Reproductive morphology of *Sargentodoxa cuneata* (Lardizabalaceae) and its systematic implications.

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Abstract:

The reproductive morphology of Sargentodoxa cuneata (Oliv) Rehd. et Wils. is investigated through field, herbarium, and laboratory observations. Sargentodoxa may be either dioecious or monoecious. The functionally unisexual flowers are morphologically bisexual, at least developmentally. The anther is tetrasporangiate, and its wall, of which the development follows the basic type, is composed of an epidermis, endothecium, two middle layers, and a tapetum. The tapetum is of the glandular type. Microspore cytokinesis is simultaneous, and the microspore tetrads are tetrahedral. Pollen grains are two-celled when shed. The mature ovule is crassinucellate and bitegmic, and the micropyle is formed only by the inner integument. Megasporocytes undergo meiosis resulting in the formation of four megaspores in a linear tetrad. The functional megaspore develops into an eight-nucleate embryo sac after three rounds of mitosis. The mature embryo sac consists of an egg apparatus (an egg and two synergids), a central cell, and three antipodal cells. The pattern of the embryo sac development follows a monosporic Polygonum type. Comparisons with allied groups show that Sargentodoxa shares more synapomorphies with the Lardizabalaceae than other Ranunculales. Characteristics of its reproductive morphology are consistent with the placement of Sargentodoxa as the sister group of the remaining Lardizabalaceae. It does not possess a sufficient number of apomorphic characters to justify its separation into a separate family or subfamily. It is best retained as a member of the Lardizabalaceae.

Keywords: sargentodoxa cuneata | lardizabalaceae | reproductive morphology | anther | ovule | sargentodoxaceae | biology | plant systemics | plant evolution | botany

Article:

Introduction

Sargentodoxa, a monotypic genus of the Lardizabalaceae (Rehder and Wilson 1913; Chen and Tatemi 2001; Soltis et al 2000; APG 2003) has often been placed in its own family, Sargentodoxaceae (Hutchinson 1973; Cronquist 1981; Dahlgren 1989; Thorne 1992). It consists of the single species, Sargentodoxa cuneata (Oliver) Rehder and E. H. Wilson. Although Qu and Min (1986) described a second species, Sargentodoxa simplicifolia S. Z. Qu et C. L. Min, based on the possession of a simple leaf and the occurrence of both male and bisexual flowers on the same individual, it has been suggested that this species is not distinct from S. cuneata (Shi et al. 1994). S. cuneata occurs on dankish and saprophytic soils of thickets (Stapf 1926) and is restricted to central and southwestern China, extending into northern Laos and Vietnam (Chen and Tatemi 2001). Paleobotanical evidence places it in North America in the Tertiary (Tiffney 1993).

The taxonomic placement of Sargentodoxa has been unstable. Stapf (1926) placed Sargentodoxa in its own family, the Sargentodoxaceae, based on its possession of female flowers with numerous carpels borne on enlarged, ovoid receptacles, and the occurrence of a single ovule in each carpel. Other members of the family have three (to nine) carpels borne on smaller receptacles and have numerous ovules (Chen and Tatemi 2001). Nowicke and Skvarla (1982) agreed with this placement, but presented palynological evidence that suggests that Sargentodoxa belongs in the Lardizabalaceae (see below). A phytochemical study of Sargentodoxa (Ying and Zhang 1994) failed to find the triterpenoidal sponins that occur in the other Lardizabalaceae and supports the placement of the genus in its own family. Based on a chromosome number of 22, which differs from that of other Lardizabalaceae, Shi et al. (1994) also supports the segregation of Sargentodoxa into its own family. Liu and Sheng (2003) and Sheng et al. (2005) describe the formation of the micro- and megaspores and the development of the male and female gametophytes in S. simplicifolia, and conclude that the Sargentodoxaceae should be accepted as a monotypic family distinct from Lardizabalaceae.

Other authors have placed Sargentodoxa in the Lardizabalaceae. Nowicke and Skvarla (1982) discovered three pollen characters that appear to be synapomorphies uniting Sargentodoxa with other members of this family. The prominent tectum, thin foot layer and columellae, and a two-unit endexine are found, within the Ranunculales, only in the Lardizabalaceae. Loconte et al. (1995) conducted a cladistic analysis of 109 morphological features from most genera of Ranunculales and conclude that Sargentodoxa and Boquila are sister groups within the Lardizabalaceae. Wu and Kubitzki (1993) and Chen and Tatemi (2001) both placed Sargentodoxa in the Lardizabalaceae in their taxonomic treatments of the family. Thorne (2000) maintained Sargentodoxa in a monotypic subfamily of Lardizabalaceae, and Stevens (2008) treats Sargentodoxa as a genus of Lardizabalaceae.

