Floral development in Anemoneae (Ranunculaceae)

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The floral development of two Clematis species and four Anemone species (including Pulsatilla) (Anemoneae, Ranunculaceae) is described. Shared features are: (1) sepals shortly after initiation broad, crescent-shaped, as opposed to the other organs, which are narrow and hemispherical; (2) outermost organs of the androecium often smaller than the others and sometimes sterile; (3) carpels ascidiate, with distinctive stalk, stigma papillate, decurrent; the carpels have one median fertile ovule and a few lateral sterile ovules in all species studied; the fertile ovule appears before the carpel closes. Generic differences are: (1) In Clematis, four sepals are initiated in two pairs; sometimes one of the sepals in the second pair appears to be divided into two organs (double position) resulting in a pentamerous perianth; the first eight stamens are positioned in two alternating whorls, the outer whorl alternating with the four sepals. In Anemone, the perianth organs, if five, are initiated in spiral sequence; in the Pulsatilla group of Anemone, six sepals are initiated in two whorls; the first three organs of the androecium (staminodes) alternate with the inner sepals. (2) Further androecial organs are mostly in complex whorls (i.e. including double positions) in Clematis, but in an irregular spiral or in irregular complex whorls in Anemone. (3) Anther maturation is largely centripetal in Clematis, but centrifugal or bidirectional in Anemone. In Clematis macropetala, the outermost organs of the androecium lack anthers and the filaments expand and become petal-like. In contrast, in the Pulsatilla group of Anemone, these organs retain sterile anthers and become small, capitate organs. © 2010 The Linnean Society of London, Botanical Journal of the Linnean Society, 2010, 162, 77–100.

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INTRODUCTION

The tribe Anemoneae is a well-supported clade in Ranunculoideae (Ranunculaceae) based on molecular phylogenetic studies and morphological data (Hoot, 1995b; Jensen et al., 1995; Johansson, 1995; Tamura, 1995; Ro, Keener & McPheron, 1997; Wang et al., 2009). Jensen et al. (1995) recognized two subtribes, Clematidinae (including Clematis L. and Clematopsis Boj. ex Hook.) and Anemoninae (including Anemone L., Hepatica Mill., Pulsatilla Mill. and Knowltonia Salisb.). Phylogenetic studies have shown that Hepatica, Pulsatilla and Knowltonia are all nested in Anemone, and thus should be included within Anemone s.l. (Hoot & Palmer, 1994; Hoot, Reznicek & Palmer, 1994; Ehrendorfer, 1995; Hoot, 1995a; Johansson, 1995; Ehrendorfer & Samuel, 2001; Schuettpe1z et al., 2002). Likewise, Archiclematis (Tamura) Tamura, Clematopsis Boj. ex Hook. and Naravelia DC. are nested in Clematis L., and thus should be sunk in Clematis s.l. (Mikeda et al., 2006). However, Hepatica appears to be sister to Anemone s.l. plus Clematis s.l. in Wang et al. (2009). Anemoneae shares floral features, such as coloured sepals, lack of petals [but petals present in part of Clematis, or outer staminodes that are much smaller than sepals present in some species of the Pulsatilla group (section Pulsatilla) of Anemone and nectariferous in some], and numerous, often slender and hairy carpels with a single fertile median ovule and two or more sterile lateral ovules (Schaeppi & Frank, 1962). Floral phyllostaxis in Anemone s.l. is unusually diverse and...
encompasses spiral and whorled patterns (Schöffel, 1932). The presence and structure of ‘petals’ or ‘stamino-odes’ in Anemoneae appears to be evolutionarily complex. Miikeda et al. (2006) assumed that the petals in Clematidinae are probably not homologous. The sequence of stamen maturation is centripetal in most Ranunculaceae (Song, Tian & Ren, 2007; Gu & Ren, 2007), but centrifugal in Aquilegia L. (Tepfer, 1953; Feng et al., 1995). However, in Anemone rivularis Buch.-Ham. ex DC. of Anemoneae, the sequence is bidirectional: largely centripetal, but with the outermost stamens retarded and microsporogenesis in the outermost stamens slower than in the next inner stamens (Chang, Ren & Lu, 2005). Bidirectional development is also known from the Pulsatilla group of Anemone since Eichler (1878). Is this bidirectional pattern common in Anemoneae?

The unusual diversity in floral morphology in Anemoneae and Ranunculaceae also renders comparative studies on floral development and molecular developmental genetic studies especially attractive (Endress, 1995; Endress & Igersheim, 1999; Kramer & Irish, 1999; Kramer, Di Stilio & Schluter, 2003; Chang et al., 2005; Di Stilio, Kramer & Baum, 2005; Lee et al., 2005; Tucker & Hodges, 2005; Cui et al., 2006; Irish, 2006, 2009; Kramer & Zimmer, 2006; Gu & Ren, 2007; Jaramillo & Kramer, 2007; Song et al., 2007; Hileman & Irish, 2009; Kramer, 2009; Rasmus- sen, Kramer & Zimmer, 2009; Ren et al., 2009). Recent developmental studies on the tribe Anemoneae include Anemone coronaria L. (Ben-Hod, Kigel & Steinitz, 1988), Anemone rivularis (Chang et al., 2005) and Clematis montana Buch.-Ham. ex DC. (only carpels; van Heel, 1981, 1984). In the present paper, one of a series of studies on Ranunculaceae, the floral structure and development of Clematis (two species) and Anemone (four species, including one species of former Pulsatilla) are described. It is the first comparative developmental study encompassing several species of Anemoneae and covering both sub-tribes. Our main focus is on early flower development, floral phylotaxis, stamen maturation and carpel development.

