COMPARATIVE FLORAL ONTOGENY IN WINTERACEAE

Andrew N. Doust

ABSTRACT

The flowers of Winteraceae have often been considered representative of the floral morphology of the earliest angiosperms. The diversity in number and arrangement of the (mostly) free floral organs gives an opportunity to critically examine floral developmental processes in this basal angiosperm family. Comparative ontogeny and morphology of flowers of eight species from six genera of Winteraceae were examined using scanning electron microscopy (SEM) and light microscopy. Results were compared with the floral morphology of Takhtajaniania perrieri (Capuron) Baranova & J-F. Leroy, the recently rediscovered species of Winteraceae from Madagascar. The analysis showed that the basic floral architecture in the family is one of varying numbers of decussate organs followed by whorls with four (or more) organs. Spiral and irregular patterns also occur, with irregularities in floral organ arrangement often the result of initiation of primordia on an asymmetrical floral meristem. The first organs initiated are a pair of sepals that are usually lateral relative to the subtending bract of the flower. Further growth of the calyx is via a ring of meristematic tissue encircling the floral meristem, producing a tubular calyptra bearing the sepals at its apex. The calyptra either encloses the bud until anthesis (Tasmania and Drimys) or ruptures while the bud is still young (Zygogynum, Exospermum, Bubbia, Pseudowintera, and Takhtajaniania). Differences in the position of the sepals relative to the subtending bract and the position of the petals relative to the sepals create differences in floral architecture between taxa. These differences support Tasmania and Drimys being considered separate taxa and Zygogynum and Exospermum being considered separate from Bubbia. The decussate and whorled floral architecture of Takhtajaniania perrieri reflects the basic pattern found throughout the family.

Key words: floral morphology, floral ontogeny, Takhtajaniania, Winteraceae.

Winteraceae have long been considered to be one of the basal angiosperm families and have played an important role in our understanding of the evolutionary history of angiosperms (Bessey, 1915; Bailey & Nast, 1945; Cronquist, 1988). Their importance can be traced to the possession of a suite of morphological character states that have been presumed to be primitive for flowering plants. Foremost among these has been a simple flower construction of many free parts and the occurrence, at least in some taxa, of conduplicate carpels (Bailey & Nast, 1943). Conduplicate carpels reflect the theory that angiosperms evolved carpels by the folding of an ovule-bearing leaf (Bessey, 1915; Bailey & Nast, 1945; Cronquist, 1988). Tubular (ascidiate) carpels can also be found in Winteraceae (Tucker, 1959; Leinfellner, 1965, 1966; Frame-Purgy, 1996).

In recent angiosperm phylogenies Winteraceae are one of the first branching lineages (Qiu et al., 1993; Nandi et al., 1998), and the floral morphology of the family possesses many characteristics that may elucidate the features of floral evolution in basal angiosperms. The recent rediscovery of the only Winteraceae from Madagascar, Takhtajaniania perrieri (Schatz et al., 1998), allows examination of all extant genera in the family.

Other papers in this issue of the Annals discuss the phylogenetic placement of Takhtajaniania within Winteraceae and detail its floral and vegetative morphology. Its floral morphology is unusual in the family because of the unique syncarpellate gynoe- cium (Leroy, 1977), and recent collections have allowed the structure of the gynoecium to be comprehensively examined for the first time (Endress et al., 2000). As a complement to the detailed analysis of Takhtajaniania (other papers, this issue), floral morphology and ontogeny in Zygogynum baillonii, Bubbia howeana, Pseudowintera axillaris, Pseu- dowintera colorata, Tasmannia lanceolata, Tasman- nia xerophila, and Drimys winteri are presented. Analysis of the mature floral morphology of other species of Zygogynum, Bubbia, and Exospermum is also presented.

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Comparative analysis of ontogenetic data can help in assessing the possible homologies of mature floral structures. Data from floral ontogeny may also shed light on two persistent systematic questions within the family. One of these is whether the genera *Bubbia*, *Belliolium*, *Exospernum*, and *Zygogynum* are best regarded as separate genera or as one large and variable genus. *Bubbia* and *Belliolium* were combined by Burtt (1936). Vink (1985) combined *Bubbia* (including *Belliolium*), *Exospernum*, and *Zygogynum* into an expanded *Zygogynum* s.l., with the argument that the characters that separate the genera (degree of connation of petals and of carpels) were variable and overlapping between them. In this paper data have been recorded under *Bubbia* (including *Belliolium*), *Exospernum*, and *Zygogynum* s. str. so as to highlight possible distinctions between them. The other systematic question is whether *Tasmannia* should be considered a separate genus or a section of *Drimys* (Smith, 1969; Vink, 1970, 1988; Sampson et al., 1988). In this paper data on *Tasmannia* are presented separately from that for *Drimys*.

These results are part of a more comprehensive study of floral development in Winteraceae (Doust, 1997; Doust & Drinnan, 1999; unpublished data). The focus in this paper is on early ontogenetic patterns, especially of sepal and petal initiation.

