Floral Developmental Morphology of *Persea americana* (Avocado, Lauraceae): The Oddities of Male Organ Identity

Author(s): Matyas Buzgo, André S. Chanderbali, Sangtae Kim, Zhengui Zheng, David G. Oppenheimer, Pamela S. Soltis, and Douglas E. Soltis

Reviewed work(s):
Source: *International Journal of Plant Sciences*, Vol. 168, No. 3 (March/April 2007), pp. 261-284
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/510297
Accessed: 09/02/2013 21:46

Int. J. Plant Sci. 168(3):261–284. 2007.

1058-5893/2007/16803-0002$15.00

FLORAL DEVELOPMENTAL MORPHOLOGY OF *PERSEA AMERICANA* (AVOCADO, LAURACEAE): THE ODDITIES OF MALE ORGAN IDENTITY

Matyas Buzgo,[1,]* André S. Chanderbali,*,† Sangtae Kim,* Zhengui Zheng,* David G. Oppenheimer,*,‡ Pamela S. Soltis,† and Douglas E. Soltis*

*Department of Botany, University of Florida, Gainesville, Florida 32611, U.S.A.; †Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611, U.S.A.; and ‡University of Florida Genetics Institute, and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, Florida 32610-3610, U.S.A.

A floral developmental series was determined for *Persea americana* (Lauraceae, avocado), and the floral morphology of this species was compared with available data for other members of *Persea*. We compared the structure of the inflorescence and flower with that of vegetative shoots with respect to phyllotaxy and leaf shape. The inflorescence is a determinate thyrse (panicle) with variable numbers of lateral branches. Staminal glands in *Persea* may represent abaxial-marginal emergences rather than stamens. However, these glands are occasionally involved in transitions to pollen sacs and ovary margins. Stigmas, pollen sacs, staminal appendages, glands of staminodes, and margins of tepals share features that are subjectively associated with "androecia." In the innermost androecial whorl, staminodial glands appear united because of the reduction of the middle portion to a staminodial apex. The apex of staminodes is homologous to the filament and anther, as well as to the stigma of the carpel, and corresponds to the connective tip in other basal angiosperms. In *Persea*, the connective and the staminode apex also correspond to the body of the tepal (i.e., all but the margin). Above a constriction (stipe), the carpel forms a cross zone bearing the single ovule; this cross zone also corresponds to the thecae in stamens, similar to observations for other basal angiosperms.

Keywords: evolutionary development, homology, nectaries, sporophyll, staminal gland, staminode.

Online enhancement: appendix.

Introduction

Overview of Laurales

Laurales are part of the large magnoliid clade of basal angiosperms and comprise seven families, with Calycanthaceae sister to two clades: (1) Hernandiaceae, Monimiaceae, and Lauraceae and (2) Siparunaceae, Gomortegaceae, and Atherospermataceae, with Lauraceae harboring most of the extant species diversity and geographic distribution (Renner 1999, 2004; Renner and Chanderbali 2000; Chanderbali et al. 2001; Angiosperm Phylogeny Group II 2003). The lineage has a long fossil record, extending back to the mid-Cretaceous (Friis et al. 1994, 2000; Herendeen et al. 1994; Boyd 1998; Eklund and Kvaček 1998; Eklund 2000; Takahashi et al. 2001; Kvaček and Eklund 2003; Frumin et al. 2004).

Laurales afford the opportunity to examine many of the more conspicuous trends in floral evolution in the context of a closely related group of basal angiosperms. For example, floral phyllotaxy ranges from spiral (most families) to whorled in Lauraceae and Hernandiaceae, merosity is highly variable, predominantly trimerous only in the Lauraceae, fusion among floral parts occurs sporadically (e.g., Siparuniaceae), and both reductions and increases in the number of floral organs are evident in several lineages (Renner 1999; Doyle and Endress 2000; Renner and Chanderbali 2000; Ronse de Craene et al. 2003). Importantly, trimery also occurs in monocots, as well as in many basal angiosperm lineages, including Nymphaeales, Magnoliaceae, and Piperales (Doyle and Endress 2000), and is the ancestral character state for the large magnoliid clade (Soltis et al. 2005).

Phylogenetic Placement of Persea *within Lauraceae*

Phylogenetic relationships within Lauraceae based on plastid and nuclear sequence data differ from all published classification schemes (Chanderbali et al. 2001). The disagreement between the molecular phylogeny and morphologically based classifications may reflect frequent morphological homoplasy (Chanderbali et al. 2001), which may either result from similar selective pressures leading to similar evolutionary solutions or "point to inherent developmental constraints" (Brooks 1996, p. 9). Of the ca. 55 genera of Lauraceae, *Persea*, which is distributed in both the Old and New Worlds (Raven and Axelrod 1974; reviewed by Chanderbali et al. [2001]), is by far the best studied. *Persea* is derived within Lauraceae and may be paraphyletic, interspersed with *Apollonias*, *Phoebe*, and *Dehaasia* (Chanderbali et al. 2001). *Persea americana*, as an important crop plant (Gomez-Lim and Litz 2004), has received much attention with respect to floral morphology and development (e.g., Reece 1939, 1942; Kasapligil 1951; Endress 1972*b*; Rohwer 1993, 1994; Thorp et al. 1993; Endress and Igersheim 1997; Salazar-Garcia et al. 1998, 1999; van

[1] Author for correspondence; e-mail mbuzgo@ufl.edu.

Manuscript received July 2006; revised manuscript received September 2006.

der Werff 2002) and is one of three exemplar magnoliids of the Floral Genome Project (Albert et al. 2005).

Floral Morphology of Lauraceae

Persea americana exhibits the reproductive features typical of Lauraceae (Endress 1972*a*; Endress and Hufford 1989; Endress and Igersheim 1997; Rohwer 1993). In Lauraceae, inflorescences typically are panicles or determinate thyrses (fig. 1*C*; Weberling [1985] uses the term "thyrsoids"; also see Rohwer 1993). Flowers are small (less than 1 cm in diameter) and mostly bisexual and typically consist of seven trimerous whorls of floral organs: two perianth whorls, four androecial whorls, and one single carpel (see "Organography"; fig. 3*A*). Typically, the two perianth whorls are similar. In some species of *Persea*, the perianth is subtly differentiated into what could be referred to as sepals and petals (Endress 1972*b*; Chanderbali et al. 2006; this study; also in *Umbellularia*; Kasapligil 1951). The stamens of the third whorl bear a pair of appendages at the base (a feature also seen in some Monimiaceae; Endress and Hufford 1989). In this study, we address whether these appendages correspond to independent stamens (as a fasciculate group), to an independent structure ("emergence" *sensu* Kasapligil 1951; Rohwer 1993, 1994), or to pollen sacs (see "Discussion"). Pollen sacs open introrsely in the two outer stamen whorls but extrorsely in the third whorl (Kasapligil 1951; Endress 1972*b*, 1980*a*, 1980*b*, 1994*a*; Endress and Hufford 1989; Rohwer 1993, 1994). Anthers of Lauraceae possess either four pollen sacs in a superimposed orientation (Kasapligil 1951; Endress and Hufford 1989) or two through a variety of reductions (Endress and Hufford 1989; Rohwer 1993, 1994). Interestingly, stamens normally lack an apical connective appendage ("connective tip"), a feature typical of flowers of basal angiosperms (Endress 1972*b*; Endress and Hufford 1989; Taylor and Hickey 1996). The gynoecium is considered a single carpel (Endress 1972*b*; also in *Umbellularia*; Kasapligil 1951) and is superior in most Lauraceae (but inferior in *Hypodaphnis*, sister to all other Lauraceae, and *Cassytha*, part of a basal grade in the family; Chanderbali et al. 2001). In most Lauraceae, the receptacle is significantly elongated and may be considered a hypanthium, as in *Cinnamomum* (Endress 1972*b*). In later stages, this cup-shaped receptacle can participate in the formation of a cupule: a collar or tube inserted around the base of the fruit (this feature is homoplasious in the family; Chanderbali et al. 2001). The presence of a cup-shaped receptacle is considered a synapomorphy of Laurales (Renner 1999) but occurs variably in the order. In *Persea*, receptacle elongation is not prominent.

There are, however, remarkable exceptions to the "typical" floral scheme for Lauraceae. Members of the tribe Laureae (*sensu* Chanderbali et al. 2001) are often dioecious, and all anthers open introrsely (not only the two outermost whorls), in contrast to other Lauraceae (Kasapligil 1951; Endress and Hufford 1989). Laureae also possess distinctive "umbellate inflorescences," subtended by persistent involucral bracts (Chanderbali et al. 2001). As we show in this study, *Persea* also has a somewhat umbellate inflorescence and specialized bud scales that cover the inflorescence ("bud scales" forming an "involucrum" around the "winter bud" in Laureae, according to Rohwer 1993) but abscise at anthesis.

Dimerous phyllotaxy occurs in some Lauraceae (e.g., *Laurus*, *Neolitsea*, *Potameia*, and *Endiandra*; trimery, tetramery, and pentamery occur in *Chlorocardium*; Kasapligil 1951; Rohwer 1993; but phyllotaxy is spiral in *Endiandra montana*, according to Kubitzki [1987] and Endress [1994*a*]); dimery is also present in female flowers of *Hernandia* in the related family Hernandiaceae (Endress and Lorence 2004). In dimerous Lauraceae, it appears as if the subdecussate phyllotaxy of the lateral shoot of the inflorescence continues in the flower (in contrast to the trimerous flowers of *Persea*).

In some species, including *Persea borbonia*, the tepals of the outer whorl are smaller than those of the inner whorl and appear sepal-like (Rohwer 1993; Chanderbali et al. 2006). Occasionally, the inner tepals are smaller than those of the outer whorl, for example, in *Endiandra*, which also lacks outer stamens (Rohwer 1993, 1994). Some genera have three or more perianth whorls, putatively via conversion of outer stamens into tepals (*Dicypellium*, *Phyllostemonodaphne*, *Eusideroxylon*; Rohwer 1993; see "Androecium and Receptacle Expansion"). Stamens of *Calycanthus* (Calycanthaceae), *Aniba*, *Endlicheria* (Lauraceae), *Doryphora* (Atherospermataceae), and other Laurales bear apical structures similar to connective appendages (Endress 1972*b*, 1994*a*; Endress and Hufford 1989; Rohwer 1993, 1994).

Previous Developmental Studies

Because Lauraceae have long been considered an ancient lineage of flowering plants (part of a "Ranalean complex" or "Magnoliidae"; Bessey 1915; Takhtajan 1980, 1991, 1997; Cronquist 1988), morphology and development of several members have been thoroughly studied and compared (e.g., Reece 1939, 1942; Saunders 1939; Kasapligil 1951; Endress 1972*a*, 1972*b*, 1980*a*, 1980*b*, 1994*a*; Endress and Hufford 1989; Endress and Igersheim 1997). Floral development of *P. americana* was studied previously (e.g., Salazar-Garcia et al. 1998, 1999). A comparison of floral morphology was also conducted on *Umbellularia californica* and *Laurus nobilis* (Kasapligil 1951)—from genera that represent the *Ocotea* complex and Laureae, respectively, two distant clades within Lauraceae (Chanderbali et al. 2001)—which differ in floral gender, merosity, and phyllotaxy. In these taxa (*P. americana*, *U. californica*, *L. nobilis*), vegetative and floral shoot apical meristems (SAMs) exhibit a consistent zonation, showing that the floral shoot does not differ fundamentally from other shoots (Kasapligil 1951; Salazar-Garcia et al. 1998, 1999).

Several detailed studies of developmental morphology and embryology of Laurales were conducted by Endress and coworkers (Endress 1972*a*, 1972*b*, 1980*a*, 1980*b*; Endress and Lorence 1983, 2004; Endress and Igersheim 1997), including details on carpel development, cell division pattern, and embryology (Endress 1972*a*, 1972*b*; see "Carpel") and pollination mechanisms, such as heterodichogamy (Endress and Lorence 2004). However, no previous studies have presented a comprehensive analysis of floral development in *P. americana* that provides a suitable framework for studies of the genetic regulation of floral organ identity and evolution.

Goals

Persea americana offers opportunities for functional genomics in basal angiosperms. The species can be transformed

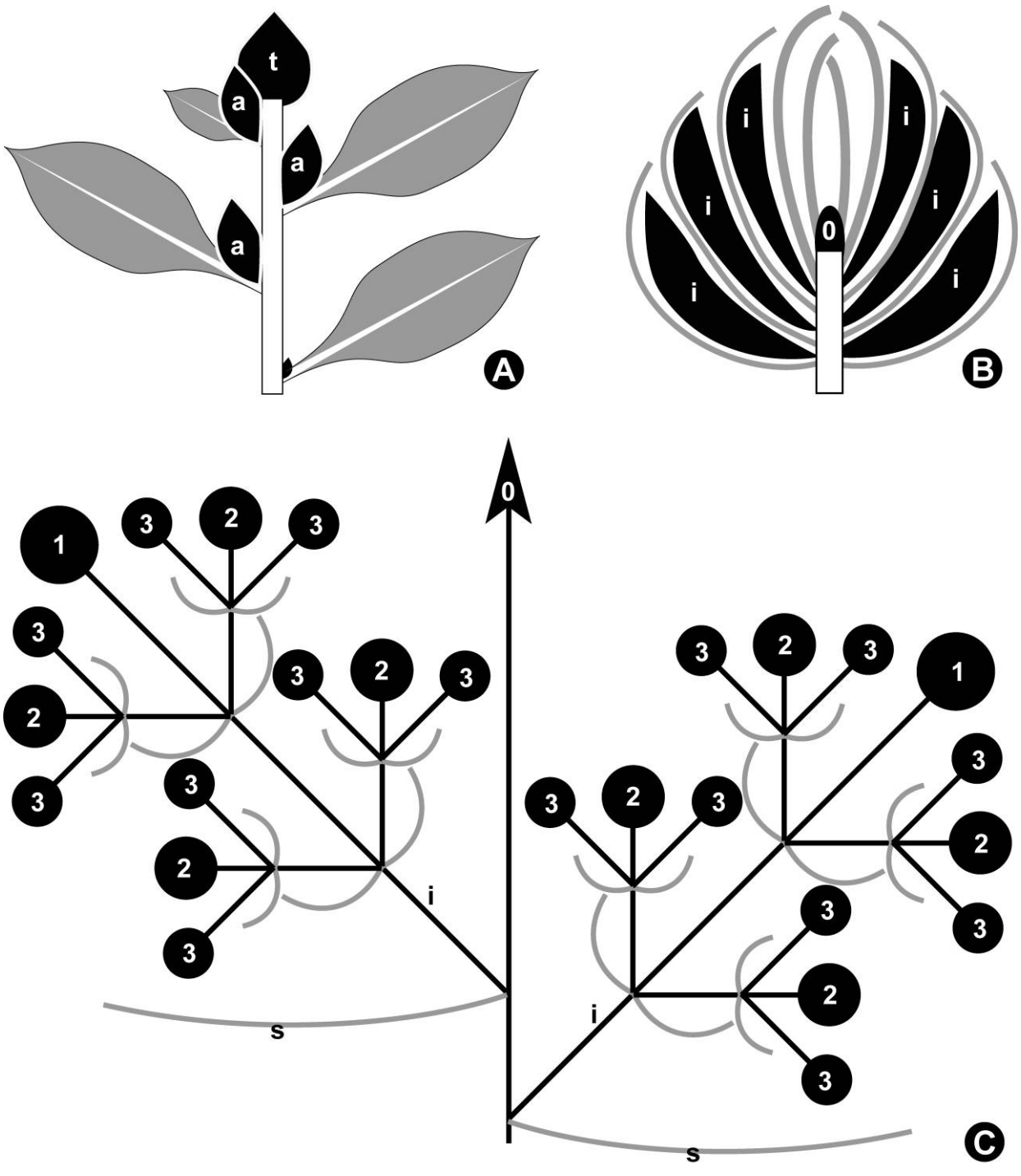

Fig. 1 Diagrams of inflorescence architecture in *Persea*. *A*, A shoot during winter with foliar leaves (gray), terminal winter bud (reproductive, *t*), axillary winter buds (reproductive, *a*), and a small vegetative axillary bud (bottom-most leaf, no index). *B*, Longitudinal section of a winter bud enclosing smaller buds (black), main shoot of order 0 (*0*), and lateral buds, the actual inflorescence (*i*) subtended by bud scales (gray). *C*, The thyrse with the continuing main shoot (order *0*), bud scales of winter buds (*s*) subtending the primary lateral shoot of the actual inflorescence (*i*, to terminal flower order *1*), membranous bracts (gray, not marked), and shoots and terminal flowers of subsequent orders (*2*, *3*); shoot axis and flowers (black), leaves (gray). *a* = axillary winter buds; *i* = inflorescence *s.s.* as system of lateral shoots of order 1 and higher; *s* = winter bud scale; *t* = terminal winter bud; 0 = main shoot; *1–3* = lateral shoots corresponding to orders.

