamens. (D) *Ceratophyllum demersum* (Ceratophyllales), male reproductive unit consisting of several stamens surrounded by bracts (or

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tepals). (E, F) *Amborella trichopoda* (Amborellales) opening flower buds from above. (E) Functionally female with seven carpels surrounded by ca. eight tepals and one staminode. (F) Male flower with numerous stamens surrounded by tepals. (G) *Trithuria occidentalis* (Hydatellaceae, Nymphaeales), female reproductive unit. (H) *T. cookeana*, female reproductive unit with most carpels abscised; 1 = outer whorl of involucre; 2 = second whorl of involucre; 3 = innermost whorl of involucre. Note that Fig. 1H cannot be interpreted as 3+3+6+1 because when the involucre is examined from the reverse side (not shown), aestivation of the six outermost bractlike phyllomes clearly shows they are neither in two whorls nor in a Fibonacci spiral. Scale bars: in A–F = 200 μm, in G, H = 1 mm.

In their influential benchmark paper on floral evolution, Arber and Parkin (1907) postulated that the ancestral angiosperm flower possessed high numbers of unfused organs and that each carpel possessed several ovules. This hypothesis contrasts with an alternative (but also popular) view that ancestral flowers possessed moderate or low numbers of unfused organs and that carpels were uniovulate or at most contained a few ovules (reviewed by Endress, 2001b). The latter opinion relies partly on the relative frequency of Chloranthaceae in the early angiosperm fossil record (e.g., Friis et al., 2006) and partly on phylogenetic placement of *Amborella* as sister to all other angiosperms (Soltis et al., 2000). However, species of Chloranthaceae differ considerably in reproductive morphology from *Amborella*, in which flowers are minute but possess larger numbers of floral parts (male flowers bear 9–11 tepals and 12–21 stamens, and functionally female flowers 7–8 tepals, a few staminodes and ca. 5 uniovulate carpels: Fig. 1E, F) (e.g., Endress and Igersheim, 2000a, b; Endress, 2001b; Posluszny and Tomlinson, 2003; Buzgo et al., 2004).

The recent recognition of a further early-divergent extant angiosperm family, Hydatellaceae (Saarela et al., 2007), prompts us to reexamine these issues. Hydatellaceae are represented by a single genus, *Trithuria* (Fig. 2), following placement of *Hydatella* in synonymy with *Trithuria* (Sokoloff et al., 2008a). Subsequent to detailed studies of comparative morphology and embryology (Rudall et al., 2007, 2008; Sokoloff et al., 2008a, 2008b), we here present novel data from terata that appeared spontaneously in both wild and laboratory-grown material of *Trithuria submersa* Hook.f. and preliminary data from immunolocalization studies on the same species using the *Arabidopsis* gene *LEAFY* (*LFY*).

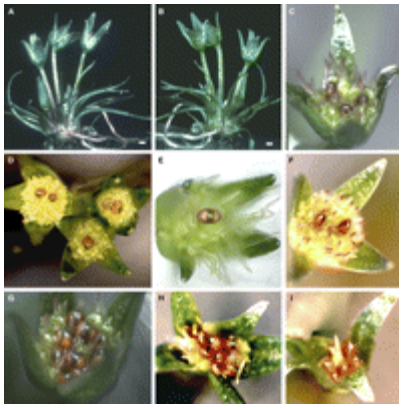

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Fig. 2. *Trithuria submersa*, photographs of plants and reproductive units. (A, B) Entire plants grown on agar, side view. (C–I) Entire reproductive units (red/black structures are undehisced anthers; yellowish structures are carpels, often with red-tipped stigmatic hairs visible). (D–E) "Typical" reproductive units with numerous carpels surrounding one or two central stamens. (C, G–I) Atypical reproductive units with relatively numerous stamens interspersed among carpels, or in one rare instance (I) surrounding one or two central carpels (though in this case carpels were also present outside the stamens). (H) Same individual as in Fig. 5B, E; this specimen is clearly fasciated. Scale bars: in A, B = 1 mm.

Hydatellaceae are significant for several reasons. The reproductive units of Hydatellaceae possess features that are characteristic of both flowers and inflorescences (Rudall et al., 2007). Their recent robust phylogenetic placement as sister to Nymphaeaceae plus Cabombaceae (Saarela et al., 2007) puts this small monogeneric family close to the root of the extant angiosperms and hence close to the angiosperm stem-group. Hydatellaceae consist of 12 species of aquatics, of which 10 are annuals; most are from Australia, though one species is from India and one from New Zealand (Sokoloff et al., 2008a). The reproductive structures of Hydatellaceae (Rudall et al., 2007) differ significantly from those of their close relatives in Nymphaeales.

In this paper, we discuss whether current hypotheses on floral construction and evolution reflect patterns observed in nature. We compare reproductive structures of Hydatellaceae with those of their close relatives in Cabombaceae. Although Hydatellaceae are equally closely related to Cabombaceae and Nymphaeaceae, we focus here on Cabombaceae because flowers of Nymphaeaceae possess more apomorphic characters than those of Cabombaceae, especially syncarpy and carpels sealed by postgenital fusion at the stigmatic region (Endress and Igersheim, 2000a). We reexamine broader questions on the origin of the flower, focusing on the spatiotemporal arrangement of floral organs, because this allows us to address models of floral origin and floral patterning, including the iconic ABC model and its derivatives (e.g., Coen and Meyerowitz, 1991; Theissen et al., 2002). We use *LFY* as a starting-point because in model angiosperms such as *Arabidopsis*, *LFY* orthologs—including *FLORICAULA* (*FLO*), the *Antirrhinum* ortholog—are implicated in specification of floral identity in indeterminate meristems (Coen et al., 1990; Schultz and Haughn, 1991; Weigel et al., 1992; Blázquez and Weigel, 2000), though *LFY* is also expressed at lower level in leaves of *Arabidopsis* (Blázquez et al., 1997) and plays an important role in compound leaf development in legumes (e.g., Gourlay et al., 2000).

