

Developmental morphology of branching flowers in *Nymphaea prolifera*

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Abstract *Nymphaea* and *Nuphar* (Nymphaeaceae) share an extra-axillary mode of floral inception in the shoot apical meristem (SAM). Some leaf sites along the ontogenetic spiral are occupied by floral primordia lacking a subtending bract. This pattern of flower initiation in leaf sites is repeated inside branching flowers of *Nymphaea prolifera* (Central and South America). Instead of fertile flowers this species usually produces sterile tuberiferous flowers that act as vegetative propagules. *N. prolifera* changes the meristem identity from reproductive to vegetative or vice versa repeatedly. Each branching flower first produces some perianth-like leaves, then it switches back to the vegetative meristem identity of the SAM with the formation of foliage leaves and another set of branching flowers. This process is repeated up to three times giving rise to more than 100 vegetative propagules. The developmental morphology of the branching flowers of *N. prolifera* is described using both microtome sections and scanning electron microscopy.

Keywords AGAMOUS · Floral reversion · Homeosis · Meristem identity · *Nymphaea prolifera* · Proliferated flowers

Introduction

Water lilies have attracted botanical systematists for at least 200 years. Nymphaeales (Nymphaeaceae and Cabombaceae) comprise eight genera and approximately 70 species of aquatic plants, with a worldwide distribution in tropical to temperate regions. Whereas the stem lineage of Nymphaeales is old based on fossil evidence (125–115 million years ago), molecular dating indicates that the extant nymphaealean genera diversified relatively recently, i.e., not earlier than approximately 46 million years ago (Gandolfo et al. 2004; Yoo et al. 2005). Features like scattered vascular bundles, embryological data, cotyledonary and floral structure have linked them to monocots, especially to the Alismatidae (Les and Schneider 1995). Molecular data corroborate the recent morphological understanding of the relationship among basal angiosperms: *Amborella* as the basal-most angiosperm is sister to all extant angiosperms, with Nymphaeales being the first branch after *Amborella* (Zanis et al. 2002; APG II 2003; Löhne and Borsch 2005; Qiu et al. 2005; Soltis et al. 2005). All Nymphaeales share several morphological characters, e.g., rhizomatous stems, solitary flowers, acropetal initiation of floral organs, radial floral symmetry, simultaneous initiation of the carpels in a whorl, and formation of a vascular plexus at the base of the flower. Floral characters vary extensively among genera, ranging from the small, simple monocot-like flowers of *Cabomba* to the large, showy, elaborate flowers of *Nymphaea* and *Victoria* (Les et al. 1999; Ronse De Craene et al. 2003; Schneider et al. 2003; Soltis and Soltis 2004; Yoo et al. 2005).

Most angiosperms show axillary branching, where a lateral bud is subtended by a leaf or bract. Nymphae-

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ales, however, show various deviating patterns with respect to flower positioning at the shoot apex. For example, flowers of *Victoria* and *Euryale* arise in nonmedian axillary positions to the associated leaf or seemingly so (Cutter 1961; Weidlich 1980), whereas in *Nuphar* and *Nymphaea* each flower replaces a leaf in the ontogenetic spiral of the shoot apex (Cutter 1961; Chassat 1962; Weidlich 1976a, 1976b; Les et al. 1999; Schneider et al. 2003).

The genus *Nymphaea* consists of approximately 45 species of aquatic herbs with perennial rhizomes, mostly floating leaves and showy flowers. The bractless flowers have mostly tetramerous calyces, whereas the other flower organs occur in whorls or rings of many members. The petals are white, blue, red or yellow in various shades (Wiersema 1987).

Nymphaea prolifera Wiersema belongs to the neotropical and night-flowering subgenus *Hydrocallis*, which according to molecular data is a basal division of the genus (Borsch et al. 1998). This species was originally described by Wiersema (1984, 1987) from Argentina (Corrientes). Since then it has also been found in Brazil: Mato Grosso and Mato Grosso do Sul (Pott 1998), and in Paraguay (Ritter et al. 2001), Ecuador (Cornejo and Bonifaz 2003), and Mexico (Olvera and Lot 1991). Fruits and seeds are rarely, if ever, produced in *N. prolifera*. Propagation seems to rely mostly, if not exclusively, on the tuberiferous (sterile) flowers. Instead of sexual reproduction, these flowers are tubers, giving rise to another set of leaves and tuberiferous flowers. The floral meristem, which starts with the development of the perianth, switches back to an indeterminate, vegetative meristem. This “sleeping” shoot apical meristem (SAM) starts the establishment of a new rhizome as soon as the detached flower has dropped to the ground. This is an effective way of asexual propagation, as hundreds of tiny tubers, already complete with juvenile leaves and roots, can be released from a single individual (Wiersema 1987). *N. prolifera* seems to occur with a disjunct pattern within the species area of *Nymphaea amazonum*, a close relative of *N. prolifera*. Overall, the morphology of *N. amazonum* and that of *N. prolifera* are very similar. Cornejo and Bonifaz (2003) even observed *N. amazonum* individuals with tuberiferous flowers. Wiersema (1987) found potential hybrids in Argentina, propagating entirely via tuberiferous flowers. They were triploid ($3n=27$) compared with the diploid ($2n=18$) mother species. Wiersema (1984, 1987) separated the two species in that *N. prolifera* lacks pubescence on the petioles. Moreover, *N. prolifera* usually has a few inconspicuous green sepaloid organs instead of white petals. Rarely, *N. prolifera* produces

completely white flowers that have sepals that are usually shorter and narrower than the outer petals, and carpellary appendages with tips that are mostly less than 1.5 mm thick (Wiersema 1987).