**Materials and Methods**

Freshly collected buds and flowers were either dissected immediately or fixed in FAA (formalin, acetic acid, 70% ethanol, 10 : 5 : 85 v/v) and stored in 70% ethanol. Preparatory to dissection fixed buds were dehydrated in an ethanol concentration series (70%, 85%, 95%) and stained for greater visibility with 1% acid fuchsin in 95% ethanol. Excess stain was removed from the buds by daily washing in 95% ethanol for three days before dissection. Several changes of 100% ethanol were used to rid the specimens of all water before critical point drying. After drying, specimens were mounted on aluminum stubs and sputter-coated with gold before being imaged in either a Jeol 840 or Phillips XL30 scanning electron microscope. Herbarium specimens of a number of species of *Zygogynum*, *Exospernum*, and *Bubbia* were examined at MO. The number of buds and flowers dissected and a list of voucher specimens are given in Table 1.

**Results**

The floral ontogeny and morphology of each species examined are described below. A generalized diagram of the pattern of floral morphology for each species is given in Figure 1.

**ZYGOGYNUM BAILLONI**

The inflorescence of *Zygogynum baillioni* is one-to three-flowered. Both terminal and lateral flowers have a tubular calycine calyptra, which encircles the young bud but ruptures as the bud expands. The remains of this calyptra are thereafter persistent around the base of the flower. The calyptra bears two sepal tips, which are oriented at right angles to the subtending floral bract in lateral flowers but which are variably oriented in terminal flowers (Fig. 2A). The next organs to be initiated are two opposite decussate sets of petals, the four bases of which are valvate and connate while the tips are free and imbricate The tips of the first initiated pair of these two pairs of petals overlap those of the second. The first pair of petals in lateral flowers is usually oriented parallel (rarely perpendicular) to the orientation of the lateral sepals (Fig. 2A). It was not possible to discern the orientation of the first petal pair relative to the sepals in terminal flowers. The two pairs of decussate petals become connate basally as they grow. They may also be more or less fused post-genuitally toward the apices of the petals. An adaxial groove is formed at the boundary between the connate regions of adjacent petals because the adjoining tissue is thinner than the petals themselves. When the petal tips are dissected away from the flower an invagination of the adaxial epidermis into the groove can be seen; epidermal tissue is also clearly evident when the boundary region is observed on the longitudinal edge of an individual petal (Fig. 2B, C). The four outer petals rupture along the boundary grooves when the flower expands at anthesis.