MATERIAL AND METHODS

Flower buds were studied in Clematis petersae Hand.-Mazz. [section Flammula DC. sensu Prantl] (alt. 600–1750 m, voucher: Bai Genlu 2004004, SANU), Clematis macropetala Ledeb. [section Atragine (L.) DC.] (alt. 2800–3100 m, voucher: Bai Genlu 2004019, SANU), Anemone taipaiensis W.T.Wang [section Omalocarpus DC.] (alt. 3100–3600 m, voucher: Bai Genlu 2004018, SANU), Anemone tomentosa (Maxim.) Péi [section Eriocapitella (Nakai) Tamura] (alt. 600–2300 m, Bai Genlu 2004017, SANU), Anemone altaica Fisch. [section Anemonanthea DC.] (alt. 900–2300 m, voucher: Bai Genlu 2004016, SANU) and Anemone chinensis Bunge [=Pulsatilla chinensis (Bunge) Regel] (alt. 600–1800 m, Bai Genlu 2004001, SANU). The material was collected from late March to early November of the years 2002–2005 at intervals of 7–10 days in the Taibaishan Mountains, Shaanxi Province, China, and fixed in formalin–acetic acid–alcohol (FAA). For scanning electron microscopy (SEM) studies, the flower buds were dehydrated in an ethanol and iso-amyl acetate series, treated with critical-point drying in CO2, vacuum evaporated and observed with a Hitachi 800 scanning electron microscope. For histological studies, the flower buds were dehydrated in an alcohol series, infiltrated with xylene and embedded in paraffin wax. The embedded material was sectioned at 8–10 µm thickness and stained with safranin and fast green. Photographs of anthetic flowers (Fig. 1) were taken with a Nikon Coolpix 990 digital camera.

RESULTS

Clematis petersae (Figs 1A, 2A–4)
The flowers are bisexual, polysymmetric, 1–1.5 cm in diameter and form multiflowered thyrsoids. The flowers are preceded by two transverse prophylls. They have four or sometimes five, white, spreading, obovate to elliptical, obtuse sepals, no petals, 70–80 stamens and 14–18 carpels (from 15 mature flowers) (Fig. 1A).

The following descriptions are based on the terminal flower in a cyme of the thyrsoid. The two transverse prophylls that precede the floral primordium are round and toothed in early development (Fig. 2A, B). The sepals are initiated in pairs. The organs of the first pair alternating with the prophylls appear almost simultaneously (Fig. 2A); the second pair alternates with the first pair (Fig. 2B). The sepal primordia are broad, crescent-shaped and obtuse. Those of a pair are slightly different in size. In flowers with five tepals, there is a double position instead of a single position in the second pair (Figs 2D, G, 3A). Four stamens alternating with the sepals are initiated in a whorl with a relatively long plastochnor after the last sepals (Fig 2C). The following four stamens are in two median double positions, that is in the same radius as the sepals of the second pair (Fig. 2D). The floral apex considerably increases in size and a large number of stamens are initiated rapidly (Fig. 2D–G). The hemispherical stamen primordia are quite small compared with the floral apex. The formation of c. 14–16 somewhat irregular orthostichies indicates that stamen phylotaxis is irregularly whorled, thus with c. 7–8 stamens in a whorl.
(Fig. 2H). When the carpels are initiated, the remaining floral apex has decreased (Fig. 2H, I). No distinctive plastochron appears to be present at the transition from androecium to gynoecium. The young carpels are slightly smaller than the young stamens (Fig. 2H). They are positioned in the same irregular orthostichies as the stamens (Fig. 2H, I). After initiation of all carpels, a small residual floral apex remains (Fig. 2I), which is later hidden by the growing carpels.

The outer two sepals grow more rapidly than the inner two (Fig. 2C). Although initiation and early development of the stamens are centripetal (Fig. 2D–G), later the outermost stamens lag somewhat behind the others and those immediately following the outermost ones become the longest, as seen in a radial series of stamens in a more advanced floral bud (Fig. 3B). The initially hemispherical carpels become concave on the ventral side, resulting in a chair-like shape with an ascidiate base, and a short stalk appears early (Fig. 3C). Carpel elongation is faster in the upper part of the carpel than the lower, so that the plicate zone becomes more pronounced, and the stalk remains short (Fig. 3D–F). An ovule primordium appears in median position at the transition between the plicate and ascidiate zone (Fig. 3D). The carpel closes to form a ventral slit in the shape of a reversed T (Fig. 3E, F). Later, three sterile lateral ovules develop above the fertile ovule (Fig. 3J). The style elongates rapidly and the prospective stigma becomes somewhat everted (Fig. 3G, H). At anthesis, the unicellular-papillate stigma is decurrent in the upper part of the style for about 1 mm (Fig. 3I).

The histology of four stamens in a longitudinal series (Fig. 4A) shows that pollen has separated from the tetrads in the outer three stamens (St1, St2, St3) (Fig. 4B–G). Pollen maturation of stamen 1 (St1) and stamen 2 (St2) is slightly ahead of that in stamen 3 (St3). Microsporogenesis of the innermost stamen (St4) is at the tetrad stage (Fig. 4H, I). Thus, stamen maturation appears to be centripetal, even though the outermost stamen (St3) is shorter than the other stamens.