The purpose of this paper is to explore the developmental morphology of the repeatedly branching flowers of *N. prolifera* using both microtome sections and scanning electron microscopy:

1. We ask if the meristem of branching flowers of *N. prolifera* mirrors the pattern of the SAM of other *Nymphaea* spp. such as *N. alba*
2. We search for comparable patterns in the Nymphaeaceae and other vascular plants
3. We discuss the observed patterns in the light of developmental genetic findings

Materials and methods

The material of *N. prolifera* Wiersema used for this study was collected in the Reserva de la Biosfera “Pantanos de Centla,” Estado de Tabasco, Mexico (1 November 1998). The material of *N. alba* L. was collected from plants cultivated in the Botanical Garden of the University of Zurich (Switzerland). The material was fixed and preserved in 70% ethanol. For scanning electron microscopy, the dissected material was critical point dried and sputter coated (Au). The micrographs were taken with scanning electron microscopes Cambridge S4 and JEOL. For microtome sections, specimens were embedded in Kulzer’s Technovit (2-hydroethyl methacrylate), as described by Igersheim and Cichocki (1996), and sectioned with a MICROM HM 355 rotary microtome and conventional microtome knife types C and D. The mostly 7- μ m thick sections were stained with ruthenium red and toluidine blue (Weber and Igersheim 1994). The permanent slides of the microtome sections are deposited at the Institute of Systematic Botany, University of Zurich (z). Fixed and dry voucher specimens (Novelo 3347) of *N. prolifera* are housed in the Herbarium of Zurich Universities (z/zT); dry material is also deposited in the Herbarium of UNAM (Mexico). The authorities of additional species names mentioned in the text are equivalent to the species and author names given by Wiersema (1987).

Results

The measurements in the following description are based on our own observations in the field (Tabasco,

Mexico). They may deviate to some degree from the measurements given by Wiersema (1987).

Leaves and branching flowers arising from rooted tubers

N. prolifera is a perennial aquatic herb, as typical for most Nymphaeaceae (Fig. 1a–d). The rhizome of this species consists of an erect and nearly spherical tuber or caudex 2–2.5 cm in diameter. Its lower side is fixed to the muddy soil by several contractile roots. Mature

leaves arising from this rooted tuber consist of cylindrical stalks (petioles) up to 1.2 m long. Their base is provided with two attached lateral stipules that are fused to some degree along the adaxial leaf insertion zone (Figs. 2a, 4e, f). Stipules of mature leaves are attached to a slightly broadened leaf sheath 4–7 cm long. The suborbicular to reniform leaf blades are up to 20 cm wide with a slight tendency towards peltation (Fig. 1d). The rooted tubers (rhizomes) also produce flowers on stalks up to 80 cm long. This first set of branching flowers will be called here mother flowers,

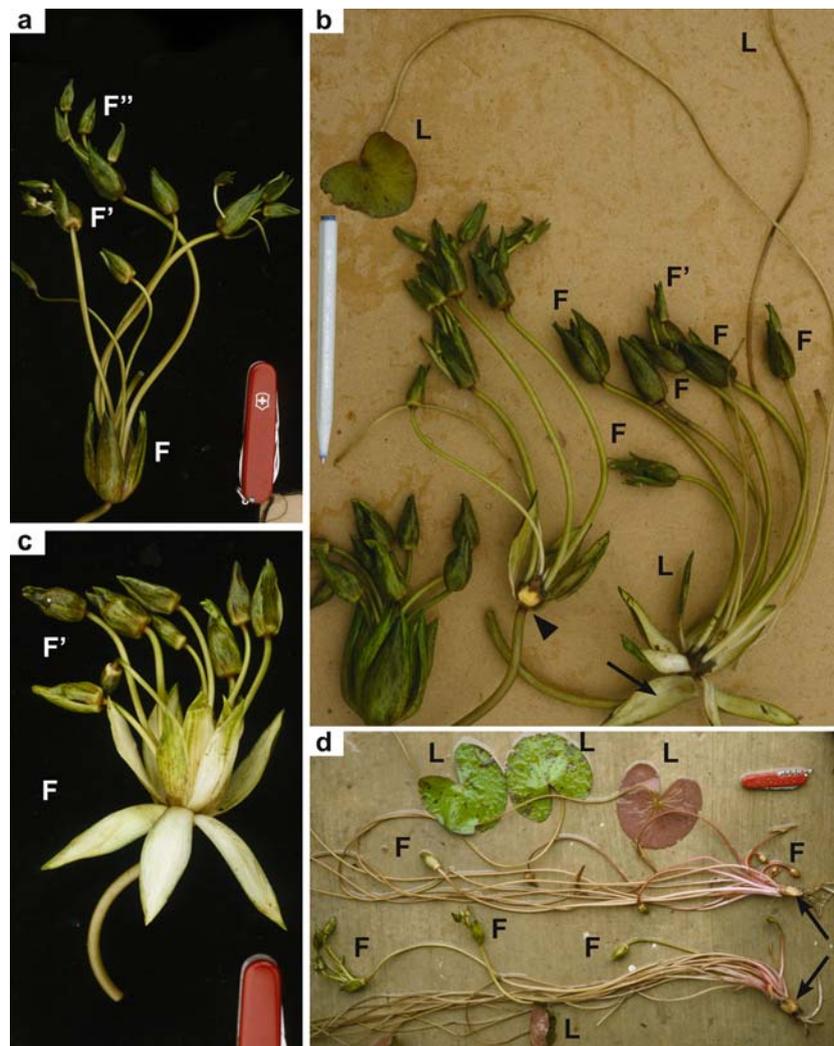


Fig. 1 *Nymphaea prolifera*. Plants photographed in the field: Reserva de la Biosfera “Pantanos de Centla,” Estado de Tabasco, Mexico (1st November 1998, photographs by R. Rutishauser). **a** Mother flower (*F*) with daughter flowers (*F'*) and granddaughter flowers (*F''*). Note that the perianth of all flowers is *green* and consists of approximately 4–8 sepal-like organs only. **b** Three mother flowers, two of them with the perianth partly removed in order to see the starch-containing tuber in the center (*arrowhead*). The right mother flower with seven perianth members, *green* on outer side, *whitish* inside

(*arrow*). This mother flower gives rise to seven daughter flowers (*F*, one removed) and three stalked daughters (*L*). One daughter flower already with outgrowing granddaughter flowers (*F'*). **c** The only flower (*F*) found to have an increased number (approximately 15) of mainly white perianth members, giving rise to nine daughter flowers (*F'*). **d** Two whole plants pulled out of the soil. *Arrows* point to the tuber-like rooted rhizomes. The tubers already produced mother flowers (*F*) and leaves (*L*) on long stalks

i.e., first-order flowers, which give rise to daughter flowers, i.e., second-order flowers, and so on (Fig. 1a–c). The stalks of the daughter flowers are usually less than 20 cm long. No sign of any stamens or carpels were found in any of the flowers inspected in the field (Tabasco, Mexico).