Following the initiation and growth of the outer two pairs of petals, two inner tetramerous whorls of petals are initiated (Fig. 2B). On rare occasions fewer or greater numbers of petals may be found in these whorls. The petals in both inner whorls have narrow bases and grow throughout as completely free structures. The arrangement of the petals is mostly regular, although the sizes of the petal primordia in the inner two whorls vary, apparently according to the space available for them. The second inner whorl of petal primordia is initiated almost at the same height on the meristem as the first inner whorl, and, with continued expansion of the meristem, members of the two whorls rapidly become indistinguishable and the petal bases appear to be in one whorl of eight. However, the middle whorl completely overlaps the inner whorl at its apex.
Table 1. Sample size and voucher list.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sample size (# buds, flowers)</th>
<th>Selected voucher specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zygogynum bicolor Tiegh.</td>
<td>50</td>
<td>NEW CALEDONIA. Near top of track from Sarra méa to Plateau de Dogny, Dec. 1994, Doust 926 (MELU); Old lumber road S of road leading from Cascade de Ciu to Koindé and La Foa, 10 Dec. 1993, McPherson 6121 (MO).</td>
</tr>
<tr>
<td>Zygogynum pomiferum Baill.</td>
<td>30</td>
<td>NEW CALEDONIA. Forested slopes of watershed of Rivière des Pittogues, N of Nouméa- Yate road, 15 July 1981, McPherson 3945 (MO); Thé River valley, ca. 12 air km NE of Nouméa, McPherson 5862 (MO).</td>
</tr>
<tr>
<td>Zygogynum veillardi Baill.</td>
<td>30</td>
<td>NEW CALEDONIA. South of Thio on coastal road, between Nimbo and Gambuti Rivers, 26 Apr. 1984, McPherson 6517 (MO); road N of Canala to Prokomé and Koaoua, 20 Apr. 1983, McPherson 5621 (MO); Prokomé Region, N of Canala, 28 Dec. 1983, McPherson 6222 (MO).</td>
</tr>
<tr>
<td>Bubbia howeana (F. Muell.) Tiegh.</td>
<td>50</td>
<td>AUSTRALIA. Lord Howe Island; 2 Nov. 1963, Green (WELTU); 8 Dec. 1966, Chinnoch (WELTU).</td>
</tr>
<tr>
<td>Bubbia amplexicaulis (Parm.) Dandy</td>
<td>30</td>
<td>NEW CALEDONIA. Mt Panié, ca. 20 air km NW of Hienghène, 31 Mar. 1981, McPherson 3695 (MO); Mandjélia, above Pouébo, 30 Jan. 1984, McPherson 6280 (MO); Pouébo: Mont Mandjélia, 6 Mar. 1979, Mackee 36654 (MO).</td>
</tr>
<tr>
<td>Bubbia comptonii (E. G. Baker) Dandy</td>
<td>30</td>
<td>NEW CALEDONIA. Ponerihuouen: Mont Aoupinié, 8 Sep. 1976, Mackee 31875 (MO); Mt Panié, ca. 20 air km NW of Hienghène, 20 July 1980, McPherson 2982 (MO); along old lumber road to top of Mt. Me Ori, above Katrikoin, 6 Sep. 1980, McPherson 3045 (MO).</td>
</tr>
<tr>
<td>Bubbia crassifolia (Baill.) Burtt</td>
<td>30</td>
<td>NEW CALEDONIA. Massif de Boulinga, ca. 8 air km N of Poya, 22 May 1980, McPherson 2692 (MO); Mont Taum: Crête Est, Mackee 38150 (MO).</td>
</tr>
<tr>
<td>Bubbia pancheri (Baill.) Burtt</td>
<td>30</td>
<td>NEW CALEDONIA. Haute Amon, 9 Oct. 1971, Mackee 24400 (MO); along trail from Sarra méa toward Plateau de Dogny, 1 Sep. 1982, McPherson 4923 (MO); Duratto 1995 (MEL).</td>
</tr>
<tr>
<td>Exaspernum stipitatum (Baill.) Tiegh. ex Morot</td>
<td>30</td>
<td>NEW CALEDONIA. Mt Panié, N of Hienghène, 3 Nov. 1983, McPherson 5929 (MO); Boguen River Valley, along lumber track above Katrikoin, ca. 20 air km E of Bourail, 21 Nov. 1979, McPherson 2110 (MO); Tehamba River valley, 1 Nov. 1983, McPherson 5916 (MO).</td>
</tr>
<tr>
<td>Pseudowintera axillaris (J. R. &amp; G. Forst.) Dandy</td>
<td>100</td>
<td>NEW ZEALAND. North Island: Kaitoki Waterworks Reserve, Wellington, 3 Nov. 1996, Doust 891 (MELU); Akatawara, Upper Hutt Valley, 4 Nov. 1996, Doust 892, 894, 895 (MELU); Kakarui Ridge Road, S. Tararua, 4 Nov. 1996, Doust 895 (MELU).</td>
</tr>
<tr>
<td>Pseudowintera colorata (Raoul) Dandy</td>
<td>100</td>
<td>NEW ZEALAND. North Island: Kakarui Ridge Road, S. Tararua, 4 Nov. 1996, Doust 896, 897, 899 (MELU); S. Tararua, 1995, Sampson (WELTU).</td>
</tr>
<tr>
<td>Tasmannia glaucifolia J. B. Williams</td>
<td>50</td>
<td>AUSTRALIA. New South Wales: Dilgry, 28 Nov. 1983, Williams (NSW); Moffat Falls, 8 Nov. 1986, Sampson (NSW).</td>
</tr>
</tbody>
</table>
Table 1. Continued.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sample size (# buds, flowers)</th>
<th>Selected voucher specimens</th>
</tr>
</thead>
</table>

Stamen initiation follows directly on from petal initiation. There are usually eight stamens in each whorl, stamens in the first whorl alternating with the whorl of eight petal bases formed from the two inner whorls of petals (Fig. 2D). Succeeding whorls of stamens alternate with each preceding whorl, and there are usually the same number of stamens in each whorl. The last whorl of organs initiated contains both stamens and carpels, and the larger size of the carpels often leads to a reduction in the total number of organs in this whorl (Fig. 2D). The carpels are connate but there are no connections between the stigmatic crests and thus no common pollen-transmitting tract (Igersheim & Endress, 1997).

**BUBBIA HOWEANA**

Each flower of *Bubbia howeana* has a calyceine calyptra that protects the bud early in development but which ruptures as the bud expands. The remains of the calyptra persist as remnants of tissue around the base of the flower. The calyptra initiates as two laterally placed free sepals but attains most of its growth by the action of an annular meristem underneath the sepal tips. Two outer deccussate pairs of petals are initiated next, with the first of these two pairs of petals oriented perpendicularly to the sepal tips. The two outer deccussate pairs of petals in the flowers of *B. howeana* are free throughout their growth, and they tightly wrap around the bud and protect it as it develops (Fig. 2E). An inner whorl of petals is next initiated with four or rarely five petals, followed by a second whorl of petals and/or stamens (this whorl is shown as the outermost whorl of stamens in Fig. 1B). Three or four alternating whorls of four or five stamens are then initiated, followed by three to six free carpels arranged either symmetrically or more irregularly.