This study was undertaken to provide new data that bears on the taxonomic placement of Sargentodoxa. Floral development and gametogenesis have been suggested as important sources of information for uncovering the relationships among eudicot taxa (Bhojwani and Bhatnagar 1978; Johri et al. 1992). Studies of these sorts have been rare on Sargentodoxa (but see Liu and Sheng 2003; Sheng et al. 2005; Wang et al. 2007; Zhang and Ren 2008). The present study addresses both of these characters through a study of the reproductive morphology of S. cuneata.

Materials and methods

Floral buds and mature flowers were collected and measured in the Nanchuan district of Chongqing city, China (altitude 997 m, 29°08.205′N, 107°13.542′E). Three natural populations were sampled every 3 to 4 days from March to May 2007. A detailed phenological study of the species was also undertaken (Wang et al. 2007).

Floral development

Material for scanning electron microscopy (SEM) was fixed in FAA [50% ethanol, 5% (v/v) acetic acid and 3.7% (v/v) formaldehyde] for at least 24 h and dehydrated through a tertiary butyl alcohol series (Jensen*1962*). The male and female flowers were dissected and observed in 95% ethanol under a dissecting microscope (PXS-2040; Hangzhou, Hui'er equipment, China), transferred through an ethanol iso-amyl acetate series (95% ethanol, 100% ethanol, 75% ethanol + 25% iso-amyl acetate, 50% ethanol + 50% iso-amyl acetate, 25% ethanol + 75% iso-amyl acetate and 100% iso-amyl acetate, 10–20 min each), critical-point dried with CO_2 in an ORION critical-point dryer, mounted on stubs, and coated with gold palladium in an SPI Module (Structure Probe) sputter coater. The samples were observed and micrographs taken with an Hitachi S-800 scanning electron microscope at 30 kV.

Gametogenesis

Fixed male and female flower buds were dehydrated in an ethanol series (70, 85, 95 and 100% ethanol twice, 2 h each) and embedded in paraffin wax. Serial sections were cut at 6–9 μm, stained for 4 h in 4% Heidenhain's iron–alum, washed for 40 min with H_2O, stained for 4 h with 0.05% hematoxylin, washed again with H_2O (30 min), and mounted on slides in a gelatin solution (1 g gelatin, 100 ml H_2O, 2 g phenol, 15 ml glycerol; Li *1978*). Photographs were taken with an Olympus SP-565UZ digital camera mounted on an Olympus BH-2 photomicroscope equipped with Nomarski optics. The tonal qualities of the images were adjusted, labels were added, and plates assembled with Adobe Photoshop CS2 and CS3.

Apomorphy determination

Apomorphic states of the characters for Sargentodoxa and the Lardizabalaceae (Table 1) were determined based on Doyle and Endress' (2000) and Endress and Doyle's (2009) character analyses. Apomorphic states were determined by mapping the characters from Endress and Doyle's (2009) paper onto their "D & E tree, Recent" using Mesqite 2.6 (Maddison and Maddison 2009). Characters not treated by Endress and Doyle (2009) were not evaluated.

Table 1

Comparison of reproductive characters in Sargentodoxa and Lardizabalaceae

Character (character number in Endress and Doyle (*2009*))	***Sargentodoxa***	**Lardizabalaceae**
Tapetum type (char. 56)	Secretory	Secretory[f,g,h]
Cytokinesis	Simultaneous	Simultaneous[f,g]
Mature pollen grains	Two-celled	Two-celled[f,g]
Number of carpels (char. 74)	Numerous	Three, up to nine in *Akebia* spp.[d]
Carpel phyllotaxis	Irregular[a]	Whorled in *Akebia quinata* [i]
Carpel form (char. 75)	*Intermediate (both plicate and ascidiate zones)[a,b]	Plicate[b]
Closure of carpels (char. 76)	*Partial postgenital sealing	*Partial postgenital sealing, completely sealed in*Sinofranchetia* [d,j]
Carpel fusion (char. 84)	Apocarpous	Apocarpous[d]
Ovule curvature (char. 93)	Anatropous[c]	Anatropous in *Decaisnea* [k] and *Stauntonia hexaphyll*[l],*Campylotropous in *Akebia* spp. [m]
Ovules per carpel (char. 90)	*1	Numerous[d]
Arrangement of megaspore tetrads	Linear	Linear[g],T-shaped, and rarely linear in *Holboellia latifolia* [n], linear in *Stauntonia hexaphylla* [m]

