EVOLUTION AND DEVELOPMENT IN THE AMORPHOID CLADE (AMORPHEAE: PAPILIONOIDEAE: LEGUMINOSAE): PETAL LOSS AND DEDIFFERENTIATION

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We used comparative developmental morphology to study the evolution of nonpapilionaceous corollas in the amorphoid clade of the tribe Amorpheae (Papilionoideae). This clade consists of five genera in which there are no papilionaceous corollas (five petals differentiated into one banner, two wing, and two keel petals). We studied the ontogenies of three nonpapilionaceous forms: corollas consisting of one petal (exemplified by *Amorpha canescens*), no petals (*Parryella filifolia*), and five petals in two types (*Errazurizia megacarpa*). We compared these to the ontogeny of a papilionaceous corolla (exemplified by the closely related *Psorothamnus scoparius*). In *A. canescens*, all petals initiated, but four did not grow beyond the primordial stage. In *P. filifolia*, no distinct petal primordia were visible. The corolla of *E. megacarpa*, which has only two types of petals, exhibits nonpapilionaceous characteristics at an early ontogenetic stage. Aside from the earliest primordial mounds, the petals of *Psorothamnus* and *Errazurizia* do not resemble each other, indicating that paedomorphosis is not responsible for the nonpapilionaceousness of *Errazurizia*. Comparing the morphological results to a phylogeny, we infer a single origin of the characteristics that differentiate *Errazurizia* petals from *Psorothamnus* petals, and we infer at least two evolutionary events leading to the reduced corolla in *Parryella*, *Amorpha*, and *Errazurizia rotundata*. When considered in the context of the remaining Amorpheae, in which additional floral diversification has occurred, and in the context of the entire papilionoid group, in which floral form is relatively conserved, our results indicate a relaxation of selective or developmental constraint within the clade Amorpheae.

Keywords: Amorpheae, corolla, flowers, Leguminosae (Fabaceae), organ loss, Papilionoideae.

Introduction

The large and cosmopolitan Leguminosae (Fabaceae) includes tremendous diversity in vegetative structures, fruit morphology, and, across subfamilies, floral form. Within subfamilies, however, flowers are relatively conserved. The largest subfamily, Papilionoideae (ca. 12,000 spp.; Polhill 1981), is characterized by pentamerous, zygomorphic flowers that have differentiated lateral and abaxial pairs of petals (wing and keel petals, respectively), fused keel petals that enclose the stamens and the style, and an adaxial petal (the banner, or standard) that is external to the other petals in bud. This floral form is nearly ubiquitous in the subfamily, but exceptions do occur, and flowers are particularly diverse in Amorpheae Borissova emend. Barneby (Barneby 1977). Flowers in this clade vary in fusion among parts and in numbers and positions of parts. In this article, we examine the morphology and development of three variants that occur in a relatively small clade within the Amorpheae: corolla lacking, corolla consisting of one petal, and corolla consisting of a banner and four undifferentiated petals.

Amorpheae comprise eight genera with ca. 240 species (Barneby 1977) native to the New World, primarily in arid and semiarid regions. Using DNA sequence data, we have confirmed the monophyly of the tribe and identified two intratribal groups, the daleoid clade and the amorphoid clade (McMahon and Hufford 2004). Flowers of the three genera in the daleoid clade, *Dalea*, *Marina*, and *Psorothamnus*, have primarily papilionaceous corollas, although exceptions occur in *Dalea*. The amorphoid clade consists of the remaining five genera. Monotypic *Parryella* and *Errazurizia rotundata* have no petals. All species in *Amorpha*, a genus of ca. 12 species (Wilbur 1975), have only one petal. The remaining taxa, *Eysenhardtia* (ca. 15 species; Lang 1972), monotypic *Apoplanesia*, and the other three species of *Errazurizia*, have five petals, but none have papilionaceous corollas. In all, the lateral and abaxial pairs of petals, corresponding to the wing and keel petals, are not differentiated from one another, and they do not enclose the androecium at anthesis (Lang 1972; Barneby 1977).

The goals of this study are to describe the developmental morphology of three nonpapilionaceous floral forms from the amorphoid clade, to compare these with the developmental morphology of a closely related papilionaceous flower, and to evaluate evolutionary transformations in the developmental morphology using phylogenetic hypotheses for the clade.

Material and Methods

Taxa were selected for inclusion in the study on the basis of their floral forms and the availability of material throughout ontogeny. Three amorphoid taxa, *Parryella filifolia*,

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Amorpha canescens, and *Errazurizia megacarpa* were included to represent three different nonpapilionaceous corollas (see table 1 for authorities and voucher specimen data). Ontogeny for *Psorothamnus scoparius* was also included to provide data on a papilionaceous corolla to which the other taxa were compared. *Psorothamnus scoparius* is a member of the clade sister to the amorphoid clade (McMahon and Hufford 2004) and is among the few Amorpheae with a standard papilionoid floral form. Mature flowers for two additional errazurizias (*Errazurizia rotundata* and *Errazurizia benthami*) were also studied.

Ca. 50 buds of each species were selected in an attempt to span the entire ontogeny, with denser sampling at smaller sizes to bracket the major ontogenetic events, such as organogenesis. Inflorescences bearing anthetic and preanthetic flowers were collected from natural populations and preserved in FAA (37% formalin, glacial acetic acid, ethanol, and water, 5 : 5 : 45 : 45). Flowers and flower buds were dissected in 50% ethanol, dehydrated in a graded ethanol series, critical-point dried, coated with gold, and examined using a scanning electron microscope (SEM) at 10–20 kV.