(Gomez-Lim and Litz 2004), and micrografting techniques considerably reduce the 7-yr juvenile period (Suarez et al. 2004; Raharjo and Litz 2005). The species is economically important, and several commercial varieties have been developed. In this article, we address five main points relative to floral development and evolution in *P. americana*. (1) We provide a complete floral developmental series of *P. americana* as a foundation for subsequent studies of gene expression and homology assessment in Lauraceae. To facilitate comparison with other taxa, we employ the developmental landmarks identified through a consensus alignment of floral developmental stages (Buzgo et al. 2004*a*). (2) We discuss the transition from vegetative shoot to inflorescence. Is the inflorescence represented best by the determinate lateral thyrses or by the entire winter bud (indeterminate thyrse along a main axis)? (3) We discuss the transition from inflorescence to flower. Is the transition distinct (as in most core eudicots) or gradual (as in many basal angiosperms)? Are there bracts on the receptacle? Are there more than six tepals (i.e., two whorls with three each)? Are there transitions in the floral shoot from a decussate phyllotaxy to a trimerous phyllotaxy, or does a trimerous whorl show a unidirectional initiation of organs (e.g., starting with the abaxial organ)? (4) We discuss the homology (organ identity) of the paired staminal appendages of the third androecial whorl. Do these appendages correspond to entire stamens, or are they emergences of stamens (Rohwer 1993, 1994)? (5) We emphasize the possible role of underlying developmental constraints as a source of homoplasy; we also examine the potential recurrence of features in diverse floral organs.

Material and Methods

Inflorescences were collected from four individual trees of *Persea americana* (Buzgo 1075, 1095, 1096, 1109), along with collections for comparative study from *Persea borbonia* (1101, 1103), *Persea palustris* (1104, 1105), and *Cinnamomum camphora* (1102) between March 2002 and late February 2004 on the campus of the University of Florida, Gainesville. Voucher specimens are deposited at the Herbarium of the Florida Museum of Natural History (FLAS). Three individual plants from which we sampled grew next to each other under almost the same conditions (1075, most sunny, hit most severely by frost; 1096, most shaded and protected; and 1095). The smallest individual (1096) had smaller fruits and earlier anthesis and suffered less from severe frost than the two larger individuals.

Studies of nectar secretion, glandular activity (neutral red staining), and stigma activity (catalase test; Ruzin 1999) were conducted on fresh material. Samples for developmental study were fixed in FAA (formalin, acetic acid, alcohol), involving a short application of vacuum (ca. 7 min) until no more bubbles appeared, incubated for ca. 6 or 13 h at 4°C, and then transferred to 70% ethanol (RNase free). Organs were dissected to appropriate size in 70% ethanol, optionally postfixed in FAA, stored in 70% ethanol at 4°C until use, and dehydrated along an ethanol series. For SEM, samples were dehydrated, critical-point dried, gold-sputtered, and observed in a Hitachi S-4000 field emission scanning electron microscope (acceleration 4.0 kV) at the University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR) Electron Microscopy Laboratory. For microtome sections, the dehydrated samples were transferred to xylene and embedded in Paraplast or methacrylate (butyl methacrylate and methyl methacrylate) with benzoin ethyl ether and DTT (DL-1,4-Dithiothreitol; a detailed protocol is available (see the appendix in the online edition of the *International Journal of Plant Sciences*). After the samples were sectioned with a rotary microtome (10-μm-thick Paraplast, 4–6-μm-thick methacrylate), the sections were placed onto microscope slides coated with poly-L-lysine or Fisherbrand SuperFrost/Plus microscope slides (Fisher Scientific, both for Paraplast) or fat-free slides (for methacrylate). Staining was by Sass's safranin–fast green or toluidine blue O (pH < 5; both Ruzin 1999). Mounting was in Cytoseal 280 (Richard Allen Scientific). Observations were made using a Leica MZ12-5 dissection microscope and a Carl Zeiss compound microscope with transmitted light. Photographs were taken with a Nikon Coolpix 995 digital camera. Image editing included linear adjustment of contrast, color temperature, frame, and resolution, using Adobe Photoshop 7.0.

Definitions of terms in "Results" are as follows. "Winter buds" are defined as buds (shoot portions with minimal internode length and scale-shaped, rigid leaves), terminal and distally lateral on strong shoots, designed to persist in winter in dormancy; "bud scales" as brown-yellow scale leaves of the winter bud and subtending the lateral inflorescences, often with remnants of the lamina; "frondose transitional leaves" as membranous inflorescence bracts (on lateral shoots subtended by bud scales) with no remnants of the lamina (Rohwer 1993); "hypophyll," or "Unterblatt," as part of the leaf base containing sheath and stipules but neither lamina nor the free portion of petiolus (Troll 1939; Roth 1949); panicle, or determinate thyrse, as a system of floral shoots (inflorescence) in which each shoot is terminated by a flower and iterates the branching pattern of the bearing shoot, a determinate monopodial "main shoot" bearing lateral shoots (Troll 1964; fig. 1*C*); "vigor" as "capacity to produce the burst of foliar leaves after anthesis" (defined by Thorp et al. [1993], p. 649); "cross zone" and "sekundärer Querwulst" as tissue forming at the adaxial side of a lateral organ (leaf or carpel) that renders the entire structure peltate or ascidiate (Endress 1972*b*), corresponding to "cross meristem" (Rudall and Buzgo 2002); and "wet stigma" as epidermis with exudation or a disrupted cuticle (Thien et al. 2003).

Results

Time Frame

Normally, the plants of *Persea americana* are evergreen, but most new foliar leaves emerged during March in Gainesville, synchronously throughout the plant (see stage 2 in "Developmental Series"). In early August, the new internodes at the end of the shoot remained short, and the new leaves remained scale shaped, forming a terminal bud. These buds were dedicated for the next season's production (winter buds; fig. 1*A*, 1*B*). In addition to the terminal bud, winter buds developed in the axils of the three to five distal foliar leaves (fig. 1*A*). By November, the winter buds reached a size

of 4–8 mm, growing to 8–12 mm in December (fig. 2*A*). Inside the winter buds, inflorescence development began at the end of the vegetation season (early September 2002, 2003; fig. 1*B*). Cold weather affected early development. A severe frost (January 24, 2003) eliminated 70% of all developing buds, and the plants of *P. americana* lost most leaves.

The initiation of flowers occurred during the winter (January 2003, December 2003, and January 2004). First flowers were initiated in December (individual 1096 in 2003) or January (all individuals in 2003; 1075 and 1095 in 2004). In early January, the winter buds resumed growth (fig. 2*B*). By late January or February, the inflorescence buds were growing rapidly and the flowers had pushed out from underneath the bud scales (fig. 2*B*).

Organography

During early inflorescence development (December–late January), the distalmost internodes of the main shoot remained short, and the last two to four foliar leaves approached a seemingly decussate phyllotaxy (initiated in early August). Distally in this portion with short internodes, the leaves changed abruptly to scale shaped, small, yellow-brown, and densely packed (fig. 2*A*, 2*B*; leaves of the winter bud, bud scales, respectively); sometimes a single intermediate leaf occurred. These bud scales occurred as pairs, two initial scales (such as prophylls in axillary winter buds), or four initial ones forming a tetrad (such as two decussate pairs in terminal winter buds of the main shoot; fig. 2*B*). The subsequent bud scales followed in a spiral, sometimes seemingly alternating with the first tetrad. This resulted in a phyllotaxy similar to alternate tetramerous whorls, with conspicuous orthostichies, in an octagonal pattern (enclosing an angle of 45°; fig. 2*B*). This phyllotaxy was most conspicuous at the base of the winter bud and became more spiral toward the tip, where the young foliar leaves for the subsequent season were formed. The basalmost bud scales were narrow and awl shaped. In the subsequent bud scales, the distal portion was ovally extended and showed a pinnate nervature, corresponding to the lamina of foliar leaves (fig. 2*G*). This observation indicates that proximal bud scales do not consist of only the hypophyll (Unterblatt). Distally in the winter bud, the bud scales lacked a distinctly extended upper part and were more triangular and thick but otherwise similar to the bracts in the lateral panicles (inflorescence bracts; see next paragraph; fig. 2*I*). The distalmost leaves of the main-shoot winter bud resumed the expression of a lamina, corresponding to the new foliar leaves of the current season.

The bud scales of a winter bud subtended a panicle, or determinate thyrse, i.e., the inflorescence in the strict sense (fig. 1*B*, 1*C*; fig. 2*C*, 2*D*, 2*G*). In the panicle, the two transverse prophylls and following median bracts were arranged in decussate phyllotaxy. The prophylls and bracts were hairy and rigid and together formed a pouch containing the panicle (fig. 2*D*–2*F*). Each of these inflorescence bracts subtended the next-higher order of partial inflorescences, i.e., a lateral determinate thyrse or cyme (dichasium) (fig. 1*C*; fig. 2*C*, 2*E*). The inflorescence bracts were membranous (frondose transitional leaves) and had no sign of a lamina remnant but were entirely scale shaped and oval-triangular. Like the distal bud scales, they appeared to consist of only the hypophyll (Unterblatt) and were similar to the organs of the perianth (see below). The prophylls had a conspicuously U- or V-shaped profile in transverse section (fig. 2*G*, 2*H*), whereas the median bracts were uncurved (fig. 2*I*, 2*J*).

Four orders of inflorescence shoots were discerned (0–3) (fig. 1*C*). Order 0 represents the vegetative shoot axis of the winter bud, whether this is a lateral shoot in the axil of a foliar leaf or the main shoot. It would resume vegetative growth after anthesis, although in a few lateral buds, it formed a terminal inflorescence corresponding to the lateral panicles. Order 1 is the first axis of the lateral panicle, which is subtended by the scale leaf of the winter bud and is terminated by a flower. Order 2 includes shoots along order 1, normally about six per panicle, each a small cyme by itself, subtended by an inflorescence bract and terminated by a flower. Order 3 consists of the shoots along order 2, normally two flowers in transverse position, subtended by the prophylls of order 2.

At anthesis, the shoot axis of the lateral panicles elongated (fig. 2*C*). The proximal portion of shoot order 1 was proportionally more elongated than the distal internodes; as a result, the cluster of each winter bud appeared umbel-like.

The inflorescence and flowers were clearly distinct from each other. Flowers had a prominent pedicel, typically without bracts or bracteoles. The receptacle was not cup shaped. The perianth consisted of two trimerous, alternate whorls of pale, cream, or green tepals, with hairs on the abaxial and (though shorter) the adaxial side (fig. 2*C*; fig. 3*A*, 3*B*, 3*D*, 3*E*). The two perianth whorls (floral whorls 1, 2) were typically only slightly differentiated (*P. americana*), but in *Persea borbonia*, the outer whorl was distinctly smaller (fig. 3*C*).

The androecium consisted of four trimerous whorls (floral whorls 3–6), the outermost whorl alternating with the inner tepals: three outer whorls of fertile stamens and an inner sterile whorl of staminodes (floral whorl 6, androecial whorl 4). The stamens of the two outer whorls (androecial whorls 1, 2) inserted almost in one series and possessed a long, hairy filament and a cylindrical or box-shaped anther with four flaplike valves opening introrsely (stoma; fig. 3*A*, 3*F*). The stamens of the next inner whorl (androecial whorl 3) differed from those of the outer ones by the extrorse anthers and by the occurrence of a pair of basal staminal appendages (fig. 3*G*). Both appendages inserted at the base of the filament and possessed a white, hairy stalk similar to the filament (fig. 3*H*). At maturity, the apices of the appendages were kidney shaped to triangular, yellow, wet-glossy, and similar to an anther and to the apical structure in staminodes (fig. 3*G*, 3*I*, 3*J*; see below). The yellow bulge of the appendage apex was more prominent toward the adaxial side (with respect to the floral axis) (fig. 3*G*, 3*H*). The staminodes (androecial whorl 4) had a shorter filament, and the swollen apex was yellow, triangular-acute with a tip similar to an apical connective appendage, and more prominently bulging adaxially, with a broader connective on the abaxial side (fig. 3*I*, 3*J*). Staminodes and paired staminal appendages appeared to secrete nectar. The type of secretion of the tepals and anthers is unknown but is likely to involve odor, although at the base of some tepals, we found yellow thickenings at the base of the margins, very similar to the nectariferous structures of the staminal appendages of third-whorl stamens (fig. 4*A*); for details of secretory functions, see "Developmental Series."

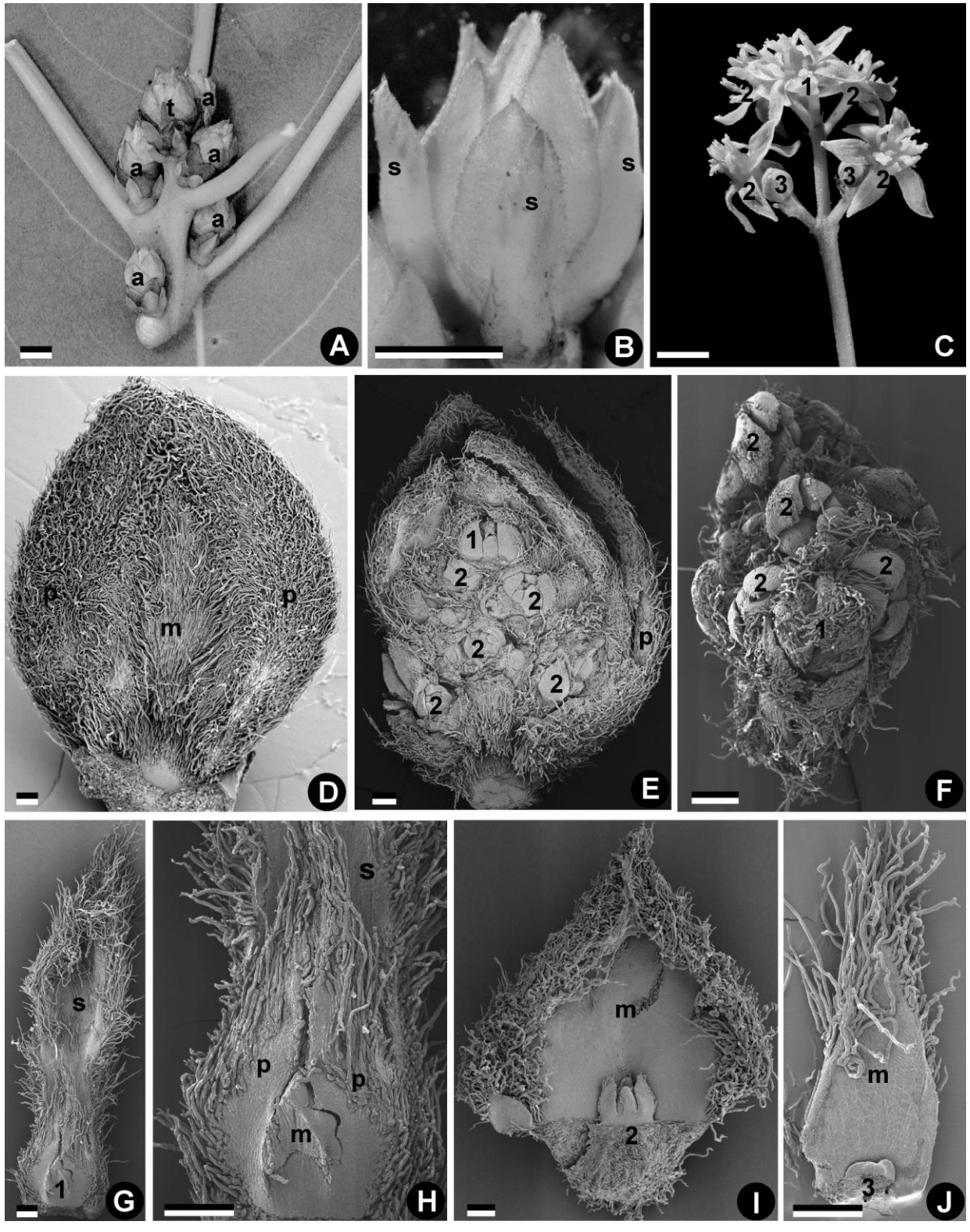

Fig. 2 Inflorescence of *Persea americana*. *A–C*, Macrophotography and dissecting microscope. *D–J*, SEM. *A*, Shoot tip with winter buds, side view. *B*, Winter bud with bud scales starting to open, side view, first series (tetrad) with pinnate portion (*s*). *C*, Inflorescence *s.s.*, order-1 shoot with terminal flower and lateral shoots (orders 2 and 3), side view. *D*, Young lateral inflorescence bud (order 1), from an axile of a peripheral scale (removed) of a winter bud, transversal prophylls laterally compressed, abaxial view. *E*, Young lateral inflorescence bud (order 1), several bracts removed, flowers exposed (orders 1–3), abaxial view. *F*, Young lateral bud (order 1), top view. *G*, Bud scale, distally in the bud, subtending a very

The single uniovulate carpel was ascidiate for up to 80% of the ovary and plicate above this and along the elongate style; the stigma was small, papillose, and slightly capitate (fig. 3*K*). The anatropous, pendent ovule possessed two integuments.

Occasionally, unusual floral formations were found, mostly in individual 1109. From the base of the margins of the inner tepals, up to 2 mm above the base, yellow knots were found (fig. 4*A*). The knots were ca. 0.5 mm long, glossy, and glabrous, strikingly similar to paired staminal appendages and staminodes in the androecium. Further, we found a few stamens that looked exactly like tepals with yellow, glandular knots (fig. 4*B*). This finding is unexpected because the adjacent outer stamens in *P. americana* normally have no paired appendages. We found stamens of the third androecial whorl with more than one appendage at the base of one or both sides. In these cases, two appendages appeared in one series along the side of the stamen base, similar to pinnae in a compound leaf (not shown). Similarly, staminodes with the lateral yellow tissues subdivided were also observed (fig. 4*C*). Additionally, staminodes (fourth androecial whorl) were observed, with the apex elongated to a structure similar to a style and stigma (figs. 4*D*, 7*E*).