**PERIANTH PATTERN IN ZYGOGYNUM AND BUBBIA**

There is a difference in orientation of sepals and petals in *Zygogynum bailloni* and *Bubbia howeana*. In *Zygogynum bailloni* the lateral sepals are followed by a pair of lateral petals (Figs. 1A, 2A), whereas in *Bubbia howeana* the lateral sepals are followed by a pair of medial petals (Figs. 1B, 2E). These contrasting patterns of perianth morphology were examined in herbarium specimens of a number of species of *Zygogynum, Bubbia*, and the related genus *Exospermum* (Vink, 1985), and the results are given in Table 2. There is a clear distinction in perianth pattern between *Bubbia* on the one hand and *Zygogynum* and *Exospermum* on the other, with lateral flowers of all species of *Bubbia* examined having lateral sepals and medial first petal pair and lateral flowers of all species of *Zygogynum* and *Exospermum* examined having lateral sepals and lateral first petal pair. The two groups of species examined also differ in petal connation, with species of *Bubbia* having free outer petals and species of *Zygogynum* and *Exospermum* (except *Z. pomiferum* and some specimens of *E. stipitatum*) having connate outer petals.

**PSEUDOWINTERA AXILLARIS AND P. COLORATA**

No material of the earliest developmental stages for either species of *Pseudowintera* was examined. However, at a somewhat later stage the buds are enclosed in a calyceine calyptra that has two lateral sepal tips. F. B. Sampson (pers. comm.) and Vink (1970) reported that three sepal tips may be present. The calyptra encloses the bud during early development but ceases growth while the bud is still small. After the calyptra is ruptured by the expansion of the developing bud two or three lobes persist around the base of the flower. The pattern of initiation of the petals is somewhat variable, and
differences in size of the petals may indicate that they are initiated sequentially (Fig. 2F). However, the petals are often in two decussate pairs with further petals in a whorl inside these first four petals. Other patterns, such as spirals and whorls of three, are also occasionally seen.

**Tasmannia lanceolata** and *T. xerophila*

The plants of *Tasmannia* are dioecious, but the pattern of sepal and petal initiation is the same in both male and female flowers (although the number of organs can differ). In *Tasmannia lanceolata* two sepalis are initiated at the lateral poles of the meristem, followed by the initiation of two more sepals situated medially (adaxially and abaxially) (Fig. 3A, B). The abaxial sepal is at first only a line of tissue, but a flap develops from this region in later developmental stages. In *Tasmannia xerophila* and all other species of *Tasmannia* examined (Table 1) only medial sepalis are initiated, the adaxial sepal initiating before the abaxial sepal (Fig. 3D, E). The continued growth of the calyx in both species occurs via a ring of meristematic tissue that encircles the floral meristem, so that the calycine calyptra grows as a cylinder, bearing the sepal tips at its apex. The calyptra encloses the bud until anthesis, when it ruptures and abscises as the flower expands.

In all species of *Tasmannia* petals are initiated soon after the sepalis and alternate with them. In *Tasmannia lanceolata* four petals alternate with the four sepals (Fig. 3B), while in other species of *Tasmannia* two lateral petals initiate perpendicular to the two medially placed sepals (Fig. 3E). In all species further petalis may be initiated alternating with the previous whorls. (Note that in *Drimys piperita* [Tasmannia] s.l. [as defined by Vink, 1970] staminate and pistillate flowers of the same entity [species] may have different numbers of petals [P. F. Stevens, pers. comm.]). In staminate flowers in *Tasmannia lanceolata* and *T. xerophila* stamen initiation follows directly on from the initiation of the petals (Fig. 3C). The arrangement of the stamens may be variable, as the shape of the meristem is often asymmetric. A single sterile carpel is the last organ to be initiated. In pistillate flowers of *T. lanceolata* a single terminal carpel is initiated after the initiation of the petals, while in other species a number of lateral carpels are usually initiated (*T. insipida* only initiates one or occasionally two carpels). The position of the first four of these lateral carpels alternates with the positions of the medial sepals and lateral petals (Fig. 3F). When more than two petalis are initiated these alternate with the “whorl” of the medial sepals and lateral petals in the same positions as illustrated for the first four carpels (Fig. 1H).

**Drimys winteri**

The inflorescences of *Drimys winteri* are composed of a number of racemose unifloroses with a terminal and up to eight lateral flowers. In terminal flowers the calycine calyptra initiates as a ring of tissue around the approximately circular floral meristem (Fig. 4A), whereas in lateral flowers two sepal tips are initiated laterally with respect to the subtending floral bract (Fig. 4B). These sepal tips are subsequently borne aloft by the growth of a calycine ring of tissue around the floral meristem, so that the growth of the calyptra is similar in terminal and lateral flowers. The calyptra eventually encloses the developing flower, and at anthesis the calyptra splits into two or three segments and the petalis unfold. The reflexed calyptra segments eventually wither and abscise.