Size, colour, and merosity of mother and daughter flowers

The largest branching flower found had a diameter of 10 cm and consisted of 15 perianth members, the usually four outermost ones (“sepals”) were spreading and green on the outer side, white inside; the inner ones (“petals”) were upright, and white on both sides (Fig. 1c). This mother flower contained a set of nine

daughter flowers, all of them green and stalked. Most mother flowers examined in the field, however, were much less attractive, being green and having a reduced number of perianth members (Fig. 1a, b). Most mother flowers investigated consisted of 4–8 greenish perianth members (i.e., without clear differentiation into sepals and petals). These mother flowers contained a starch-bearing center approximately 1 cm wide (Fig. 1b, arrowhead).

Mother flowers produce daughter flowers and additional small foliage leaves (Fig. 1b). The same also happens inside the daughter flowers. The ratio of leaves to flowers in mother and daughter flowers is approximately 1:2, i.e., there are about twice as many daughter (or granddaughter) flowers as foliage leaves per mother (or daughter) flower (Fig. 1b). Both pedi-

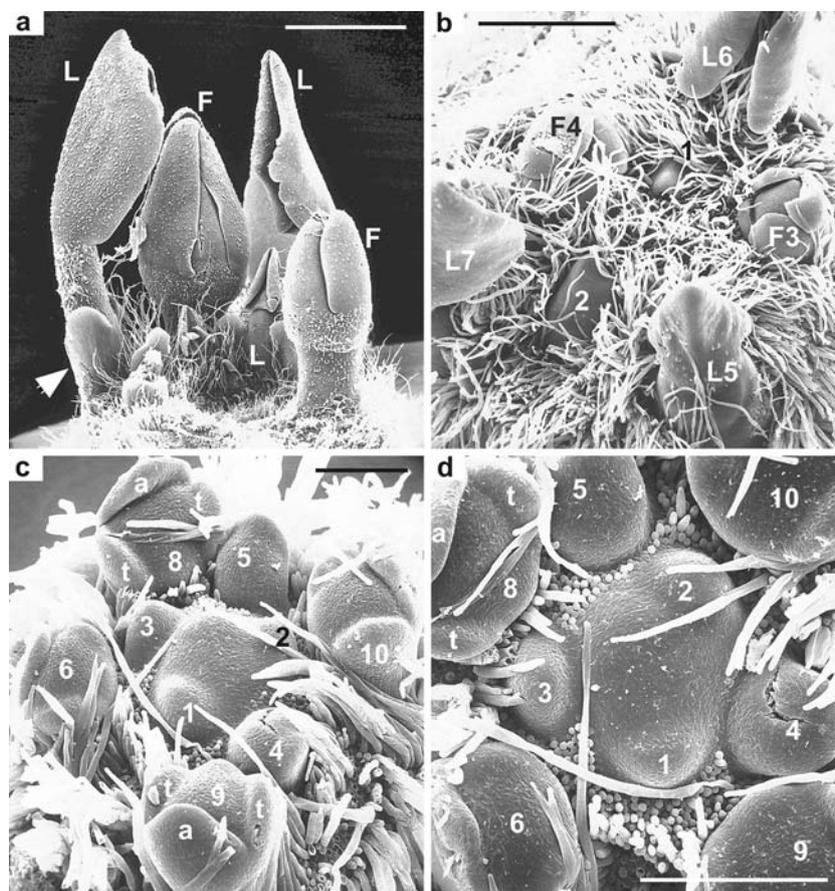


Fig. 2 *Nymphaea prolifera*. Lateral and top views of central portion of branching flower, before and after removal of protecting hairs. **a** Mother flower (perianth removed) containing leaves (*L*) and daughter flowers (*F*). *Arrowhead* points to lateral stipule at leaf base. Scale bar 2 mm. **b** Top view on central portion of mother flower. Leaves (*L*) and daughter flowers (*F*) are arranged along the same ontogenetic spiral (1–7). Note that “1” indicates the youngest primordium observable in the thicket of hairs protecting and hiding the meristematic centre. Scale bar 500 μ m. **c** and **d** Lateral and top views on central portion of

daughter flower after removal of hairs and peripheral appendages. The flower center behaves like a shoot apical meristem (SAM), showing primordia (1–10) along a Fibonacci spiral (divergence angle approximately 137.5°). Most leaf sites are occupied by floral primordia (4–6, 8–10). Dorsiventral shape of primordia 1 and 3 (next to apical meristem) indicates their putative leaf identity. Hemispherical primordium 2 appears to be the first stage of a young flower. Each floral primordium initiates first the abaxial tepal (*a*), then the two transversal ones (*t*). Scale bars 200 μ m