Figure 2. *Clematis petraea*. Floral organ initiation and phyllotaxis. A, B, Sepals. A, First pair (S1). B, Second pair (S2). C–F, Stamens. C, First whorl consisting of four stamens (St1) in two double positions (alternating with the four sepals). D, Slightly later stage, one of the sepals of the second pair divided into two. E, From the side, four approximate stamen whorls present. F, From above, phyllotaxis somewhat irregularly whorled, initiation sequence pattern of further stamens difficult to determine. G, Slightly later stage, one of sepals of the second pair divided into two. H, I, Carpels initiated. H, Carpel phyllotaxis somewhat irregularly whorled. I, Slightly later stage, carpel phyllotaxis more or less regularly whorled. B, bract; C, carpel; S, sepal; St, stamen; numbers relating to initiation sequence. Scale bars: A, 120 μm; B, 200 μm; C, E, 140 μm; D, F, 100 μm; G, 130 μm; H, 300 μm; I, 250 μm.
Figure 3. *Clematis peterae*. Floral organ development. A, Sepals enlarged and one of the second pair divided into two. B, The first (outermost) stamen in a longitudinal series of the androecium is the shortest and the second is the longest of all. C–I, Carpel development. C, Carpels becoming chair-like. D, Carpel with ovule initial at the cross zone. E, F, Carpel flanks closing as upper part of carpel elongates. G, Carpels of advanced floral bud. H, Close-up of upper part of a style of G. I, Close-up of upper part of a mature style, showing stigmatic tissue with short papillae. J, Opened ovary with one fertile and three sterile ovules. C, carpel; O, ovule; S, sepal; SO, sterile ovule; St, stamen. Scale bars: A, J, 0.3 mm; B, 0.5 mm; C, D, H, 100 μm; E, I, 120 μm; F, 180 μm; G, 0.8 mm.
CLEMATIS MACROPETALA (FIGS 1B, 5, 6) The flowers are bisexual, polysymmetric, solitary and 3–6 cm in diameter, with a long pedicel. They are preceded by two bracts. The four spreading ovate, acute sepals are blue or purple. Petals are absent. There are 20–28 staminodes, narrowly lanceolate and almost as long as the sepals (sometimes the inner ones are linear-spatulate), 60–70 stamens and 12–16 carpels (from six mature flowers) (Fig. 1B).

The early development of the floral organs of C. macropetala (Fig. 5) is similar to that of C. peterae. Floral phyllotaxis is irregularly whorled, with c. 7–8 stamens in a whorl (irregular orthostichies in Fig. 5F–J). The outer androecial organs, although they have larger anthers, soon appear shorter than the inner stamens, because of less filament elongation (Fig. 5K, L). They become staminodes, which have reduced or lacking anthers and the filaments are broadened to different degrees. There are various transitional forms from normal stamens to antherless staminodes (Fig. 6A–D). The young carpels are chair-like (Fig. 6E). The fertile ovule is enclosed by the enlarging ovary after its initiation (Fig. 6F–H). The carpels become long, slender and hairy (Fig. 6I–
Figure 6. *Clematis macropetala*. Floral organ development. A–D, Morphology of androecial organs in mature flower, including intermediate forms between stamens and staminodes (A, innermost organ; D, outermost organ). A, Stamen. B, Stamens with anthers of smaller size. C, Staminode with only rudimentary anther. D, Staminode. E–L, Development of carpels. E, Carpels in early chair-like stage. F, Carpel with ventral median ovule primordium. G, Carpels elongate and ovules are being enclosed. H, Ventral slit formed. I, Styles conspicuously elongate and hairy. J, Mature carpels. K, Upper part of mature style, showing stigmatic tissue consisting of inconspicuous papillae. L, Opened ovary with a fertile and three sterile ovules. C, carpel; O, ovule; SO, sterile ovule; St, stamen. Scale bars: A–D, 1.5 mm; E, 100 μm; F, 60 μm; G, K, 90 μm; H, 150 μm; I, 1 mm; J, 5 mm; L, 0.5 mm.
The flowers are bisexual, polysymmetric and 1.5–2 cm across, with a long pedicel and three sessile involucral leaves in an approximate whorl preceding each flower. A shoot bears one to five flowers. If several flowers are present, they form a thyrsoid. There are five white, obovate sepals, no petals, 60–64 stamens and 60–70 carpels (from eight mature flowers) (Fig. 1C).

The sepals are initiated in a spiral sequence (Fig. 7A–D). However, they are positioned in an approximate whorl with quincuncial aestivation. The first three young sepals are broad, crescent-shaped and obtuse (Fig. 7A, B), whereas the fourth and fifth are smaller and more or less hemispherical but truncate (Fig. 7C). It appears that there is a relatively long plastochron between the initiation of the last sepal and the first stamen (Fig. 7D). The first stamens appear to be initiated in the same spiral sequence as the sepals (Fig. 7D, E). The young stamens are at first hemispherical and narrow (Fig. 7D, E). When the carpels are initiated, the remaining floral apex has become smaller than it was at stamen initiation (Fig. 7F). The young carpels are hemispherical but smaller than the stamens (Fig. 7F–H). However, it is difficult to distinguish the last stamens and first carpels in early stages. After carpel initiation, a residual floral apex remains (Fig. 7I), which is later hidden by the growing carpels. Phyllotaxis of stamens and carpels is variable. The different patterns were studied in 15 young flowers, in which carpel initiation was just or almost finished. Only one flower was found with an almost regular Fibonacci spiral pattern (Fig. 7F).