MATERIALS AND METHODS

Material

We examined material of *Trithuria submersa* Hook.f. grown in the Micropropagation Unit at the Royal Botanic Gardens, Kew, from seed collected by one of us (*Tuckett & Macfarlane 016*), together with fixed material from the population from which the seed was originally collected (from Mersa road swamp, Western Australia, 27 October 2006). Other comparative material was obtained from several sources: (1) material fixed in the field: *Amborella trichopoda* Baill. (*Pillon s.n.*, New Caledonia); (2) material fixed from living collections at the Royal Botanic Gardens, Kew (HK): *Kadsura* sp. (Lem.) A.C.Sm. (HK 1985–4488); (3) material fixed from living collections at Munich Botanic Garden: *Ceratophyllum demersum* L. (Ceratophyllaceae), *Chloranthus elatior* R.Br. (Chloranthaceae); (4) fixed material in the spirit collections at K: *Brasenia schreberi* J. F. Gmel. (K: 15395, *Drummond and Hemsley 4628*, Uganda), *Cabomba aquatica* Aubl. (K: 56443, *Haase 342*, Bolivia; K: 7405, *Philcox 4636*, Brazil); (5) herbarium collections at K and DNA: *Trithuria occidentalis* Benth. (K: Western Australia, 22 Nov. 1899, *Morrison s.n.*), *T. cookeana* D. D. Sokoloff, Remizowa, T. D. Macfarl. & Rudall (DNA: Australia, Northern Territory, 22 Aug. 1995, *Cowie 5934*).

Methods

All fixed material was transferred to 70% ethanol prior to examination. For SEM, material was dissected in 70% ethanol. Material examined at RBG Kew (Figs. 1, 3) was dehydrated through absolute ethanol and critical-point dried using an Autosamdri-815B CPD (Tousimis Research, Rockville, Maryland, USA), then coated with platinum using an Emitech (Kent, UK) K550 sputter coater and examined using a Hitachi (Wokingham, UK) cold-field emission SEM S–4700–II at 1 kV. Material examined at Moscow University (Figs. 4, 5) was dehydrated through absolute acetone and critical-point dried using a Hitachi HCP–2 critical point dryer, then coated with gold and palladium using a Giko (Tokyo, Japan) IB–3 ion-coater, and observed using a JSM–6380LA SEM (JEOL, Tokyo, Japan) under 20 kV. Reproductive units were photographed using a Leica (Wetzlar, Germany) photomacroscope fitted with a Leica DC500 digital camera (Fig. 2C–I). Some serial images were merged and some images were colored using Adobe (San Jose, California, USA) Photoshop.

Fig. 3. Flowers of Cabombaceae (Nymphaeales), SEM. (A–G) *Cabomba aquatica*. (A) Young flower with all organs already initiated; carpels are clearly ascidiate. (B) Slightly later stage. (C) Young undissected flower; as in (A) and (B), petals are developmentally retarded and much smaller than stamens. (D–F) Preanthetic flowers at approximately the same stage as each other, (D, E) with sepals removed and (F) intact; note at this later stage petal/stamen length ratio is larger than in (A–C). (G) Free carpels of just preanthetic flower. (H, I)

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Brasenia schreberi, (H) top view and (I) side view of the same nearly anthetic flower with perianth removed. The flower has a perianth of 3+3 elements, 20 stamens, and eight carpels. The six first-whorl stamens are inserted in the gaps between adjacent outer- and inner-whorl tepals. The second whorl of the androecium contains eight stamens; there is one stamen on the radius of each inner whorl-tepal, one stamen on the radius of one of three outer-whorl tepals, and two stamens above each of two other outer-whorl tepals. The third whorl of the androecium contains six stamens on the same radii as the first-whorl stamens. Of eight carpels, six alternate with the third-whorl stamens, while two others appear to form another whorl. The outer perianth whorl (calyx in *Cabomba*) is colored deep green, the inner perianth whorl (corolla in *Cabomba*) pale green, stamens yellow (in *Brasenia,* first- and third-whorl stamens are pale yellow; second-whorl stamens are deep yellow), carpels red. Green dots in (A) and (B) and yellow dots in (H) show positions of organs (perianth members in [A] and [B], stamens in [H]) that are hidden by other structures (petals in [A], [B]) or removed and not visible on the images. Scale bars: in A = 100 µm, in B = 150 µm, in C–F = 250 µm, in G = 500 µm, in H, I = 1 mm.

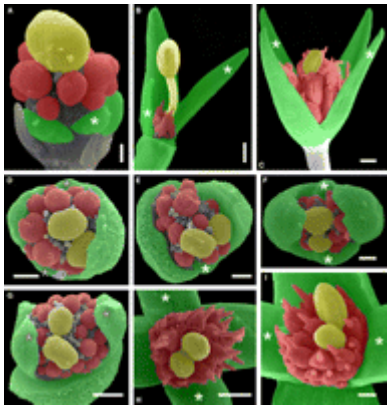

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Fig. 4. Reproductive units (RUs) of *Trithuria submersa*, SEM, showing four bractlike phyllomes and one or two stamens, an organ number that is typical for wild populations of *T. submersa* in Western Australia. (A–C) Wild-source material. (D–I) Material grown at Kew from seeds collected from the same population in Western Australia. (A) Very young RU with a single stamen; centrifugal carpel initiation is clearly visible. (B) Slightly preanthetic RU with single stamen; one of the two phyllomes of the outer involucral whorl has been turned back during dissection. One of the two inner-whorl phyllomes (the left one) is much smaller than the other. (C) Just postanthetic RU with all four involucral organs the same size. (D–I) RUs with two stamens. (D) One stamen in the center of RU,

another stamen could be regarded as being in the axil of an outer-whorl phyllome (though the stamen does not lie exactly on the median radius of the scale). (E) One of the two outer-whorl involucral organs (left in the figure, removed) was much bigger than all other scales; only one inner-whorl phyllome is visible, though it is possible that another would appear later in development. Both stamens are close to the RU periphery. (F) One (larger) stamen is in the center of the RU, while another stamen lies close to the radius of an inner-whorl phyllome. (G) Both outer-whorl involucral phyllomes removed; one stamen in the center of the RU, another (slightly larger) stamen lies on the radius of an outer-whorl involucral scale. (H) RU with one stamen in center, while another stamen cannot be regarded as occurring in the axil of any involucral phyllome. (I) Structure similar to G, but later developmental stage. The stamen closer to the RU center is smaller and younger than the other stamen. Green, involucre; red, carpels; yellow, stamens; asterisks, phyllomes of the inner whorl of the involucre. Scale bars: in A = 30 µm, in B, C, H = 300 µm, in D, E, F = 50 µm, in G, I = 100 µm.