We studied character evolution using parsimony as implemented in MacClade, version 4.0 (Maddison and Maddison 2003). Phylogenetic trees were reconstructed using the nuclear ribosomal ITS region, including 5.8S, and the chloroplast *trnK* intron, including the *matK* gene. These data were reported in a previous analysis (McMahon and Hufford 2004 for voucher information), which provided resolved relationships among many, but not all, lineages in the amorphoid clade. Therefore, we considered multiple phylogenetic hypotheses for evaluating character state changes. Sensitivity of amorphoid relationships to choice of outgroup taxa was studied by varying both the constituents of the outgroup set and the number of outgroup taxa selected. We randomly selected two, five, eight, 11, 14, 17, and 20 outgroup taxa, 10 times each, from among the 41 sequenced members of the daleoid clade and the four non-Amorpheae selected previously as outgroups (McMahon and Hufford 2004). Sensitivity of amorphoid relationships to alignment was also assessed using five alignment protocols, each on two data sets: ITS sequences from 22 taxa (11 amorphoids, 11 daleoids) and *trnK* sequences from 17 taxa (six amorphoids, 11 daleoids). These two data sets were aligned manually and by using

Clustal W (Thompson et al. 1994), combining gap opening costs of 15 and 30 with gap extension costs of 6.66 and 3. All data sets were analyzed using parsimony and likelihood with estimated transition-transversion ratio and gamma-distributed rate heterogeneity (HKY + Γ; Hasegawa et al. 1985; Yang 1994) using PAUP* (Swofford 2003). Difference in phylogenetic signal between the data sets was evaluated using the partition homogeneity test (Farris et al. 1994), as implemented in PAUP* (Swofford 2003).

Results

Developmental Morphology

Psorothamnus scoparius. After sepal initiation, petals are initiated at the corners of a pentagonal meristem, and the antesepalous stamens initiate along the sides (fig. 1*A*). The carpel is prominent as the final members of the antesepalous stamen whorl are initiated (fig. 1*A*). Antepetalous stamens are initiated last (fig. 1*B*). The adaxial antepetalous stamen is not in the median plane; instead, it initiates to the left of the position in front of the banner petal (fig. 1*B*). The petals begin as primordial mounds, then undergo dorsiventral flattening as they elongate (fig. 1*A*, 1*B*). When the petals are ca. 20 μm high, a slight longitudinal asymmetry in the wing petals is visible (fig. 1*C*). Unequal lateral expansion over the length of petals leads to the differentiation of a blade and a claw by the time they reach ca. 250 μm in height (fig. 1*D*). Longitudinal asymmetry is pronounced as the petals elongate, broaden, develop distal glands (fig. 1*E*), and begin to overlap one another (fig. 1*F*). The petal blades elongate and expand laterally, overlapping one another and enclosing the reproductive organs (fig. 1*G*, 1*H*). The overlapping margins of the keel petals cohere. In later ontogeny, the claws elongate extensively (figs. 1*I*, 2*A*). Before anthesis, the petals all strongly overlap, and the banner nearly encloses the entire flower (fig. 2*A*, 2*B*). At anthesis (fig. 2*C*, 2*D*), the banner blade reflexes, the wing petals spread, and the keel petals remain coherent by their abaxial margins.

Errazurizia megacarpa. A calyx is initiated first, and the abaxial sepal is the most prominent (fig. 3*A*). Petals initiate on the corners of the pentagonal meristem, with the more abaxial petals becoming largest first (fig. 3*B*). Two whorls of stamens initiate, with the antesepalous stamens generally enlarging earlier than the antepetalous stamens (fig. 3*B*). All flowers that we examined initiated only nine stamens; no stamen formed opposite the banner petal. Each petal apparently elongates at approximately the same rate during this stage (fig. 3*C*, 3*D*). Early in development, the antesepalous stamens become larger than the petals (fig. 3*E*) and remain so through the formation of thecae (fig. 3*C*–3*H*). As the petals enlarge, their shape changes from oblong to ovate (fig. 3*F*). Petal expansion is longitudinally symmetrical, and the petals taper gradually from a widest point just distal to midlength toward both base and apex (fig. 3*H*, 3*I*). When the petals are ca. 0.39 mm long, they have surpassed all of the stamens in length (fig. 3*I*). As the bud develops, the banner petal becomes distinctly more broad and cucullate than the others (fig. 4*A*, 4*B*). The other four remain similar, and they loosely overlap but do not fuse (fig. 4*B*, 4*C*). The proximal region of