Developmental Series

To facilitate comparison with other taxa, we employ the developmental landmarks identified through a consensus alignment of floral developmental stages (table 1; see also "Material and Methods").

Stage 1. From September to late December, the winter buds (order 0) contained primordia of the future lateral inflorescences (order 1). At this stage, the future lateral inflorescences were indistinguishable from vegetative buds. The lateral inflorescences started with two prophylls in transverse position, slightly toward the adaxial side of the primordium with respect to the order-0 shoot (fig. 5*A*, 5*C*, 5*D*). The initiation of prophylls was not completely synchronous (fig. 5*A*, 5*C*, 5*D*). At this stage, the SAM of the winter bud (order 0) was stout; the leaf primordia were initiated with a divergence angle between pairwise (almost decussate) and 138° (Fibonacci spiral) (fig. 5*A*).

In the axils of bud scales of the winter bud, lateral shoots started as transversely expanded primordia (fig. 5*B*–5*D*). The transversely outermost portions of a primordium formed the two prophylls. Shortly after the initiation of the prophylls, the abaxial bract of the median pair of bracts was initiated (fig. 5*C*–5*E*). The SAM expanded on the abaxial side, forcing the prophylls to a more adaxial position than at initiation.

The adaxial median bract appeared later than the abaxial one but soon became equal in size to the abaxial bract. Meanwhile, the prophylls elongated. They were longitudinally folded by the pressure of the neighboring bracts of the winter bud. Each prophyll and median bract had the potential to subtend a lateral shoot, forming a determinate panicle. The initiation of prophylls and median tepals iterated with each lateral inflorescence of the subsequent orders. Trichomes developed first along the abaxial median, where the pressure of adjacent leaves was weak (fig. 5*E*).

Stage 2. By the time of the initiation of the first tepals, the apical meristem of the proliferation shoot had changed its appearance. The previously stout SAM elongated (fig. 5*F*), gaining more capacity to produce the burst of foliar leaves after anthesis (vigor; stage 10 and later). Initially, the distal leaves in the winter bud were still bractlike, but the axillary meristems remained small. Later, the distal portion of the distal leaves assumed features of a lamina (midrib and lateral expansion, corresponding to the foliar leaves after anthesis) (fig. 5*F*).

The transition between the subdecussate phyllotaxy of the inflorescence and the trimerous perianth was abrupt. The three outermost floral organs (tepals) appeared simultaneously (fig. 5*G*). In contrast to other lateral shoots, the first transverse organs (lateral tepals) of the floral shoot were more abaxial than adaxial (prophylls were more adaxial), and the median tepal was on the adaxial side (not abaxial, in contrast to the first median bracts, which were abaxial). Only in some cases was perianth development slightly unidirectional from abaxial to adaxial.

Stage 3. The primordia of the second perianth whorl followed immediately and showed a weak unidirectionality (fig. 5*G*). The plastochron between the first and second whorls was short, and the gaps between the outer tepals were wide. As a result, the inner tepals initiated almost in one series with the outer tepals. This may explain, in part, the similarity of the two perianth whorls in *P. americana*. In contrast, in *P. borbonia* the relative space between the outer perianth primordia was more narrow, forcing the inner tepals to initiate in a more distinctly inner whorl. The resulting stronger developmental distinction may be a contributing factor for the later, more differentiated perianth of *P. borbonia*. In *P. americana*, the tepals soon became broad, and the receptacle expanded perpendicularly to form a club-shaped structure with a flat apex (central to the tepals) (fig. 5*G*, 5*H*). The tepals arched over the center, which thereby became slightly concave (fig. 5*H*). At this stage, large amounts of tannins were present in the epidermal cells of the bracts (and later in tepals as well). Tannin-containing cells absorbed histological stains intensively and would not release them during preparation. Later, the outer-whorl tepals became valvate.

Stage 4. The floral apex expanded laterally and became flat. Stamen primordia were distinguished from tepal primordia by a clear plastochron (as the flower broadens) and by a more narrow, cylindrical shape. The first two whorls of stamens were initiated almost simultaneously (fig. 5*H*), which may explain their similarity. The third androecial whorl

young axillary shoot bud (order 1, inflorescence or vegetative), flowers exposed (orders 1–3), adaxial view. *H*, Very young lateral shoot bud (order 1, close-up of *G*; inflorescence or vegetative, very distal), adaxial view. *I*, Median inflorescence bract on order-1 shoot, subtending axillary shoots (order 2) with transversal prophylls and a median bract (no index), adaxial view. *J*, Small distal bract on order-1 shoot of an inflorescence bud, initiation of axillary lateral shoot meristem (order 3); note prophyll on the right side ahead of the one on the left side (no index), adaxial view. *a* = axillary winter buds; *s* = winter bud scale; *t* = terminal winter bud; *1–3* = lateral shoots corresponding to order. Scale bars: *A–C*, 5 mm; *D–J*, 0.2 mm.

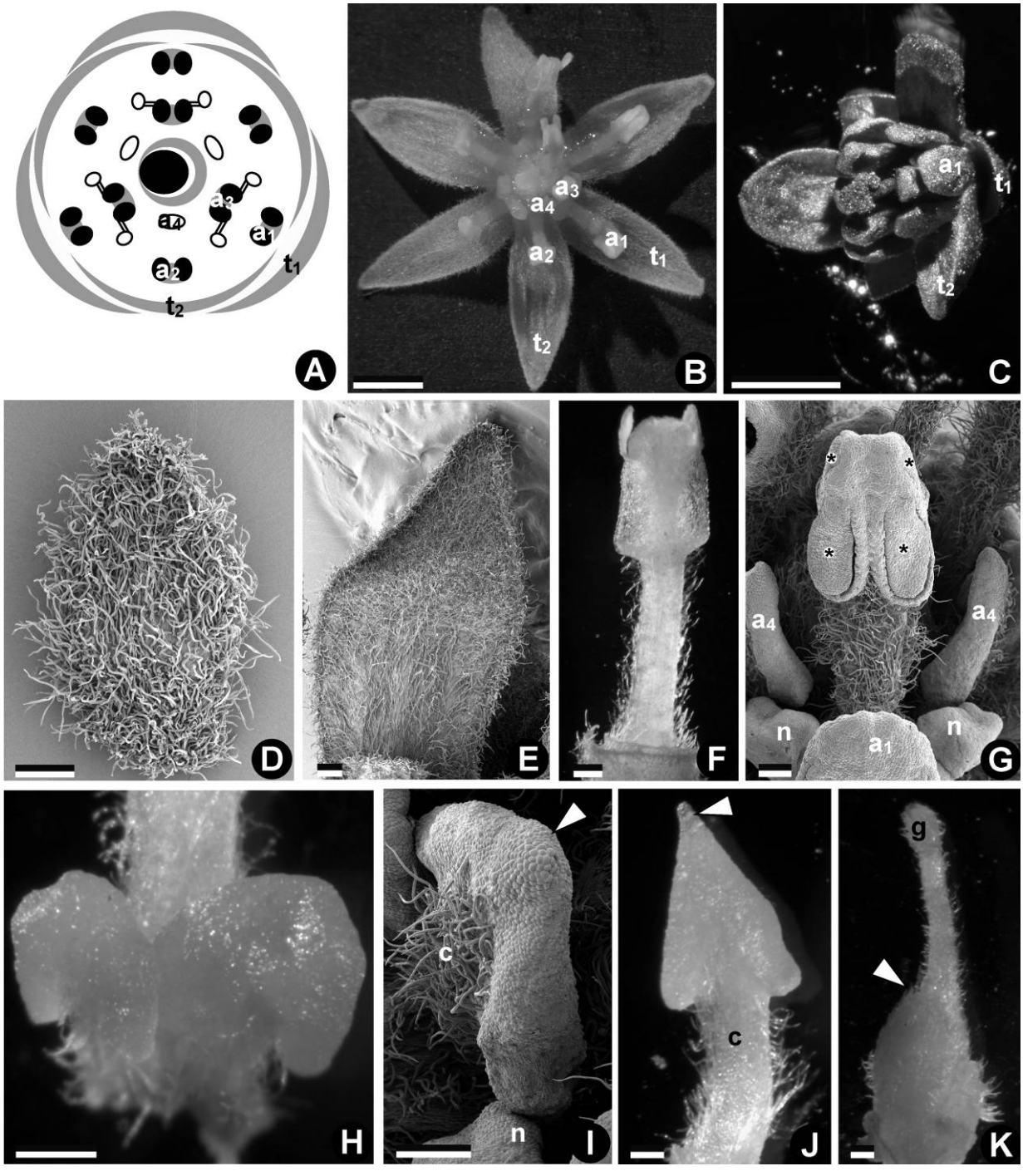

Fig. 3 Flower and floral organs of *Persea*. *B*, *D–K*, *Persea americana*. *A*, Schematic transverse section of a flower: ovule and thecae are in black; staminodes and staminal appendages are outlined; ovary wall, connectives, and tepals are in gray. *B*, Flower at male stage of anthesis, apical view, dissecting microscopy (DM). *C*, Flower of *Persea borbonia*, shorter outer tepals ("sepals," t_1), lateral-oblique side view, DM of sputtered object. *D*, First tepal, hairy abaxial side, SEM. *E*, Second tepal, adaxial side with finer hair, SEM. *F*, First-whorl stamen at anthesis (with open stoma flaps); the base has no trace of additional structures, abaxial view, DM. *G*, Stamen of the third whorl at anthesis, with a pair of paired staminal appendages, abaxial view, SEM. *H*, Close-up of the pair of appendages at the base of a third-whorl stamen, abaxial view, DM. *I*, Close-up of a staminode, with midportion (connective) and tip (arrowhead), abaxial-apical view, SEM. *J*, Staminode with a prominent tip (arrowhead), adaxial view, DM. *K*, Carpel at anthesis, with stigma and plicate style, adaxial upper rim of ascidiate ovary (secondary cross zone; arrowhead), adaxial view, DM. a_1–a_3 = stamen whorls; a_4 = staminodes; c = connective, including midportion of staminodes supposedly comprising filament and stalks of nectaries; g = stigma or corresponding structure; n = paired staminal appendages; t_1 = outer tepals; t_2 = inner tepals; asterisk = pollen sacs or stoma. Scale bars: *B–C*, 2 mm; *D–K*, 0.2 mm.

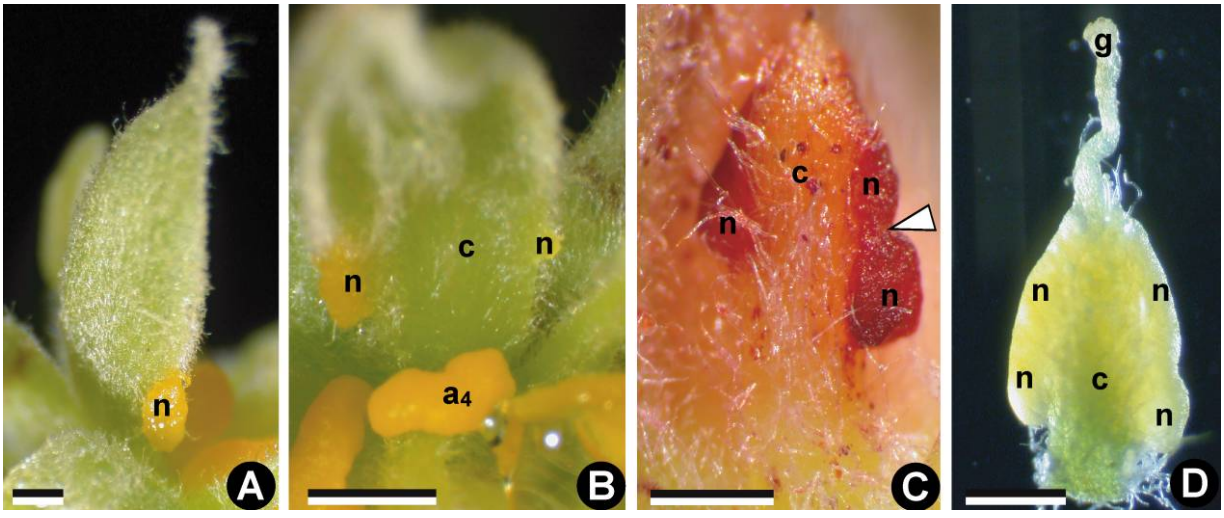

Fig. 4 Aberrant floral organs of *Persea americana*, dissecting microscopy. *A*, Aberrant inner tepal at anthesis, with yellow, putatively nectariferous structure on the margin, side view. *B*, Aberrant stamen of the second whorl (an "outer staminode"), with putatively nectariferous structures but without anther, adaxial view. *C*, Slightly aberrant staminode (fourth androecial whorl) at anthesis, with lateral nectary tissue separated by a constriction (arrowhead), reminiscent of pollen sacs, apically separated by a prominent midportion ("connective," including filament and stalks of nectaries), and stained with neutral red, abaxial view. *D*, Aberrant staminode at anthesis, with serial thickenings of the lateral yellow tissue (*n*, like pollen sacs) and an apical stigmalike formation (similar to a carpel), abaxial view. *c* = connective, including midportion of staminodes supposedly comprising filament and stalks of nectaries; *g* = stigma or corresponding structure; *n* = paired staminal appendages and nectaries. Scale bars = 0.5 mm.

followed distinctly later, filling the broad floral apex almost completely (fig. 5*I*). However, initially, the stamens on the abaxial side were at a more advanced stage of development, still reflecting the unidirectional development of the flower (fig. 5*H*). This unidirectionality contributes to the almost simultaneous initiation of the primordia of the three stamen whorls.

After the stamens were initiated, their future anthers elongated to a triangular or clublike shape (fig. 5*K*). Only after the stamen primordia were already longer than broad, and their filaments could be recognized, was a fourth androecial whorl with three staminodes initiated (fig. 5*I*; fig. 6*A*, 6*B*). This occurred just before or simultaneous with the rise of the center of the flower to form the carpel primordium (see "Stage 5").

The outer tepals elongated until they almost touched apically, while the lateral margins remained separate (fig. 5*J*). At the apex of the outer tepals, a tuft of trichomes developed (fig. 6*G*). The trichomes at the tip and along the margins interweaved and stabilized the position of the tepals, similar to a Velcro lock. Basipetally, more trichomes developed, starting with the median portion. Between the outer tepals, the median abaxial portions of the inner tepals remained exposed. Here also trichomes developed, while trichomes were lacking on the inner tepals where outer tepals were pressed toward the inner tepals.

Stage 5. Carpel initiation followed shortly after the formation of staminodes and consumed the remainder of the SAM. The single carpel commenced as a solid primordium (fig. 6*B*). The base of the carpel primordium elongated vertically. As a result, a solid stipe remained below the ovary cavity (fig. 6*C*, 6*D*). On the top of the carpel primordium, one side grew faster, leaving a slope on the other side that soon became depressed; this depression forms the future ovary cavity. The rim at the bottom of the slope corresponds to the cross zone (fig. 6*C*–6*G*). The cross zone was later responsible for the ascidiate portion of the carpel, corresponding to the ovary (fig. 6*D*, 6*E*, 6*H*).

Stages 6, 7. The lateral rims of the depression leading to the carpel apex bent inward and eventually fused secondarily, forming the style suture and enclosing the pollen transmission tract. The cross zone curved inward, initiating the ovule primordium (fig. 6*E*, 6*F*) and eventually subdividing to produce a rising rim that formed the adaxial ovary wall (fig. 6*F*, 6*G*). In most cases, the median symmetry plane of the carpel was transversely oriented to the median symmetry plane of the flower (especially orders 2 and 3).

While the cross zone in the carpel curved inward, stamens of the outer two whorls turned their stoma and pollen sacs from latrorse to introrse; those of the third whorl remained introrse-latrorse (fig. 6*E*). Staminodes remained small for a considerable time, whereas carpel and fertile stamens continued development (fig. 6*C*–6*E*, 6*G*, 6*J*). In the late stages of androecial initiation, the developmental stages of the three orders of flowers started to equalize with those of the first order. As a result, practically all flowers of an entire winter bud entered anthesis within a short time period.

After carpel initiation, as the anthers and sporangia differentiated and slightly later than the development of staminodes, the paired staminal appendages on the third whorl of stamens were initiated. These appendages appeared laterally or abaxially at the very base of the stamen, adjacent to the receptacle (fig. 6*E*). In *P. borbonia*, the primordia of each of the paired staminal appendages were oriented laterally and distant from each other. However, in *P. americana*, we observed only one initial bulge on each stamen, in a median or slightly lateral position (possibly due to space limitations).