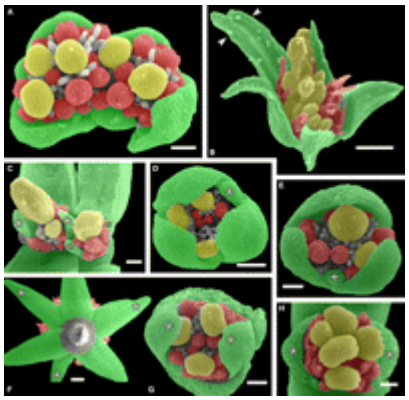

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Fig. 5. Atypical reproductive units (RUs) of *Trithuria submersa*, with increased organ number and/or unusual spatial arrangement, SEM. (A, B, D, E, G, H) Material grown at Kew from seeds collected from the same population in Western Australia. (C, F) From wild-source material. (A) Very young stage of fasciated RU with five stamens, six involucral phyllomes, and numerous carpels, some of which are inserted between stamens. (B) Just preanthetic reproductive unit with at least 13 stamens, at least 10 carpels, and six involucral phyllomes (two phyllomes marked by arrowheads are congenitally united to the top). (C) Two RUs developed on the same stalk (a kind of fasciation). (D) Young RU with involucre of three phyllomes in the outer whorl and only one phyllome visible in the inner whorl (possibly two more inner-whorl phyllomes would develop later); three stamens are inserted closer to the periphery, and young carpels are in the RU center. (E)

Involucre of three unequal phyllomes in the outer whorl and one visible phyllome of the inner whorl. The two stamens lie on the radii of the two largest involucral phyllomes. (F) RU seen from the side of its stalk, the involucre with two regular trimerous whorls. (G) RU with involucre of two dimerous whorls (the outer whorl removed); young carpels are visible at the RU center. (H) RU with involucre of two regular dimerous whorls (the inner whorl removed), stamen number is increased. Green, involucre; red, carpels; yellow, stamens; asterisks, phyllomes of the inner involucral whorl (used only when the two whorls are clearly delimited). Scale bars: in A, E = 50 µm, in B = 500 µm, in C, D, G, H = 100 µm, in F = 300 µm.

For immunolocalizations, entire plants of *Trithuria submersa* were fixed in 4% paraformaldehyde (solid in 1x phosphate-buffered saline [PBS], pH 7.4) for 4 h under vacuum. The tissue was dehydrated in an ethanol series, moved to Histo-Clear (AGTC Bioproducts, Wilmington, Massachusetts, USA), and embedded in paraffin (Paraplast). Embedded tissue was sectioned longitudinally with a rotary microtome (Reichert-Jung 2040; Leica). Sections (8 µm thick) were affixed to Polysine microscope slides. Slides were deparaffinized in Histo-Clear, rehydrated in an ethanol series, and treated as follows: 10 min in 20 $\mu g \cdot mL^{-1}$ proteinase K; diluted in TE (0.1 M Tris/0.05 M EDTA), 2 x 5 min PBS, 30 min BTX (100 mM Tris-HCl pH 7.5, 400 mM NaCl, 1% BSA, 0.3% Triton X-100). Slides were transferred into a humid box and treated as follows: 3 h incubation in blocking solution (10% goat serum in BTX), 12 h incubation in 1:300 dilution of *LFY* antibody at 25°C, 3 x15 min rinse in BTX, 60 min incubation in 1:1500 dilution of goat antirabbit alkaline phosphatase-conjugated secondary antibody (Promega, Southampton, UK), 3 x 15 min rinse in BTX, 20 min incubation in detection buffer (100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$). NBT/BCIP stock solution (Roche Diagnostics, Burgess Hill, UK) was diluted in detection buffer (NBT, 0.15 $mg \cdot ml^{-1}$; BCIP, 0.075 $mg \cdot ml^{-1}$). Staining times varied between 30 min and 135 min. Staining was stopped with 1x TE, after which samples were dehydrated in an ethanol series, incubated in Histo-Clear and mounted with DPX mounting medium. Sections were imaged using a Leitz Diaplan photomicroscope fitted with a Leica DC500 digital camera, using differential interference contrast (DIC) for improved contrast.

RESULTS

Comparison of reproductive structures in Cabombaceae and Hydatellaceae

Cabombaceae consist of two extant genera, *Cabomba* and *Brasenia*. *Cabomba* is adapted for insect pollination, whereas *Brasenia* is wind-pollinated. *Trithuria* (Hydatellaceae) probably combines wind- and water-pollination. There is strong evidence for apomixis in some members of both families. In Cabombaceae, the flowers appear solitary and laterally on shoots with vegetative leaves. Reproductive units (RUs) of Hydatellaceae,

like flowers of Cabombaceae, are borne on shoots with unmodified vegetative leaves, though details of their arrangement are unclear (Rudall et al., 2007). Flowers of *Cabomba* and *Brasenia* are polysymmetric, bisexual, and cyclic, with free carpels in the center, surrounded by stamens and ultimately by the perianth.