In both terminal and lateral flowers there is a considerable delay between initiation of the calycine calyptra and the corolla; petal primordia appear only when the calyptra has almost enclosed the floral meristem. At this stage the floral meristem is large and domed, and petalis initiate low down on the flank of the dome (Fig. 4C). The initiation of stamens follows the petalis without delay. A number of tiers of stamens are initiated on the almost vertical flanks of the floral meristem. The initiation of carpelis follows the stamens without delay and, as the growth of the floral meristem ceases, the carpels eventually develop all over the flattened apex of the dome (Fig. 4D). The arrangement of the pet-

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Figure 1. Generalized floral diagrams for each of the taxa examined. These diagrams give only the main outline of morphological patterns, as much variation can exist in the pattern of floral initiation for any species. In all diagrams the axis is toward the top of the diagram and the subtending floral bract is toward the bottom. Organs positioned laterally are toward the sides of the diagrams, and organs positioned medially are at the top and bottom of the diagrams. Many variations on these patterns can be found, especially in *Drimys, Pseudowintera*, and in the numbers of stamens and carpelis in all taxa. Where there are numerous whorls of stamenis only the first two or three whorls have been shown and the others have been indicated by broken circles. Drawings are not drawn to scale relative to each other.
Figure 2. —A. Zygogynum bailloni: a terminal and lateral bud, with the lateral bud (left) showing the lateral position of both the sepal tip (LS) and the first pair of outer petals (1). The second pair of outer petals, perpendicular in position to the first pair, is also visible (2). In the terminal bud the position of the first and second pair of outer petals relative to the sepal tips is not clear. Scale = 5 mm. —B. Z. bailloni: a young bud with calyptra and outer four petals removed. The invagination of the adaxial epidermis between the scars of each of the outer four petals (OP) can easily be seen. The two alternating whorls of inner petals (IP1, IP2) have just been initiated. Scale = 200 µm. —C. Z. bailloni: a longitudinal view of the basal region of the edge of an outer petal showing the smooth covering of epidermis (E) on the adaxial side (ADX) of the petal and the broken cells of the connate portion of the petal toward the abaxial side (ABX). Scale = 500 µm. —D. Z. bailloni: late bud stage with calyptra and petals removed, showing regular whorls of stamens and the somewhat irregular arrangement of the carpels as they begin to enlarge (outer petal scars = OP, scars of first whorl of inner petals = IP1, scars of second whorl of inner petals = IP2). Scale = 500 µm. —E. Bubbia houseiana: side view of a bud showing the lateral sepal tip (LS) and the first pair of outer petals (1) oriented perpendicular to the sepal tips. A small section of one of the second pair of outer petals (2) is also visible. Scale = 1.5 mm. —F. Pseudowintera colorata: a young bud with the calycine calyptra removed showing the arrangement of the five petals. Petals 1 and 2 (P1, P2) are opposite each other and petal 3 (P3) is alternate with these two. Petal 4 (P4) is almost opposite petal 3 but petal 5 (P5) appears to be either transitional to a spiral pattern or in the first position of the next whorl. Scale = 100 µm.
Table 2. Perianth pattern and petal connation in some species of Zygogynum, Exospermum, and Bubbia. These were observed from the fresh and herbarium specimens detailed in Table 1. Positions noted for the sepals and first pair of petals are relative to the subtending floral bract.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Position of sepals</th>
<th>Position of first pair of petals</th>
<th>Petal connation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zygogynum baillonii</td>
<td>lateral</td>
<td>lateral</td>
<td>connate</td>
</tr>
<tr>
<td>Zygogynum bicolor</td>
<td>lateral</td>
<td>lateral</td>
<td>connate</td>
</tr>
<tr>
<td>Zygogynum veillardi</td>
<td>lateral</td>
<td>lateral</td>
<td>connate</td>
</tr>
<tr>
<td>Zygogynum pumiferum</td>
<td>lateral</td>
<td>lateral</td>
<td>(mostly) free</td>
</tr>
<tr>
<td>Exospermum stipitatum</td>
<td>lateral</td>
<td>lateral</td>
<td>(mostly) connate</td>
</tr>
<tr>
<td>Bubbia hoveana</td>
<td>lateral</td>
<td>medial</td>
<td>free</td>
</tr>
<tr>
<td>Bubbia amplexicaulis</td>
<td>lateral</td>
<td>medial</td>
<td>free</td>
</tr>
<tr>
<td>Bubbia comptonii</td>
<td>lateral</td>
<td>medial</td>
<td>free</td>
</tr>
<tr>
<td>Bubbia crassifolia</td>
<td>lateral</td>
<td>medial (rarely lateral)</td>
<td>free</td>
</tr>
<tr>
<td>Bubbia pancheri</td>
<td>lateral</td>
<td>medial</td>
<td>free</td>
</tr>
</tbody>
</table>

als, stamens, and carpels may either be in more or less regular whors or spirals.