Character (character number in Endress and Doyle (*2009*))	***Sargentodoxa***	**Lardizabalaceae**
Embryo sac type	*Polygonum*	*Polygonum* in *Stauntonia hexaphylla* [f] and *Decaisnea*[o]
Antipodals	Small, ephemeral	Small, ephemeral in *Akebia* spp.[m] and *Holboellia latifolia* [n], small and persistent in *Decaisnea* [o]
Fruit wall (char. 97)	Fleshy (an aggregate of many drupes)[d]	Dry or fleshy[b,p] (follicle or follicular berry)[d]
Sex of flowers (char. 26)	*Unisexual; developmentally bisexual	*Unisexual[q], both bisexual and male flowers occur in*Decaisnea* [q,r,s]
Perianth whorls (char. 34)	More than two	*One (rarely two) in *Akebia* [r,s], *two in *Decainea* and*Archakebia* [r,s], two (rarely > 2) in *Stauntonia* [r,s], > 2 in*Holboellia* and *Sinofranchetia* [r,s]
Tepal differentiation (char. 35)	*All petaloid[a,e]	*All petaloid[e]
Pollen sacs (char. 51)	Protruding	Protruding[t]
Orientation of anther dehiscence (char. 53)	Extrorse	Extrorse[t]

Apomorphic states are marked with an asterisk, unmarked states in characters described by Endress and Doyle (2009) are either plesiomorphic or their status is ambiguous; the status of other unmarked states was not evaluated

aZhang and Ren (2008)

bEndress and Doyle (2009) and references therein

cSheng et al. (2005)

dQin (1989)

eChen and Tatemi (2001), but petals sometimes small and nectariferous

[f]Johri et al. (1992)

[g]Sastri (1969, Table 1), based on observations in an unpublished Ph.D. dissertation (Sastri 1957)

[h]An amoeboid tapetud is reported in Stauntonia hexaphylla (Yoshida and Nakajima 1978), but with figures that appear to show a secretory tapetum

[i]Van Heel (1983)

[j]Endress (1995) reports carpels closed in Decaisnea and completely open in Akebia but without documentation. Qin (1989) figures partial postgenital sealing in Akebia

[k]Swamy (1953)

[l]Yoshida and Nakajima (1978)

[m]Johri et al. (1992) reports this, but provide no supporting evidence

[n]Bhatnagar (1965)

[o]Swamy (1953)

[p]Endress (1995)

[q]Doyle and Endress (2000)

[r]Qin (1997)

[s]Chen and Tatemi (2001)

[t]Endress and Doyle (2009)

Results

Inflorescence

Individual plants of S. cuneata are either dioecious or monoecious (Fig. 1–4). The inflorescence is a raceme (Fig. 1, 2) with female flowers borne above the male (Fig. 2). Each mature inflorescence bears 25 flowers (n = 30, SE = 3.4) arranged spirally (Fig. 2). The young flowers are also borne spirally on inflorescence axes (Fig. 5) and are likely initiated spirally. The young flower buds are globose and are subtended by obtuse bracts that gradually become ellipsoid before the sepals open.

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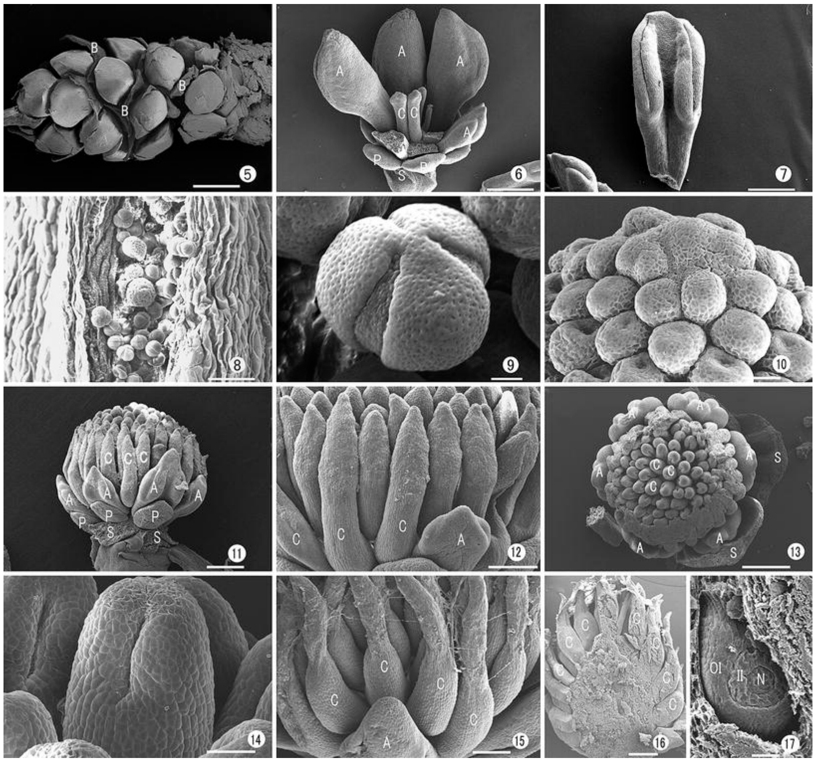