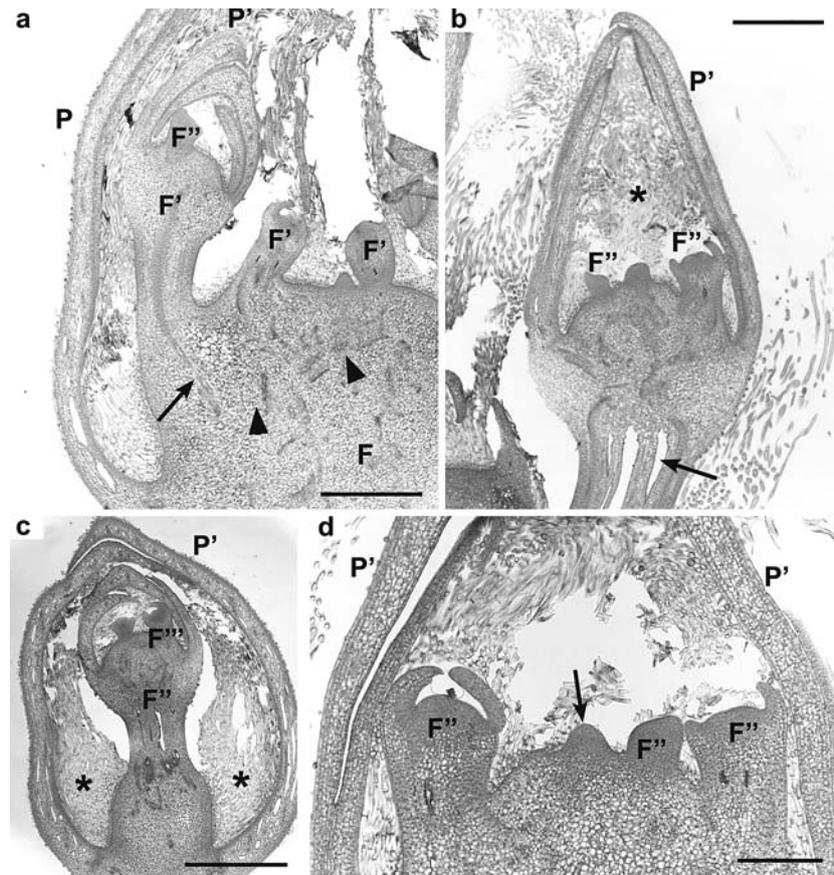


Fig. 3 *Nymphaea prolifera*. Longitudinal sections of mother flowers (*F*) and daughter flowers (*F'*). **a** Portion of mother flower with sepal-like perianth member (*P*), showing aerenchyma. Tuber-forming center of mother flower (*F*) with scattered vascular bundles (*arrowheads*) in starch-containing parenchyma. *Arrow* points to vascular bundle supplying daughter flower (*F'*). This flower (with perianth *P'*) contains primordial granddaughter flower (*F''*). Scale bar 500 μm . **b** Another daughter flower with granddaughter flowers (*F''*). *Arrow* points to aerenchyma in

pedicel (flower stalk). Note plenty of hairs (*asterisk*) inside perianth (*P'*). Scale bar 500 μm . **c** Oblique longitudinal section of daughter flower (with perianth *P'*). Note stalked granddaughter flower (*F''*) containing great-granddaughter flowers (*F'''*). Interior of daughter flower filled with hairs (*asterisks*). Scale bar 1 mm. **d** Close-up of meristematic zone of daughter flower. Inside its perianth (*P'*) are granddaughter flowers (*F''*). *Arrow* points to obliquely cut leaf primordium next to apical center. Scale bar 200 μm

cells (floral stalks) and petioles (leaf stalks) have a cylindrical cross-section, but differ slightly in their pattern of aerenchyma (Fig. 4d, f). Moreover, only petioles are provided with lateral stipules, which are slightly fused along the adaxial insertion line (Fig. 4e, f).

Initiation order of flowers and leaves in shoot tips of *N. prolifera* and *N. alba*

The SAM of *N. prolifera* produces floral primordia and leaf primordia as part of the same ontogenetic spiral, as is typical of other *Nymphaea* species such as *N. alba* (Fig. 5a, b). All flower buds are occupying leaf sites (i.e., completely replacing leaf primordia) along a spiral approaching Fibonacci angles of 137.5°. There is no sign of bracts subtending flower primordia. They

arise as hemispherical bumps whereas leaf primordia originate as dorsiventral bulges that are soon provided with stipules. SAMs (i.e., rhizome tips) of *N. alba* and *N. prolifera* usually produce fewer flowers than leaves (Fig. 5a, b).

Initiation order of flowers and leaves in the branching flowers of *N. prolifera*

The spiral initiation pattern of lateral organs as described above is found again inside the tuberiferous flowers of *N. prolifera*. Instead of producing sexual organs (stamens, carpels) the meristematic center of these flowers remains indeterminate and repeats the growth behavior of a SAM with new flower buds (arising in leaf sites) and leaves as part of the same ontogenetic spiral (Fig. 2a–d). The empty spaces be-