Table 1

Comparison of Landmarks of Floral Developmental Stages

Landmark	*Persea*	*Arabidopsis*	*Acorus*
Stage 1:			
Inflorescence formation and flower initiation	Convex shoot apex meristem, 1–2 lateral shoot meristems; shortening of internodes and formation of winter bud and bud scales (fig. 5*A*, 5*B*); winter bud diameter 5–10 mm, bud of order-1 shoot transverse diameter ≤1.5 mm		Shoot apex meristem grows to a hemispherical body without initiating leaves
Later	Outgrowth of axillary panicles (fig. 2*G*, 2*H*; fig. 5*C*–5*E*) and higher-order shoots (fig. 2*I*, 2*J*); bud scale 3.5 mm, bud of order-1 shoot transverse diameter ≤.4–1 mm		
Later: flower initiation	1–2 lateral shoot meristems (order 1), 4 lateral shoot meristems (order 2), 10 lateral shoot meristems (order 3); lateral shoots of orders >1 as conical meristems already in decussate-panicle positions; terminal meristem is largest (not shown); bud of order-1 shoot transverse diameter ≤1.8 mm	Flower primordium visible; flower primordium delimited by a groove	Globular floral primordia appear spirally in quick succession in several parastichies
Stage 2:			
Initiation of outermost perianth organs	Initiation of perianth of oldest flowers; tepals as broad primordia on broad receptacle (fig. 5*G*); bud of order-1 shoot transverse diameter ≤2.1 mm; flower diameter ≤.4 mm	Initiation of sepals	Initiation of abaxial outer tepal
Later		Sepals overlie flower meristem	Initiation of adaxial outer tepals simultaneously with initiation of adaxial inner tepal
Stage 3:			
Initiation of inner perianth organs	Shortly after outer whorl, as broad primordia on broad receptacle (fig. 5*G*); bud of order-1 shoot transverse diameter ≤2.1 mm; flower diameter ≤.4 mm	Petal primordia arise simultaneously with	Initiation of adaxial inner tepal; initiation of abaxial inner tepals simultaneously with initiation of adaxial outer stamens
Stage 4:			
First stamens	Stamens as conical-hemispheric primordia (fig. 5*H*); bud of order-1 shoot transverse diameter ≤2.5 mm; flower diameter ≤.5 mm	Initiation of 4 inner stamens	Adaxial outer stamens
Initiation of later stamens (centrifugally, centripetally) or primordia subdivision	Stamens as conical-hemispheric primordia in short, centripetal succession, with staminodes slightly delayed (fig. 5*H*–5*K*); bud of order-1 shoot becomes unreliable; flower diameter ≤.7 mm	Initiation of 2 lateral stamens (short, outer stamens)	Initiation of rest of stamens
Stage 5:			
Carpel initiation	Shortly after staminodes (fig. 6*A*–6*C*); flower diameter ≤1 mm	Sepals enclose bud; carpel initiation	Carpel initiation, visible pollen sac contour
Later	Complete perianth; anthers with sporogenous tissue	Long stamens stalked at base; carpel elongation, ovary locules present	
Stage 6:			
Microsporangia	Early anther formation; almost simultaneous with carpel initiation (fig. 6*B*–6*E*); flower bud diameter ≤1 mm	Pollen sacs (locules) appear in long stamens, then in short ones	Formation of sporogenous tissue in anthers and carpel closure
Stage 7a:			
Ovule initiation 1	We found ovule initiation (a separation of the cross zone into two transverse meristems) to be earlier than male meiosis; third-whorl nectaries appear (fig. 6*E*); flower diameter ≤1 mm	Ovule initiation	
Stage 8:			
Male meiosis	Pollen mother cells and tapetum differentiated (fig. 6*G*)	Petal primordia stalked (male meiosis)	Rise of gynoecial center, external thickening of carpels and placenta swelling, beginning meiosis in anthers and tapetum differentiation
Stage 7b:			
Ovule initiation 2			Basal ovary elongation, ovule initiation
Other developments before female meiosis	Integument formation (fig. 6*G*–6*I*); flower bud diameter ≤2.2 mm	Petals level with short stamens; stigmatic papillae appear; petals level with long stamens	Tapetum differentiation; massive ovary secretion, microspore formation
Stage 9:			
Female meiosis	Exine in microspores, ovule anatropous, with megaspore (fig. 6*I*–6*K*); flower bud diameter ≥2.5 mm	Megasporogenesis	
Stage 10:			
Anthesis	Stigma receptive, pollen shed; proterogynous (figs. 2*C*, 3, 4); flower bud diameter ≥3 mm	Bud opens, petals visible	Rise of gynoecial center, stigma receptive
Later		Long stamens extend above stigma	Drying of stigma secretion, pollen release, starting adaxially
Later		Stigma extends above long anthers	

Note. Stages for *Persea* are combined after Buzgo et al. (2004*a*) and this study. Stage numbers correspond to those in the text.

This single primordium may give rise to both staminal appendages, but we observed no point at which the appendage primordium would actually divide.

When the microsporangia became visible on the contour of the stamens, the third whorl of stamens developed paired staminal appendages, and anther valves started to become extrorse in orientation. The secondary cross zone on the adaxial side of the carpel was now tightly appressed to the base of the plicate portion, closing the suture of the style secondarily (fig. 6*G*, 6*I*). An external portion of the cross zone ("sekundärer Querwulst") continued to rise; an inner portion represents the ovule primordium (fig. 6*E*, 6*G*, 6*I*).

The paired staminal appendages at the base of the stamens of the third whorl were in a lateral position (fig. 6*J*, 6*L*). Later, the staminodes (fourth androecial whorl) expanded simultaneously with the sterile paired staminal appendages. The tip of each staminode was pronounced (in contrast to the blunt tip in fertile stamens). Subsequently, the third-whorl paired staminal appendages also developed an antherlike, more globular structure (fig. 6*I*, 6*J*).

At this same stage, the abaxial side of the outer tepals was covered with long, rigid hairs. On the inner tepals, only the median, exposed portion developed hair (fig. 6*K*). The third whorl of stamens became more extrorse.

Stages 6–9. As the lateral carpel margins fused postgenitally, closing the plicate style (fig. 6*H*), the ovule primordium curved downward into the ovary cavity (fig. 6*I*). Finally, the papillae on the stigma expanded, appearing from the inner surface of the carpel (fig. 6*K*). The stigma was restricted to the uppermost portion of the style, whereas the suture of the style was naked (fig. 6*H*, 6*K*). Once the carpel had assumed a bottle shape, development appeared to slow down, as if the plant "waited" for the right time for anthesis.

Stage 10. The entire process of anthesis took more than a day and strongly depended on temperature and light conditions; cold, cloudy days tended to delay anthesis, resulting in a higher number of open flowers on sunny days. However, the free access and reward for pollinators lasted for only ca. 12 h. A flowering schedule is presented in table 2. Between 24 and 36 h before flower opening, the inner tepals apically elongated, and a tuft of hair emerged at the tip of the outer tepals. This elongation was correlated with the elongation of the style, pushing the stigma above the anthers into the space under the tepal tips. At this stage, the upper pollen sacs of the outer stamens were clearly introrse, but the lower ones were still latrorse. Anthers were receptive to neutral red stain at the borders of stoma (see below for discussion of anther development). The pollen sacs of the third androecial whorl were not stained. In some flowers (e.g., one of the two opposite lateral flowers), the distal portion of the staminodes was stained by neutral red (but the staminal appendages were not stained), and nectar was present at the base of the stamens and staminodes (but not expanding into the upper half of the bud because of water-repellent surfaces); in other flowers, nectar was absent.

At this early stage, the stigma already appeared to be receptive (staining with neutral red yielded a positive catalase reaction). It was difficult to determine whether the stigma was really wet (exudation or disrupted cuticle), but we found minuscule traces of slowly evaporating liquid when tipping the stigma onto a clean microscope slide. However, neutral red staining of the stigma was minimal at this stage compared with that of the anthers and the antherlike structures.

Flowers opened more than 1 h before sunrise; no pollinators were observed in the dark. Early in the morning, the first visitors observed were honeybees (*Apis mellifera*) and bumblebees, and later in the day, flowers were visited by syrphid dipterans; additional visitors included collemboles. The observed putative pollinators were active all day, and the reason for flowers opening in the dark just before sunrise is not understood. At early anthesis, the yellow apices of the appendages and also the yellow portion of the staminode stained strongly with neutral red, indicating secretory function (or surface cell decay). Because the epidermis appeared intact, these portions were considered to be nectariferous structures. The anthers, the stigma, and the margins of tepals also stained with neutral red. The staining of anthers commenced around the seam of the stoma flaps (dehiscence zone) or their hinge. At full anthesis, large amounts of nectar gathered in the androecium, in direct contact with the third-whorl appendages and the staminodes. Staining expanded throughout the pollen sacs.

Until noon or afternoon of the day of anthesis (1200–1600 hours), outer tepals were curving backward (parallel to the pedicel), the outermost stamens were elongated beyond the inner tepals, and pollen was shed. The margins of tepals in both whorls stained with neutral red. When the stoma flaps had opened completely, all of the thecae were stained, except for the connective and the inner wall of the pollen sac cavity toward the connective. Frequently, however, the anthers and paired staminal appendages of androecial whorl 3 and staminodes (androecial whorl 4) were not stained by neutral red (implying that secretion had ceased). The stigma was stained by neutral red and showed a positive catalase reaction. Finally, after 1400 hours, the tepals began to fold back into a budlike formation, closing the flower. Tepals were, at that point, elongate, straight, and yellowish. Soon thereafter, most flowers abscised.

Discussion

From Vegetative Growth to Flower

The flower is generally considered to represent a structure very different from the vegetative body of the plant, starting with a distinct whorl of outer perianth members (Coen and Meyerowitz 1991; Ng and Yanofsky 2000; Dong et al. 2005; Irish 2006). However, in classical morphology, the flower is considered a short shoot (shoot axis and leaves) or shoot system and therefore just a special case of the general plant structure (von Goethe 1790; Weberling 1989; Endress 1994*a*; von Balthazar et al. 2000; von Balthazar and Endress 2002). Indeed, in basal angiosperms, the distinction between vegetative and floral shoot portions occurs gradually rather than suddenly (Endress 1994*a*, 1994*b*; Posluszny and Tomlinson 2003; Buzgo et al. 2004*b*, 2005; see also Buzgo et al. 2006). Furthermore, when the flowers of basal angiosperms occur in inflorescences of more than a single flower (shoot systems with a major reproductive commitment), the inflorescence shoot system often shows intermediate features of vegetative and floral shoots, such as a gradual transition from bracts to

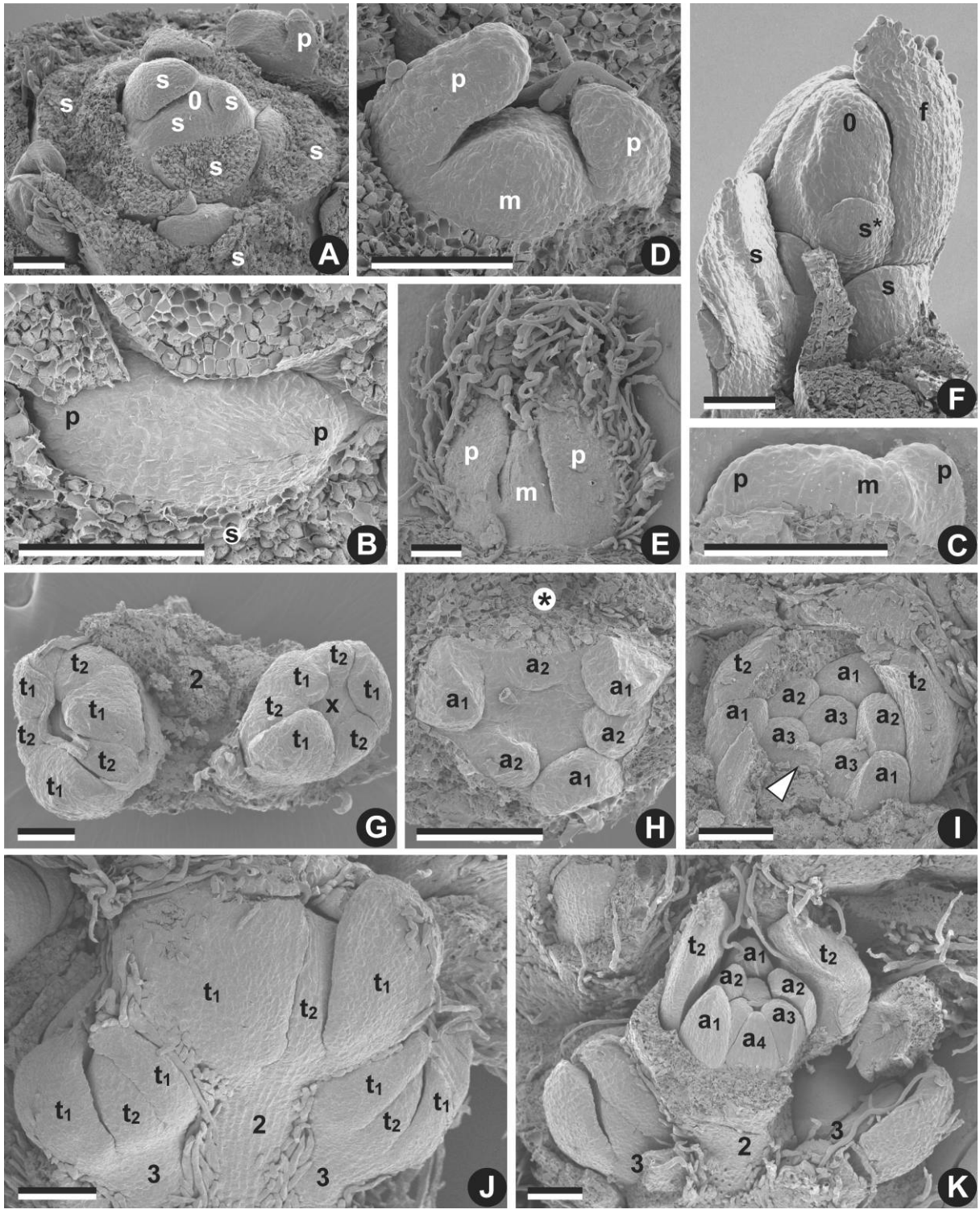

Fig. 5 *A–F*, SEM pictures from inside the winter bud: main and axillary shoot meristems. *A*, Very young winter bud (order-0 shoot) with a flat apical meristem between leaf primordia (*s*, bud scales) subtending primordia of order-1-shoots, apical view. *B*, Primordium of an axillary inflorescence shoot of order 1; prophylls not yet distinct, apical view. *C*, Initiation of axillary shoot of order 1; transversal prophyll primordia are unequal; shoot apical meristem (SAM) and median abaxial membranous bracts as central rib, abaxial view. *D*, Lateral shoot primordium of order 1 in an inflorescence bud; now the first median floral bract is also initiated abaxially, oblique abaxial view. *E*, Lateral shoot primordium of order 1 in an inflorescence bud, with adaxial first median membranous bract and remarkable hairs along the abaxial midrib of prophylls; up to this stage, there is no difference between floral and vegetative axillary buds; adaxial view. *F*, Apex of a terminal bud (order 0 *s.s.*) with inflorescence bracts and massive apical meristem determined for vegetative growth; note that the scale leaves (*s*) are older (below), and yet smaller, than the nearest foliar leaf, with a

tepals (Endress 1994*a*, 1994*b*; Posluszny and Tomlinson 2003; Buzgo et al. 2004*b*).

In *Persea americana*, foliar leaves, bud scales, and membranous bracts in inflorescences differ in phyllotaxy and shape. Changes from foliar leaves to bud scales are more abrupt than changes within the bud from proximal bud scales in the winter bud to distal leaves. Distally in the winter bud, the transition from distal membranous bud scales to foliar leaves may include few foliar leaves with a distinct but small lamina.

We found that foliar leaves are initiated and arranged in a spiral phyllotaxy at maturity (not alternate or opposite, in contrast to Rohwer 1993), whereas on the inflorescence of order 1 and higher, bracts occur in a subdecussate pattern (not spiral, in contrast to Rohwer 1993). The transition from spiral to subdecussate phyllotaxy occurs at the base of the winter buds (along order 0). Principally, all lateral shoots start with two scalelike, transverse prophylls, followed by median, reduced leaves. In vegetative lateral shoots, the subsequent leaves are separated by relatively long plastochrons. The bases of lateral organs are oriented at an angle of 138°, and the organs gradually increase in elaboration until they assume a complete foliar leaf shape with a fully expanded petiole and lamina. In contrast, in the proximal portion of the winter buds, the diameter of the young shoot axis is broad with respect to lateral organs, leading to the basally apparent fourfold orthostichies of mature bud scales. Distally in the winter bud, the diameter is relatively small and more similar to vegetative shoots.

Inflorescence phyllotaxy in *Umbellularia* was described as generally spiral but decussate in lateral shoots (Kasapligil 1951), similar to our observations for *P. americana*. On the basis of the observed change of phyllotaxy within one individual of *P. americana*, we propose that phyllotaxy in Lauraceae is a flexible character; the occurrence of purely decussate phyllotaxy in some Lauraceae (e.g., *Laurus*, *Neolitsea*, *Potameia*, *Endiandra xanthocarpa*; Kasapligil 1951; Rohwer 1993; but spiral in *Endiandra montana*, according to Kubitzki [1987] and Endress [1994*a*]) may be homoplastic, through omission of the spiral phase of the shoot. The presence of decussate phyllotaxy may depend directly on the relative size of the SAM and the lateral primordia. Also, in other basal angiosperms, transitions of phyllotaxy from vegetative to floral are often gradual and intermediate in the inflorescence (Cutter 1957*a*, 1957*b*; Endress 1977, 1994*a*, 1994*b*, 2003; Buzgo et al. 2004*b*; but see Moseley et al. 1993). For example, in *Amborella trichopoda*, a subdecussate phyllotaxy can change to (1) an alternate phyllotaxy in vegetative shoots, (2) a unidirectionally trimerous phyllotaxy in the higher orders of the inflorescence branches and at the base of flowers, or (3) a spiral phyllotaxy in the flower (Buzgo et al. 2004*b*).