Figs. 5−17

SEMs of immature male and female flowers of Sargentodoxa cuneata. Fig. 5 Young inflorescence with spirally arranged bracts (B) and globose flower buds. Bar 1 mm. Fig. 6 Male flower with rudimentary carpels (C). A Stamen, P petal, S removed sepal. Bar 0.3 mm. Fig. 7 Anther showing protruding thecae, which open by longitudinal slits. Bar 0.3 mm. Fig. 8 Close up of anther in Fig. 7, showing dehiscence. Bar 0.3 μm. Fig. 9 Tricolporate, subspheroidal pollen grain with perforate exine. Bar 5 μm. Fig. 10 Developing carpels arranged on the receptacle. Bar 250 μm. Fig. 11 Lateral view of a female flower. A Rudimentary stamen, C carpel, P petal, S removed sepal. Bar 50 μm. Fig. 12 Cylindrical young carpels (C) with pointed apices. A Rudimentary stamen. Bar 0.5 mm. Fig. 13 Apical view of a female flower. Note the mucilage covering the carpels. A Rudimentary stamen, S sepal, C carpel. Bar 0.5 mm. Fig. 14 Conduplicate tip of a developing carpel. Bar 10 μm. Fig. 15 Intermediate carpels with both plicate and ascidiate zones (C), at the time of ovule maturation. A Rudimentary stamen. Bar 100 μm. Fig. 16 Longitudinal section of an ovoid receptacle bearing carpels (C). Bar 100 μm. Fig. 17 Ovule structure at the same stage as in Fig. 16, showing nucellus (N) and inner (II) and outer (IO) integuments. Bar 250 μm

Male flowers

The mature male flowers are 1.79 cm (n = 30, SE = 0.025) in diameter and actinomorphic (Fig. 3). The sepals have mean length and widths of 1.2 × 0.4 cm (length SE = 0.8, width SE = 0.1; n = 5), are usually trimerous, and are arranged in two imbricate series (Fig. 3). Six small petals have mean length and widths of 1.0 × 1.0 mm (length SE = 0.3, width SE = 0.2; n = 5) and occur inside the sepals. The flowers produce a slightly sweet odor when they are in full bloom. The androecium consists of six antepetalous stamens (Fig. 6, 7) with short filaments, protruding pollen sacs, and longitudinal dehiscence slits (Fig. 7, 8). Tricolporate pollen grains are shed when the anthers are mature (Fig. 8, 9). The mature pollen grains are subspheroidal and from 22.5 to 30.0 μm in diameter (mean = 25.1 μm, SE = 4.9; n = 30). Some male flowers bear two to four rudimentary carpels at the center of the flower (Fig. 6).