The other 14 flowers showed some irregularities in the presence of incomplete parastichies in addition to the complete ones. Three flowers showed irregular whorls with sets of nine, 11 or 12 parastichies in both directions (irregular because of the presence of additional partial parastichies) (example with two sets of nine parastichies in Fig. 7H). This pattern probably results by double positions at the onset of androecium development after whorls or series of three or five organs. Eleven flowers were irregularly spiral but not with a Fibonacci pattern. Parastichy sets recorded in these were 8/9 (four flowers; example in Fig. 7I), 8/11 (three flowers), 8/12 (two flowers; example in Fig. 7G), 9/10 and 12/13, but always with additional partial parastichies or single surplus organs. The enlarging sepals enclose the other floral organs in later development. Development of the stamens is faster than that of the carpels (Fig. 8A–C). Before the stamen filaments elongate, the second and third of the four stamens in a longitudinal series become larger than the first and the fourth, and this pattern is also present in later stages (Fig. 8A–C). The enlarging carpels first become chair-like (Fig. 8D). The concavity deepens and gives rise to the ascidiate zone. With the elongating upper part, the plicate zone becomes pronounced. An ovule appears in median position at the transition between the plicate and ascidiate zone (Fig. 8E, F). When the carpels close, they conspicuously elongate, including the stalk (Fig. 8F, G). The median region at the base of the ventral slit becomes irregularly bumpy (Fig. 8H, I). Two sterile (lateral) ovules are formed above the fertile ovule after carpel closure (Fig. 8K). The uppermost part of the style becomes recurved (Fig. 8I). Unicellular-papillate stigmatic tissue differentiates on the ventrally everted upper portion of the carpel and is recurrent for most of the length of the style (Fig. 8I, J).

Of the four stamens of a longitudinal series studied (Fig. 9A), stamen 1 (St1) (Fig. 9E) and stamen 4 (St4) (Fig. 9B) were at the end of meiosis, stamen 2 (St2) contained mature pollen (Fig. 9D) and stamen 3 (St3) young pollen (Fig. 9C). Thus, anther maturation appears to be bidirectional, because the outermost anther is retarded.

Anemone taipaiensis (Figs 1C, 7–9)
The flowers are bisexual, polysymmetric and 3–4.5 cm across, and have a long pedicel; they are preceded by three petiolate involucral leaves in an approximate whorl. Each shoot forms a thyrsoid of several flowers. The five or rarely six sepals are white or pinkish, obovate or broadly elliptic. Petals are absent. There are 186–201 stamens and 450–470 carpels (from five mature flowers) (Fig. 1D).

Early flower development (Figs 10, 11) differs in some respects from that of A. taipaiensis. All young sepals are crescent-shaped (Fig. 10A–C), and rarely a sixth sepal appears (Fig. 10C). There are conspicuously more stamens, and the initiation sequence of stamens is more difficult to evaluate because it proceeds very rapidly (Fig. 10D–F). There are considerably more carpels (Fig. 10G–I), and the carpels have longer stalks (Fig. 11E, F). The size of the stamens and carpels is more distinctly different in early stages (Figs 10H, I, 11B, C). The carpel tip is not recurved, but rather incurved from early on (Fig. 11D–G). The stigma is shorter and appears quadrangular and pyramidal (Fig. 11G–I). Floral phyllotaxis appears to be more or less whorled. This is shown by the approximate orthostichies that are
Figure 7. *Anemone taipaiensis*. Floral organ initiation and phyllotaxis. A–C, Sepals initiated spirally, the outer ones broad, crescent-shaped. A, Third sepal initiated. B, Fourth sepal initiated. C, Fifth sepal initiated. D, E, First stamens initiated, hemispherical. F–I, Young flowers with later stamens and carpels initiated, in (I) carpels chair-like. Each flower with a different phyllotaxis pattern in androecium and gynoecium. F, Flower with almost regular Fibonacci spiral phyllotaxis (sets of eight and 13 parastichies and one surplus organ). G, Flower with irregular spiral phyllotaxis (sets of eight and 12 parastichies and surplus organs). H, Flower with irregular whorls (two sets of nine parastichies and surplus organs). I, Flower with irregular spiral phyllotaxis (sets of eight and nine parastichies and surplus organs). B, bract; C, carpel; S, sepal; St, stamen. Scale bars: A, B, D, E, 120 μm; C, 140 μm; F, 260 μm; G, 350 μm; H, 420 μm; I, 400 μm.
Figure 8. *Anemone taipaiensis*. Floral organ development. A–C, Development of stamens in a longitudinal series, the central ones developing faster than the outer and inner ones. A, Filaments not yet evident. B, Anthers and filaments differentiated. C, Filaments elongate. D–J, Development of carpels. D, Carpels in early chair-like stage. E, Later chair-like stage. F, Carpels with ovule primordium. G, Ventral slit forming, uppermost part of carpel appearing round in transverse section, not conduplicate. H, Carpels elongate, basal end of closure with irregular, bumpy surface. I, Mature carpel. J, Everted upper part of style, showing stigmatic tissue. K, Opened ovary with a fertile and two sterile ovules. C, carpel; O, ovule; SO, sterile ovule; St, stamen. Scale bars: A, 140 μm; B, 0.3 mm; C, 1.5 mm; D, E, 90 μm; F, J, 120 μm; G, 200 μm; H, 0.6 mm; I, 0.9 mm; K, 0.4 mm.
present in the flowers shown in Figure 10F and 10G. Each whorl has numerous organs (>20) (Fig. 10I). In addition to the median fertile ovule, four lateral sterile ovules are present (Fig. 11J). Anther maturation is centrifugal in the androecium (not figured).