Table 1

Voucher Information, with Herbarium Location in Parentheses

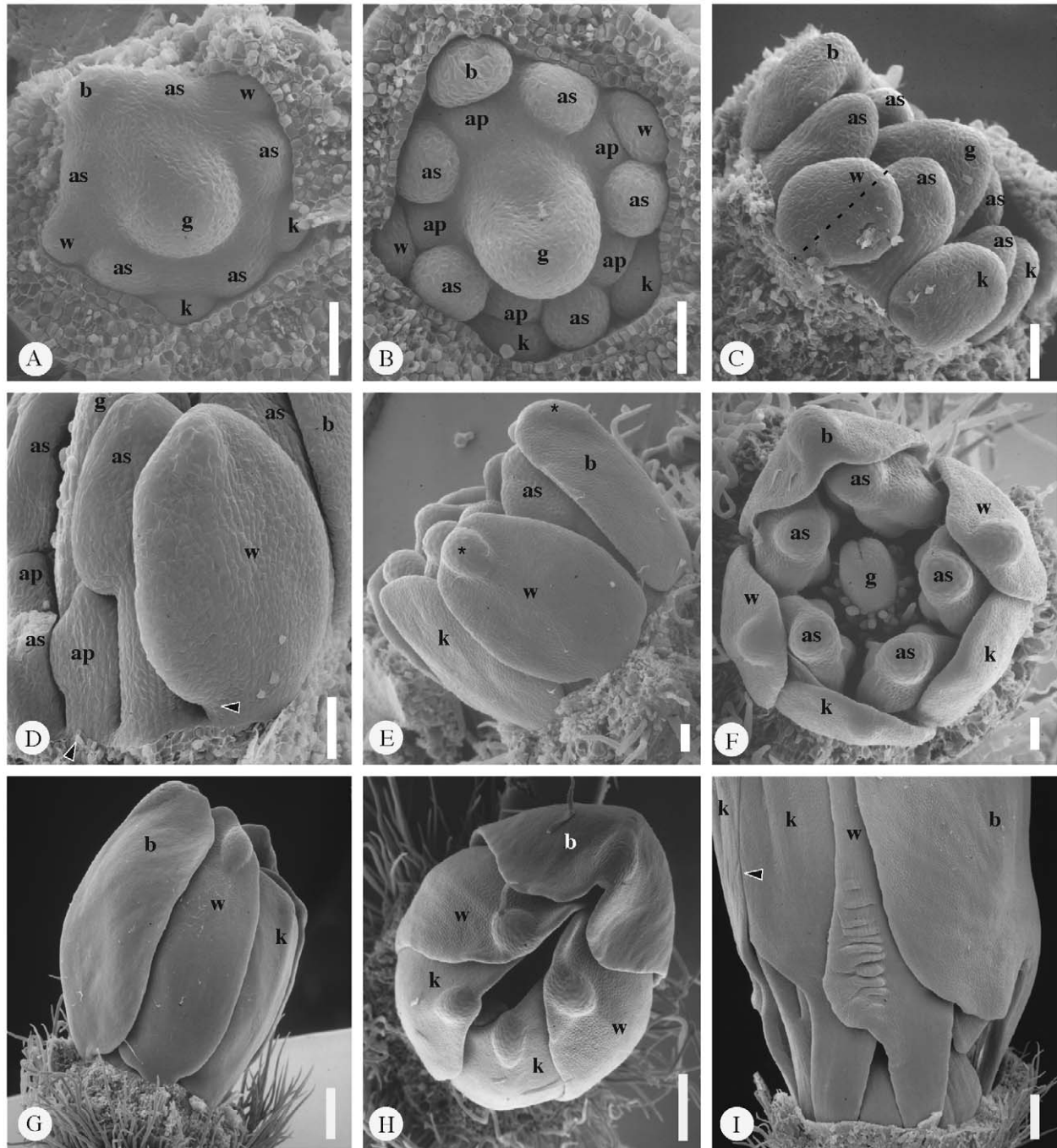| Taxon	Voucher
Amorpha canescens Pursh	McMahon 683 (WS)
Errazurizia benthami (Brandegee) I. M. Johnst.	McMahon 438 (WS)
Errazurizia megacarpa (S. Watson) I. M. Johnst.	Fishbein 3773 (WS)
Errazurizia rotundata (Wooton) Barneby	McMahon 452 (WS)
Parryella filifolia Torr. & A. Gray ex A. Gray	McMahon 333 (ARIZ)
Psorothamnus scoparius (A. Gray) Rydb.	McMahon 688 (WS)

Fig. 1 Early floral ontogeny of *Psorothamnus scoparius*; calyx removed in all. *A*, Polar view of floral meristem on which five petals have initiated between the sepal lobes (removed). The carpel is a distinct mound in the center, and the antesepalous whorl of stamens has initiated. *B*, Polar view of floral meristem on which all organs have initiated. The adaxial antepetalous stamen primordium is not in the median plane. *C*, Oblique lateral view of flower in which the petals have undergone dorsiventral flattening and the wing petal shows asymmetry. Dashed line indicates approximate plane around which the petal is asymmetric. *D*, Lateral view of a wing petal, showing a sharp distinction between the blade (above horizontal arrowhead) and the claw (below horizontal arrowhead). A keel petal was removed (vertical arrowhead). *E*, Lateral view of an asymmetrical wing petal with a distinct claw; prominent distal glands (asterisks) are visible on wing and banner petals. *F*, Polar view of bud, showing overlap of wing and keel petal margins. *G*, Lateral view of bud, showing expanded blade regions of the petals. *H*, Polar view of corolla, showing petal aestivation. *I*, Lateral view of proximal region of corolla, showing petal sculpturing on the wing petal and region of coherence in the overlapping margins of the keel petals (arrowhead). Scale bars = 50 μm. *ap* = antepetalous stamen; *as* = antesepalous stamen; *b* = banner petal; *g* = gynoecium; *k* = keel petal; *w* = wing petal.
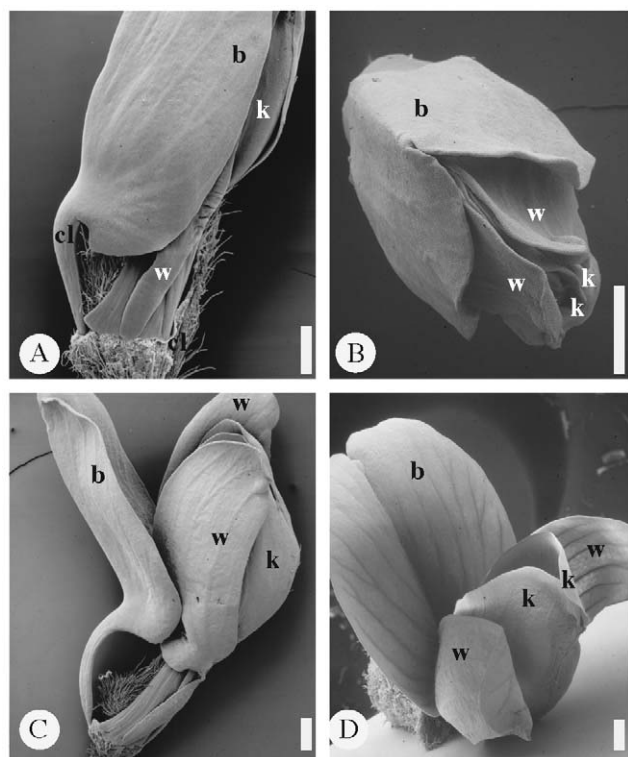
Fig. 2 Late ontogeny of *Psorothamnus scoparius*; calyx removed in *A* and *C*. *A*, Preanthetic flower, lateral view, showing expanded claw regions and a banner petal nearly enclosing the remaining petals. *B*, Preanthetic flower, oblique polar view, showing aestivation of petals. *C*, Anthetic flower, lateral view, in which the banner is reflexed and the wings are spreading. *D*, Anthetic flower, oblique polar view, showing papilionaceous floral form. Scale bars = 0.5 mm. *b* = banner petal; *cl* = claw; *k* = keel petal; *w* = wing petal.