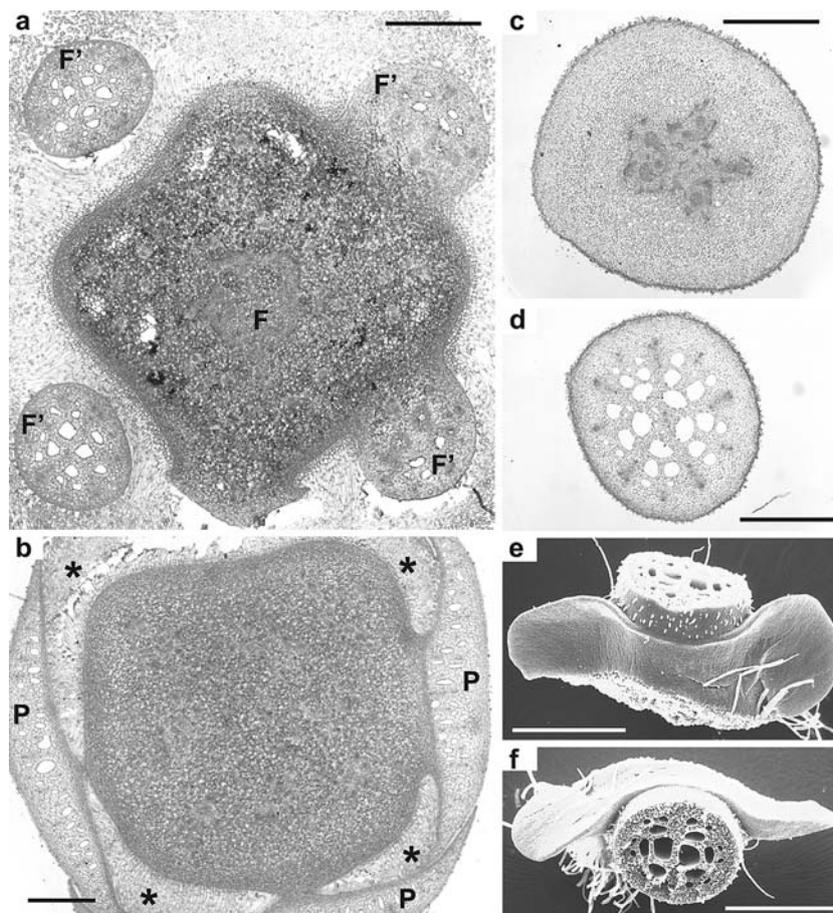


Fig. 4 *Nymphaea prolifera*. Basal portions of flowers (transversal microtome sections) and leaves (SEM graphs). **a–d** Series of four transversal sections of the same flower, from its tuberiferous center down to its pedicel. **a** Level of tuberiferous flower center (*F*) surrounded by the pedicels of four daughter flowers (*F'*). Scale bar 500 μ m. **b** Base of tuberiferous flower with insertion of sepals (*P*) containing aerenchyma. Gaps between sepals and flower center are completely filled with hairs (*asterisks*). Scale

bar 500 μ m. **c** Level below perianth insertion. Note central group of vascular tissue and lack of aerenchyma. Scale bar 1 mm. **d** Floral stalk (pedicel) with symmetrically arranged aerenchyma and vascular bundles forming a ring (besides inconspicuous central bundles). Scale bar 1 mm. **e, f** Base of leaf stalk (petiole) with two lateral stipules, which are fused along the adaxial side of the petiole base, seen from ventral side and from above, respectively. Scale bars 500 μ m

tween the perianth members and the solid center in floral buds are completely filled with hairs that grow out next to the apical meristem. The very same pattern of flower initiation in leaf sites is repeated inside daughter flowers, granddaughter flowers (Fig. 3a–d), and perhaps also inside great-granddaughter flowers. Each floral bud produces some perianth-like leaves, then it switches back to the developmental behavior of the SAM, with the formation of small foliage leaves and another set of branching flowers. As a consequence, more than 100 branching flowers acting as vegetative propagules are produced by a single mother flower (Fig. 3a–d). The floral center gives rise to a tuber that mainly contains starch-filled parenchyma with scattered vascular bundles (vascular plexus), but without aerenchyma, whereas in the flower stalk there is a prominent lacunae system (Fig. 4a–d).

Discussion

Flowers and vegetative buds arising from leaf sites in *Nymphaea* spp.

As in *Nuphar*, floral primordia in *Nymphaea* are produced by the rhizome apex (SAM) as members of the same ontogenetic spiral approaching the Fibonacci angle of 137.5°. There are no bracts subtending the flowers (Schneider et al. 2003). Thus, flower primordia are occupying leaf sites (i.e., replacing whole leaf primordia) in *Nymphaea*, as already described by Cutter (1957a, 1957b, 1961) and Chassat (1962). In *Nymphaea* spp. the ratios of leaves to flowers for entire plants vary from 1:1 to 4:1. Thus, there are usually fewer flowers than leaves arising from a SAM (Cutter 1957a; Weidlich 1976a, 1976b). Very occasionally, vegetative buds

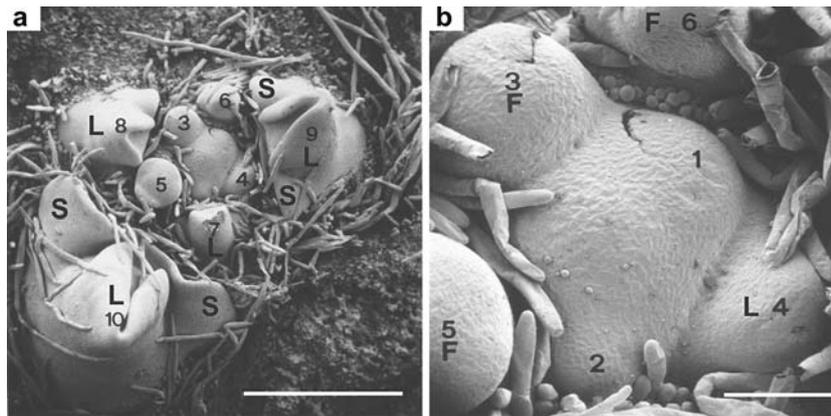


Fig. 5 *Nymphaea alba*. Rhizome tip with shoot apical meristem (SAM) (photographs received from Matthias Wolf, Zurich). **a** Top view of SAM with primordia arranged along a Fibonacci spiral (3–10). Positions 4 and 7–10 are occupied by young leaves (L); positions 3, 5, and 6 are occupied by flower primordia. Note

prominent lateral stipules (S) at the base of leaf 10. Scale bar 500 μm . **b** Close-up of same SAM, showing youngest primordia 1–6 (abbreviations as above). Note difference in primordial shape between initial stages of young flower (position 1) and young leaf (position 2) bordering the SAM. Scale bar 200 μm

are also formed. In *Nymphaea* they occupy leaf positions, i.e., positions in which flowers are also expected. In *Nuphar*, however, vegetative buds are axillary and subtended by a leaf (Cutter 1961; Chassat 1962).

In branching flowers of *N. prolifera* a small number of tepals and, instead of stamens and carpels, new leaves and flowers are produced along a spiral. Compared with the rhizome tips of *Nymphaea* in general the ratio of leaves to flowers (approximately 1:2) is considerably smaller inside the branching flowers of *N. prolifera*.