**ANEMONE ALTAICA (FIGS 1E, 12)**

The flowers are bisexual, polysymmetric and 2–3 cm across, with a long pedicel; they are preceded by three petiolate involucral leaves in an approximate whorl. Each shoot has a single (terminal) flower and sometimes a second flower in the axil of an involucral leaf. There are five or, rarely, up to eight white, bluish or reddish violet, oblong to narrowly elliptic sepals. Petals are absent. There are 34–40 stamens and 7–13 carpels (from nine mature flowers) (Fig. 1E).

Organ initiation and development (Fig. 12A–D) are similar to those of *A. taipaiensis*, but there are many fewer stamens and carpels (Fig. 12A), and the stigmatic tissue consists of longer papillae (Fig. 12E, F). In addition to the median fertile ovule, several lateral sterile ovules are present (Fig. 12G). The sequence of anther maturation in a longitudinal series of stamens is approximately centrifugal (not figured).
Figure 10. Anemone tomentosa. Floral organ initiation. A–C, Sepals (crescent-shaped) initiated spirally. A, Sepals 1–3. B, Sepal 4. C, Sepals 5 and 6. D–F, Stamens (hemispherical) initiated. D, First stamens; as they are much smaller than sepals and compared with the floral apex, determination of the initiation sequence is not possible. E, Same, from side. F, More stamens initiated, approximate orthostichies visible. G–I, Carpels initiated, slightly smaller than stamens. G, Outer carpels initiated, approximate orthostichies visible. H, Somewhat later stage, borderline between androecium and gynoecium more distinct. I, Floral centre with most of the carpels initiated. B, bract; C, carpel; S, sepal; St, stamen. Scale bars: A, F, 150 μm; B, G, 180 μm; C, E, 120 μm; D, 100 μm; H, 240 μm; I, 200 μm.
Figure 11. *Anemone tomentosa*. Floral organ development. A, Sepals (crescent-shaped) in spiral sequence. B, C, Stamens development, stamens in the centre of a longitudinal series develop faster than outer and inner ones. B, Stamens in the centre begin their differentiation. C, Stamens in the centre much larger than the outer and inner ones. D–I, Carpel development. D, Carpels in chair-like stage. E, Stalk elongate, ovule initiated. F, Ventral slit formed, ovule enclosed. G, Slightly immature carpels, with quadrangular pyramidal stigma. H, I, Stigma of mature carpel with inconspicuous papillae. J, Opened ovary, with one fertile and four sterile ovules. C, carpel; O, ovule; S, sepal; SO, sterile ovule; St, stamen. Scale bars: A, 230 μm; B, 200 μm; C, 400 μm; D, 250 μm; E, 100 μm; F, J, 150 μm; G, 90 μm; H, I, 80 μm.
**ANEMONE CHINENSIS** (= *PULSATILLA CHINENSIS*)

(Figs 1F, 13–15)

The flowers are solitary, bisexual, polysymmetric and 5–7 cm across, with a long pedicel; they are preceded by three approximately whorled, basally connected involucral leaves. The six sepals are arranged in two whorls; they are violet and oblong-ovate. Petals are absent. There are 30–35 staminodes with short stalks and enlarged heads, 225–239 stamens and 240–253 carpels (from ten mature flowers) (Fig. 1F).

Three sepals are initiated successively to form the first whorl (Fig. 13A), and three additional ones form the second whorl (Fig. 13B). The young sepals are broad, crescent-shaped and truncate. The outer three are slightly larger than the inner three. It appears that there is a distinctive plastochron between the initiation of the second sepal whorl and the first staminodes (Fig. 13C). The initiation sequence of the staminodes and stamens is difficult to judge because initiation takes place rapidly and the hemispherical young staminodes and stamens are small compared with the floral apex (Fig. 13D). The floral apex is exceedingly convex and becomes almost spherical, so that the outer stamens are not visible from above in the youngest stages (Fig. 13E, F). The carpels are initially difficult to distinguish from the stamens (Fig. 13G). Carpels are initiated until they cover the entire remaining floral apex (Fig. 13H–J). At this stage, the stamens can be distinguished from the carpels by their larger size (Fig. 13J). When the floral apex becomes almost spherical, the androecial organs located at the equator become larger than the more basal ones (Fig. 13G). The result is that there is a conspicuous decrease in organ size from the upper to the lower part of the androecium (Fig. 14A–C). The outermost organs, although differentiated into anther...
and filament, are only one-quarter to one-third of the length of the inner ones (Fig. 14D). Some of them will become staminodes.

The young carpels become chair-like (Fig. 14E, F). The base elongates into a stalk and, with the deepening of the ventral cavity, an ascidiate and a plicate zone are formed (Fig. 14G). A median ovule appears at the border between these two zones (Fig. 14G). When the carpel has closed, the style elongates exceedingly (Fig. 14H, I). The stigmatic region differentiates on the everted ventral surface of the upper part of the style (Fig. 14J, K). The stigma consists of unicellular papillae and is slightly decurrent (Fig. 14L).

The study of ten stamens in a longitudinal series (Fig. 15A) shows the following sequence of anther maturation (staminodes not considered): stamen 1...