**TAKHTAJANIA PERRIERI**

The flower of *Takhtajania perrieri* has a two-lobed calycine calyptra with the lobes oriented laterally with respect to the subtending floral bract. Although no young buds at early stages were available it is likely that the calycine calyptra initially encloses the bud before rupturing as the bud increases in size. Fragments of the calycine calyptra are persistent around the floral base in flowering and fruiting stages. The initiation of this calyptra is followed by initiation of the outer petals in two decussate pairs. After the initiation of the outer two pairs of petals an inner whorl of four petals is initiated, the positions of the inner petals alternating with the positions of the four outer petals. This is followed by the initiation of a further tetramerous whorl of petals. The outer petal pairs are imbricate and fused to each other along their lateral overlapping edges (as shown by Endress et al., 2000), and the basal adaxial face of the outer petals is partially fused to the abaxial faces of the petals in the first inner whorl of petals (Fig. 4E, F). Occasionally more than four petals may occur in each of the inner two whors of petals; sometimes this is because the fifth petal is in the position of one of the stamens of the first androecial whorl. Usually two complete whors of four or five stamens are initiated, as well as one or two stamens of a third whorl before initiation of the gynoecium. The syncarpous gynoecium is terminal, and its morphology is fully described by Endress et al. (2000). The overall structure of the flower comprises three sets of opposite decussate whors followed by alternating tetramerous or pentamerous whors, and ending in the formation of the terminal gynoecium.

**DISCUSSION**

**SEPAL INITIATION**

In all species of Winteraceae the calyx forms a tubular calyptra, which completely encloses the bud for at least a short time during development. The presence of the calyptra appears to be a synapomorphy for Winteraceae, as neither of the likely outgroups of Canellaceae or Degeneriaceae (Suh et al., 1993; Nandi et al., 1998; Karol et al., 2000; Endress et al., 2000) possess such a tubular structure enclosing the bud.

In *Drimys winteri*, *Tasmannia lanceolata*, and *T. xerophila* the calyptra grows with and encloses the bud until anthesis, abscising as the flower opens. However, in *Zygogynum baillonii*, *Bubbia hoveana*, *Pseudowintera axillaris*, and *P. colorata* the calyptra encloses the bud only during early development and is ruptured as the bud enlarges. The remnants of the ruptured calyptra persist around the base of the flower and can still be seen in flowering and fruiting stages. Early stages of the flowers of *Takhtajania perrieri* were not available for study, but the mature flower shows remnants of the calyptra around the base of the flower similar to those found in *Zygogynum*, *Bubbia*, and *Pseudowintera*. This implies that in *T. perrieri* the calyptra encloses the bud only in early development. Other species of *Zygogynum*, *Exospermum*, *Bubbia*, and *Pseudowintera* also have a calyptra that encloses the bud only during early development (Sampson, 1963; Vink, 1970, 1977, 1983, 1988).

The first stage in the development of the calyptra in nearly all flowers in the family is the initiation of two or more sepals. The exception is in the terminal flower of *Drimys winteri* (and possibly other species of *Drimys* as well), where the calyx initiates as an annular primordium surrounding
Figure 3. —A. *Tasmannia lanceolata*: two lateral sepals (LS) have been initiated, and the adaxial sepal (ADS) can just be seen. The abaxial sepal at this stage is typically merely a line of tissue. Scale = 100 μm. —B. *T. lanceolata*: petals (P) are being initiated in positions alternate with the lateral (LS) and medial (adaxial = ADS, abaxial = ABS) pairs of sepals. Scale = 100 μm. —C. *T. lanceolata*: semi-mature bud with calyptra removed showing the initiation of four petals (P) and a number of whorls of stamens (S). A sterile carpel will be the last organ to be initiated. Scale = 200 μm. —D. *Tasmannia xerophila*: early sepal initiation with only the adaxial sepal (ADS) and the meristem (M) visible. The position where the abaxial sepal will initiate is marked in parentheses as (ABS). Scale = 100 μm. —E. *T. xerophila*: both adaxial (ADS) and abaxial (ABS) sepals have been initiated and two petals (P) have now been initiated in lateral positions, alternate with the sepals. Scale = 100 μm. —F. *T. xerophila*: a young bud with the sepals removed, showing four carpels (C) that have been initiated in positions alternate with the medial sepals and lateral petals (P). Note the tilted meristem (M). Scale = 100 μm.

the floral meristem. In the lateral flowers of *D. winteri* two sepal tips lateral to the subtending floral bract are commonly initiated before the bulk of the growth of the calyptra takes place via an annular meristem. The initiation of sepals lateral to the subtending floral bract is also seen in lateral flowers of *Zygogynum, Exospermum, Bubbia*, and *Pseudowintera*. A calyptra also forms in terminal
flowers in these genera, but here the orientation of the sepals is unclear. In *Tasmania lanceolata* lateral sepals are initiated as well as a further pair of medial sepals. In *T. xerophila* only two medial sepals are initiated. Inspection of semi-mature buds and flowers of *Takhtajania perrieri* indicates that two lateral sepals are initiated. Thus the pattern of initiation of the sepals has three states; two lateral sepals, two medial sepals, or two lateral sepals followed by two medial sepals. By itself,
the pattern of initiation allies Drimys with Takhtajanica, Zygogynum, Bubbia, and Pseudowintera, rather than with Tasmannia. The pattern shown by Tasmannia lanceolata (two lateral sepals and two medial sepals) is intermediate between the flowers of other Tasmannia species and those of the rest of the genera in the family.