the petals tapers toward the base, and the distal region is paddle shaped; this subtle shape transition occurs about midlength. At anthesis, the distal ends of the lateral and abaxial petals reflex (fig. 4D, 4E). Immediately before anthesis, zonal growth occurs below the stamen sheath and the petals, creating a unified basal tube below the androecium and corolla (fig. 4E, 4F). The result of zonal growth is that the petals in the mature flower are inserted ca. 0.15 mm above the rim of the hypanthium (fig. 4F), forming a minute "stemonozone," i.e., tissue that has the appearance of stamen tube between the insertion point of the petals and the top of the hypanthium.

Amorpha canescens. The calyx initiates first, and the abaxial sepal in the median position is the first to enlarge (fig. 5A). The carpel initiates as a mound in the center of the pentagonal meristem, the corners of which form petal primordia (fig. 5B, 5C), apparently simultaneously. The antesepalous stamens initiate along the sides of the meristem (fig. 5C), with the abaxial stamen appearing first (fig. 5B), followed by the lateral pair (fig. 5C) and then the adaxial pair, which differ in size (fig. 5D). The antepetalous stamens form last (fig. 5D–5F). The lateral petals are smaller than the banner petal at an early stage (fig. 5D–5F), and subsequent floral enlargement encompasses the arrested lateral petal primordia, leading to their disappearance and leaving only the ban-

ner petal (fig. 5G, 5H). From initiation, most antesepalous stamens are larger than the banner petal and the antepetalous stamens (fig. 5D–5I). They remain larger as the thecae form and the other floral organs enlarge (fig. 6A–6C), including style elongation (fig. 6C). The banner surpasses the stamens by the time it is 2 mm in height (fig. 6C), although at anthesis, filament elongation results in anther exsertion (fig. 6D). The stamens are fused at the base in a short sheath (fig. 6D). The banner petal is cucullate and does not reflex at anthesis (fig. 6D). The petal is broadest distally and tapers toward the base without an abrupt transition to the narrower claw region (fig. 6D, 9).

Parryella filifolia. A calyx initiates, and the abaxial sepal becomes the most prominent (fig. 7A). By the time the sepals have begun to overarch the floral meristem, a calyx tube is visible, stamens have initiated, and the carpel is a prominent mound in the center (fig. 7B). Although distinctive petal primordia are not clearly visible at any stage, the early pentagonal floral meristem has pronounced protrusions at the corners (fig. 7A), which precede the initiation of petal primordia in other examined taxa (fig. 5C). However, the meristem becomes circular as the second whorl of stamens initiate in antepetalous positions (fig. 7C), corresponding to the corners of the earlier pentagon. The primordia that become stamens are the only primordia visible in the antepetalous positions (fig. 7C–7E). As the carpel and stamens elongate, the antesepalous stamens remain larger than the others (fig. 7F–7H). As the thecae are forming, the carpel closes (fig. 7H). Before anthesis, zonal growth occurs under all 10 stamens, forming a tube (fig. 7G–7I). At anthesis, the stamens are exserted from the tubular calyx.

Other amorphoids. We examined mature flowers from herbarium specimens of several species of *Eysenhardtia* and the single *Apoplanesia* species and found their corollas to be consistent with published descriptions; all have five petals in two types (a banner and four nearly identical petals), with no corolline fusion. In addition, we examined preserved anthetic and preanthetic flowers of *Errazurizia benthami* and *Errazurizia rotundata*. We found that the flowers of *E. benthami* are similar to those of *Errazurizia megacarpa*, with the exception of the stemonozone; in *E. benthami* the petals attach directly to the rim of the hypanthium (fig. 8A, 8B). *Errazurizia rotundata* has a filament tube and no evidence of petals in our collections (fig. 8C, 8D).