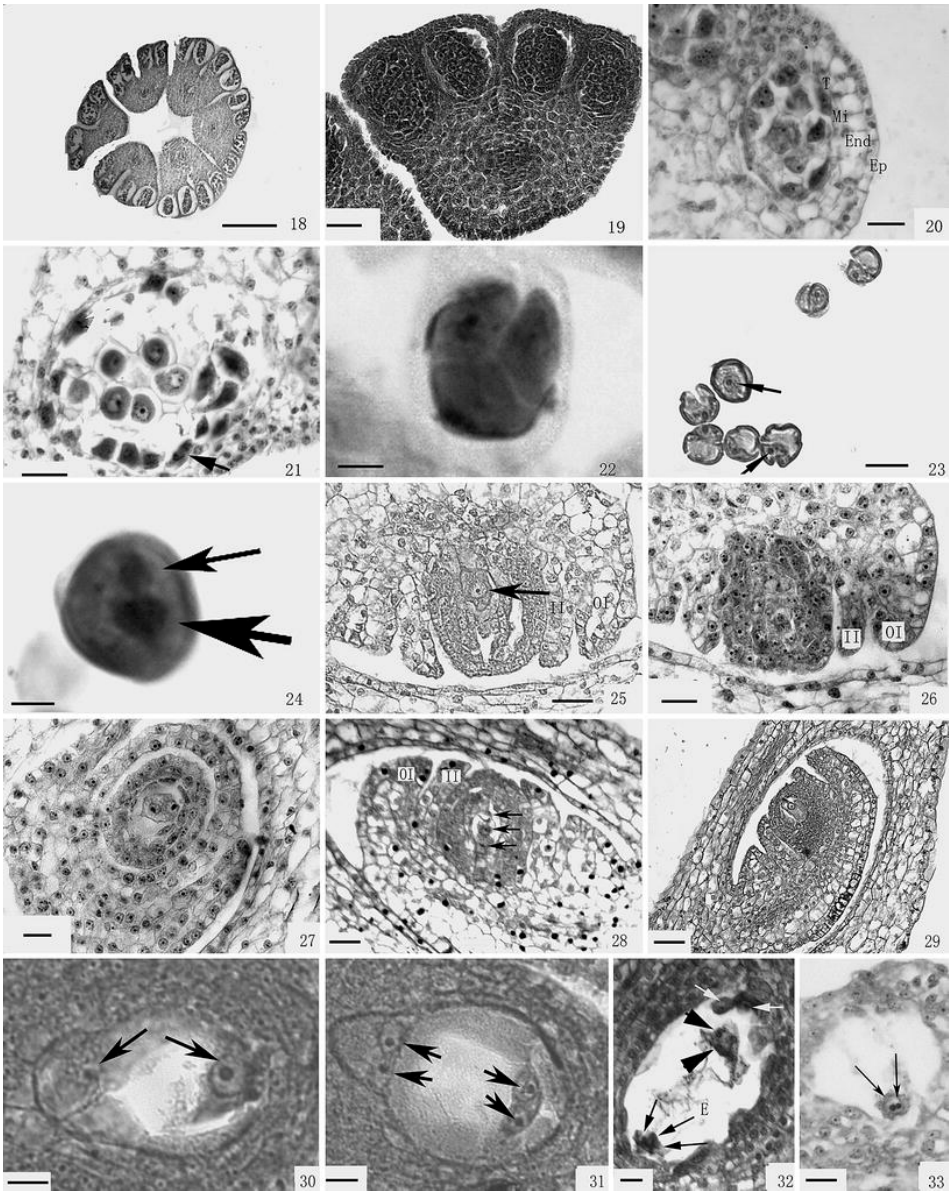
Female flowers

Compared to the number of male flowers, each inflorescence contains few female flowers. The sepals and petals of these flowers are similar to those of the male, but unlike the male, the petals remain erect at anthesis (Fig. 4). Six rudimentary anthers occur interior to petals (Fig. 11–13). The apices of the petals are nectariferous and can produce a slightly sweet odor at anthesis. The anthers are smaller than those in the male flowers and produce fewer pollen grains. Numerous apocarpous carpels are borne on an axiolitic receptacle at the center of the flower (Figs. 10, 11, 13, 16). The number of carpels ranges from 61 to 123 (mean = 83, SE = 45.2; n = 30).

Soon after initiation, the carpels become conduplicate through the formation of a groove on their adaxial surface (Fig. 10). As they develop, the carpels become tubular and develop pointed apices, which will become the stigma and style (Fig. 11–15). A layer of mucilage occurs on the surface of the gynoecium at this stage (Fig. 13). A slit in the apex marks the entrance to the style (Fig. 14). The carpels are intermediate in structure between ascidiate and conduplicate carpels at the stage of ovule maturation (Fig. 15–17), when the layer of mucilage disappears. Intermediate carpels have both ascidiate and conduplicate zones.

Microspores and male gametophyte

The anthers are tetrasporangiate with abaxially borne locules (Fig. 18, 19). The archesporium is hypodermic and undergoes a periclinal division resulting in a primary parietal layer and a primary sporogenous layer. The parietal layer divides periclinally to form two layers. The inner functions as the tapetum, while the outer undergoes another periclinal division to produce an outer endothecium and a middle layer (Fig. 20). When mature, the anther wall consists of five layers: a single-layered epidermis, a single-layered endothecium, two middle layers, and a single-layered tapetum (Fig. 20). The endothecium develops fibrous thickenings prior to anther anthesis. At the tetrahedral pollen stage, the tapetal cells elongate radially and protrude into the anther loci. Some become binucleate (Fig. 21). Following microsporogenesis (Fig. 21), the tapetal cells degenerate at their original sites, indicating that the tapetum is of the glandular type.



Figs. 18−33

Light micrographs of anther and ovule development. Figs. 18–24 Cross-sections of anther and pollen development. Fig. 18 The androecium of a male flower with six anthers. Bar 0.3 mm. Fig. 19 A young anther with four ipsilateral microsporangia. Bar 60 μm. Fig. 20 Locule with sporocytes at meiosis I. The anther wall consists of a single-layered epidermis (Ep), a single-layered endothecium (End), two middle layers (Mi), and a single-layered tapetum (T). Bar 20 μm. Fig. 21 Locule at prophase I of meiosis. At least some cells of the tapetum are binucleate (arrow). Bar 20 μm. Fig. 22 A tetrahedral tetrad formed from simultaneous microsporogenesis.