Cabomba typically has three sepals alternating with three petals (rarely the flowers are dimerous; Orgaard [1991]). The petals are developmentally retarded (Fig. 3A–C; see also Raciborski, 1894; Tucker and Douglas, 1996; Endress, 2001a). Stamens of *Cabomba* are typically arranged in a single whorl of six, occurring at radii between adjacent petals and sepals (Fig. 3A, B, D, E), though sometimes just three stamens alternate with the petals (e.g., Wettstein, 1924; Williamson and Schneider, 1993). Some authors have described the six stamens of *Cabomba* as belonging to two whorls of three (e.g., Dahlgren et al., 1985), but we have never observed this pattern, nor have other authors (e.g., Ito, 1986; Tucker and Douglas, 1996; Endress, 2001a; Schneider et al., 2003). Carpel number is variable in *Cabomba*, but most often there are three carpels on the radii of the petals (Fig. 3A–E). We have observed flowers of *C. aquatica* that were intermediate between trimerous and dimerous, possessing two sepals, two petals, one perianth member intermediate between sepal and petal, five stamens, and two or three carpels.

The perianth of *Brasenia* has six organs in two whorls. Early in development, it resembles the perianth of *Cabomba* (e.g., Schneider et al., 2003), but outer and inner whorl organs are alike at anthesis and thus usually described as tepals (though Richardson [1969] and Schneider et al. [2003] termed them sepals and petals). The number and position of stamens and carpels are variable in *Brasenia* (e.g., Raciborski, 1894; Richardson, 1969; Schneider et al., 2003), but generally both carpels and stamens are more numerous than in *Cabomba*. The first stamen whorl includes six stamens in the same position as in *Cabomba* (Fig. 3H, I), i.e., on radii between adjacent outer and inner whorl perianth elements (see also Ito, 1986; Ronse De Craene, 1992). Doubling of organ number in the third whorl of the flower (regardless of which organ type develops in the third whorl) is characteristic for both Cabombaceae and Nymphaeaceae (Endress, 2001b). Carpels initiate after the last stamens are formed; this is also a common pattern of Cabombaceae and Nymphaeaceae (Richardson, 1969; Tucker and Douglas, 1996; Endress, 2001a; Schneider et al., 2003).

Reproductive units of *Trithuria* (Rudall et al., 2007; Sokoloff et al., 2008a) are either bisexual (in five species) or unisexual (in seven species, of which four are dioecious). They are surrounded by an involucre of 2–30 bractlike phyllomes; in all dioecious species, male RUs have fewer and longer involucral phyllomes than female RUs (Sokoloff et al., 2008a). Phyllome arrangement is whorled. Involucres of four phyllomes are common; in this case they are typically arranged in two dimerous whorls. In *T. submersa* (the only species in which this pattern has been studied developmentally), the second whorl of the involucre is developmentally retarded (Rudall et al., 2007). Phyllotaxy in involucres with numerous phyllomes is imperfectly known due to absence of fixed material. At least in female RUs of *T. occidentalis* (Fig. 1G), where the first and the second whorls are dimerous, the third whorl is tetramerous (Sokoloff et al., 2008a). Figure 1 H shows an example of a female RU of *T. cookeana* with 13 bractlike

phyllomes; here six plus six phyllomes form two alternating whorls, and the final, innermost phyllome is inserted on a radius between two adjacent phyllomes of the inner and outer whorls. The relatively stability of this pattern in *T. cookeana* is so far unknown.

In bisexual RUs of *Trithuria*, stamens lie in the center and are surrounded by carpels (Fig. 2D–F). Carpel and stamen (when stamens are numerous) initiation sequence is centrifugal in both unisexual and bisexual reproductive units (Rudall et al., 2007). In bisexual RUs, carpels appear only when all stamens are initiated. Stamens are typically numerous in male RUs: up to 6–8 in *T. australis*, *T. inconspicua*, and *T. filamentosa*; up to 10–17 in the four dioecious species (Sokoloff et al., 2008a). When RUs are bisexual, one or two stamens are often present. These are the only conditions described so far in *T. konkanensis*, *T. lanterna*, and *T. bibracteata*, and the presence of only one stamen is characteristic for the vast majority of studied reproductive units of these three species (Cooke, 1987; Yadav and Janarthanam, 1994; Gaikwad and Yadav, 2003; Sokoloff et al., 2008a). *Trithuria cowieana* possesses 1–3 stamens, and *T. submersa* typically has one or two stamens, though in some populations in Tasmania and southeastern Australia up to six stamens per RU are observed (Hooker, 1858; Cooke, 1987; Sokoloff et al., 2008a). The material used for the only previous developmental study of *T. submersa* (Rudall et al., 2007) had two stamens.

Variation in reproductive units in Trithuria submersa

We report here novel data on variation in organ number and spatiotemporal arrangement in RUs of *Trithuria submersa*, based mainly on material cultivated at Kew (Figs. 2, 4, 5). Because the variation observed is very high and the degree of variation is greater than previously described for this species (Sokoloff et al., 2008a), we are tempted to classify all the unusual morphotypes as terata. However, material (admittedly limited) of the same origin collected from its original habitat also displayed unusually high morphological variation, indicating that in vitro methods are not the primary causal factor. It is unlikely that the different morphotypes are genetically fixed, representing several mutant lineages, because we observed radically different RU structures within the same individual. For example, images for Figs. 4A–C and 5C–F are from the same individual collected in the wild. Figures 4F–I and 5D, G, and H are from the same cultivated individual; Figs. 2H, 5B, and E from another cultivated individual; and Fig. 4D and E from a third cultivated individual.

Figure 4 consists of RUs with four involucral phyllomes in two whorls and one or two stamens. These are typical organ numbers in RUs of *T. submersa* (Sokoloff et al., 2008a). However, neither of the two-staminate RUs (Fig. 4D–I) has the same spatial arrangement of organs as that previously described by Hieronymus (1888) and Rudall et al. (2007), specifically two stamens in the RU center, each stamen lying on the radius of one of the two outer-whorl involucral phyllomes. In Fig. 4D and F, one of the two stamens is clearly in the center of the RU, whereas another lies on the periphery, close to the involucre, at least in the younger stages. In Fig. 4I, the larger stamen is closer to the periphery, which complicates the concept of centrifugal stamen initiation. In Fig. 4E, both stamens are shifted from the center of the reproductive unit. In Fig. 4F, attachment of the

two stamens occurs almost in the plane of two inner-whorl involucral phyllomes, while in Fig. 4H stamen insertion is oblique.