Figure 13. *Anemone chinensis*. Floral organ initiation. A, B, Sepal initiation. A, First three sepals (crescent-shaped) initiated to form first perianth whorl. B, Sepals 4–6 initiated to form second perianth whorl. C–F, Stamen initiation. C, Outermost stamens initiated. D–F, Centripetal initiation of outer stamens. D, Several outer stamens initiated, inner sepals still much smaller than outer ones. E, F, Shape of floral apex changing from hemispherical to almost spherical so that, in a view from above, the outermost stamens are no longer visible. F, Sequence of stamen initiation cannot be determined because stamen primordia are very small compared with the floral apex. G–J, Initiation of first carpels, stamen and carpel primordia difficult to distinguish. G, From the side. H, From above. I, Most carpels initiated. J, All carpels formed and now much smaller than stamens. B, bract; C, carpel; S, sepal; St, stamen. Scale bars: A–C, 180 μm; D, 140 μm; E, F, 200 μm; G, 240 μm; H, 270 μm; I, 380 μm; J, 0.5 mm.
Figure 14. *Anemone chinensis*. Floral organ development. A–D, Stamen development. A, Outer stamens in a longitudinal series retarded. B, Slightly older stage, retardation more pronounced. C, Retardation strongly pronounced. D, Androecium viewed from the periphery: outermost, smallest androecial organs will develop into staminodes. E–L, Carpel development. E, Carpels in chair-like stage. F, Chair-like carpel from ventral side. G, Young carpel with ovule initiated. H, Ventral slit formed, ovules enclosed, styles elongate. I, Later stage, hairs initiated. J, Almost mature carpels. K, Upper part of a style, with stigma differentiating. L, Everted papillate stigma of mature carpel. M, Opened ovary, with one fertile and one sterile ovule visible. C, carpel; O, ovule; St, stamen. Scale bars: A, 230 μm; B, 270 μm; C, 0.6 mm; D, 0.5 mm; E, 140 μm; F, 60 μm; G, 100 μm; H, 380 μm; I, 0.8 mm; J, 1.2 mm; K, 180 μm; L, 150 μm; M, 80 μm.
Figure 15. *Anemone chinensis*. Microsporogenesis in a longitudinal series of ten stamens marked as St₁ to St₁₀ (staminodia not represented). A, Overview. B–D, Close-up of pollen sacs of St₁–St₂ at stage of meiocytes. From St₁ to St₅, meiocytes successively (slightly) more advanced. E, Close-up of pollen sac of St₄ at stage of dyads. F–K, Close-up of pollen sacs of St₅–St₁₀ at stage of tetrads. In St₁₀, tetrads slightly less advanced than in St₅–St₉. C, carpel; St, stamen. Scale bars: A, 1 mm; B–D, 200 μm; E, K, 40 μm; F–J, 20 μm.
DISCUSSION

FLORAL PHYLLOTAXIS

In the older literature, floral phyllotaxis in Anemone and Clematis is often uncritically and incorrectly stated as spiral, as in many other angiosperm taxa with numerous stamens and carpels. In fact, the floral phyllotaxis of Anemoneae is diverse. This was first established by Schöffel (1932), who worked with microtome transverse sections of advanced floral buds. Although Payer (1857) performed developmental studies on a Clematis species and illustrated young floral stages with detailed drawings, which showed orthostichies in the androecium, only later, with microtome sections (Schöffel, 1932), and with SEM, did better founded floral phyllotaxis studies become possible (e.g. Endress, 1987, 2006). As shown by Schöffel (1932), in Anemone, floral phyllotaxis is spiral (Fibonacci or rarely Lucas pattern) in some species and irregularly whorled in others. Whorled patterns include complex whorls, i.e. including double or multiple positions. Such diversity was also found in our material, and the younger stages studied show this still more clearly. Both irregularly spiral and irregularly whorled floral phyllotaxis are even present in the same species: A. taipaiensis (this study). In the two species with the highest floral organ numbers of this study, A. tomentosa and A. chinensis (= Pulsatilla chinensis), phyllotaxis is irregularly whorled. Whorled flowers also occur in the highly polystemonous and highly multicarpellate A. coronaria (Ben-Hod et al., 1988).

In Clematis, floral phyllotaxis is whorled; usually, there are complex whorls with an increase in organ number per whorl at the transition from the perianth to the androecium by double or multiple organ positions (this study; Schöffel, 1932; Tobe, 1976; for a general discussion of complex whorls, see also Staedler & Endress, 2009). Payer (1857) figured young stages of C. calycina Aiton [= C. cirrhosa L.], apparently with a whorled androecium (but without describing this feature).

Phyllotaxis in the perianth is easier to determine. The most common patterns in Anemone are two whorls of three sepals or one whorl of a series of five. In contrast, in Clematis, the most common pattern is two whorls of two sepals; if five sepals occur, the inner pair may have a double position on one side; in this case, pentamery is not based on a spiral developmental sequence or spiral phyllotaxis, but on a modification of a dimerous whorled pattern (see also ‘Perianth’ below).

Such lability of floral phyllotaxis as found in Anemoniniae is perhaps only known from some families of early branching angiosperms (Atherospermataceae, Monimiaceae; Laurales), in which whorled, spiral and irregular floral phyllotaxis patterns are also present (Staedler & Endress, 2009).

Irregularly whorled phyllotaxis tends to be present in flowers with numerous stamens and carpels (Endress, 2006) and also in perianthless flowers. Both conditions are present in several genera of Ranunculales, the former in some Ranunculaceae (e.g. Anemone and, perhaps, Glaucidium Siebold & Zucc.) (this study; Schöffel, 1932) and some Papaveraceae (e.g. Papaver L., Romneya Harv.) (Murbeck, 1912; Karrer, 1991), and the latter in Eupteleaceae (Ren et al., 2007) and Achlys DC. (Berberidaceae; Endress, 1989). To characterize tentatively different cases of irregularly whorled or irregularly spiral flowers, we determined the number of complete parastichies of two subsequent antidromous parastichy sets in each flower and noted the presence of supernumerary organs.