Bar 20 µm. Fig. 23 Pollen grains at the single-nucleate stage when the vacuoles enlarge and displace the nuclei to the peripheries of the cells (arrows). Bar 30 µm. Fig. 24 Two-celled pollen grains with a larger vegetative cell (large arrow) and a smaller generative cell (small arrow). Bar 10 µm. Figs. 25–33 Ovule development. Fig. 25 Longitudinal section of a carpel with a hemitropous ovule and a megasporocyte (arrow). II Inner integuments, OI outer integuments. Bar 10 µm. Fig. 26 Longitudinal section showing an earlier stage of integument formation. II Inner integuments, OI outer integuments. Bar 10 µm. Fig. 27 Oblique section of an ovule with a megasporocyte following meiosis I. Bar 10 µm. Fig. 28 Linear tetrad of megaspores (arrows). Bar 100 µm. Fig. 29 Early formation of the embryo sac, before meiosis. Fig. 30 Two-nucleate (arrows) embryo sac. Bar 50 µm. Fig. 31 Four-nucleate (arrows) embryo sac. Bar 50 µm. Fig. 32 Mature embryo sac with an egg cell (E), two synergids (black arrows), a central cell with two nuclei (black arrowheads), and two antipodal cells (white arrows). Bar 10 µm. Fig. 33 Central cell with two nuclei (arrows). Bar 50 µm

Each microsporangium contains numerous sporogenous cells, which enlarge and differentiate into microspore mother cells. The microsporocytes originate from the primary sporogenous layer and become enclosed in a thick callose wall at meiosis I. Meiosis is followed by simultaneous cytokinesis to produce tetrahedral microspore tetrads (Fig. 22). The tetrads enlarge and acquire thick walls. With the breakdown of the callose walls, the microspores are released from the tetrad. They enlarge and acquire thick walls, after which vacuole formation displaces the nucleus toward the cell wall (Fig. 23). The microspore divides asymmetrically to form a large vegetative cell and a small generative cell (Fig. 24). The generative cell moves into the cytoplasm of the vegetative cell. Pollen grains are shed at this two-celled stage. At shedding, the grains are packed with granular contents. The exine pattern is perforate (Fig. 9).

Macrosporgenesis and megagametophyte development

The unilocular carpel contains a single ovule borne on a marginal placenta (Figs. 17, 25–29). The ovule is surrounded by inner and outer integuments (Figs. 17, 25–29), which are well formed by the megasporocyte stage (Fig. 25). The micropyle is formed only by the inner integument. The archesporium is hypodermal and cuts off a primary parietal cell and a sporogenous cell. The sporogenous cell undergoes repeated divisions to form a massive nucellus, resulting in the megasporocyte being deeply seated within the ovule (Fig. 26). The megasporocyte undergoes meiosis, resulting first in a dyad (Fig. 27), and following the second meiotic division, a linear tetrad (Fig. 28). There is a single functional megaspore. The three other microspores degenerate and are crushed at the micropylar end of the ovule. The functional megaspore lies adjacent to the chalaza and divides to form the embryo sac (Fig. 29–33). The first meiotic division produces two haploid nuclei, which move to opposite poles of the embryo sac (Fig. 30). Meiosis II results in a four-nucleate embryo sac (Fig. 31). Each nucleus now undergoes a mitotic division resulting in the formation of a mature, eight-nucleate embryo sac (Fig. 32). One nucleus from the micropylar end and one from the chalazal end move to the center of embryo sac to form polar nuclei. The mature embryo sac is of the Polygonum-type and contains two synergids, an egg, a central cell with two polar nuclei (Fig. 33), and three antipodals at the chalazal end. The two synergids degenerate before the arrival of the pollen tube. The antipodals are small and ephemeral.

Discussion

Habitat and growth characteristics

Sargentodoxa cuneata is a typical sun plant (Bao et al. 2003). It prefers to grow on forested land where there is sufficient sun, in well-watered, acidic soils (Wang et al. 2007). Its vining habit allows it to climb to the top of tall trees where the insolation is higher. It grows better and blossoms earlier in higher altitudes than lower. Even in the same individual, the branches grow faster and blossom earlier in sunny places than in shade.