The bud scales are attributes of the winter bud main axis (order 0) and are proximally similar to foliar leaves in the appearance of a remnant of the lamina. In contrast, the bracts along the lateral shoots of the inflorescence (order 1 and higher) lack a distinct lamina and appear to consist of the hypophyll only (Unterblatt; Troll 1939; Roth 1949). This similarity of outer bud scales to juvenile cataphylls has also been reported for *Umbellularia* (Lauraceae; Kasapligil 1951). In the tribe Laureae, the outer bud scales characteristically persist as an involucrum (Chanderbali et al. 2001; in all other members of Lauraceae, an involucrum has been considered to be absent). However, distally in the winter bud (order 0), the bud scales subtending the lateral inflorescences also lack a distinct lamina and appear to consist of the hypophyll only, similar to the membranous bracts. Is this because the main shoot is reduced to a smaller inflorescence shoot? Above this, the main shoot returns to a fate as a vegetative shoot, resuming the production of foliar leaves. Candidate genes for the regulation of this shoot determination are genes responsible for shoot meristem maintenance, e.g., the *CLAVATA* complex (Crone and Lord 1993; Clark 2001; Fletcher 2002) and *WUSCHEL* (Clark 2001); genes responsible for the determination of meristem identity (floral determination), e.g., *LEAFY* (Schultz and Haughn 1991; Shannon and Meeks-Wagner 1991; Weigel 1998; Clark 2001); and genes of the A and C classes (Coen and Meyerowitz 1991; Soltis et al. 2006).

The flowers of *P. americana* are clearly distinct from the inflorescence. We found no gradual transition between extrafloral bracts and tepals, in contrast to some other magnoliids (Endress 1987, 1990, 1994*b*), basal monocots (Buzgo and Endress 2000; Buzgo 2001), basal eudicots (von Balthazar and Endress 2002), and basal angiosperms (Endress 1980*c*, 1983, 2001; Endress and Igersheim 2000; Buzgo et al. 2004*b*). In contrast to tepals, bracts of *P. americana* develop axillary meristems and are separated from the flower by a distinct plastochron and internode (pedicel). We did not find any floral organs to be initiated as a spiral. Our observation of a whorled initiation of perianth organs differs from descriptions of the trimerous flowers of *Umbellularia*, where sepal initiation was described as following in short sequence, i.e., not in a whorl in the strict sense but in a spiral with extremely short plastochrons (Kasapligil 1951).

Trimerous flowers occur throughout basal angiosperms (e.g., Nymphaeales, Magnoliaceae, Annonaceae, Piperales), as well as in monocots and some basal eudicots (e.g., Berberidaceae), and trimery may be correlated with the formation of whorled floral organ phyllotaxy (instead of spiral; Endress 1994*a*; Buzgo et al. 2004*b*). In *Amborella*, the transition from subdecussate phyllotaxy of the inflorescence to a seemingly trimerous outermost whorl was described in detail (Buzgo et al.

distinct lamina portion forming (*f*; *s** indicates a leaf primordium with unknown fate above the insertion of the foliar leaf); side view. *G–K*, Developing flowers of *Persea americana*, SEM. *G*, Young flowers, order 3, tepal initiation, slightly unidirectional (adaxially delayed, order-2 shoot removed), floral SAM is plane, receptacle thickened, apical view. *H*, Young flower, order 3, initiation of first and second stamens; adaxially (asterisk) delayed, apical view. *I*, Young basal lateral flower, order 2, with bract, first tepals, and one second stamen removed, all stamens initiated, with staminode just initiated (arrowhead) using almost all of the floral SAM, oblique side view. *J*, Young basal lateral flowers, bracts removed, abaxial view. *K*, Young flowers, lateral-basal, stamens exposed, bracts and abaxial tepals removed, oblique side view. f = foliar leaves; m = abaxial membranous bracts; a_1–a_4 = stamen whorls and staminodes; p = prophylls; r = receptacle; s = winter bud scales on order-0 shoot, subtending order-1 axillary shoots; t_1 = outer tepals; t_2 = inner tepals; x = floral shoot apex; 0 = apical meristem; *1–3* = orders of shoots. Scale bars = 0.1 mm.

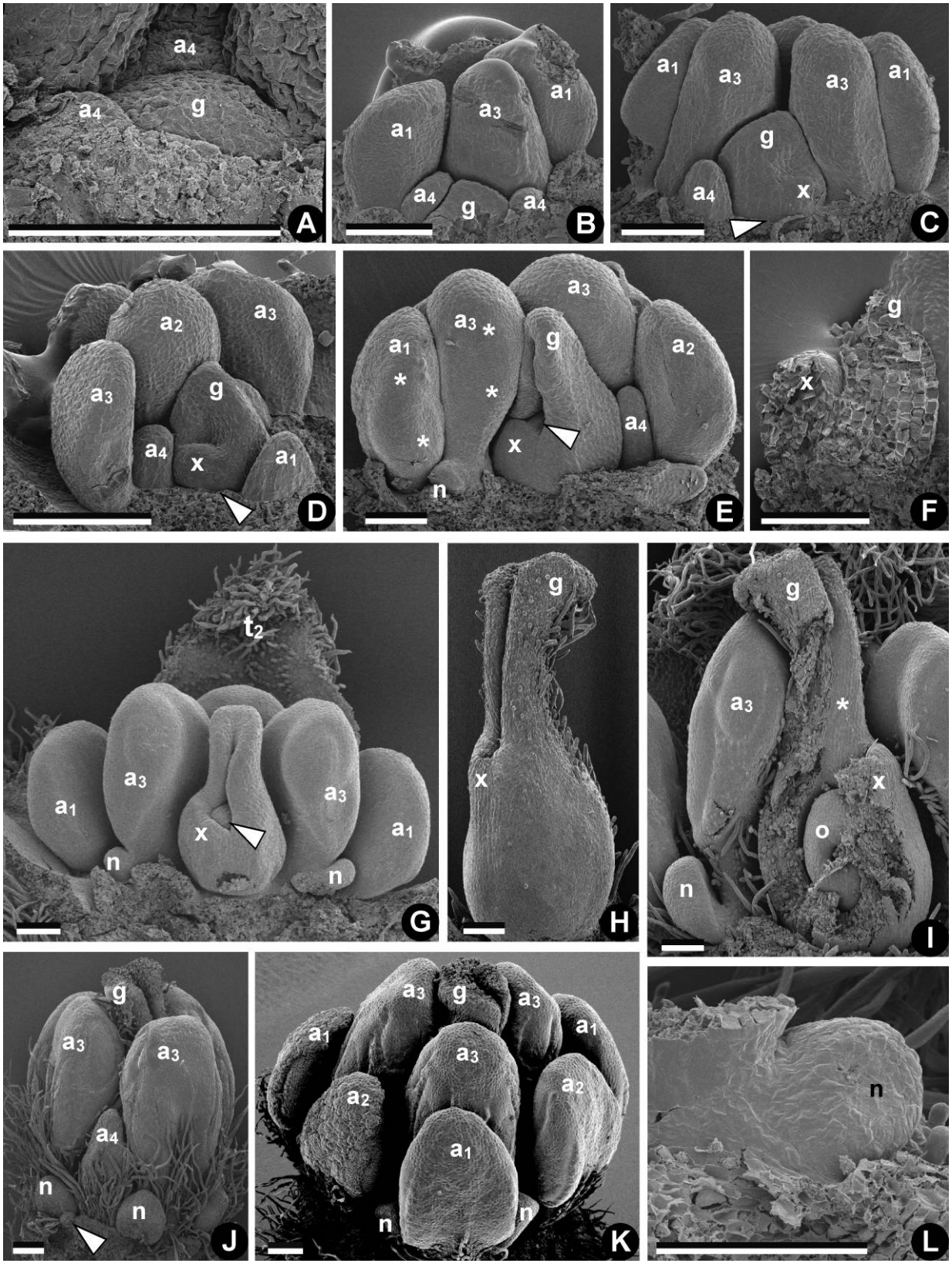

Fig. 6 Flower development of *Persea americana*, gynoecium and sporogenesis; all lateral view and SEM, where not stated differently. *A*, Young flower, perianth and some stamens removed, initiation of staminodes (a_4) and carpel (g). *B*, Primordia of staminodes and carpel just rising; pollen sacs outside not visible (but megaspores inside; not shown). *C*, Third-whorl stamen development (pollen sacs not yet visible), carpel now with a

Table 2

Schedule of Anthesis

	March 16, 2005	March 17, 2005	March 17, 2005	March 18, 2005
Weather	Cloudy, cool	Cloudy, warm	Cloudy, cool	Cloudy, cool
Time	0800 hours	0800 hours	1400 hours	1400 hours
Flower opening		Next day	Same day	Next day
Perianth (inner and outer tepals)	Open, margins stained	Inner tepals elongating, bud still tight; not stained	Open or closed; margins not stained	Outer tepals starting to gap, along with outermost stamen elongation; inner tepals closed, elongating; margins stained
Outer stamens	Close to gynoecium; filament stained and some dots on anthers (valve hinge), but pollen sacs closed	Close to gynoecium; pollen sacs/stoma stained, closed	Stamens spreading; anther valves stained, opening	Filament elongating, some outside of inner tepals; some pollen sacs stained
Inner stamens	Close to gynoecium, not stained; closed	Close to gynoecium; pollen sacs/stoma not stained, closed	Narrow; anther (calves) stained	Elongating
Paired staminal appendages	Dry, not stained	Wet or dry, not stained	Wet or dry, stained	Dry, not stained
Staminodes	Wet, stained	Wet or dry, not stained	Wet or dry, not stained	Dry, not stained
Stigma	Above inner stamens; stained, catalase reaction (+)	Above inner stamens; stained, catalase reaction (+)	Above inner stamens; stained, catalase reaction (+)	At inner stamens; stained; catalase reaction (+)

Note. "Stained" means stained by neutral red.

2004*b*): Distally in inflorescences of *Amborella*, plastochrons are short, which would result in a tetramerous whorl; however, the adaxial organ is reduced along with the plastochron, rendering the whorl trimerous. In *Persea*, there is less correlation between unidirectional floral development and bractlike features of the outermost floral organs. However, in *Laurus* (Lauraceae), the transition from the subdecussate inflorescence to the dimerous flower (Kasapligil 1951; Rohwer 1993) is direct, suggesting that the phyllotaxy of the inflorescence is restrained in the flower. Both systems of phyllotaxy can coexist: in *Hernandia* (Hernandiaceae, Laurales), male flowers are trimerous (like *Persea*), and female flowers are dimerous-tetramerous (similar to *Laurus*; Endress and Lorence 2004).

It appears that the core of the inflorescence of *Persea* and most Lauraceae consists of a floral shoot (terminal flower, order 1) bearing lateral floral shoots. The similarity between flower buds and inflorescence buds in Lauraceae was noted by previous authors (Weberling 1985; Rohwer 1993). Also, in some taxa of Lauraceae, the entire inflorescence is reduced to only a single, terminal flower (*Mezilaurus*, *Litsea*, *Dodecadenia*; Weberling 1985; Rohwer 1993). Is the production of additional, lateral flowers correlated with the relative size or vigor (Thorp et al. 1993) of the terminal flower SAM? Is there a specific size threshold at which the flower meristem identity of the SAM is determined, or does the inflorescence always bear some partial flower meristem identity? A putative threshold is likely to be regulated by genes that affect the proportions of the SAM and lateral organs and the separation of inflorescence and terminal flower, such as meristem maintenance genes, e.g., *CLAVATA1*, *CLAVATA2* (Crone and Lord 1993; Clark 2001; Fletcher 2002), and *WUSCHEL* (Clark 2001), or flower identity, e.g. *TFL1*, A-class genes (*APETALA1*, *APETALA2*), and *LEAFY* (Schultz and Haughn 1991; Shannon and Meeks-Wagner 1991; Weigel 1998; Clark 2001).

distinct cross zone (*x*) above stipe (arrowhead), and a future plicate portion (triangular meristem edge [*g*] surrounding future ovary cavity). *D*, Third-whorl stamen development (pollen sacs not yet visible), carpel now with a distinct cross zone above stipe, and a future plicate portion (triangular meristem edge surrounding future ovary cavity). *E*, Third-whorl stamen pollen sacs (asterisk) now visible in latrorse orientation behind the rim of the cross zone, below the opening of the plicate fold; the curved base of the ovule is visible (arrowhead). *F*, Young carpel longitudinally opened with a plicate apex, and cross zone (primary, ovule primordium), longitudinally sectioned. *G*, Young flower, lateral view, tepals and stamens partially removed, exposing stamen of first and second whorls, third whorl and elevated paired staminal appendages (*n*), carpel cross zone not yet closed toward plicate portion, but exposing base of ovule (arrowhead). *H*, Young carpel; style has now fused secondarily (between *x* and *g*); the stigma (*g*) broadens. *I*, Paired staminal appendages of the third whorl now become stalked; young carpel longitudinally opened, exposing the normally closed style-suture (plicate portion between *x* and *g*); note the relatively broad, naked portion of the crevice (asterisk) leading into the ovary behind the cross zone and above the ovule, longitudinally sectioned. *J*, Young flower, lateral view, perianth, first and second androecial whorls removed, third-whorl stamens, basal staminal appendages (arrowhead indicates a supernumerary one). *K*, Young flower, tepals removed, thecae and stoma forming, carpel style elongating; anthers have assumed a moderate introrse (first and second whorls) and extrorse (third whorl) orientation. *L*, Third-whorl stamen base appendage rising along with stamen margin base. a_1–a_3 = stamens; a_4 = staminodes; *g* = plicate portion of carpel (style and stigma) and carpel primordium; *n* = single, abaxial staminal appendage; *o* = ovule; *x* = carpel adaxial side and cross zone. Scale bars = 0.1 mm.

Perianth

In several members of Lauraceae, a differentiation into shorter outer and longer inner perianth whorls (sepals and petals, *s.l.*, respectively) has been reported: *Anaueria*, *Caryodaphnopsis*, *Notaphoebe*, *Williamodendron*, *Cassytha* (Rohwer 1993; Chanderbali et al. 2001), some species of *Persea* (e.g., *Persea borbonia*; fig. 3*C*), *Aniba*, and *Dehaasia* (Rohwer 1993). The tepals are of equal size in *Umbellularia* but were considered dimorphic on the basis of vasculature (Kasapligil 1951). One component of sepal-petal distinction in angiosperms generally is the number of vascular strands: sepals typically have three to five vascular strands, and petals have one. In *Umbellularia*, outer tepals ("sepals") and stamens are reported to have three (sometimes four or five) strands, and inner tepals ("petals") have one (Kasapligil 1951). However, the differentiation seems highly flexible throughout *Umbellularia*, where the number of strands may be reduced in sepals and increased in petals (Kasapligil 1951). In *P. borbonia*, the differentiation of outer and inner tepals becomes pronounced only late in development (stages 6–8). What is the mechanism responsible for the distinction of outer and inner tepals? The outer and inner tepals in *P. americana* initiate in fast succession and almost in one whorl, which may explain their similarity, while in *P. borbonia*, the flower is smaller and the relative space between the inner and outer perianth primordia is larger than in *P. americana*. This implies that perianth differentiation in the family may depend on a few genes affecting the size of the floral SAM (similar to the branching of the inflorescence). The relation of the floral SAM to floral organ plastochrons and primordia size differs between *P. americana* and *P. borbonia* and may affect the downstream expression of genes responsible for organ identity. In fact, the expression patterns of *AG* and *SEP3* homologues in *P. americana* and *P. borbonia* correlate with a differentiation of the perianth: in *P. borbonia*, these genes are not expressed in the outermost whorl, which consists of smaller, "sepaloid" tepals, whereas in *P. americana*, these genes are expressed in all floral organs (Chanderbali et al. 2006). Whereas genetic studies in model organisms show a conserved role for *AG* homologues in specifying stamen and carpel identity, expression in the perianth has been observed not only in *Persea* but also in the inner tepals of *Illicium* (Kim et al. 2005). Expression of *AG* in the perianth of *Persea* may result either from retained ancestral expression, perhaps reflecting a staminal origin of tepals ("andropetals") in *Persea* and other Lauraceae, or from expansion of *AG* expression into the perianth (see Chanderbali et al. 2006 for further discussion). The function of *AG* in the perianth, if any, is currently unknown.

Androecium and Receptacle Expansion

In several members of Laurales (below) and other basal angiosperms (Buzgo et al. 2004*b*), the receptacle is extended at anthesis or fruit development. It then forms a concave cup bearing perianth and stamens (hypanthium) or a collar or cup-shaped ring at the base of the gynoecium or fruit (cupule; Rohwer 1993). Although the occurrence of such a concave receptacle is considered a synapomorphy for Laurales, it must have been lost in some members (Renner 1999; Renner and Chanderbali 2000; Chanderbali et al. 2001). We propose below that the formation of an expanded floral SAM is related to the formation of a concave receptacle in Lauraceae (or even Laurales).