This high lability of floral phyllotaxis also still impedes a reconstruction of floral phyllotaxis evolution in Ranunculaceae. Optimization studies based on a morphological dataset of early branching angiosperms and early branching eudicots show a whorled perianth as plesiomorphic for the family, but for the androecium the basal state is equivocal (Endress & Doyle, 2007, 2009).

PERIANTH

Sepal number shows a range in both genera: Clematis mostly has four sepals, some species six (rarely eight), whereas, in Anemone, five and six sepals are common (Schöffel, 1932; Tamura, 1995) and, in some species, the normal sepal number is ten or more (up to 30) (Eichler, 1878; Tamura, 1995). In Clematis, the sepal number sometimes fluctuates between four and five in the same species. In C. petersiae, in young flowers with five sepals, we found a double position in the inner sepal pair (see ‘Floral phyllotaxis’). A similar configuration was reported from C. tosaensis (Tobe, 1976). It is not known whether this is the normal developmental pathway in Clematis flowers with five sepals, or whether a normal spiral sequence resulting in quincuncial aestivation may also occur.

Presence or absence of petals: the coloured perianth organs in Anemoneae are commonly interpreted as sepals because they are the outermost organs and
have three vascular traces (Hiepko, 1965, 1975). However, between the sepals and fertile stamens, there are sterile organs of different shape and/or size in some species of Clematis and some member of the Pulsatilla group within Anemone. In Clematis, they are commonly longer and broader than the stamens and may equal the sepals in length, whereas, in the Pulsatilla group of Anemone, they are smaller than but similar in shape to the stamens. Therefore, they are commonly called staminodes in the Pulsatilla group, but petals (or staminodes) in Clematis (for a review of the diversity of the organs between sepals and stamens, see Tamura, 1965). The difference in the terminology for these organs is arbitrary. According to our observations, there may be two ways in Anemoneae by which the outermost stamens become petal-like: by expansion of the filaments, as in C. macropetala, and by forming club-shaped staminodes, as in A. (Pulsatilla) chinensis.

Based on molecular systematic studies on Clematis, Miikeda et al. (2006) assumed that the petals of Clematis (section Atragene) and of Naravelia are ‘probably not homologous’. As the two clades are not directly related and their closest relatives do not have petals, non-homology is likely in the sense of cladistic homology. However, the arguments for non-homology given by Miikeda et al. (2006) are not convincing in view of the great plasticity of petal/staminode shape in Ranunculaceae, if homology is viewed in a biological sense (Wagner, 1989, 2007), as the genetic background may be the same in both cases (Rasmussen et al., 2009).

**ANDROECIUM**

Although stamen initiation is centripetal in all species of Clematis s.l. and Anemone s.l. (and all other Ranunculaceae) studied so far, anther maturation is not always centripetal, as centrifugal or bidirectional maturation also occurs. From our results, anther maturation is more or less centripetal in Clematis, but centrifugal or bidirectional in Anemone (see also Chang et al., 2005), and the centrifugal pattern is most conspicuous in the Pulsatilla group of Anemone (see also Sun & Wang, 1984). That the sequence of pollen release is not centripetal, but proceeds centrifugally and centripetally, starting in an intermediate zone, has long been known for the Pulsatilla group (Eichler, 1878). Associated with the centrifugal maturation is a progressive delay of filament elongation towards the periphery of the androecium in older buds and at anthesis (see also Voelter & Weber, 1962). Often the outermost organs of the androecium in Anemoneae are sterile, but are nectariferous in some species (see ‘Nectaries’ below). These outer sterile organs may be larger (some Clematis) or smaller (some subgroups of Anemone s.l., such as Pulsatilla or Barneoudia) Gay; Hiepko, 1965, 1975). A similar pattern of centrifugal stamen maturation to that in the Pulsatilla group occurs in Glaucidium, which was perhaps wrongly interpreted as having centrifugal stamen initiation by Tamura (1972), and therefore thought to be related to Paeonia L. Molecular studies clearly place Glaucidium in Ranunculaceae (Hoot & Crane, 1995) and not with Paeonia, which is positioned in Saxifragales of core eudicots (Soltis et al., 2000).

**NECTARIES**

In some species of Clematis, nectar is produced on the ventral side of the lower part of the stamen filaments or lower part of the staminodes (Schaffnit, 1904; Daumann & Slavíková, 1968). For Clematis stans Siebold & Zucc., an origin from the ‘base of the calyx tube’ has been mentioned, but without a precise indication of the site of the secretory tissue (Dohzono & Suzuki, 2002; Dohzono, Suzuki & Murata, 2004).

Within Anemone s.l., nectaries are only present in part of the species of the Pulsatilla group (Eichler, 1878; Hiepko, 1965; Zimmermann, 1965/1974). Nectar is secreted from the thickened filament of small outer staminodes in A. pulsatilla (Kratochwil, 1988) or from the entire surface of the thickened upper portion (species not indicated; Schaffnit, 1904; Hiepko, 1965). Nectar production was recorded in other species of the Pulsatilla group without location of the secretory tissue (Torvik, Borgen & Berg, 1998; Huang, Takahashi & Dafni, 2002).

In other Ranunculaceae, nectar is secreted by nectaries on the ventral surface of petals (staminodes) (e.g. Erbar, Kusma & Leins, 1998). This is also the case in some other families of Ranunculaceae (Berberidaceae, Menispermacaceae, Lardizabalaceae, Circaestercaceae). However, in Fumarioideae (Papaveraceae) (Dahl, 1989) and Decaisnea Hook.f. & Thomson (Lardizabalaceae) (Endress, 1995), nectar is secreted on the stamen filaments.