Flowers or vegetative buds (shoots) arising from leaf sites in Nymphaeaceae and other vascular plants

Flowers occupying leaf sites are known from other nymphaeaceous genera such as *Nuphar* and *Ondinea* among basal angiosperms (Cutter 1957a, 1961; Schneider et al. 2003). Bractless flowers arising directly from the SAM are also known from eudicots, such as wild-type *Arabidopsis* and other Brassicaceae (Venugopala Reddy et al. 2004; Dornelas and Rodriguez 2006), also *Theligonum* (Rubiaceae), with regard to male flowers (Rutishauser et al. 1998). Vegetative buds occupying leaf sites are (besides *Nymphaea*) also observable in angiosperms such as *Utricularia* spp. (Rutishauser and Isler 2001) and in club mosses such as *Huperzia* (Stevenson 1976). Cutter (1957b, p. 93) called the gemmae forming short shoots in *Huperzia* and the floral buds in *Nymphaea* “topographically homologous with leaf primordia.” Thus, a certain kind of homology (i.e., homotopy) exists between leaves and shoots (also between leaves and whole flowers), as

proposed by adherents of the continuum morphology (Lacroix et al. 2005; Rutishauser and Moline 2005). The topological correspondence of shoots (including flowers) and leaves in various vascular plants (e.g., *Nymphaea* flowers in leaf sites) may be due to uncommitted primordia that only subsequently become differentiated into shoot and leaf (as known from certain ferns), or it may be due to homeosis, i.e., ectopic expression of a shoot (or flower) completely replacing a leaf. According to Theissen (2005) there is increasing evidence that homeotic transitions played a role in plant evolution.

Branching flowers, floral reversion, and asexual propagation in various *Nymphaea* spp.

We hypothesize that there is a reversion of floral meristem identity back to the SAM in *N. prolifera*. The observational evidence for this hypothesis was not recognized by earlier *Nymphaea* students. For example, Wiersema (1987, p. 88) mentioned *N. prolifera* as: “Tuber globose, densely woolly, producing alternating whorls of tuberous flowers and leaves in (3’s) 4’s.” We did not observe such alternating whorls except for the outermost organs (Fig. 4a, b). Each branching flower of *N. prolifera* first produces some perianth-like leaves, then it switches back to the SAM identity, with the formation of spirally arranged foliage leaves and several daughter flowers arising again from leaf sites. This process is repeated up to three times giving rise to a branched structure of more than 100 vegetative modules. The detachment of these modules results in many vegetative propagules as products of branching floral meristems.

Nymphaea prolifera is not the only species of this genus that exhibits flower reversion as described in this article. It seems that the transition from sexual hermaphrodite flowers to sterile tuberiferous flowers serving as vegetative propagules is known from four other *Nymphaea* species (*N. lasiophylla*, *N. mexicana*, *N. pubescens*, *N. rubra*), at least occasionally or as teratology. *N. lasiophylla*, as described by Wiersema (1987, pp. 90–94) in his monograph on the subgenus *Hydrocallis*, has regular flowers, but there are “tuberiferous flowers usually present” and “propagation in most populations is by means of abortive tuberiferous flowers.” He found seeds in only 1 out of 20 populations. This species from northeastern Brazil is linked to *Nymphaea lingulata* and not to *N. amazonum* or *N. prolifera*. *N. mexicana* is another species with flower reversion. Mohan Ram and Nayyar (1974) reported tuberiferous flowers from a few *N. mexicana* plants, cultivated in a pond of the Botanical Garden of Delhi (India). The regularly yellow flowers were replaced in these cultivated plants by tuberiferous flowers with roots. *N. pubescens* and *N. rubra* are two other *Nymphaea* species in which tuberiferous flowers have also been observed. It seems that in *N. rubra* propagation is achieved mainly asexually with tuberiferous flowers (Conard 1905; Debbarman 1934). Mitra and Subramanyam (1982) found fruits neither in natural populations examined in West Bengal and Bangladesh nor as result of an intensive herbarium search. The tuberiferous flowers are described as bearing leaves and tuberiferous flower buds in the position of floral parts, as well as roots. In *N. pubescens*, Bose (1961) described a specimen with tuberiferous flowers. Here the floral organs were completely suppressed, a few bract-like structures subtended the terminal portion of the shoot, where several regular flowers and leaves developed.

Branching flowers of *N. prolifera* compared with proliferated flowers in other angiosperms

Proliferated flowers are flowers that return completely or partially to the vegetative and indeterminate meristem identity, after having started the floral developmental program. Proliferated flowers have been recorded for more than 2,000 years (Meyerowitz et al. 1989). The first records date back to Theophrastus 286 BC, who described double roses, i.e., flowers containing more than the normal number of petals. Well-known are the unbranched proliferated rose and the branched proliferated pink described by Goethe (1790; see translation and comments by Arber 1946). Flowers in angiosperms are—by definition—determinate and

unbranched short-shoots. Thus, the indeterminate branching flowers of *N. prolifera* violate this flower definition twice, as long as we do not accept overlapping concepts such as “inflorescence flowers” with mixed developmental programs, as proposed and discussed by Baum and Donoghue (2002), Lacroix et al. (2005), and Rutishauser and Moline (2005).

Nymphaea prolifera and the molecular models of floral development

The isolation and induction of specific mutations and their products have given a new boost to the unraveling of the development of the flower. Currently, two widely accepted models explain nicely how floral development is controlled molecularly. The ABCDE model introduces floral homeotic functions, which are represented by groups of homeotic genes. In this model one organ, like the stamen, is influenced by several homeotic functions, i.e., B, C, and E. The more recent floral quartet model attributes four homeotic genes to each of the four floral organs (Theissen 2001). As both models are widely applicable to the angiosperms, including basal angiosperms such as Nymphaeaceae (Goto et al. 2001; Kim et al. 2005; Soltis et al. 2005; Theissen 2005) they help to find candidates for genes responsible for the peculiar phenotype of *N. prolifera*. The most promising will be found in the *AGAMOUS* (*AG*)- or *SEPALLATA* (*SEP*)-like MADS-box genes. The class C genes (*AG*-like genes) specify the carpels and also play a role in floral meristem determinacy (Bowman et al. 1989; Zahn et al. 2006). For example, the sepals and petals of *ag* mutants in *Arabidopsis* are as in the wild type. The stamens, however, are replaced by additional petals, and in the position of the gynoecium a daughter flower develops showing the same characteristics as the mother flower. This scheme is repeated up to five times along the same floral axis (Bowman et al. 1991; Goto et al. 2001). However, since according to the ABCDE model class E genes are required for the specification of reproductive organ identity and floral determinacy, changes in class E genes (such as *SEP*) may also underlie the branching flowers of *N. prolifera*. The *SEP* homologues act on petals, stamen, and carpels as well as the determination of the floral meristem (Theissen 2001; Zahn et al. 2006). For example, transgenic plants of *Gerbera hybrida* (Asteraceae) with strongly reduced *SEP* homolog expression have been studied (Uimari et al. 2004). The capitula of these plants lose their determinacy, and they continue to produce new florets as long as they live. Within the single florets, there is a reversion of ovaries to inflorescences. Moreover, meristem identity