During the initiation of the perianth in *P. americana* and *P. borbonia*, the floral SAM expanded faster than organ initiation proceeded and reached its maximum just before stamen initiation. Thereby, a bare plane was formed in the center of the flower, accompanied by the dilatation of the tissue below, resulting in an expanded receptacle, on which the stamens were initiated. In *P. americana*, and even more pronouncedly in *P. borbonia*, the expansion of the receptacle then slowed down, and organ initiation caught up and consumed the SAM. However, a subsequent intercalary growth, or simple cell expansion, would be sufficient to form a concave receptacle (as in *Amborella*; Buzgo et al. 2004*b*). The tissue required for this formation is provided by the previous dilatation of the floral SAM (fig. 5*G*, 5*H*).

Cup-shaped receptacles occur in most Laurales, except *Persea* and its allies (Chanderbali et al. 2001). In *Umbellularia*, the receptacle starts from a plateau of the floral SAM, similar to that in *Persea*, which later becomes concave, i.e., "crateriform" (Kasapligil 1951). Interestingly, the expansion of the receptacle can coincide with a semi-inferior gynoecium (*Eusideroxylon* and *Potoxylon*; Chanderbali et al. 2001) or an inferior gynoecium (*Hypodaphnis* and Hernandiaceae; Chanderbali et al. 2001). Monimiaceae and Himantandraceae are also considered to possess an "invaginated receptacle," and Eupomatiaceae are considered to possess a "hypanthium" (Endress 1977).

In basal angiosperms in general, the extension of the floral SAM and later the receptacle appears to be linked with the formation of the androecium. The androecium is often initiated as an androecial ring meristem, a torus-shaped meristem elevation that surrounds the center of the flower. This androecial ring meristem is particularly well developed in cases with a high number of organs (namely, stamens) in basal angiosperms (below; Ronse De Craene and Smets 1993) but is also found in eudicots (Ronse De Craene and Smets 1998; "ring primordium": Ronse De Craene et al. 2003). For example, the receptacle tends to be concave in *Nymphaea*, *Amborella* (male flower), *Calycanthus*, and Monimiaceae, all with many organs (typically stamens), whereas invagination is less obvious in related taxa with fewer series of organs, such as *Amborella* (female flower) and *Cabomba* (Kubitzki 1987; Buzgo et al. 2004*b*). The relationship between an expanded receptacle and a high number of stamens is also illustrated in *Nuphar* (Nymphaeaceae), although in a different manner than in other Nymphaeaceae: *Nuphar* has a convex, cone-shaped androecium and obtains an invaginated periphery by "diffuse growth" in the receptacle (intercalary; Moseley 1972). Other examples in which this *Nuphar*-like method achieves an expanded receptacle are *Eupomatia* (Eupomatiaceae), *Duguetia* (Annonaceae), and other Annonaceae (with a distinct androecial ring, "Androecialringwulst," a gynoecium cone, "Gynoecialkegel," and a broad receptacle with spiral phyllotaxy; Endress 1977). In Winteraceae (Canellales), the receptacle and sepal bases are involved in androecial development (Doust 2000). *Tasmannia* possesses a ring meristem with irregular stamens. The terminal flower of *Drimys* differs from

the lateral flowers in having a ring meristem, whereas lateral flowers lack a ring meristem (Endress 1977).

Two questions arise regarding organ identities in the androecium: (1) Is there a sharp or gradual transition between perianth and androecium, and is there a pattern of transition within the androecium? (2) What do the paired staminal appendages represent, i.e., what are their homologues in other organs and other taxa?

1. Transition between perianth and androecium. In *P. americana*, the transition from perianth to androecium is abrupt. The androecium was separated from the perianth by the distinct plastochron before androecial initiation and by the more narrow shape of the stamen primordia. This distinction allowed the first two androecial whorls to initiate in very fast succession, as if almost in one whorl, emphasizing the separation between tepals and stamens. Interestingly, the third androecial whorl again followed a distinct plastochron, as did the fourth whorl (staminodes) and then the carpel. The delay of organ initiation is parallel to changes of stamen features: paired appendages (androecial whorl 3) and sterilization (innermost androecial whorl). Is this delay the result of the reduction of the SAM? Does it render the change of stamen features gradual (rather than distinct)? Does the delay point to a change of organ identity, along with the change of morphological and functional features?

Apparently, there is some flexibility in terms of whorl numbers and organ features among Lauraceae. The pattern of three whorls of fertile stamens and consistently one innermost whorl of staminodes is common among Lauraceae (*Persea* group; Chanderbali et al. 2001). However, in *Umbellularia*, occasionally up to five whorls were found in the androecium, the innermost generally consisting of staminodes (Kasapligil 1951).

The two outermost stamen whorls and the third whorl are not very different at initiation. In most Lauraceae, the two outer whorls of the androecium are introrse, whereas the third whorl is extrorse (Endress and Hufford 1989; Chanderbali et al. 2001). We found that all pollen sacs initiated in a latrorse manner and that the extrorse position of the stoma consolidates only later (stage 7), as the abaxial side of the connective remains narrow while the thecae expand (although the pollen sacs in the third whorl initiated in a more latrorse position than those of the two outer whorls). The third whorl of stamens was accompanied by two staminal glands at the base. However, they arose only late in development, and similar structures were found occasionally in the outermost stamens and tepals. Staminodes of the fourth androecial whorl initiated after another distinct plastochron. The sterility of this whorl is probably the result of a reduction of the entire filament-anther complex. This may simply be the result of a reduction of SAM capacity, resulting in fewer or smaller organs (vigor; Thorp et al. 1993). However, it may also be the result of an overlapping of complementary developmental signals: the organ identity of the supposedly reduced filament-anther portion is not clear because it occasionally can develop into a style and stigma–like structure.

These observations indicate that organ features change gradually. It is likely that the size of the floral SAM determines the effect of genes regulating organ identity and specific features.

2. Paired staminal appendages. What do the paired staminal appendages represent, i.e., to what structures in other organs, if any, are they homologous? The paired appendages occur in many members of Laurales and are nectariferous structures like inner staminodes. The correlation of staminodes and nectar production in Lauraceae was noted in previous papers (e.g., Kasapligil 1951; Endress and Lorence 2004), including a review of the morphological and evolutionary significance of staminodes by Ronse de Craene and Smets (2001). In *Litsea*, the paired staminal appendages are elevated on the filament (Endress and Hufford 1989), similar to intermediate stages between stamen and staminodes observed by Kasapligil (1951). It is interesting to compare the nectar glands to similar, paired staminal appendages in male flowers of other Laurales. Monimiaceae also have paired staminal appendages, superficially looking similar to pollen sacs but disconnected from anthers (Endress and Hufford 1989). In *Peumus*, the two staminal appendages are at the base of the stamen, with a broad insertion. In *Hernandia* (Hernandiaceae, Laurales; Endress and Lorence 2004), three small basal protrusions develop in an alternate position with the stamens; these appendages correspond to staminodes or stamens. Female flowers have four small basal protrusions that develop in alternation with the tepals; these protrusions are similar to the protrusions in male flowers and occupy the position of stamens. These mounds develop later than do other floral organs (Endress and Lorence 2004). In female flowers, the staminodes are considered nectariferous structures (Endress and Lorence 2004). In male flowers, nectar was reported sporadically, but its production could not be reconfirmed (Endress and Lorence 2004). In male flowers, the tissue of mounds and appendages does not appear secretory (P. K. Endress, personal communication), although the vascular supply of the appendages is significant (Endress and Lorence 2004).

In Lauraceae, the paired stamen glands are consistently restricted to the third whorl of stamens, although exceptions occur in *P. americana* (outer aberrant structures; this study), *Laurus* (Kasapligil 1951), *Brassiodendron*, *Chlorocardium*, *Phyllostemonodaphne*, and *Urbanodendron* (Rohwer 1993, 1994). The paired staminal appendages with a stalk and a yellow, glandular, apical portion are similar to fertile stamens, with their filament and anther, and to staminodes because both yellow apical portions are putative nectariferous structures. Further, in this study, both fourth-whorl staminodes and the third-whorl paired staminal appendages appeared relatively late in floral development. What is the relationship between fourth-whorl staminodes and the third-whorl paired staminal appendages in terms of organ identity and homology? Do the staminal glands correspond to individual stamens? Does the yellow nectariferous structure in paired staminal appendages correspond to the nectary gland in staminodes? Do these nectariferous structures, or one of them, correspond to an anther (or microsporangium)? Or are the staminal glands accessory emergences of the third-whorl stamens?

Our developmental studies suggest that the staminal glands are not independent structures but attributes of the third-whorl stamens, in agreement with earlier interpretations of the staminal glands as emergences in *Umbellularia* and *Laurus* (Kasapligil 1951). The staminal glands are found on a common base with the third-whorl stamens. Initiation of the staminal

glands is on the base of the stamen, not directly on the receptacle. The paired staminal appendages appeared later than the innermost staminodes. This position and timing alone are not sufficient to deny the structures an identity as stamens on their own because stamens or androecial meristems may give rise to accessory stamens by fasciculation or branching, often in a centrifugal pattern (the outer stamens appear later than the inner ones, contrarily to the expected direction of organ initiation along the floral shoot axis; Rohwer 1993; Ronse De Craene and Smets 1993, 1998; Ronse De Craene et al. 2003). Indeed, polyandry does occur in Lauraceae, e.g., in *Actinodaphne*, *Chlorocardium*, *Cinnadenia*, *Dodecadenia*, *Laurus*, *Lindera*, and *Litsea* (Rohwer 1993, 1994). However, in light of these examples, polyandry is probably obtained by the increase of floral whorls, not by fasciculation or centrifugal initiation of stamens, and is therefore different from the seemingly centrifugal initiation of staminal appendages (Ronse de Craene et al. 2003). Also in *Persea*, no distinct ring primordium or initial androecial meristem was detected from which stamen fasciculation could derive. In other Laurales with higher numbers of stamens (Monimiaceae, Calycanthaceae), organs initiate in a continuous spiral, not by fractioning meristems or primordia (Ronse de Craene et al. 2003).

We found cases with more than one staminal appendage at the base of the stamen. In these cases, two staminal appendages appeared in one series along the side of the stamen base, sometimes sessile or on a very short stalk, similar to pinnae of a compound leaf (not shown); sometimes, this subdivision of lateral appendages occurred in the yellow gland of staminodes, above the stalk (which could correspond to pollen sacs, in contrast to the interpretation by Kasapligil [1951]; see below). On the basis of our observations, paired staminal appendages are likely to represent emergences of the stamen margin but with some relation to pollen sacs (partial homology).

In male flowers of *Laurus nobilis*, all stamens normally bear paired staminal appendages (Kasapligil 1951). Support for the paired staminal appendages as marginal emergences also comes from teratologies in inner-whorl tepals and outer-whorl stamens in *P. americana* (this study). At the base of the margins of inner tepals, or up to 2 mm above it, yellow knots were found; the tepal body may correspond to the filament (base of tepals) and the connective. Apparently, the putatively nectariferous structures are structures of the margin and may be related to the indication of secretion of the tepal margin by neutral red staining (this study). In tepal-like structures of other basal angiosperms, pollen sacs or their remnants are found on the adaxial surface of the structure rather than along the margins (*Amborella*, Buzgo et al. 2004*b*; "laminar stamens" in *Austrobaileya*, Endress 1980*c*, 1994*a*; also Annonaceae and *Calycanthus*), which again suggests that the staminal appendages do not correspond to pollen sacs (or stamens) but represent independent marginal structures. It is noteworthy that in *Amborella*, *Austrobaileya*, and *P. americana*, the outer staminodes differ strongly from the normal, inner staminodes: instead of a reduced connective, their connective is enlarged, representing the tepal scale (tepal in the strict sense), and the filament and stalks of the glandular appendages are absent. In Lauraceae, outer staminodes are also found in *Dicypelium* and *Phyllostemonodaphne* (Rohwer 1993, 1994).

How do the staminodes compare with stamens and staminal appendages? Staminodes look similar to stamens, including the anther (yellow apical gland), but secrete nectar like staminal appendages. *Laurus* exhibits a reduction series (Kasapligil 1951), with stages ranging from fertile stamens bearing staminal appendicular glands to staminodes with unified apical glands. Intermediate stages include staminodes on which the apical gland is split into two, similar to the staminal appendages. From between parts of this split gland, the actual filament, sometimes bearing an anther, protrudes. The yellow gland of staminodes therefore appears to correspond to the two congenitally fused staminal appendages; the acute tip of the normal staminodes corresponds to the actual stamen (filament and anther). On the basis of this homology assessment in *Laurus*, we recognize a gradual transition from occasional staminodes and tepals (filament fused with appendage stalk and short, appendages and sporangia reduced, connective expanded) to outer stamens (filament elongate, appendages reduced, sporangia differentiated, connective narrow and short), inner stamens (filament elongate, appendages differentiated and freely stalked, sporangia differentiated, connective narrow and short), and staminodes (filament fused with appendage stalk and short, appendages differentiated and fused, sporangia reduced, connective reduced; fig. 7).

Surprisingly, in some staminodes of *P. americana*, we found that the acute apex sometimes obtains the shape of a style and a rudimentary stigma (figs. 4*D*, 7*E*); similar organs showing features of stamen and carpel were reported in other studies (e.g., Endress 1972*b*). This sporadic occurrence of staminal and carpellary features supports the view that staminal features gradually change across the entire flower, including the perianth and carpel. It also implies that carpel and stamen are fundamentally homologous organ identities (details on corresponding parts in the following paragraphs), in agreement with similar findings in *Amborella* (Buzgo et al. 2004*b*). In *Amborella*, connective tips correspond to the abaxial portion of the stigma and are located above the anther, which corresponds to the ovary.

We described above why the staminal appendages may be better considered marginal appendages than independent stamens. However, including the aberrant staminodes of *Persea* in a comparison of organ parts will confound this simple, and preliminary, inference. Some of the staminode glands showed a subdivision into elements similar to pollen sacs, and some even exhibited a differentiated internal tissue similar to sporogenous tissue. Are the nectariferous staminal appendages related to pollen sacs after all?

The homology of the staminal appendages remains ambiguous: they share features and position with pollen sacs and with the rim of the style and stigma (below). Therefore, they may originate from sites corresponding to the basal margin of tepal, stamen, and style, including the secondary cross zone. Antherlike paired staminal appendages with sterile thecae, including valvate stomia, were also reported in other studies (Saunders 1939; Kasapligil 1951). Also, reduction of the nectariferous structures appears to parallel pollen sac reduction (from four to two), and the number of lateral vascular strands correlates with the pollen sacs and paired staminal appendages (Kasapligil 1951). In fully developed stamens of *Umbellularia*, five vascular strands were found, three central ones that are grouped together lead into the anther, and two lateral ones

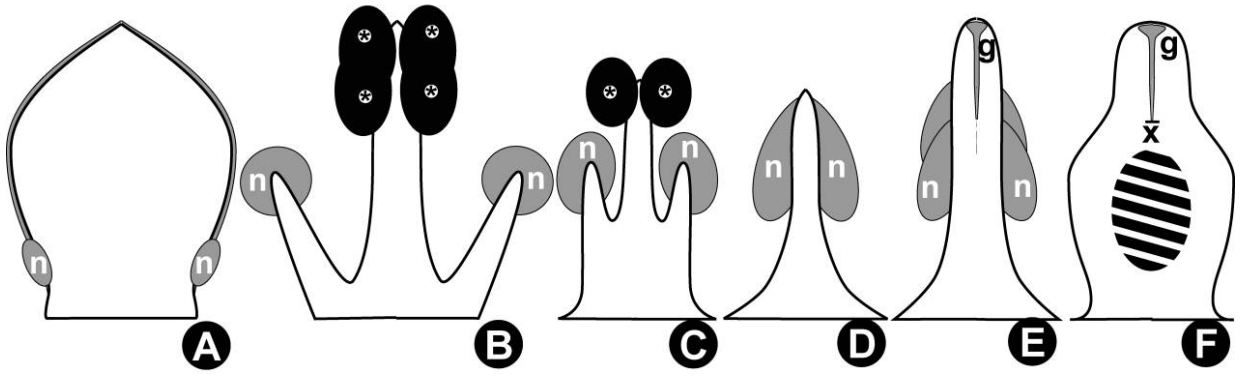

Fig. 7 Schematic comparison of floral organs of *Persea americana*, all adaxial view. *A*, Tepal with basal putatively nectariferous structure. *B*, Stamen of the third androecial whorl with four pollen sacs and staminal appendages. *C*, Intermediate stamen with a reduced central portion (two pollen sacs, staminal glands still separate; after Kasapligil 1951). *D*, Staminode of the fourth androecial whorl, nectaries united and central portion reduced to a small tip. *E*, Abnormal staminode with stigmalike structure formed by the tip (corresponding to fig. 4*D*). *F*, Carpel; the ovule is marked by a pattern indicating its location behind the adaxial ovary wall (cross zone); white = portion corresponding to filament, connective, ovary wall; gray = secretory tissue; black = sporogenous tissue; striped black region = ovule inside of ovary cavity (hidden by cross zone); *g* = style and stigma; *n* = staminal appendage or nectariferous structure; *x* = cross zone; asterisk = pollen sac.

each lead into a lateral nectariferous structure. Reduction of pollen sacs was accompanied by dissociation of the three central strands and loss of one or two central strands. Likewise, reduction of the nectariferous structures was accompanied by the loss of the corresponding lateral strands. It is unclear in cases of pollen sac reduction in *Persea* whether the proximal pollen sacs (Rohwer 1993, 1994) or the distal ones (Endress and Hufford 1989) are reduced. Pollen sac reduction is frequent in Lauraceae and may even coincide with the evolution of anther openings as "circular or elliptic flaps" (suggested for Monimiaceae by Endress and Hufford 1989), involving the reduction of pollen sacs to two and then an increase to four (now independent ones). However, we found no report of more than four sporangia per anther that could point to the staminal appendages being "additional, vestigial pollen sacs" (*sensu* Endress and Hufford 1989). In *Persea*, neutral red staining occurred in tepal margins, anthers, staminal glands, staminode glands, and stigma. Similar staining was also observed in *P. borbonia* and *Cinnamomum camphora* (not shown). In other basal angiosperms, secretory activity has been reported for stamens (nectariferous structure in *Illicium*; White and Thien 1985; Williams et al. 1993; M. Buzgo, personal observation; basalmost angiosperms, magnoliids, basal eudicots, and former "hamamelidids"; Endress and Hufford 1989) and particularly anthers (including odor secretion; Vogel 1963, 1990; Buzgo 1998).