Phylogenetic Hypotheses

The amorphoids were consistently monophyletic, regardless of alignment technique, choice of outgroup, or optimality criterion. Within amorphoids, however, relationships varied, depending on the data source and the outgroups but not on the alignment or the optimality criterion (fig. 10). The chloroplast *trnK* intron, including the *matK* gene, placed *Parryella* as sister to the rest with reasonable support (82% bootstrap support in the analysis shown; fig. 10). This result was also found in 61 out of 70 experiments in which we randomly selected smaller sets of outgroups. When *Parryella* is sister to the rest, *E. rotundata* is either sister to or nested within *Amorpha*. The ITS data, however, consistently support *E. rotundata* and *Parryella* as sisters, variously sister to or nested within *Amorpha* (fig. 10). Using these data,
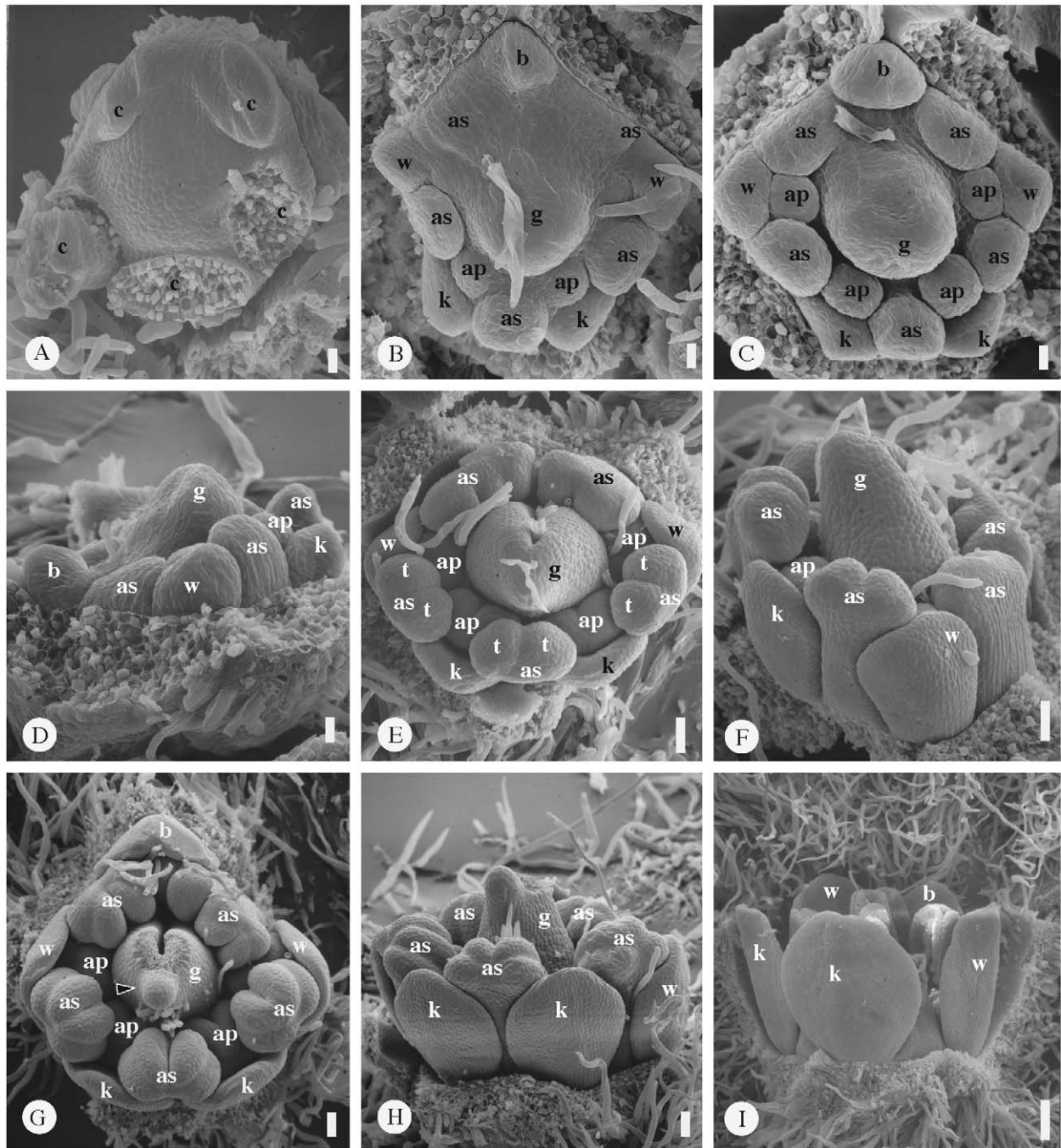
Fig. 3 Early floral ontogeny of *Errazurizia megacarpa*; calyx removed in *B–I*. *A*, Polar view of floral meristem on which five sepals have initiated (two removed). *B*, Polar view of floral meristem on which petals, the carpel, all antesepalous stamens, and the two antepetalous stamens opposite the keel petals have initiated. *C*, Polar view of meristem on which all organs have initiated. *D*, Lateral view of a meristem on which the petal primordia are elongated mounds, slightly flattened dorsiventrally. *E*, Polar view, showing the carpel nearly closed, thecae forming, and the petals dorsiventrally flattened. *F*, Oblique lateral view of bud, showing symmetrically expanding wing petal and no evidence of a claw. *G*, Polar view of bud, showing laterally expanded petals and a prominent stylar gland (arrowhead). *H*, Lateral view, showing expanded symmetric petals. *I*, Lateral view, showing symmetric petals. Scale bars = 20 μm in *A–D*; 50 μm in *E–H*; 100 μm in *I*. *ap* = antepetalous stamen; *as* = antesepalous stamen; *b* = banner petal; *c* = calyx lobe; *g* = gynoecium; *k* = keel petal; *t* = theca; *w* = wing petal.