**GYNOECIUM**

Carpels are slender, often conspicuously hairy, with a commonly well-developed stipe and often long styles. The ovary is ascidiate and the style plicate. The closing carpel becomes bumpy at the site of closure in A. taipaiensis (for this phenomenon in other angiosperms, see also Endress, 2006).

Each carpel has a single median fertile ovule. Additional lateral sterile ovules, often three or more, above the fertile ovule have been reported for a number of species in Clematis [Payer, 1857; Baillon, 1863, 1866; Prantl, 1887; Lonay, 1901; Souèges, 1910–1914 (1910); Chute, 1950; Rassner, 1931; Brouland,
1935; Schaeppi & Frank, 1962; Vijayaraghavan, 1962; Bhandari, 1968; Fish, 1970; Tobe, 1980; Wang & Ren, 2008] and in Anemone [Bailon, 1863, 1866; Prantl, 1887; Lonay, 1901; Souèges, 1910–1914 (1911); Chute, 1930; Rassner, 1931; Brouland, 1935; Schaeppi & Frank, 1962; Shukova, 1965; Rohwedder, 1967; Salisbury, 1973; Tobe, Adachi & Yoshida, 1975; Wang & Ren, 2008]. Of earlier studies in the Pulsatilla group of Anemone, only Souèges [1910–1914 (1911)] drew a figure with minute sterile ovules but without mentioning them, and Bessey (1898) globally mentioned them together with Anemone and Clematis. Voelter & Weber (1962) referred to a report of occasional additional sterile ovules in the Pulsatilla group by Zimmermann (1938); however, we could not find this in Zimmermann (1938). Zimmermann (1938) described and illustrated only a single ovule in photographs of microtome sections of A. pulsatilla (as Pulsatilla vulgaris). The present study appears to be the first clear report of additional ovules in the Pulsatilla group of Anemone, and thus adds to the consistency of this character in Anemone s.l. and Anemoneae as a whole.

**UNUSUAL FLORAL FEATURES IN ANEMONEAE**

Although stamen initiation is centripetal in Anemo- neae, the outermost stamens in Anemoninae are retarded in development, so that stamen maturation is neither centripetal, as found in most genera of Ranunculaceae (Gu & Ren, 2007; Song et al., 2007), nor completely centrifugal, as found in Aquilegia L. of Thalictridoideae (Tepfer, 1953; Feng et al., 1995), but bidirectional.

The upper part of the carpel becomes conspicuously everted and the stigmatic tissue on the everted part has long papillae. This feature was also observed in Isopyrum [Bailon, 1863, 1866; Prantl, 1887; Souèges, 1910–1914 (1911); Chute, 1930; Rassner, 1931; Brouland, 1935; Schaeppi & Frank, 1962; Shukova, 1965; Rohwedder, 1967; Salisbury, 1973; Tobe, Adachi & Yoshida, 1975; Wang & Ren, 2008]. Of earlier studies in the Pulsatilla group of Anemone, only Souèges [1910–1914 (1911)] drew a figure with minute sterile ovules but without mentioning them, and Bessey (1898) globally mentioned them together with Anemone and Clematis. Voelter & Weber (1962) referred to a report of occasional additional sterile ovules in the Pulsatilla group by Zimmermann (1938); however, we could not find this in Zimmermann (1938). Zimmermann (1938) described and illustrated only a single ovule in photographs of microtome sections of A. pulsatilla (as Pulsatilla vulgaris). The present study appears to be the first clear report of additional ovules in the Pulsatilla group of Anemone, and thus adds to the consistency of this character in Anemone s.l. and Anemoneae as a whole.

**Differences in floral structure between Anemone and Clematis**

The perianth organs are commonly initiated in two pairs in Clematis, and the floral phyllotaxis is whorled or irregularly whorled (Schöffel, 1932; Hiepko, 1965; this study). If the perianth has five instead of four organs, one position of the inner pair is doubled (this study). In contrast, in Anemone, floral phyllotaxis is more diverse: spiral, whorled or irregular (Schöffel, 1932; Hiepko, 1965; this study). In three of the Anemone species studied here, the commonly five sepals are initiated in spiral sequence, whereas, in one (A. chinensis), the six tepals are initiated in two whorls. The first eight stamens in Clematis can be interpreted as two alternating pairs, each with double positions (for this pattern, see also fig. 3D in Staedler & Endress, 2009). In Anemone, the detailed sequence of stamen initiation is difficult to determine, as their primordial size is exceedingly small and the initiation sequence rapid. Stamen maturation is centripetal in Clematis (except for the outermost stamens, which are retarded), but is centrifugal or bidirectional in Anemone (only the innermost stamens may be retarded), the centrifugal pattern being especially pronounced in the Pulsatilla group of Anemone.

**CONCLUSIONS**

Species with excessive lability in floral phyllotaxis patterns, such as A. taipaiensis, lend themselves to more detailed studies in the search for boundary
conditions of the different phyllotaxis patterns, which are still unknown (see also Staedler & Endress, 2009). Such studies could include the comparison of flowers at different positions in the ramification system of the same inflorescence and in different individuals at the population level.

It is still not known whether developmental retardation in the maturation of the outer stamens, as in Anemoneae, and retardation of the petals before anthesis, as in other Ranunculaceae and many other eudicots, are directly functionally connected, and whether and how this could be another indicator of the evolutionary relationship between androecium and corolla in the family and other eudicots. Although floral structure and development have been studied in relatively many Ranunculaceae, as compared with other angiosperm families, new comparative studies encompassing the many still unstudied genera and discussions based on new phylogenetic and molecular developmental results are needed to better understand floral evolution at the base of the eudicots.

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