Figures 2C, G–I, and 5 show RUs with strongly altered Bauplan. In Fig. 5C, two more or less typical RUs are closely aggregated on the same stalk. This atypical spatial arrangement is clearly a case of fasciation. The structures in Figs. 2H, 5A, and B are also fasciated, and the fasciation is more pronounced because individual reproductive units cannot be recognized. Reproductive units in Fig. 5D–F possess a trimerous involucre. In the young stages illustrated in Fig. 5D and E, only one inner-whorl involucral phyllome (of potentially three) is visible; it is not clear whether two other phyllomes will initiate later. In the anthetic RU shown in Fig. 5F, the involucre possesses two regular trimerous whorls. In Fig. 5D and G, stamens do not form a compact group in the RU center; instead, young carpels are present at the center, between the stamens. These central carpels are younger than some other carpels closer to the RU periphery. The RU in Fig. 5H shows increased stamen number (five), though the involucre of 2+2 scales is "normal" for *T. submersa*. Reproductive units with numerous stamens surrounding a central carpel were also observed; in this case, other carpels were present between stamens and the involucre (Fig. 2I). Also, a few RUs were found with multiple stamens forming an incomplete ring surrounded outside and inside by numerous carpels (Fig. 2C, G). A rare case of an organ combining features of a stamen and a carpel (a "hybrid" stamen/carpel) was observed (Fig. 6G–J). In this RU, one central organ possessed two pollen-bearing anther locules (though with relatively fewer pollen grains than typical anther locules), yet bore stigmatic hairs. An additional smaller locule, present above the two pollen-bearing locules, contained a single prominent nucleus and some degenerate cells, in some respects resembling a partial embryo sac (Fig. 6H). All other reproductive organs in this RU were carpels; no typical stamen was present.

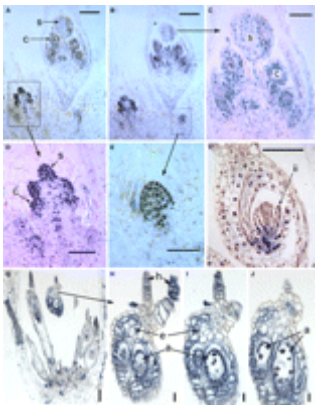

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Fig. 6. Immunolocalization of *LFY* protein in reproductive units of *Trithuria submersa* (longitudinal sections of reproductive units, DIC images). (A) Two reproductive units, the larger one with male and female structures (stamen and carpels) formed; both show immunostaining for *LFY* protein. The smaller structure shows initials for male and female structures (stamen and carpels), which also have strong signals for *LFY* protein (details shown in [D]). Note that the surrounding tissue, including the bractlike phyllomes, have almost no staining. (B) Similar stage as in (A), but with a third site of strong immunostaining (rectangle, detail shown in [E]), which is the primordium of an entire reproductive unit. Upper arrow indicates detail of same stamen in (C), but in different optical section. (C) Reproductive unit showing developing male and female structures (stamen and

carpels) with distinct immunostaining. (F) Details of female structure (carpel) showing some immunostaining at the chalazal region of the ovule. (G–J) Specimen with hybrid stamen/carpel in center, surrounded by normal carpels. (H–J) Optical sections of the stamen/carpel. a = pollen-bearing locule; c = carpel; e = abnormal locule; h = stigmatic hairs; ii = inner integument; s = stamen. Scale bars: in A–G = 100 µm, in H–J = 20 µm.

Immunolocalization

To clarify the distribution of *LFY* proteins in normally developed ("typical") RUs of *Trithuria submersa*, we performed immunolocalizations with anti-LFY antibodies (Fig. 6A–F). We detected highest protein concentrations at the youngest developmental stages (Fig. 6A, B, D, E), both in the primordium of the entire reproductive unit (Fig. 6B, E) and the primordia of carpels and stamens of somewhat later developmental stages (Fig. 6A, B, D). At these later stages, no *LFY* protein could be detected in the surrounding tissues, including the involucral phyllomes. The protein level remains high in older developmental stages in which the stamens and carpels are already differentiated (Fig. 6A–C). Within the carpel, a signal for *LFY* protein was present in the chalazal region of the ovule (Fig. 6F).

DISCUSSION

Flowers, inflorescences, and nonflowers

In some angiosperm reproductive structures, there is a loss of flower individuality because certain floral organs (e.g., stamens and carpels) cannot be readily assigned to individual flowers. This "fuzzy" type of pseudanthium could provide insights into the inflorescence–flower boundary, and hence into the origin(s) of the flower. (Note that "fuzzy" pseudanthia should not be confused with pseudanthia of another type, such as condensed inflorescences of Asteraceae, in which individual flowers generally remain clearly recognizable, e.g., Classen-Bockhoff, 1990). For example, in some angiosperm groups pseudanthial terminal flower-like structures could have given rise to what we normally term "true" (i.e., euanthial) flowers (Sokoloff et al., 2006). Furthermore, loss of floral identity can provoke morphological novelties, including unusual tubular and filamentous structures and unusual patterns of connation (Sokoloff et al., 2006; Prenner and Rudall, 2007; Rudall, 2008).

Examples of "fuzzy" pseudanthia include the cyathium of the eudicot *Euphorbia*, which is widely interpreted as an inflorescence (Prenner and Rudall, 2007; see also Prenner et al., 2008a, b), and female or hermaphrodite reproductive units in the monocot family Triuridaceae (including the much-discussed "inside-out flower" of *Lacandonia*), which are traditionally regarded as flowers, but have also been interpreted as inflorescences (see Rudall, 2003, 2008; Vergara-Silva et al., 2003; Ambrose et al., 2006; Rudall and Bateman, 2006). In several other angiosperms, including some putative stem-group and/or early-divergent taxa, interpretation of the reproductive units—whether as flowers or inflorescences—is ambivalent. For example, in *Ceratophyllum*, female reproductive

units possess a single uniovulate carpel, whereas male units (Fig. 1D) might be interpreted as pseudanthia, consisting of 3–46 stamens, though they are widely described as flowers. Both male and female units are surrounded by leaflike structures that have been interpreted as either tepals or bracts (Schleiden, 1837; Endress, 1994; Iwamoto et al., 2003). Male reproductive units of *Hedyosmum* (Chloranthaceae), which consist of numerous lateral stamens along an axis lacking any bracts or tepals, have been interpreted either as cone-like prefloral structures (Leroy, 1983) or as an abracteate inflorescence of perianthless unistaminate male flowers (reviewed by Endress, 1987). The attenuated reproductive unit of the Cretaceous fossil *Archaefructus* has been considered to be either a "prefloral structure," possibly homologous with an angiosperm flower (Sun et al., 2002), or an inflorescence (Friis et al., 2003).