Apoplanesia is placed as sister to *Errazurizia* s. str. + *Eysenhardtia*, or as sister to *Parryella + E. rotundata + Amorpha*. Although these trees differed across well-supported nodes, the two markers were not significantly dif-ferent when the partition homogeneity test was used. Combin-ing the data gave nearly the same result as for the ITS data, except that we found support for placement of *Apoplanesia* with the other taxa that have five petals, *Errazurizia* s. str. and
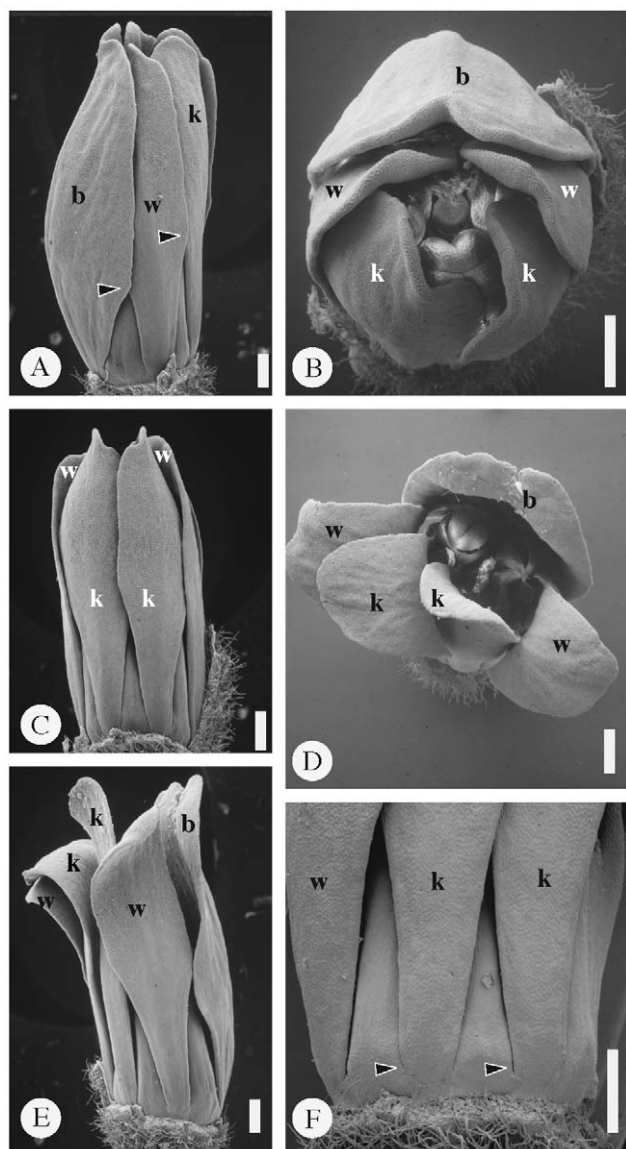
Fig. 4 Late ontogeny of *Errazurizia megacarpa*; calyx removed in all. *A*, Lateral view of preanthetic flower, showing petals without distinct claw regions; i.e., the change from parallel margins to tapering margins (arrowheads) is not abrupt (cf. fig. 2*A*). *B*, Polar view of preanthetic flower, showing petal aestivation. *C*, Abaxial view of overlapping, unfused keel petals. *D*, Polar view of anthetic flower, showing banner not reflexed and wing and unfused keel petals spread. *E*, Oblique lateral view of anthetic flower. *F*, Lateral view of proximal region of flower, showing petals attached (arrowheads) above the rim of the hyanthium. Scale bars = 0.5 mm. *b* = banner petal; *k* = keel petal; *w* = wing petal.

Eysenhardtia. However, this result was sensitive to outgroups, and there were some instances in which *Apoplanesia* was sister to all amorphoids.

Discussion

Members of the papilionoid legume clade are characterized as having corollas with strong zygomorphy and five petals in

three types (Kalin Arroyo 1981; Polhill 1981). Among the ca. 12,000 species (Polhill 1981), most have this floral form. However, exceptions occur and have been largely relegated to what were considered two plesiomorphic tribes, Swartzieae and Sophoreae, with a few additional cases in Dalbergieae (Polhill 1981). Recent phylogenetic work has shown that neither Sophoreae nor Swartzieae are monophyletic (Ireland et al. 2000; Pennington et al. 2001; Wojciechowski et al. 2004), that the nonpapilionaceous Dalbergieae are isolated in the dalbergioid clade (Lavin et al. 2001), and that some cases of nonpapilionaceous corollas have evolved secondarily, i.e., from papilionaceous ancestors (Pennington et al. 2000; McMahon and Hufford 2004). Amorpheae is phylogenetically distinct from the lineages that involve Sophoreae and Swartzieae (Ireland et al. 2000; Kajita et al. 2001; Pennington et al. 2001; Wojciechowski et al. 2004) and the few nonpapilionaceous taxa nested within the dalbergioids (Lavin et al. 2001; Wojciechowski et al. 2004), yet it too has exceptional, nonpapilionaceous members that evolved from a papilionaceous ancestor (McMahon and Hufford 2004). Furthermore, the flowers of Amorpheae have become nonpapilionaceous in several different ways.

Corolla Reduction

In the amorphoid clade, several species have fewer than the plesiomorphic five petals. All species of *Amorpha* have just one petal (the banner), monotypic *Parryella* has no corolla, and one of four errazurizias, *Errazurizia rotundata*, also has no corolla. By comparing developmental trajectories, we can ask whether the cases of corolla reduction develop in the same way. We have shown that *Amorpha canescens* initiates all five petals, but the lateral and abaxial pairs of petal primordia are small bumps on the side of the meristem and are no longer clearly visible by the time the banner is ca. 50 μm long. This is similar to the result for *Amorpha fruticosa* (Tucker 1988), in which the four lateral and abaxial petals are initiated but do not elongate. Examining our data for *Parryella filifolia*, one of two species that have no petals at maturity, we find no clear evidence that petals are initiated. After the sepals have initiated, the remaining floral meristem is pentagonal, just as in *Amorpha* and other papilionoids (Picklum 1954; Sattler 1973; Derstine 1988; Crozier and Thomas 1993; Klitgaard 1999). We see two possibilities for the regions that are in the points of the pentagonal meristem. Either the points of the pentagon are precursors to primordia, or they are the geometric result of the five sepals initiating. If the former is the case and the regions are truly primordia, they could be either petal primordia or antepetalous stamen primordia. In *Amorpha*, the identification of primordia was clear because we saw two sets of primordia alternating with the sepals; the internal set becomes the antepetalous stamens, and the external set becomes undetectable. In *Parryella*, we have no clear evidence of two separate sets of primordia and cannot conclude that the petals are initiated. Because petal primordia are not visible when the stamens are of comparable height (cf. figs. 5*F*, 7*F*), we conclude instead that petals are not initiated.