Therefore, even if paired staminal appendages do not correspond to entire additional stamens, it appears that the staminal glands are related to pollen sacs. Homology of entire organs (on the basis of positional and structural homology) may not stand up to a thorough assessment of features that define organ identity. Some part of development is shared between organs, and it cannot be determined at this point whether these shared features are due to an ectopic effect (outside of its original range) or a reflection of evolutionary developmental history (process homology; Sattler 1984, 1988).

It is important to test whether genes responsible for anther differentiation are expressed in the sterile antherlike structures, such as the glands of staminal appendages and staminodes. How do gene expression patterns in sterile antherlike structures compare with those in fertile anthers, and do they differ from those in the basal stalk of the paired staminal appendages and filaments? Candidates for developmental genetic studies are transcription factors, such as B-class MADS-box genes (orthologues of *Arabidopsis APETALA3* and *PISTILLATA*) and C-class genes (orthologues of *Arabidopsis AGAMOUS*), and genes involved in microsporogenesis in other angiosperms (Pelaz et al. 2000; Zhao et al. 2001). B-class genes are correlated with the formation of structures associated with petals and stamens (color, tissue consistency, thin basal insertion, possibly odor or other secretion) (Hill and Lord 1989; Coen and Meyerowitz 1991; Jack et al. 1992, 1994; Schwarz-Sommer et al. 1992; Tröbner et al. 1995; Zachgo et al. 1995; Cacharrón et al. 1999; Jenik and Irish 2001; Kramer et al. 2003). Differential activity of B-class genes within anthers, the tips of paired staminal appendages, and staminodes could be involved in the observed morphological similarities. For example, in *Aquilegia* (Ranunculaceae), differential expression of *APETALA3* paralogues may be responsible for the differences between sepals and petals (Kramer et al. 2003, 2004), although sepals of *Aquilegia* are also partially petaloid. Both petals and stamens are often secretory or olfactory, and secretion may be regulated by B-class genes in these organs. C-class genes play a specific role in the regulation of sexual functions (sporophores, stamens, and carpels), in contrast to perianth organs, where C-class genes are typically not expressed, at least in eudicot models (Yanofsky et al. 1990; Mizukami et al. 1996; Riechmann and Meyerowitz 1997; Kang et al. 1998). However, in *Persea*, homologues of *AGAMOUS* are also expressed in the perianth and receptacle (Chanderbali et al. 2006). Is *AGAMOUS* (the "C-class" homologue) responsible for these "stamen-petal" features in *Persea*? Does the reduced activity of *AGAMOUS* homologues cause the sterility in the antherlike staminodes, or are *AG* homologues expressed even in the glands of staminal appendages?

Carpel

Carpel initiation and putative pseudomonomery in Laurales were compared by Endress and co-workers (Endress 1972*b*, 1994*a*; Endress and Lorence 2004; "pseudomonomery" is defined as a gynoecium consisting of seemingly only one carpel but actually representing several carpels merged and partially reduced). Important findings are that Lauraceae (including *Persea*) are truly unicarpellate, not pseudomonomerous, and that the singular carpel forms terminally on the floral SAM, not laterally (Endress 1972*b*, 1994*a*; Endress and Lorence 2004). This corresponds to the findings of this study: the primordium of the carpel first formed a cylindrical, massive body, resulting in a constriction below the ovary. The cross zone delimiting the ascidiate portion was formed from a tissue that is above the stipe and therefore separate from the receptacle; the cross zone connected the initially separate bases of the two lateral margins. In *Persea*, the carpel primordium entirely consumed what was left of the floral SAM, and no initial horseshoe shape could be detected. This differs from descriptions of *Umbellularia* (Kasapligil 1951), where the carpel was interpreted as clearly lateral and forming a horseshoe-shaped primordium at initiation, partially surrounding the shoot apex.

The cross zone in ascidiate carpels plays an important role, primarily giving rise to the ovule; hence, it corresponds to the sporogenic tissue. A secondary outgrowth will "overtop" it and form the adaxial stigma margin. Although we agree with the developmental pattern of the carpel described by Endress (1972*b*, 1994*a*), we do not consider the adaxial stigma surface to be fundamentally different from the ovule-bearing placenta; the tissue appears rather continuous, as the adaxial stigma margin in *Persea* is very close to the base of the funiculus. Endress (1972*b*) noted the continuity between the cross zone and the lateral margin of the carpel, forming the seam of the plicate style and stigma. In *Amborella*, the cross zone of the carpel is also continuous with the lateral portions forming the stigma and corresponds to the sporogenic tissue in both carpels and stamens (Buzgo et al. 2004*b*).

Carpel development of Laurales has been described in detail (Endress 1972*b*; reviewed by Endress [1972*a*, 1994*a*]; Endress and Igersheim [1997]). Some have considered carpels as derived from simple lateral organs ("leaves"; Hagemann 1970; Endress 1972*b*). According to Endress, in Laurales the apex of the carpels is radial, and in Lauraceae the base is as well (Endress 1972*b*, 1994*a*). The unifacial carpel apex is directly derived from a primordium before its dorsiventral polarization and is therefore similar to the precursor tip in monocot leaves (Rudall and Buzgo 2002). Endress notes that edges or rims in carpels can form in several locations and may not correspond to "leaf margins" in the conventional sense (Endress 1972*b*), as in *Amborella* (Buzgo et al. 2004*b*), where the lateral edges of the carpel (stigma) and stamen (outer pollen sac) correspond to the formation of the adaxial surface in tepals, not the actual leaf margin. This flexibility of edge formation and delimitation of dorsiventrality makes it difficult to assess the homology of marginal or submarginal structures, such as ovary (primary cross zone vs. secondary cross zone), pollen sacs (margin or adaxial), nectariferous structures, and staminal appendages.

In agreement with Endress (1972*b*, 1994*a*), the carpel primordium initially appeared as a solid body in a pyramidal shape, responding to the space conditions between the stamens and staminodes. However, when the tip of this pyramid reached the level of the anthers, it formed a triangular meristematic edge ("Ringwulst" in Endress 1972*b*). Thereby, it lost its radial symmetry: the largest point of the pyramid became the carpel apex (future stigma), the two other corners became the bases of the lateral edges of the carpel (style), and the side opposite the highest corner started to proliferate as cross zone. There was no enlarged carpel apex that would correspond to a radial, unifacial precursor tip as found in monocot leaves (Rudall and Buzgo 2002) and described for the carpel apex of some Lauraceae (Endress 1972*b*).

There is a transition between staminodes (androecium) and gynoecium. In other basal angiosperms, the differentiation between carpel and stamen is also flexible. In *Zygonium* (Winteraceae), the last whorl contains both stamens and carpels (Doust 2000). In *Tasmannia* (Winteraceae), the gynoecium is distinct from the androecium (meristem ring); however, carpels replace (i.e., take the position of) stamens in *Tasmannia lanceolata* (Doust 2000). In *Nuphar* (Nymphaeaceae), stamens have been reported to emerge from carpel walls (Moseley 1972). A gradual transition from bract to tepal, staminode, stamen, and carpel was found in *Amborella* (Buzgo et al. 2004*b*). A similar series of transitions can also be seen in *P. americana*.

In a comparison of all floral organs of *P. americana* (table 3), the solid, constricted carpel base (stipe) corresponds to the basal filament (including the appendage stalks and the filament-like base of staminodes) and the base of tepals. The correspondence of nectariferous tissue (staminal appendages and staminodes) and the staminode tip is ambiguous. The nectariferous tissue plus the filament-connective-like tissue between the two glands of the staminode correspond to the ovary of the carpel; the nectary glands proper as adaxial portion correspond to the primary cross zone bearing the ovule. We also found a strong similarity between the yellow nectary glands and the pollen sacs of a functional stamen. However, in staminodes, the acute tip also represents the modified pollen sacs (actually, filament and anther; Kasapligil 1951). In tepals, as suggested by the mutants noted here, the adaxial surface may correspond to the pollen sacs. The abaxial wall of the ovary corresponds to the connective of stamens, the stalk, and the acute tip of staminodes and the main portion of the tepal (tepal *s.s.*). The style rim and the stigma are represented by the acute staminode tip and possibly the tip of the tepals. A cylindrical, massive carpel primordium, a constriction below the ovary (stipe), a stigma formed by the apex of the carpel margin, and an ascidiate ovary are also found in carpels of other basal angiosperms and are likely to represent plesiomorphic character states for angiosperms (Buzgo et al. 2004*b*).

In previous discussions of the origin (organ identity) of carpels, two concepts have been considered: the carpel as a simple leaf (sporophyll, "phyllospory") or the carpel as a compound organ, the ovule borne on a shoot axis ("stachyspory"; Endress 1972*a*, 1972*b*). Of importance is the formation of cross zones at diverse positions on "leaves" and the median position of the ovule on the cross zone (Endress 1972*b*). In Lauraceae, edges or rims on carpels can form in several locations and may not correspond to "leaf margins" in the conventional sense (Endress 1972*b*). The carpel is considered

Table 3

Comparison of Corresponding Portions between Organs

Carpel	Staminode	Abnormal staminode	Stamen	Tepal
Style and stigma	Tip	Style and stigma and filament and anther	Filament and anther	Margin, tip
Primary cross meristem and ovule	Glands	Pollen sac–like glands	Pollen sacs and glands	Glands or adaxial surface
Abaxial wall	Stalk to tip	Stalk and portion between nectaries	Connective	Body
Stipe	Glandular stalk	Glandular stalk	Filament and glandular stalk	Base

Note. Note the ambiguous situations in "Abnormal staminode" (first row) and "Stamen" (second row).

primarily peltate (Endress 1972*b*), with an adaxial cross zone, which then raises the question, Which portion of the leaf forms the cross zone? In monocots, the formation of cross zones and the leaf tip is linked and complex (Rudall and Buzgo 2002). The apex of the carpel (i.e., stigma) also exhibits similarities to the precursor of monocot leaves (Endress 1972*b*) and can be extended to *Amborella* (Buzgo et al. 2004*b*) and *Persea* (Endress 1972*b*; this study). Recent studies of floral developmental genetics and carpel development in particular have paid great attention to transcription factors of the *YABBY* gene family because they are involved in leaf polarity (Bowman 2000; Golz et al. 2004; Yamada et al. 2004; Fourquin et al. 2005; Lee et al. 2005; Scutt et al. 2006).

As detailed floral developmental series for basal angiosperms accumulate, similarities and differences are beginning to emerge. For example, similarities between stamens and carpels and associated homologies of their structures are now evident, as are transitional features between perianth and androecial organs. Such patterns are important for ultimately inferring plesiomorphic angiosperm features and for generating and testing hypotheses relating morphology to gene function.

Acknowledgments

We would like to thank Jack B. Fisher (Fairchild Tropical Garden, Miami, FL), Peter K. Endress (University of Zurich, Switzerland), and Richard Abbott (University of Florida, Gainesville) for providing us with plant material. We thank Samuel Salazar-Garcia (Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias/Campo, Santiago Ixcuintla, Nayarit, Mexico), Hong Ma, Claude W. dePamphilis, and John E. Carlson (all of Pennsylvania State University, State College), James Leebens-Mack (University of Georgia, Athens), Elena M. Kramer (Harvard University, Cambridge, MA), and Amy Litt (New York Botanical Garden, Bronx) for critical discussions; Matthew A. Gitzendanner, Paris H. Grey, and the staff of Florida's Interdisiplinary Center for Biotechnology Research ICBR Electron Microscopy Laboratory for technical assistance; and two anonymous reviewers for their critical and helpful review. This study is part of the Floral Genome Project and would not have been possible without its support (National Science Foundation grant PGR-0115684 and supplement).

Literature Cited

Albert VA, DE Soltis, JE Carlson, WG Farmerie, PK Wall, DC Ilut, LA Mueller, et al 2005 Floral gene resources from basal angiosperms for comparative genomics research. BMC Plant Biol 5:5.

Angiosperm Phylogeny Group II 2003 An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot J Linn Soc 141:399–436.

Bessey CE 1915 The phylogenetic taxonomy of flowering plants. Ann Mo Bot Gard 2:109–164.

Bowman JL 2000 The *YABBY* gene family and abaxial cell fate. Curr Opin Plant Biol 3:17–22.

Boyd A 1998 Cuticular and impressional angiosperm leaf remains from the early cretaceous of west Greenland. Palaeontogr Abt B Palaeophytol 247:1–53.

Brooks DR 1996 Explanations of homoplasy at different levels of biological organization. Pages 3–36 *in* MJ Sanderson, L Hufford, eds. Homoplasy: the recurrence of similarity in evolution. Academic Press, San Diego, CA.

Buzgo M 1998 Odour differentiation in *Lagenandra ovata* (Araceae). Acta Bot Yunnanica Suppl 10:76–88.

——— 2001 Flower structure and development of Araceae compared with alismatids and Acoraceae. Bot J Linn Soc 136:393–425.

Buzgo M, PK Endress 2000 Floral structure and development of Acoraceae and its systematic relationships with basal angiosperms. Int J Plant Sci 161:23–41.

Buzgo M, DE Soltis, PS Soltis, S Kim, H Ma, BA Hauser, J Leebens-Mack, B Johansen 2006 Perianth development in the basal monocot *Triglochin maritima* (Juncaginaceae). *In* JT Columbus, EA Friar, JM Porter, LM Prince, MG Simpson, eds. Monocots: comparative biology and evolution. 2 vols. Rancho Santa Ana Botanic Garden, Claremont, CA. ALISO 22:105–123.

Buzgo M, DE Soltis, PS Soltis, H Ma 2004*a* Towards a comprehensive integration of morphological and genetic studies of floral development. Trends Plant Sci 9:164–173.

Buzgo M, PS Soltis, S Kim, DE Soltis 2005 The making of the flower. Biologist 52:149–154.

Buzgo M, PS Soltis, DE Soltis 2004*b* Floral developmental morphology of *Amborella trichopoda* (Amborellaceae). Int J Plant Sci 165: 925–947.

Cacharrón J, G Theißen, H Saedler 1999 Expression of MADS box genes *ZMM8* and *ZMM14* during inflorescence development of *Zea mays* discriminates between the upper and the lower floret of each spikelet. Dev Genes Evol 209:411–420.