We use here a speculative term "nonflower" for structures that contain typical angiosperm carpels and stamens but do not allow recognition of a typical angiosperm flower. As defined here, the term nonflower could combine cases of secondary loss of flower identity and cases of a prefloral condition, similar to those that gave rise to the angiosperm flower.

It is tempting to interpret the reproductive units of Hydatellaceae as flowers because the bractlike phyllomes enclosing them show similarities with the perianth organs (sepals and petals, or tepals) in flowers of their closest relatives in Cabombaceae (Fig. 3) and Nymphaeaceae. (Note that in the context of floral organ identity, "bractlike phyllomes" represents a more neutral term than "bracts" or "tepals": Balthazar and Endress, 2002.) For example, RUs of Hydatellaceae and flowers of Cabombaceae and Nymphaeaceae share whorled phyllotaxy. Cabombaceae typically have a trimerous perianth, and many Nymphaeaceae are even tetramerous. Dimery was traditionally considered the basic condition in the involucre of Hydatellaceae. However, the current study shows examples of trimerous dicyclic involucres and even hexamerous patterns in *Trithuria*. Dimerous flowers and flowers that are intermediate between dimery and trimery are rarely found in *Cabomba*. Richardson (1969) reported sporadic occurrence of dimerous and tetramerous flowers in *Brasenia*. Both involucres of Hydatellaceae and flowers of Cabombaceae and Nymphaeaceae show frequent increase of organ number in the third whorl. In both groups, organs of the second whorl are developmentally retarded in two-whorled perianth or involucre systems. However, the RUs of Hydatellaceae cannot be regarded as typical flowers for two main reasons: (1) carpels (pistils) surround the stamens in Hydatellaceae, whereas they occupy a central position in typical flowers (i.e., they have "inside-out" morphology with respect to typical flowers); (2) multiple carpels develop centrifugally in Hydatellaceae, whereas they develop either centripetally or simultaneously in typical flowers. These two factors combined distinguish the reproductive units of Hydatellaceae from flowers of almost all other angiosperms. One significant exception is the "inside-out flower" of *Lacandonia* (Triuridaceae), in which later-formed carpels surround three central early-formed stamens (e.g., Vergara-Silva et al., 2003; Ambrose et al., 2006).

Conversely, there are several problems with an inflorescence interpretation for RUs of Hydatellaceae. (1) The complex stamen and carpel arrangement would require interpretation as groups of cymose partial inflorescences. However, the symmetry planes of the carpels (i.e., presumed reduced female flowers) are often all almost parallel to each

other or, in other cases, apparently chaotically organized in Hydatellaceae. In contrast, a regular zig-zag configuration would be expected in a cincinnus (a type of cyme interpreted previously in reproductive units of Hydatellaceae: Hieronymus, 1888; Rudall et al., 2007). Furthermore, (2) evidence from ontogenetic data provides limited help in addressing this issue because during early ontogeny each carpel is not readily assignable to a particular cyme. Finally, (3) accumulation of data on the diversity of spatial arrangement of organs in reproductive units of Hydatellaceae leads us to hypothesize that none of the involucral bractlike phyllomes subtend next-order inflorescence branches. In other words, *even if we accept* the presence of two or more cymose partial inflorescences, they may *not* be situated in the axils of the involucral organs.

We demonstrate this conundrum with data from RU variation in *Trithuria submersa*. If we accept the presence of two or several cymes, then the first reproductive organs to initiate should belong to the first flowers of each corresponding cyme. When there are two stamens per RU, and these are the first reproductive organs to form, it is logical to accept two first-formed cincinni, each started with a male flower and continued by female flowers (Hieronymus, 1888). Since Hieronymus (1888) and Rudall et al. (2007) found these two stamens lying on the radii of the outer-whorl bractlike phyllomes, it was reasonable to consider that the two cincinni develop in their axils. However, the current study shows that virtually any relative position of 2+2 involucral organs and two stamens can be found. Thus, if accepting a pseudanthial interpretation, it is now logical to argue that the real subtending bracts of the cincinni are reduced or deleted. When involucral organs are numerous (as in *T. cookeana*, *T. polybracteata*; Sokoloff et al., 2008a), at least the lower ones definitely lack axillary structures. This new data lead us to reject the concept of branching in the axils of the involucral organs. Thus, in this respect the involucre resembles a typical perianth. It is important to note in this context that precise delimitation between bracts and tepals is also problematic in some early-divergent angiosperms with "typical" flowers (e.g., Balthazar and Endress, 2002; Endress, 2002, 2003; Buzgo et al., 2004; Kim et al., 2005).

Fasciation

Superficially, the novel teratological forms presented here undermine our earlier report of a "fixed" inside-out morphology in Hydatellaceae (Rudall et al., 2007) because we have now observed reproductive units with central carpels (Figs. 2C, G–I, 5). However, since some of the multistaminate reproductive units in our material of *T. submersa* could readily be interpreted as products of fasciation, we suspect that all of the cases of unusual carpel and stamen position and increased stamen number are due to a tendency to fasciation. One problem with this interpretation is the lack of a clear and useful definition of the term fasciation (see also Sokoloff et al., 2007). The term is normally applied to cases of incompletely divided axes and less frequently applied to multiple partially fused axes (Masters, 1869; Worsdell, 1915; Sokoloff et al., 2006). There is a risk of using it as a "catch-all" to cover a wide range of nonorthodox, teratological morphologies.