genes like *LEAFY* (*LFY*) also influence the fate of the meristem. *lfy* mutations of *Arabidopsis* show proliferation of inflorescence meristems and the formation of shoot–flower intermediates in the place of flowers (Yoon and Baum 2004).

The switch from a sexually producing ancestral *Nymphaea* sp. (possibly *N. amazonum* as the next relative) to *N. prolifera* with repeating vegetative propagules inside the flower may be due to a single mutation of a gene homologous to, for example, *AG*, *SEP*, or *LFY*. To verify this hypothesis, expression patterns of the aforementioned genes should be investigated for *N. prolifera*. Any developmental genetic hypothesis explaining the branching flowers of *N. prolifera*, however, should also explain the replacement of leaf primordia by floral buds as being typical of *Nymphaea* and *Nuphar* in general, and the nonmedian axillary positions of flowers with regard to their “subtending” leaves in *Victoria* and *Euryale* (Schneider et al. 2003).

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References

- APG II (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Bot J Linn Soc* 141:399–436
- Arber A (1946) Goethe’s botany. *Chron Bot* 10:63–126
- Baum DA, Donoghue MJ (2002) Transference of function, heterotopy and the evolution of plant development. In: Cronk QCB, Bateman RM, Hawkins JA (eds) *Developmental genetics and plant evolution*. Taylor and Francis, London, pp 52–69
- Borsch T, Hilu KW, Wilde V, Bartlott W (1998) Inferring the phylogeny of *Nymphaea*: evidence from the trnT–trnF region of the chloroplast genome. *Am J Bot* 86:116–117
- Bose RB (1961) A note on an abnormal *Nymphaea pubescens* Willd. *Bull Bot Surv India* 3:41
- Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in *Arabidopsis*. *Plant Cell* 1:37–52
- Bowman JL, Smyth DR, Meyerowitz EM (1991) Genetic interactions among floral homeotic genes of *Arabidopsis*. *Development* 112:1–20
- Chassat JF (1962) Recherches sur la ramification chez les Nymphaeacées. *Mém Soc Bot Fr* 42:72–95
- Conard HS (1905) The water lilies. A monograph of the genus *Nymphaea*. The Carnegie Institution of Washington, Washington
- Cornejo X, Bonifaz C (2003) Nymphaeaceae. *Flora Ecuador* 70:5–24
- Cutter EG (1957a) Studies of morphogenesis in the Nymphaeaceae. I. Introduction: some aspects of the morphology of *Nuphar lutea* (L.) Sm. and *Nymphaea alba* L. *Phytomorphology* 7:45–56
- Cutter EG (1957b) Studies of morphogenesis in the Nymphaeaceae. II. Floral development in *Nuphar* and *Nymphaea*: bracts and calyx. *Phytomorphology* 7:57–73
- Cutter EG (1961) The inception and distribution of flowers in the Nymphaeaceae. *Proc Linn Soc Bot* 172:93–100
- Debbarman PM (1934) A case of axial floral proliferation of the flower of *Nymphaea rubra* Roxb. *Indian Bot Soc* 3:66–67
- Dornelas MC, Rodriguez APM (2006) The tropical cedar tree (*Cedrela fissilis* Vell., Meliaceae) homolog of the *Arabidopsis LEAFY* gene is expressed in reproductive tissues and can complement *Arabidopsis leafy* mutants. *Planta* 223:306–314
- Gandolfo MA, Nixon KC, Crepet WL (2004) Cretaceous flowers of Nymphaeaceae and implications for complex insect entrapment pollination mechanisms in early angiosperms. *Proc Natl Acad Sci USA* 101:8056–8060
- Goto K, Kyojuka J, Bowman JL (2001) Turning floral organs into leaves, leaves into floral organs. *Curr Opin Genet Dev* 11:449–456
- Igersheim A, Cichocki O (1996) A simple method for microtome sectioning of prehistoric charcoal specimens embedded in 2-hydroxyethyl methacrylate (HEMA). *Rev Palaeobot Paly-nol* 92:389–393
- Kim S, Koh J, Yoo MJ, Kong H, Hu Y, Ma H, Soltis PS, Soltis DE (2005) Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators. *Plant J* 43:724–744
- Lacroix C, Jeune B, Barabé D (2005) Encasement in plant morphology: an integrative approach from genes to organisms. *Can J Bot* 83:1207–1221
- Les DH, Schneider EL (1995) The Nymphaeales, Alismatidae and the theory of an aquatic monocotyledon origin. In: Rudall PJ, Cribb P, Cuttler DF, Humphries CJ (eds) *Monocotyledons: systematics and evolution*. Royal Botanic Gardens Kew, Kew, pp 23–42
- Les DH, Schneider EL, Padgett DJ, Soltis PS, Soltis DE, Zanis M (1999) Phylogeny, classification and floral evolution of water lilies (Nymphaeaceae: Nymphaeales): a synthesis of non-molecular, *rbcL*, *matK*, and 18s rDNA data. *Syst Bot* 24:28–46
- Löhne C, Borsch T (2005) Molecular evolution and phylogenetic utility of the *petD* Group II intron: a case study in basal angiosperms. *Mol Biol Evol* 22:317–332
- Meyerowitz EM, Smyth DR, Bowman JL (1989) Abnormal flowers and pattern formation in floral development. *Development* 106:209–217
- Mitra RL, Subramanyam K (1982) Is *Nymphaea rubra* Roxb. ex Andrews an apomict? *Bull Bot Surv India* 24:83–86
- Mohan Ram HY, Nayyar VL (1974) A case of reversion of flower of *Nymphaea mexicana* to vegetative condition. *Curr Sci* 43:290–291
- Olvera M, Lot A (1991) New record of *Nymphaea prolifera* in Mexico. *Bol Soc Bot Mex* 51:93–94
- Pott VJ (1998) A Família Nymphaeaceae no Pantanal, Mato Grosso e Mato Grosso do Sul, Brasil. *Acta Bot Bras* 12:183–194