The pattern of initiation of the sepals in Tasmannia and Drimys may help decide whether these two taxa should be considered as one genus or two (Smith, 1943a, b; Vink, 1988; Sampson et al., 1988). Smith (1969) segregated Tasmannia as a separate genus from Drimys because of differences in chromosome number (Tasmannia, n = 13, Drimys, n = 43; Ehrendorfer et al., 1968), and sexuality of the flowers (Drimys is bisexual, Tasmannia is unisexual and dioecious). The two genera also differ markedly in flavonoid composition (Williams & Harvey, 1982). However, Vink (1970, 1988, 1993) disagreed with the segregation of Tasmannia and recombined it with Drimys, citing the calyptra that protects the bud until anthesis, the monopodial construction of both Tasmannia and Drimys, and the observation that sporadic plants of Tasmannia have bisexual flowers. In the molecular phylogenies presented by Suh et al. (1993) and Karol et al. (2000) Tasmannia and Drimys do not appear as sister taxa, but as sequentially branching lineages on the tree. The differences in position of sepal initiation between the two genera supports the branching pattern of the molecular phylogenies, and is consistent with Tasmannia being a separate genus from Drimys. This implies that the possession of a calyptra that encloses the bud until anthesis may have evolved independently in these two genera.

FLORAL ORGAN ARRANGEMENT

Flowers in Winteraceae generally have organs arranged in pairs and whorls. In most cases the pattern in the flowers is determined by the position of the sepals, and, in general, succeeding pairs or whorls of organs alternate with the previous pair or whorl. This is the case in Bubbia and Takhtajanica, where a lateral pair of sepals is followed by medial and lateral pairs of petals (Table 2; Figs. 1B, D, 2E). A number of whorls of petals, stamens, and carpels are then initiated. A similar situation occurs in Zygogynum and Exospermum, except that the sepals and the first pair of outer petals are parallel with each other (they are both lateral with respect to the subtending floral bract) (Table 2; Figs. 1A, 2A). In the large flowers of Z. baillonii the two innermost tetramerous whorls of petals initiate almost simultaneously and act as one pseudo-whorl of eight petals (Fig. 2B). Succeeding whorls of eight stamens each alternate with the pseudo-whorl of eight petals (Figs. 1A, 2D). Irregularities in floral organ arrangement are often seen in the larger flowers of species of Zygogynum and Exospermum (pers. obs.; Endress, 1986). These have previously been explained as the result of the combination of asymmetry of the floral apex and the initiation of small primordia relative to the size of the floral apex (Endress, 1986). Another cause of irregular arrangements is that the changeover from petal to stamen and from stamen to carpel production can occur within one whorl. The different sizes of the primordia in a whorl can then perturb the pattern of primordial position.

The difference in sepal and petal initiation in Zygogynum and Exospermum (lateral sepals, lateral first pair of petals) versus that in Bubbia (lateral sepals, medial first pair of petals) was also noted by Vink (1977). This difference does not support the decision by Vink (1985) to combine Zygogynum s. str., Exospermum, and Bubbia into an expanded Zygogynum s.l. Vink made his decision after observing that the main characters used to separate the genera (degree of connation of the petals and carpels) were variable within each genus and transitional between genera (Vink, 1985). Such variability was observed in the present study for species of Zygogynum (Table 2) and Exospermum. However, the position of the sepals and first pair of petals indicates that the three genera fall into two groups; (Zygogynum + Exospermum) and (Bubbia). This suggests that it might be advantageous to use floral ontogeny to reexamine the homology of petal and carpel connation in the different genera.

A basic pattern of sets of decussate and whorled organs is also found in flowers of Tasmannia. There are two distinct variations in this basic pattern of floral organ arrangement within flowers of Tasmannia. In Tasmannia lanceolata a lateral pair of sepals is initiated followed by a medial pair. A tetramerous whorl of petal primordia is then initiated, with the positions of the four petals alternating with those of the four sepals. Further petals, stamens (male flowers), or a carpel (female flowers) are then initiated. In Tasmannia xerophila two sepal primordia are initiated medially, followed by two petal primordia initiated laterally. A whorl of four organs (which may be petals, stamens [male flowers], or carpels [female flowers]) are then initiated alternating with the “whorl” comprised of the pair of sepals and the first pair of petals. More organs can also be initiated. The similarity in floral organ arrangement between the flowers of these two species of Tasmannia is that in both, two sets of decussate
organs are followed by a tetramerous whorl of organs. The differences are in the position of the first pair of organs (lateral sepals in *T. lanceolata* vs. medial sepals in *T. xerophila*) and in the identity of the second pair of organs (medial sepals in *T. lanceolata* vs. lateral petals in *T. xerophila*). All other species of *Tasmannia* examined conform to the pattern shown by *T. xerophila*.

The initiation of the perianth in *Pseudowintera* is consistent with the general pattern described above. There is usually at least one pair of decussate organs, the laterally positioned sepals, but there may also be one or two decussate pairs of petals alternate with the sepal tips. Vink (1970) and F. B. Sampson (pers. comm.) reported that three sepal tips may also occur, along with trimerous whorls of petals and stamens, as illustrated by Vink (1970, fig. 3e). A transition to spiral arrangements of the stamens and carpels starting with the innermost petals also has been observed.