Floral development

Sargentodoxa cuneata may be either monoecious or dioecious (Zhang and Ren 2008). Although its flowers are functionally unisexual, they are usually morphologically bisexual. In the flowers observed in this study, both male and female organs are initiated during early floral development. At maturity, rudimentary carpels persist in some mature male flowers, and rudimentary stamens occur in all female flowers. Thus, all of the female, and some of the male flowers observed here are morphologically bisexual. The anthers of the female flowers are indehiscent, and the pollen grains are abortive (Shi et al. 1994). The carpels of the male flowers are fewer and smaller than those of female flowers and lack ovules. The morphologically bisexual flowers are thus functionally unisexual. This is also the case in the genera of Lardizabalaceae that have been studied (Wang and Li 2002).

Zhang and Ren (2008) report the presence of bisexual flowers on otherwise monoecious plants. They find bisexual flowers occurring between the unisexual male and unisexual female flowers in at least some inflorescences. It is unclear from their report if these flowers bear both functional pollen and ovules. Flowers that are morphologically intermediate between male and female flowers occur in the monoecious inflorescences of bananas (Musaceae; Kirchoff personal observation) but do not function as bisexual flowers.

The perianth of S. cuneata is differentiated into sepals and petals, as is the perianth of Holboellia, Parvatia, and Sinofranchatia (Chen and Tatemi 2001), while the perianth is not differentiated in the genera Decaisnea, Stautonia, Akebia, and Archakebia (Chen and Tatemi 2001). Zhang and

Ren (2008) found that the number of perianth members varies in the female flowers of Sargentodoxa, but not in the male. They found four to nine sepals and five to seven petals in the female flowers.

Zhang and Ren (2008) also studied floral development and found minor developmental differences among male, female, and morphologically bisexual flowers. The reader is referred to their paper for a detailed account of these differences. They also clarified the sequence of carpel initiation in female flowers, which was found to be irregular (Zhang and Ren 2008). In their treatment of the mature female flowers, they describe the sterile, rudimentary stamens as "petaloid staminodes" (Zhang and Ren 2008). While these stamens do resemble the petals of Sargentodoxa, the use of the term "petaloid" suggests that they resemble more or less normal petals of a more typical flower and is probably best avoided for these staminodes (Kirchoff 2001; Kirchoff et al. 2009).

Anther development in comparison with the Lardizabalaceae

On the basis of the formation of the middle layers, Davis (1966) classified the development of anther walls into four types: basic, dicotyledonous, monocotyledonous, and reduced. The development of the anther wall in Sargentodoxa is of the basic type. The tapetum is secretory in Sargentodoxa, as in Decaisnea (Swamy 1953), and as it is reported to be in Holboellia latifolia (Bhatnagar 1965). At least some tapetal cells contain two nuclei in Sargentodoxa as in Decaisnea (Swamy 1953), while they are reported to contain two to four nuclei in H. latifolia (Bhatnagar 1965). The microspore mother cells form simultaneously, resulting in tetrahedral tetrads in Sargentodoxa, while the tetrads are reported to be either tetrahedral or decussate in H. latifolia (Bhatnagar 1965). The mature pollen grains are tricolpate and two-celled at the time of shedding, as in H. latifolia (Bhatnagar 1965). In this study, we found linear tetrads of megaspores in Sargentodoxa, although Sheng et al. (2005) reported T-shaped tetrads in this genus. Bhatnagar (1965) found the tetrads of megaspores are mostly T-shaped, though rarely linear, in H. latifolia (Table 1).

Ovule development and carpel form in comparison with the Lardizabalaceae

The mature ovules are anatropous or hemitropous (Akebia, Boquila, Lardizabala) in the Lardizabalacae (Endress and Igersheim 1999). Bhatnagar (1965) reported orthothropous ovules in H. latifolia, but the ovule he pictures is too young for this determination to be made. The development of the embryo sac of Sargentodoxa conforms to the Polygonum type as in most Lardizabalaceae that have been studied (Bhatnagar 1965; Yoshida and Nakajima 1978). The antipodals are small and ephemeral in Sargentodoxa as in H. latifolia (Bhatnagar 1965). The antipodals are persistent in Decaisnea (Swamy 1953).

Several reproductive features of Sargentodoxa differ from those found in Lardizabalaceae (Table 1). These include number of carpels, carpel phyllotaxis, carpel form, and number of ovules per pistil. According to the definitions of Endress and Doyle (2009), Sargentodoxa has carpels that are intermediate in form between plicate and ascidiate (Zhang and Ren 2008—although they term these carpels ascidiate). In intermediate carpels "the stigma is plicate but some or all of the ovary is ascidiate and all ovules are attached to the ascidiate zone" (Endress and Doyle 2009). This is the first use of the term intermediate carpels in the Lardizabalaceae, which have previously been reported to have plicate (Qin 1989) or ascidiate carpels (Zhang and Ren 2008). The possession of intermediate carpels may be an autapomorphy of Sargentodoxa.