Fasciation probably occurs when apical meristem shape and/or size are radically altered. Studies of *Arabidopsis* have suggested a role for the *CLAVATA* gene family in the appearance of fasciated phenotypes (e.g., Clark et al., 1993, 1995). It is unlikely that

the material of *T. submersa* examined here was significantly genetically heterogeneous, but it contains two radically different morphotypes (though connected by many intermediates): RUs that are orthodox for Hydatellaceae, with central stamens and peripheral centrifugal carpels, and unorthodox RUs with central carpels and more-or-less peripheral stamens. This apparent switch from the typical condition means that the primary structural feature that separates RUs of Hydatellaceae from orthodox angiosperm flowers (i.e., the inside-out carpel vs. stamen arrangement) can be at least partially altered. Even more surprisingly, this alteration could be environmentally influenced.

In general, the material studied here documents the remarkable morphological plasticity of reproductive organs of *Trithuria submersa*. The "hybrid" stamen/carpel organ in Fig. 6G–I is one of the manifestations of this plasticity, probably resulting from a mosaic of morphogenetic programs of stamens and carpels. Similar teratological structures intermediate between carpels and stamens have been described in a wide range of angiosperms (for review, see Shealy and Herr, 1973), though transformation of stamens to carpels is possibly more common than the contrary condition. Vergara-Silva et al. (2003) also reported plasticity in reproductive organ identity in some teratological specimens of *Triuris brevistylis* and *Lacandonia schismatica* (Triuridaceae).

Hierarchical shifts: An emerging paradigm for bidirectional homoplastic origins of flowers and inflorescences

Uniaxial (euanthial) models of floral origin, which are currently employed almost universally, consider the flower to be uniquely derived from a single, unbranched, condensed axis bearing sporophylls with proximal microsporophylls (stamens) and distal megasporophylls (carpels) (Arber and Parkin, 1907). In contrast, pseudanthial and other polyaxial theories perceive the flower as derived from a condensed, multiaxial structure, and each organ as a condensed axis rather than a leaflike structure (Wettstein, 1901, 1924; Melville, 1960; Meeuse, 1975; Doyle, 1994; Hickey and Taylor, 1996). Meeuse's "anthocorm theory" (Meeuse, 1975) postulated that the ancestral angiosperm reproductive structure (anthocorm) was biaxial; in some lineages, lateral branches produced uniaxial (euanthial) flowers, whereas in others the entire biaxial (pseudanthial) system was transformed into a flower.

The degree to which these two models (uniaxial and polyaxial) and their numerous variants require different types and degrees of homeosis to achieve a flower, or merely require suppression of branching and axial condensation, will be the subject of further review. It seems possible that the transition to bisexuality is especially problematic in uniaxial hypotheses, resulting in theories that invoke homeosis, such as the "mostly male" theory (Frohlich and Parker, 2000), the "out-of-male/out-of-female" model (Theissen et al., 2002), and Meyen's (1988) gamoheterotopy theory, though the latter is equally applicable to the origin of bisexual or unisexual flowers from bisexual or unisexual bennettitalean cones, respectively (see also Bateman et al., 2006; Baum and Hileman, 2006; Frohlich and Chase, 2007; Sokoloff and Timonin, 2007). However, switches between unisexual and bisexual reproductive structures, and vice versa, were relatively frequent during the evolution of the early angiosperms and Bennettitales (Meyen, 1988). Thus, the origin of the carpel is perhaps more enigmatic than the origin of bisexuality.

Our observations on Hydatellaceae raise the possibility of nonhomology of flowers and inflorescences among early-divergent angiosperms. However, this does not necessarily imply multiple transitions from a nonfloral to a floral condition (i.e., multiple origins of flowers). It is likely that the closest common ancestor of all extant angiosperms already possessed a structure that could readily be described as a flower (but see Bateman et al. [2006] for a review of definitions of the flower). Nonhomology between some angiosperm flowers could be due to iterative hierarchical shifts between flowers and inflorescences (developmental heterochrony), as suggested for terminal flower-like structures in racemose inflorescences by Sokoloff et al. (2006).

Several authors have postulated secondary derivation of flower-like structures from inflorescences (i.e., a secondary pseudanthial origin), mostly based on ontogenetic evidence. For example, conversion of an entire terminal pseudanthium into a true flower was proposed for two lineages within the early-divergent monocot order Alismatales (Sokoloff et al., 2006). Within the magnoliid order Piperales, terminal structures in inflorescences of perianthless taxa are similar to terminal flowers of perianth-bearing taxa, though the direction of possible evolutionary transformation (if any) remains difficult to determine (Remizowa et al., 2005; Sokoloff et al., 2006). Multiple origins of flower-like structures are implicit in these pseudanthial hypotheses. Similar processes could have occurred in other seed plants. For example, ontogenetic studies have indicated that flower-like reproductive units in extant members of Gnetales are derived from reduced compound cones (e.g., Hickey and Taylor, 1996; Mundry and Stützel, 2004).

A hierarchical-shifts model has been invoked in other studies, either directly or indirectly. Albert et al. (1997) proposed a "babushka doll" of internested developmental programs among complex branching structures, including inflorescences, flowers, and ovules. Classen-Bockhoff (1992) discussed the developmental similarity between flowers and inflorescences as a simple shift from one organizational level to another. Rutishauser et al. (2008) described "fuzzy" boundaries in several plant structures that cannot readily be categorized into discrete hierarchical units. Sokoloff et al. (2006) compared the occurrence of terminal pseudanthia and associated anomalous structures in racemose inflorescences of early-divergent angiosperms. They concluded that pseudanthium formation can provoke emergence of intermediate structures and morphological novelties that could result from amalgamation of developmental pathways (including expression zones of regulatory genes and/or new spatial constraints), leading to "developmental mosaics" between structures that are normally assumed to have contrasting and well-defined identities.