The floral arrangement in *Drimys winteri* is unlike that in the other genera because there appears to be no relationship between position of the lateral sepals or calyx and the succeeding petals. The lack of relationship in lateral flowers may be because the delay between initiation of the two organ types may deprive the petals of positional information from the sepals. There is, of course, no positional information given by the calyx in terminal flowers, as the calyx is initiated as an annular primordium encircling the floral meristem. There is also a variety of whorled and spiral floral organ arrangements in *D. winteri*, implying that petal, stamen, and carpel arrangement is not constrained by sepal position. Irregularities in the position of the primordia have been correlated with asymmetry of the floral meristem (unpublished results; Erbar & Leins, 1983).

**AFFINITIES OF TAKHTAJANIA**

*Takhtajania perrieri* was originally described as a species of *Bubbia* by Capuron (1963), and its floral architecture is similar to that of *Bubbia howeana*. Both species share a decussate pattern of the sepals and first two pairs of petals, followed by a whorl or whorls of petals and several whorls of stamens in fours or fives. However, the basic pattern of decussate pairs of organs followed by whorls of organs has been shown to be common throughout the family, with exceptions being found only in the relationship between the sepals and the first pair of petals in *Zygogynum* (parallel to each other), the terminal flowers of *Drimys winteri* (no decussate organs), the lateral flowers of *D. winteri* (opposite sepals followed by various arrangements of petals, stamens, and carpels), and some flowers of *Pseudowintera* spp. (opposite petals alternating with the sepals but further petals either in whorls or spirals).

A basic pattern in the family of four petals alternating with the sepal tips was also postulated by Vink (1988). Thus the similar patterns of floral organ arrangement in *Takhtajania perrieri* and *Bubbia howeana* show the general relationship in the family rather than being evidence of a special shared relationship.

Both *Zygogynum bailloni* and *Takhtajania perrieri* have fused outer petals. Such fusion does not generally occur in the corolla of the flowers of the other genera in the family and may provide evidence of a shared relationship. The method of fusion of outer petals in *Z. bailloni* is both through connation in the basal regions as well as variable amounts of post-genital fusion in the otherwise free apical regions. In the species of *Zygogynum* that display basally connate petals the mode of connation may be the result of coalescence of the margins of the petal primordia as they grow, whereupon the epidermis in the region of connation disappears. However, in *T. perrieri* the petals cohere only through post-genital fusion of the cuticles, the fusion occurring by the interdigitation of the cuticles in the overlapping petal areas (as documented by Endress et al., 2000). Interdigitation of the cuticles was also observed to occur between the bases of the outer petals and the basal portions of the next inner whorl of petals. Post-genital fusion as it occurs in *T. perrieri* may be likened to a cohesion of the petals rather than a true connation as found in the basal portion of the outer petals of *Zygogynum*. This makes it possible that the morphological similarity between the fused outer petals of the two species is not homologous.

**CONCLUSIONS**

The study of the ontogeny of the flowers of Winteraceae reveals both unifying patterns and unexpected complexity. The underlying pattern of floral organ arrangement is one of sets of decussate and whorled organs, occasionally turning to spirals in *Drimys* and *Pseudowintera*. Differences within the basic pattern include the number and position of sepals and the position of the petals relative to the sepals. In all species irregularities in floral apex symmetry and in the size of organs in a whorl may perturb the regular whorled or spiral pattern, leading to flowers with more irregular floral organ arrangements. There is a clear morphological division in the family between those taxa that retain the
calycine calyptra until anthesis (Tasmania and Drimys) vs. those where the calyptra is ruptured early in development (Bubbia, Zygozynum, Exospermum, Pseudowintera, and Takhtajania). However, differences in the position and number of sepalas between Tasmania lanceolata, T. xerophila, and Drimys winteri suggest that the two genera should not be combined into one. Differences in the position of the first pair of petals relative to the sepalas suggest that Zygozynum and Exospermum are distinct from Bubbia. The decussate and whorled arrangement of the floral organs in Takhtajania perrieri is closest to that of species of Bubbia, but this pattern is common throughout the family and does not serve as evidence of a special relationship between the two taxa. The manner of fusion of the outer petals in T. perrieri is strictly post-genital and by interdigitation of the cuticles, whereas that in Z. baillonii is primarily by connotation of the basal region of the petals. These differences do not support a close relationship between Takhtajania and Zygozynum. These observations are congruent with the molecular phylogeny of Karol et al. (2000), where Takhtajania is basal, and its unique syncarpellate gynoeicum autapomorphic.

The ontogenetic data presented in this paper are a step toward understanding the evolution of floral form in Winteraceae. On the basis of robust interpretations of phylogenetic relationships within the family (other papers, this issue), an understanding of the evolution of diverse and variable floral forms within such a well-defined monophyletic group will also advance our understanding of the evolution of the flower in basal angiosperms.

Literature Cited