Unlike the condition reported by Qin (1989), but in agreement with Zhang and Ren (2008), the carpels are incompletely closed. The same condition is found in the Lardizabalaceae. The numerous carpels are irregularly arranged (Zhang and Ren 2008) on an axiolitic receptacle, while they are arranged in whorls in Decaisnea (Zhang and Ren 2008 claim this based on unpublished data), Akebia quinata (Van Heel 1983). Pictures by Zhang et al. (2005) of the mature flowers also appear to show whorls of carpels in Sinofranchetia. Only one ovule is contained per carpel in Sargentodoxa, while numerous ovules occur in the carpels of most Lardizabalaceae (Qin 1989). The possession of a single ovule is likely an autapomorphy of Sargentodoxa.

Carpel phyllotaxis and number of ovules per pistil are distinctive features that are different in Sargentodoxa from other Lardizabalaceae. However, floral phyllotaxis and number of floral organs are very flexible in the basal angiosperms (Endress and Doyle 2007), which downplays the importance of these differences.

Comparison of reproductive morphology with related families

We selected families for comparison with Sargentodoxa based on phylogenetic analyses of the basal angiosperms by Doyle and Endress (2000) and Endress and Doyle (2009), and phylogenies of the Ranunculidae and Lardizabalaceae by Hoot et al. (1995a, b).

The anther is tetrasporangiate with a secretory tapetum in Sargentodoxa, as in Ranunculaceae, Berberidaceae, and Menispermaceae (Johri et al. 1992). The number of middle layers is two in Sargentodoxa, while it is two to three in Ranunculaceae and Berberidaceae (Johri et al. 1992). Cytokinesis is simultaneous as in Ranunculaceae and Menispermaceae (Johri et al. 1992), while it is successive in Berberidaceae (Sastri 1969), as in Circaeasteraceae (Mu 1983; Ren et al. 2003). The number of ovules per carpel is only one, whereas there are numerous ovules in Ranunculaceae, the ovules are few to numerous in Berberidaceae, and there is only one functional and one degenerate ovule in Menispermaceae (Endress and Igersheim 1999; Sastri 1969). The ovule is anatropous or hemitropous in Sargentodoxa, while it is anatropous in Circaeasteraceae, Ranunculaceae, and Berberidaceae (Endress and Igersheim 1999).

The integument is bitegumic in both Sargentodoxa and in Berberidaceae, while both bitegumic and unitegmic ovules are found in Ranunculaceae and Menispermaceae (Endress and Igersheim 1999). The ovule is unitegmic in Circaeasteraceae (Endress and Igersheim 1999). The ovule is crassinucellate in Sargentodoxa as in Berberidaceae and Menispermaceae, while it is "almost tenuinucellar" in Circaeasteraceae, and crassinucellate or tenuinucellar in Ranunculaceae (Endress and Igersheim 1999). All of the embryo sacs in these families are of the Polygonum type.

Phylogenetic placement and taxonomy of Sargentodoxa

Literature review and reconstruction of apomorphic states demonstrates that Lardizabalaceae and Sargentodoxa share a number of derived features: twining habit; unisexual flowers; four, often trimerous, perianth whorls with a petaloid outer series (Endress and Doyle 2009); petals, if present, often apically nectariferous; carpel closure of a type with a complete secretory canal and partly postgenitally fused periphery; and fleshy fruit walls. Nowicke and Skvarla (1982) also reported three pollen synapomorphies uniting these taxa: a prominent tectum, thin foot layer and columellae, and a two-unit endexine.

Hoot et al.'s (1995a, b) analyses support the recognition of a clade of core Lardizabalaceae comprising Akebia, Stauntonia, Holboellia, Lardizabala, and Boquila. Sargentodoxa is placed as the sister group to the whole family (Hoot et al. 1995b, 1999; Loconte et al. 1995). This placement is consistent with previous results based on traditional data and classification schemes (Loconte and Estes 1989; Qin 1989). Our results are also consistent with this placement. Although Sargentodoxa is more closely related to Lardizabalaceae than other Ranunculales, its unique morphological characters (e.g., intermediate carpel structure, irregular carpel phyllotaxis, one ovule per carpel) make it distinct. It could be treated as a "satellite family" (Nowicke and Skvarla 1982) of the Lardizabalaceae, although doing so would obscure its close relationship with this family. Placing it in its own subfamily, Sargentodoinae, as suggested by Thorne (2000) would only be justified if Sargentodoxa possessed a large number of apomorphic character states. Our failure to find more than three apomorphic states suggests that Sargentodoxa is best retained as a member of the Lardizabalaceae.

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