A distinction between developmental organ loss and organ suppression has been emphasized (Tucker 1988, 1989;
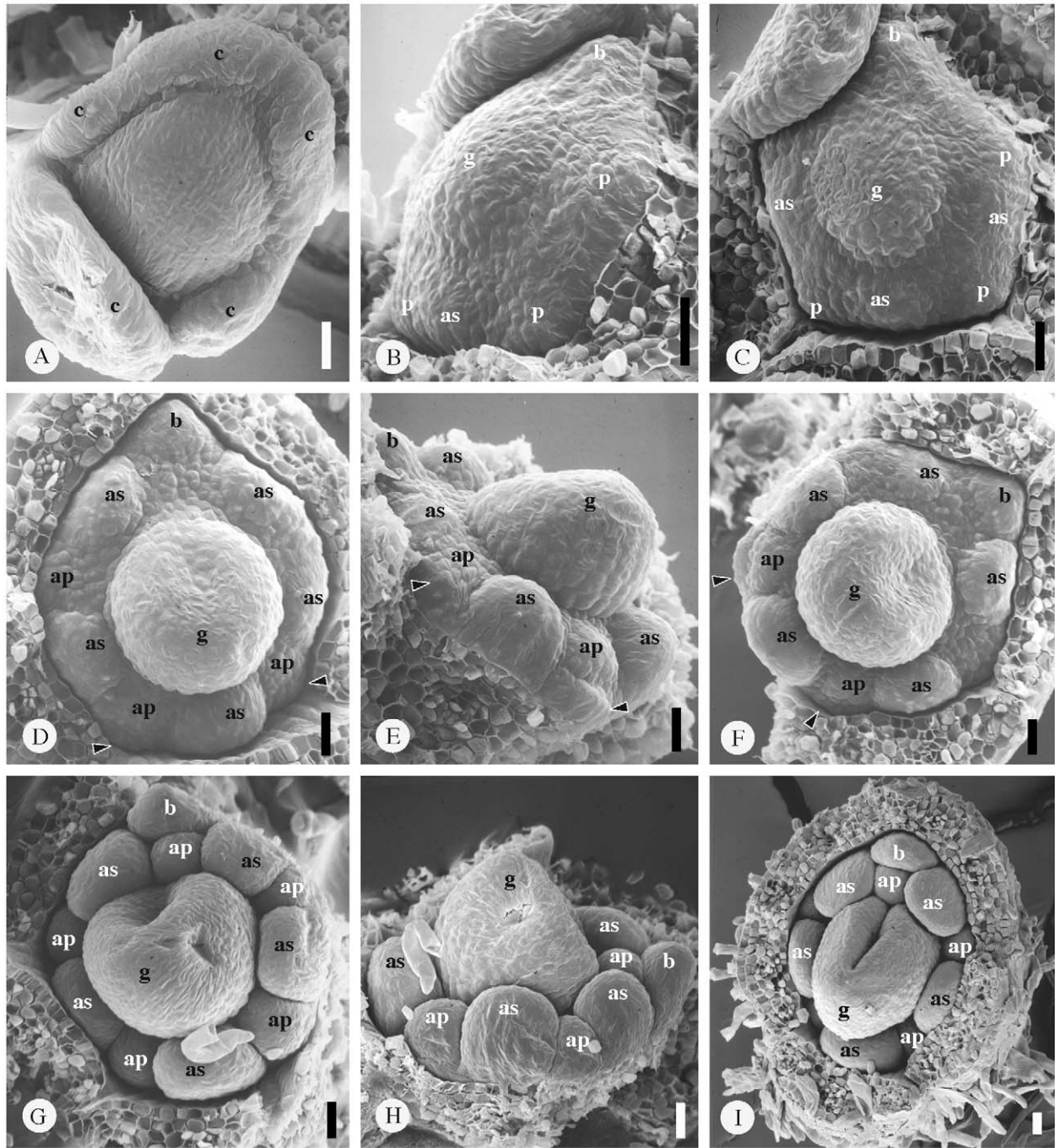
Fig. 5 Early floral ontogeny of *Amorpha canescens*; calyx completely or partly removed in *B–I*. *A*, Polar view of floral meristem on which five sepals have initiated. *B*, Oblique view of meristem after petal and carpel initiation; antesepalous stamens have begun to form. *C*, Polar view of meristem on which four petals, three stamens, and the carpel primordia are visible. *D*, Polar view of meristem on which the antesepalous stamens and three of the antepetalous stamens have initiated. The banner primordium has enlarged, but the other petal primordia are visible as small bumps centrifugal to the antepetalous stamens (arrowheads). *E*, Lateral view of bud, showing stamen primordia and diminutive petal primordium (arrowheads). *F*, Polar view of bud in *E*, showing petal primordia (arrowheads). *G*, Oblique polar view of bud after all stamen primordia have formed; no petal primordia except the banner are visible. *H*, Lateral view, showing enlarged stamen primordia and no apparent petal primordia except the banner. *I*, Oblique polar view, showing carpel, 10 stamens (not all labeled), and one banner petal. Scale bars = 20 μm. *ap* = antepetalous stamen; *as* = antesepalous stamen; *b* = banner petal; *c* = calyx lobe; *g* = gynoecium; *p* = petal.
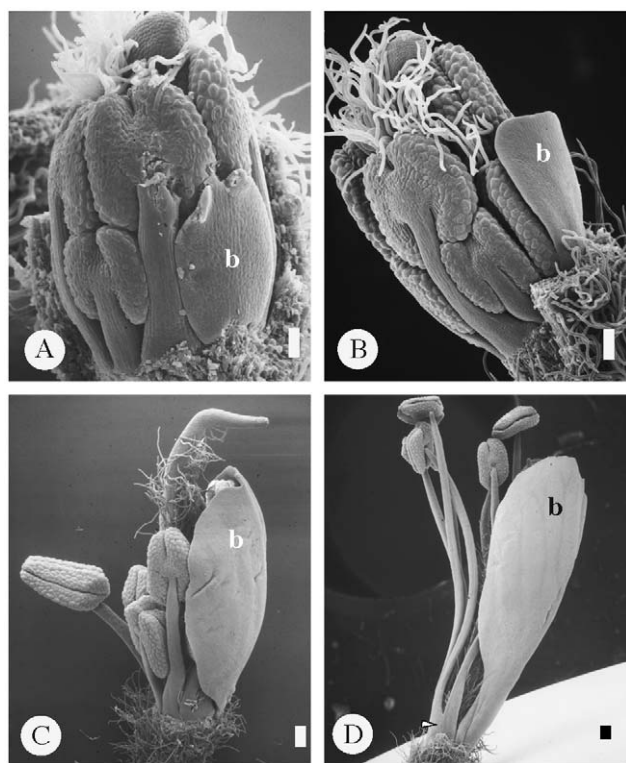
Fig. 6 Late ontogeny of *Amorpha canescens*; calyx removed in all. *A*, Lateral oblique adaxial view, showing banner petal shorter than the developing stamens. *B*, Lateral view, showing enlarged banner petal. *C*, Lateral view of preanthetic bud, showing banner enclosing only part of the androecium and the style elongated. *D*, Lateral view of anthetic flower, showing a short filament sheath (arrowhead), exserted stamens, and banner not reflexed. The banner tapers toward the base and does not have an abruptly distinct claw region. Scale bars = 50 μm in *A*; 100 μm in *B*; 200 μm in *C*, *D*. *b* = banner petal.