- Qiu YL, Dombrowska O, Lee J, Li L, Whitlock BA, Bernasconi-Quadroni F, Rest JS, Davis CC, Borsch T, Hilu KW, Renner SS, Soltis DE, Soltis PS, Zanis MJ, Cannone JJ, Gutell RR, Powell M, Savolainen V, Chatrou LW, Chase MW (2005) Phylogenetic analyses of basal angiosperms based on nine plastid, mitochondrial and nuclear genes. *Int J Plant Sci* 166:815–842
- Ritter NP, Crow GE, Wiersema JH (2001) *Nymphaea* (Nymphaeaceae) in Bolivia: notes on several species, three new country records, and a key to species. *Rhodora* 103:326–331
- Ronse De Craene LP, Louis P, Soltis PS, Soltis DE (2003) Evolution of floral structure in basal angiosperms. *Int J Plant Sci* 164:S329–S363
- Rutishauser R, Isler B (2001) Fuzzy Arberian Morphology: *Utricularia*, developmental mosaics, partial shoot hypothesis of the leaf and other FAMOUS ideas of Agnes Arber (1879–1960) on vascular plant bauplans. *Ann Bot* 88:1173–1202
- Rutishauser R, Moline P (2005) Evo-devo and the search for homology (“sameness”) in biological systems. *Theory Biosci* 124:213–241
- Rutishauser R, Ronse De Craene LP, Smets E, Mendoza-Heuer I (1998) *Theligonum cynocrambe*: the developmental morphology of a peculiar rubiaceous herb. *Plant Syst Evol* 210:1–24
- Schneider EL, Tucker SC, Williamson PS (2003) Floral development in the Nymphaeales. *Int J Plant Sci* 164:S279–S292
- Soltis PS, Soltis DE (2004) The origin and diversification of angiosperms. *Am J Bot* 91:1614–1626
- Soltis DE, Albert VA, Kim S, Yoo MJ, Soltis PS, Frohlich MW, Leebens-Mack J, Kong H, Wall K, dePamphilis C, Ma H (2005) Evolution of the flower. In: Henry RJ (eds) *Plant diversity and evolution: genotypic and phenotypic variation in higher plants*. CAB International, Wallingford, pp 165–200
- Stevenson DW (1976) Observations on phyllotaxis, stelar morphology, the shoot apex and gemmae of *Lycopodium lucidulum* Michaux (Lycopodiaceae). *Bot J Linn Soc* 72:81–100
- Theissen G (2001) Development of floral organ identity: stories from the MADS house. *Curr Opin Plant Biol* 4:75–85
- Theissen G (2005) Birth, life and death of developmental control genes: new challenges for the homology concept. *Theory Biosci* 124:199–212
- Uimari A, Kotilainen M, Elomaa P, Yu D, Albert VA, Teeri TH (2004) Integration of meristem fates by a *SEPALLATA*-like MADS-box gene. *Proc Natl Acad Sci USA* 101:15817–15822
- Venugopala Reddy G, Heisler MG, Erhardt DW, Meyerowitz EM (2004) Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis at the shoot apex of *Arabidopsis thaliana*. *Development* 131:4225–4237
- Weber M, Igersheim A (1994) ‘Pollen buds’ in *Ophiorrhiza* (Rubiaceae) and their role in Pollenkitt release. *Bot Acta* 107:257–262
- Weidlich WH (1976a) The organization of the vascular system in the stems of the Nymphaeaceae. I. *Nymphaea* subgenera *Castalia* and *Hydrocallis*. *Am J Bot* 63:499–509
- Weidlich WH (1976b) The organization of the vascular system in the stems of the Nymphaeaceae. I. *Nymphaea* subgenera *Anechya*, *Lotos* and *Brachyceras*. *Am J Bot* 63:1365–1379
- Weidlich WH (1980) The organization of the vascular system in the stems of the Nymphaeaceae. III. *Victoria* and *Euryale*. *Am J Bot* 67:790–803
- Wiersema JH (1984) Systematics of *Nymphaea* subgenus *Hydrocallis* (Nymphaeaceae). I. Four new species from the neotropics. *Brittonia* 36:213–232
- Wiersema JH (1987) A monograph of *Nymphaea* subgenus *Hydrocallis* (Nymphaeaceae). *Syst Bot Monogr* 16:1–112
- Yoo MJ, Bell CD, Soltis PS, Soltis DE (2005) Divergence times and historical biogeography of Nymphaeales. *Syst Bot* 30:693–704
- Yoon HS, Baum DA (2004) Transgenic study of parallelism in plant morphological evolution. *Proc Natl Acad Sci USA* 101:6524–6529
- Zahn LM, Leebens-Mack J, Arrington JM, Hu Y, Landherr LL, DePamphilis CW, Becker A, Theissen G, Ma H (2006) Conservation and divergence in the *AGAMOUS* subfamily of MADS-box genes: evidence of independent sub- and neofunctionalization events. *Evol Dev* 8:30–45
- Zanis MJ, Soltis DE, Soltis PS, Mathews S, Donoghue MJ (2002) The root of the angiosperms revisited. *Proc Natl Acad Sci USA* 99:6848–6853