Use of evolutionary-developmental genetics in evaluating floral origins

To what extent are these ideas testable using evolutionary-developmental genetics? Several factors, largely reflecting pre-Cenozoic extinctions of gymnosperms and stem-group angiosperms, make such an approach problematic. These include profound uncertainties surrounding the phylogenetic context of the angiosperms and considerable phenotypic and genotypic lacunae separating early-divergent extant angiosperms from their closest relatives among extant gymnosperms (Bateman et al., 2006). Doyle (1994) predicted that the pseudanthial/euanthial anthophyte debate of floral origin would

ultimately be resolved by comparison of expression in various seed plants of A-function genes such as the floral transcription factor *APETALA1* (*AP1*); such methodology would allow comparison of the angiosperm perianth with the outer integument of Gnetales and the seed-bearing and subtending scales of conifers. However, this approach would not test whether hierarchical shifts can occur. For example, Li et al. (2005) found that in the perianthless, early-divergent angiosperm *Chloranthus*, the gene *CsAP1* is expressed broadly in the flower, thus supporting earlier suggestions (e.g., Lawton-Rauh et al., 2000) that the ancestral function of *AP1* could be specification of meristem identity.

The low-copy gene *LFY* also has a role in specification of meristem identity, at least in *Arabidopsis*, in which *LFY* is expressed in the (determinate) floral primordia but not in the (indeterminate) primary inflorescence meristem (Weigel et al., 1992; Sessions et al., 2000). In *Arabidopsis leafy* mutants, lower inflorescence nodes form secondary short shoots rather than flowers (Ratcliffe et al., 1998). Thus, if the reproductive unit of *Trithuria* is an inflorescence, we would expect *LFY* /*FLO* orthologs to be expressed only in individual organ primordia (i.e., putative flowers). However, this is not the case; we clearly observed localization of LFY protein in reproductive primordia at several different hierarchical levels (individual organs and RU primordia) in *Trithuria*. Thus, our preliminary immunolocalization results fail to resolve the identity of the *Trithuria* reproductive unit, though they demonstrate that *LFY* is expressed in developing reproductive meristems.

Many more comparative expression studies, especially on early-divergent angiosperms, are required to establish the ancestral function of *LEAFY*-like genes. The expression patterns of *LEAFY*-like genes vary widely among the different angiosperm and gymnosperm species in which *LFY* orthologs have been identified (for review, see Allnutt et al., 2007). Among gymnosperms, Vázquez-Lobo et al. (2007) found complex spatiotemporal expression patterns of *LFY* /*FLO* and *NEEDLY* orthologs in reproductive structures of a heterogeneous set of conifers: *Picea*, *Podocarpus*, and *Taxus*. In angiosperms, the majority of *LFY* expression studies have been carried out on *Arabidopsis* and *Antirrhinum*, which are both eudicots placed in the rosid and asterid clades, respectively (APG II, 2003). However, in these taxa the inflorescence is racemose; the main axis of the raceme (where *LFY* is not expressed) is therefore fundamentally different from the lateral, flower-bearing axes, where *LFY* is expressed in the floral apices. In species with a cymose inflorescence, which could be the case in *Trithuria*, it may be futile to try to identify a "flower identity gene" such as *LFY* to distinguish between a flower and inflorescence meristem, because each apical meristem will ultimately produce a flower. There are currently few expression studies of *LFY*-like genes in species that are commonly regarded as cymose (Benlloch et al., 2007); among the best examples are the asterid eudicot family Solanaceae (e.g., tomato and *Petunia*: Souer et al., 1998; Molinero-Rosales et al., 1999) and the core eudicot family Caryophyllaceae (*Silene*: Allnutt et al., 2007), but inflorescence types in these two groups, though both cymose, are markedly different from each other and have different *LFY* expression patterns. Future work will examine the structure and function of *LFY* orthologs in *Trithuria*.

Concluding remarks

We recognize that it is impossible to resolve the origin of the angiosperm flower using evidence from Hydatellaceae (or any other early-divergent angiosperm) alone. Even less ambitious tasks such as evolutionary interpretation of morphological floral diversity within a given family can prove to be as problematic as elucidating the primitive type of angiosperm flower. A good example is the early-divergent angiosperm family Chloranthaceae. Phylogenetic relationships among extant species of Chloranthaceae are now well resolved using molecular methods (e.g., Zhang and Renner, 2003), and the results do not conflict with a morphological cladistic analysis (Eklund et al., 2004). Comparative and developmental morphology of extant Chloranthaceae are well studied (Endress, 1987, 2001b; Kong et al., 2002 and references cited therein). Even floral homeotic genes are relatively well characterized in *Chloranthus* (Li et al., 2005). Finally, Chloranthaceae is among very few extant families that are extensively represented in the earliest sediments that contain indisputable angiosperm flowers and fruits (reviewed by Doyle et al., 2003; Friis et al., 2001, 2006). One might think that this combined wealth of information would help in producing a detailed picture of diversification of the family, but this is not the case. Even if we reject the widely criticized view of Leroy (1983) that multistaminate male reproductive units of *Hedyosmum* could be compared directly to gymnosperm cones, there remain contrasting hypotheses about the morphology of the closest common ancestor of extant Chloranthaceae (Doyle et al., 2003). It is not clear whether the common ancestor had unisexual or bisexual flowers, whether the androecium was initially trimerous or monomerous, and whether the ovary was initially superior or half-inferior. Similar debates could be held regarding any of the other basal angiosperm lineages.

Our current data demonstrate that the inside-out structure, which represents the primary feature that separates RUs of Hydatellaceae from more orthodox angiosperm flowers, can be at least partially modified in teratological forms, thus producing a morphology that is closer to an orthodox flower. The *Trithuria* RU therefore does not allow recognition of a typical angiosperm flower, and could be described as a "nonflower." It could represent an inflorescence-like structure that is derived from a secondarily modified flower, as recently proposed for the remarkable reproductive unit of the monocot family Triuridaceae (Rudall, 2008). Alternatively, it could be a prefloral structure, perhaps sharing some features with the structure that gave rise to the angiosperm flower. Despite remaining ambiguities, our data show that reproductive structures of some early angiosperm lineages could have combined (or not yet adequately separated) features of flowers and inflorescences, allowing for evolutionary experimentation before establishment of the complex set of structures that we now collectively, and sometimes oversimplistically, term a flower.

FOOTNOTES

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