Chanderbali AS, S Kim, M Buzgo, Z Zheng, DG Oppenheimer, DE Soltis, PS Soltis 2006 Genetic footprints of stamen ancestors guide

perianth evolution in *Persea* (Lauraceae). Int J Plant Sci 167: 1075–1089.
Chanderbali AS, SS Renner, H van der Werff 2001 Phylogeny and historical biogeography of Lauraceae: evidence from the chloroplast and nuclear genomes. Ann Mo Bot Gard 88:104–134.
Clark S 2001 Meristems: start your signaling. Curr Opin Plant Biol 4:28–32.
Coen ES, EM Meyerowitz 1991 The war of the whorls: genetic interactions controlling flower development. Nature 353:31–37.
Crone W, EM Lord 1993 Flower development in the organ number mutant *clavata 1-1* of *Arabidopsis thaliana* (Brassicaceae). Am J Bot 80:1419–1426.
Cronquist A 1988 The evolution and classification of flowering plants. 2nd ed. New York Botanical Garden, Bronx. 555 pp.
Cutter EG 1957*a* Studies of morphogenesis in the Nymphaeaceae. I. Introduction: some aspects of the morphology of *Nuphar lutea* (L) SM and *Nymphaea alba* L. Phytomorphology 7:45–56.
——— 1957*b* Studies of morphogenesis in the Nymphaeaceae. II. Floral development in *Nuphar* and *Nymphaea*: bracts and calyx. Phytomorphology 7:57–73.
Dong Z-C, Z Zhao, C-W Liu, J-H Luo, J Yang, W-H Huang, X-H Hu, TL Wang, D Luo 2005 Floral patterning in *Lotus japonicus*. Plant Physiol 137:1272–1282.
Doust AN 2000 Comparative floral ontogeny in Winteraceae. Ann Mo Bot Gard 87:366–379.
Doyle JA, PK Endress 2000 Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. Int J Plant Sci 161(suppl):S121–S153.
Eklund H 2000 Lauraceous flowers from the Late Cretaceous of North Carolina, U.S.A. Bot J Linn Soc 132:397–428.
Eklund H, J Kvaček 1998 Lauraceaous inflorescences and flowers from the Cenomanian of Bohemia (Czech Republic, Central Europe). Int J Plant Sci 159:668–686.
Endress PK 1972*a* Aspekte der Karpelontogenese. Verh Schweiz Naturforsch Ges Wiss Teil 1972:126–130.
——— 1972*b* Zur vergleichenden Entwicklungsmorphologie, Embryologie und Systematik bei Laurales. Bot Jahrb Syst 92:331–428.
——— 1977 Über Blütenbau und Verwandtschaft der Eupomatiaceae und Himantandraceae (Magnoliales). Ber Dtsch Bot Ges 90: 83–103.
——— 1980*a* The floral structure and relationships of *Hortonia* (Monimiaceae). Plant Syst Evol 133:199–221.
——— 1980*b* Ontogeny, function and evolution of extreme floral construction in Monimiaceae. Plant Syst Evol 134:79–120.
——— 1980*c* The reproductive structures and systematic position of the Austrobaileyaceae. Bot Jahrb Syst 101:393–433.
——— 1983 The early development of *Austrobaileya*. Bot Jahrb Syst 103:481–497.
——— 1987 Floral phyllotaxis and floral evolution. Bot Jahrb Syst 108:417–438.
——— 1990 Evolution of reproductive structures and functions in primitive angiosperms (Magnoliidae). Mem N Y Bot Gard 55:5–34.
——— 1994*a* Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge. 511 pp.
——— 1994*b* Floral structure and evolution of primitive angiosperms: recent advances. Plant Syst Evol 192:79–97.
——— 2001 The flowers in extant basal angiosperms and inferences on ancestral flowers. Int J Plant Sci 162:1111–1140.
——— 2003 Early floral development and nature of the calyptra in Eupomatiaceae (Magnoliales). Int J Plant Sci 164:489–503.
Endress PK, LD Hufford 1989 The diversity of stamen structures and dehiscence patterns among Magnoliidae. Bot J Linn Soc 100: 45–85.
Endress PK, A Igersheim 1997 Gynoecium diversity and systematics of the Laurales. Bot J Linn Soc 125:93–168.
——— 2000 Reproductive structures of the basal angiosperm *Amborella trichopoda* (Amborellaceae). Int J Plant Sci 161(suppl): S237–S248.
Endress PK, DH Lorence 1983 Diversity and evolutionary trends in the floral structure of *Tambourissa* (Monimiaceae). Plant Syst Evol 143:53–81.
——— 2004 Heterodichogamy of a novel type in *Hernandia* (Hernandiaceae) and its structural basis. Int J Plant Sci 165: 753–763.
Fletcher JC 2002 Shoot and floral meristem maintenance in *Arabidopsis*. Annu Rev Plant Biol 53:45–66.
Fourquin C, CP Scutt, M Vinauger-Douard, B Fogliani, C Dumas 2005 Evidence that *CRABS CLAW* and *TOUSLED* have conserved their roles in carpel development since the ancestor of the extant angiosperms. Proc Natl Acad Sci USA 102:4649–4654.
Friis EM, H Eklund, KR Pedersen, PR Crane 1994 *Virgininanthus calycanthoides* gen. et sp. nov.: a calycanthaceous flower from the Potomac group (Early Cretaceous) of eastern North America. Int J Plant Sci 155:772–785.
Friis EM, KR Pedersen, PR Crane 2000 Reproductive structure and organization of basal angiosperms from the Early Cretaceous (Barremian or Aptian) of western Portugal. Int J Plant Sci 161(suppl):S169–S182.
Frumin S, H Eklund, EM Friis 2004 *Mauldinia hirsuta* sp. nov., a new member of the extinct genus *Mauldinia* (Lauraceae) from the Late Cretaceous (Cenomanian-Turonian) of Kazakhstan. Int J Plant Sci 165:883–895.
Golz JF, A Hudson, M Roccaro, R Kuzoff 2004 *GRAMINIFOLIA* promotes growth and polarity of *Antirrhinum* leaves. Development 131:3661–3670.
Gomez-Lim MA, RE Litz 2004 Genetic transformation of perennial tropical fruits. In Vitro Cell Dev Biol Plant 40:442–449.
Hagemann W 1970 Studien zur Entwicklungsgeschichte der Angiospermenblätter: ein Beitrag zur Klärung des Gestaltungsprinzips. Bot Jahrb Syst 90:297–413.
Herendeen PS, WL Crepet, KC Nixon 1994 Fossil flowers and pollen of Lauraceae from the upper Cretaceous of New Jersey. Plant Syst Evol 189:29–40.
Hill JP, EM Lord 1989 Floral development in *Arabidopsis thaliana*: a comparison of the wild type and the homeotic *pistillata* mutant. Can J Bot 67:2922–2936.
Irish VF 2006 Duplication, diversification, and comparative genetics of angiosperm MADS-box genes. Pages 129–161 *in* DE Soltis, JH Leebens-Mack, PS Soltis, eds. Developmental genetics of the flower. Vol 44. Advances in botanical research. Elsevier, London.
Jack T, EM Meyerowitz, LL Brockman 1992 The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. Cell 68:687–697.
Jack T, EM Meyerowitz, GL Fox 1994 *Arabidopsis* homeotic gene *APETALA3* ectotopic expression transcriptional and post transcriptional regulation determine floral organ identity. Cell 76: 703–716.
Jenik PD, VF Irish 2001 The *Arabidopsis* floral homeotic gene *APETALA3* differentially regulates intercellular signaling required for petal and stamen development. Development 128:13–23.
Kang H-G, S An, JS Jeon, S Lee 1998 Identification of class B and class C floral organ identity genes from rice plants. Plant Mol Biol 38:1021–1029.
Kasapligil B 1951 Morphological and ontogenetic studies of *Umbellularia californica* Nutt and *Laurus nobilis* L. Univ Calif Publ Bot 25:115–239.
Kim S, J Koh, M-J Yoo, H Kong, Y Hu, H Ma, PS Soltis, DE Soltis 2005 Expression of floral MADS-box genes in basal angiosperms: implications on evolution of floral regulators. Plant J 43: 724–744.

Kramer EM, VS Di Stilio, PM Schlüter 2003 Complex patterns of gene duplication in the *APETALA3* and *PISTILLATA* lineages of the Ranunculaceae. Int J Plant Sci 164:1–11.

Kramer EM, MA Jaramillo, VS Di Stilio 2004 Patterns of gene duplication and functional evolution during the diversification of the *AGAMOUS* subfamily of MADS box genes in angiosperms. Genetics 166:1011–1023.

Kubitzki K 1987 Origin and significance of trimerous flowers. Taxon 36:21–28.

Kvaček J, H Eklund 2003 A report on newly recovered reproductive structures from the Cenomanian of Bohemia (Central Europe). Int J Plant Sci 164:1021–1039.

Lee J-Y, JL Bowman, SF Baum, J Alvarez, A Patel, DH Chitwood 2005 Activation of *CRABS CLAW* in the nectaries and carpels of *Arabidopsis*. Plant Cell 17:25–36.

Mizukami Y, H Ma, H Huang, M Tudor, Y Hu 1996 Functional domains of the floral regulator *AGAMOUS*: characterization of the DNA binding domain and analysis of dominant negative mutations. Plant Cell 8:831–845.

Moseley MF 1972 Morphological studies of Nymphaeaceae. VI. Development of flower of *Nuphar*. Phytomorphology 21:253–283.

Moseley MF, PS Williamson, EL Schneider 1993 Phylogenetic interpretations from selected vasculature characters in Nympheaceae *sensu lato*. Aquat Bot 44:325–342.

Ng M, MF Yanofsky 2000 Three ways to learn the ABCs. Curr Opin Plant Biol 3:47–52.

Pelaz S, MF Yanofsky, GS Ditta, E Baumann, E Wismann 2000 B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature 405:200–203.

Posluszny U, PB Tomlinson 2003 Aspects of inflorescence and floral development in the putative basal angiosperm *Amborella trichopoda* (Amborellaceae). Can J Bot 81:28–39.

Raharjo SH-T, RE Litz 2005 Micrografting and *ex vitro* grafting for somatic embryo rescue and plant recovery in avocado (*Persea americana*). Plant Cell Tissue Organ Cult 82:1–9.

Raven PH, DI Axelrod 1974 Angiosperm biogeography and past continental movements. Ann Mo Bot Gard 61:539–673.

Reece PC 1939 The floral anatomy of avocado. Am J Bot 26: 429–433.

——— 1942 Differentiation of avocado blossom buds in Florida. Bot Gaz 104:323–328.

Renner SS 1999 Circumscription and phylogeny of the Laurales: evidence from molecular and morphological data. Am J Bot 86: 1301–1315.

——— 2004 Variation in diversity among Laurales, Early Cretaceous to present. K Dan Vidensk Selsk Biol Skr 55:441–458.

Renner SS, AS Chanderbali 2000 What is the relationship among Hernandiaceae, Lauraceae, and Monimiaceae, and why is this question so difficult to answer? Int J Plant Sci 161(suppl):S109–S119.

Riechmann JL, EM Meyerowitz 1997 Determination of floral organ identity by *Arabidopsis* MADS domain homeotic proteins *AP1*, *AP3*, *PI*, and *AG* is independent of their DNA-binding specificity. Biol Cell 8:1243–1259.

Rohwer JG 1993 Lauraceae. Pages 426–437 *in* K Kubitzky, J Rohwer, V Bittrich, eds. The families and genera of flowering plants. Springer, Berlin.

——— 1994 A note on the evolution of stamens in the Laurales with emphasis on the Lauraceae. Bot Acta 107:103–110.

Ronse De Craene LP, EF Smets 1993 The distribution and systematic relevance of the androecial character polymery. Bot J Linn Soc 113: 285–350.

——— 1998 Notes on the evolution of androecial organization in the Magnoliophytina (angiosperms). Bot Acta 111:77–86.

——— 2001 Staminodes: their morphological and evolutionary significance. Bot Rev 67:351–402.

Ronse De Craene LP, DE Soltis, PS Soltis 2003 Evolution of floral structures in basal angiosperms. Int J Plant Sci 164(suppl): S329–S363.

Roth I 1949 Zur Entwicklungsgeschichte des Blattes, mit besonderer Berücksichtigung von Stipular- und Ligularbildungen. Planta 37: 299–336.

Rudall PJ, M Buzgo 2002 The evolutionary history of the monocot leaf. Pages 432–458 *in* QCB Cronk, RM Bateman, JA Hawkins, eds. Developmental genetics and plant evolution. Taylor & Francis, London.

Ruzin SE 1999 Plant microtechnique and microscopy. Oxford University Press, New York. 322 pp.

Salazar-Garcia S, CJ Lovatt, EM Lord 1998 Inflorescence and flower development of the 'Hass' avocado (*Persea americana* Mill) during "on" and "off" crop years. J Am Soc Hortic Sci 123:537–544.

——— 1999 Inflorescence development of the 'Hass' avocado: commitment to flowering. J Am Soc Hortic Sci 124:478–482.

Sattler R 1984 Homology: a continuing challenge. Syst Bot 9: 382–394.

——— 1988 Homoeosis in plants. Am J Bot 75:1606–1617.

Saunders ER 1939 Floral morphology. Vol 2. Heffer, Cambridge.

Schultz EA, GW Haughn 1991 *LEAFY*, a homoeotic gene that regulates inflorescence development in *Arabidopsis*. Plant Cell 3: 771–781.

Schwarz-Sommer Z, H Sommer, I Hue, P Huijser, PJ Flor, R Hansen, F Tetens, W-E Lönnig, H Saedler 1992 Characterization of the *Antirrhinum* floral homeotic MADS-box gene *DEFICIENS*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. EMBO J 11:251–263.

Scutt CP, M Vinauger-Douard, C Fourquin, C Finet, C Dumas 2006 An evolutionary perspective on the regulation of carpel development. J Exp Bot 57:2143–2152.

Shannon S, DR Meeks-Wagner 1991 A mutation in the *Arabidopsis TFL1* gene affects inflorescence meristem development. Plant Cell 3: 877–892.

Soltis DE, MW Chase, PS Soltis, PK Endress 2005 Phylogeny and evolution of angiosperms. Sinauer, Sunderland, MA. 370 pp.

Soltis PS, DE Soltis, S Kim, A Chanderbali, M Buzgo 2006 Expression of floral regulators in basal angiosperms and the origin and evolution of ABC function. Pages 483–506 *in* DE Soltis, JH Leebens-Mack, PS Soltis, eds. Developmental genetics of the flower. Vol 44. Advances in botanical research. Elsevier, London.

Suarez IE, RA Schnell, DA Kuhn, RE Litz 2004 Micrografting of ASBVd-infected avocado (*Persea americana* Mill.) plants. Plant Cell Tissue Organ Cult 80:179–185.

Takahashi M, PS Herendeen, PR Crane 2001 Lauraceous fossil flower from the Kamikitaba locality (lower Coniacian; upper Cretaceous) in northeastern Japan. J Plant Res 114:429–434.

Takhtajan A 1980 Outline of the classification of flowering plants (Magnoliophyta). Bot Rev 46:225–359.

——— 1991 Evolutionary trends in flowering plants. Columbia University Press, New York. 241 pp.

——— 1997 Diversity and classification of flowering plants. Cambridge University Press, New York. 643 pp.

Taylor DW, LJ Hickey 1996 Evidence for and implications of an herbaceous origin of angiosperms. Pages 232–266 *in* DW Taylor, LJ Hickey, eds. Flowering plant origin, evolution and phylogeny. Chapman & Hall, New York.

Thien LB, J-L Dupre, TL Sage, T Jaffré, P Bernhardt, V Pontieri, PH Weston, et al 2003 The population structure and floral biology of *Amborella trichopoda* (Amborellaceae). Ann Mo Bot Gard 90: 466–490.

Thorp TG, M Sedgley, D Aspinall 1993 Influence of shoot age on floral development and early fruit set in avocado (*Persea americana* Mill) cv. Hass. J Hortic Sci 68:645–651.

Tröbner W, Z Schwarz-Sommer, L Ramirez, P Motte, I Hue, P Huijsser, W-E Lönnig, H Saedler, H Sommer 1995 *GLOBOSA*: a homeotic gene which interacts with *DEFICIENS* in the control of *Antirrhinum* floral organogenesis. EMBO J 11:4693–4704.

Troll W 1939 Vergleichende Morphologie der höheren Pflanzen. II. Vegetationsorgane. Bornträger, Berlin. 957 pp.

——— 1964 Die Infloreszenzen, Typologie und Stellung im Aufbau des Vegetationskörpers. Vol 1. Gustav Fischer, Stuttgart. 615 pp.

van der Werff H 2002 A synopsis of *Persea* (Lauraceae) in Central America. Novon 12:575–586.

Vogel S 1963 Duftdrüsen im Dienste der Bestäubung: Über Bau und Funktion der Osmophoren. Akad Wiss Lit Mainz Abh Math-Naturwiss Kl 10:559.

——— 1990 The role of scent glands in pollination. Smithsonian Institution Libraries, Washington, DC. 202 pp.

von Balthazar M, PK Endress 2002 Development of inflorescences and flowers in Buxaceae and the problem of perianth interpretation. Int J Plant Sci 163:847–876.

von Balthazar M, PK Endress, Y-L Liu 2000 Phylogenetic relationships in Buxaceae based on nuclear internal transcribed spacers and plastid *ndhF* sequences. Int J Plant Sci 161:785–792.

von Goethe JW 1790 Versuch die Metamorphose der Pflanzen zu erklären. Ettinger, Gotha.

Weberling F 1985 Zur Infloreszenzmorphologie der Lauraceae. Bot Jahrb Syst 107:395–414.

——— 1989 Morphology of flowers and inflorescences. Cambridge University Press, Cambridge. 405 pp.

Weigel D 1998 From floral induction to floral shape. Curr Opin Plant Biol 1:55–59.

White DA, LB Thien 1985 The pollination of *Illicium parviflorum* (Illiciaceae). J Elisha Mitchell Sci Soc 101:15–18.

Williams EG, TL Sage, LB Thien 1993 Functional syncarpy by intercarpellary growth of pollen tubes in a primitive apocarpous angiosperm, *Illicium floridanum* (Illiciaceae). Am J Bot 80: 137–142.

Yamada T, M Kato, M Ito 2004 *YABBY2*-homologue expression in lateral organs of *Amborella trichopoda* (Amborellaceae). Int J Plant Sci 165:917–924.

Yanofsky MF, EM Meyerowitz, H Ma, JL Bowman, GN Drews, KA Feldman 1990 The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. Nature 346: 35–39.

Zachgo S, Z Schwarz-Sommer, E de Andrade Silva, P Motte, W Tröbner, H Saedler 1995 Functional analysis of the *Antirrhinum* floral homeotic *DEFICIENS* gene in vivo and in vitro by using a temperature sensitive mutant. Development 121: 2861–2875.

Zhao D, H Ma, Q Yu, M Chen 2001 The *ASK1* gene regulates B function gene expression in cooperation with *UFO* and *LEAFY* in *Arabidopsis*. Development 128:2735–2746.