Tucker and Douglas 1994; Prenner 2004*c*). Both are examples of a reduced number of apparent mature parts in a descendant relative to the ancestor, but the difference lies in the timing of cessation of growth. In organ loss, the part that is missing at maturity is also missing at organogenesis; i.e., a primordium does not form. In organ suppression, the missing organ initiates—i.e., a primordium forms—but does not enlarge. Tucker (1988, 1989; Tucker and Douglas 1994) has found that organ loss, but not organ suppression, was correlated with major developmental changes, establishing that the distinction is important developmentally. Our data on *Parryella* demonstrate that it can be difficult to assess with certainty that an organ does not initiate. Lack of a visible primordium may be considered an extreme case of suppression or size reduction. Therefore, it may be better in some cases to consider loss and suppression as two different expressions of heterochrony. In the Amorpheae studied, no other major developmental changes accompany the missing petals in either *Amorpha* or *Parryella*, so the correlation observed elsewhere does not hold here. However, it is evident that multiple evolutionary changes are required to explain the diversity among amorphoids.

Nonpapilionaceous Corolla

Four morphological characteristics can be used to characterize the papilionaceous corolla (Polhill 1981; Endress 1994; Westerkamp 1997): zygomorphy, five petals in three types (banner, wing, keel), petal asymmetry (in the latter two types), and petals with distinct blades and abruptly delimited claws. Corollas of all amorphoids are zygomorphic, even if subtly so, because the adaxial petal is generally larger than the other four. However, amorphoid flowers depart from papilionaceous flowers in all three remaining characteristics. The amorphoid corolla, in the taxa with all five petals, has only two types of petals: a broad petal and four narrow petals. The broad petal occupies the position of the banner in papilionaceous flowers; i.e., it is median and adaxial. However, differing from the common type of papilionoid flower, the amorphoid banner does not reflex at anthesis. The remaining petals are very similar to one another. Likewise, a common papilionaceous corolla has petals that have a narrow proximal portion (claw or limb) and an abruptly broader distal portion (blade). In contrast, the petals in the amorphoid clade taper gradually to the base, without an abrupt delimitation between distinct claw and blade regions (fig. 9). Symmetry within the petals also differs: papilionaceous wing and keel petals are asymmetrical, whereas the amorphoid lateral and abaxial petals are bilaterally symmetrical or nearly so (fig. 9).

How does this nonpapilionaceous floral form arise developmentally? Comparing the development of *Psorothamnus scoparius* to the development of *Errazurizia megacarpa*, we can see that the differences are apparent early in ontogeny, after organogenesis. Among these species, organogenesis is approximately the same and corresponds to that of many other papilionoids (Payer 1857; Picklum 1954; Sattler 1973; Tucker 1989, 1993, 1994, 2003). Amorpheae androecia are asymmetrical, but this has been recently found in several papilionoids (39 of 91 species examined; Prenner 2004*a*). The order of primordia formation also varies among papilionoids (Tucker 1984, 2003; Prenner 2004*b*). Order of initiation can only be determined when buds that have some primordia but not others are discovered. Because organs can grow unevenly, size is an unreliable indicator of which organ initiated first. Therefore, we do not have sufficient data to distinguish the precise order for each organ initiated in Amorpheae. However, our data are consistent with the general papilionoid pattern of unidirectional initiation within whorls, beginning with the abaxial side of the flower, with some overlap between whorls (Tucker 2003).

The primary difference between nonpapilionaceous and papilionaceous types, as found in Amorpheae, is in the shape of the petals. This is evident early in the "differentiation" phase of development, in which the organs take on their distinctive shapes. Early asymmetry of the wing petals is clear; further data need to be collected to determine precisely the onset of asymmetry in the keel petals. The early wing petal of *P. scoparius* does not resemble the early lateral petal of *E. megacarpa* by the time they are ca. 110 and ca. 150 μm long, respectively. A distinct claw is also evident in *P. scoparius* at this early stage, again setting the forms apart.

If the ancestor of the amorphoids developed as does *P. scoparius*, are the amorphoid petals examples of paedomorphosis
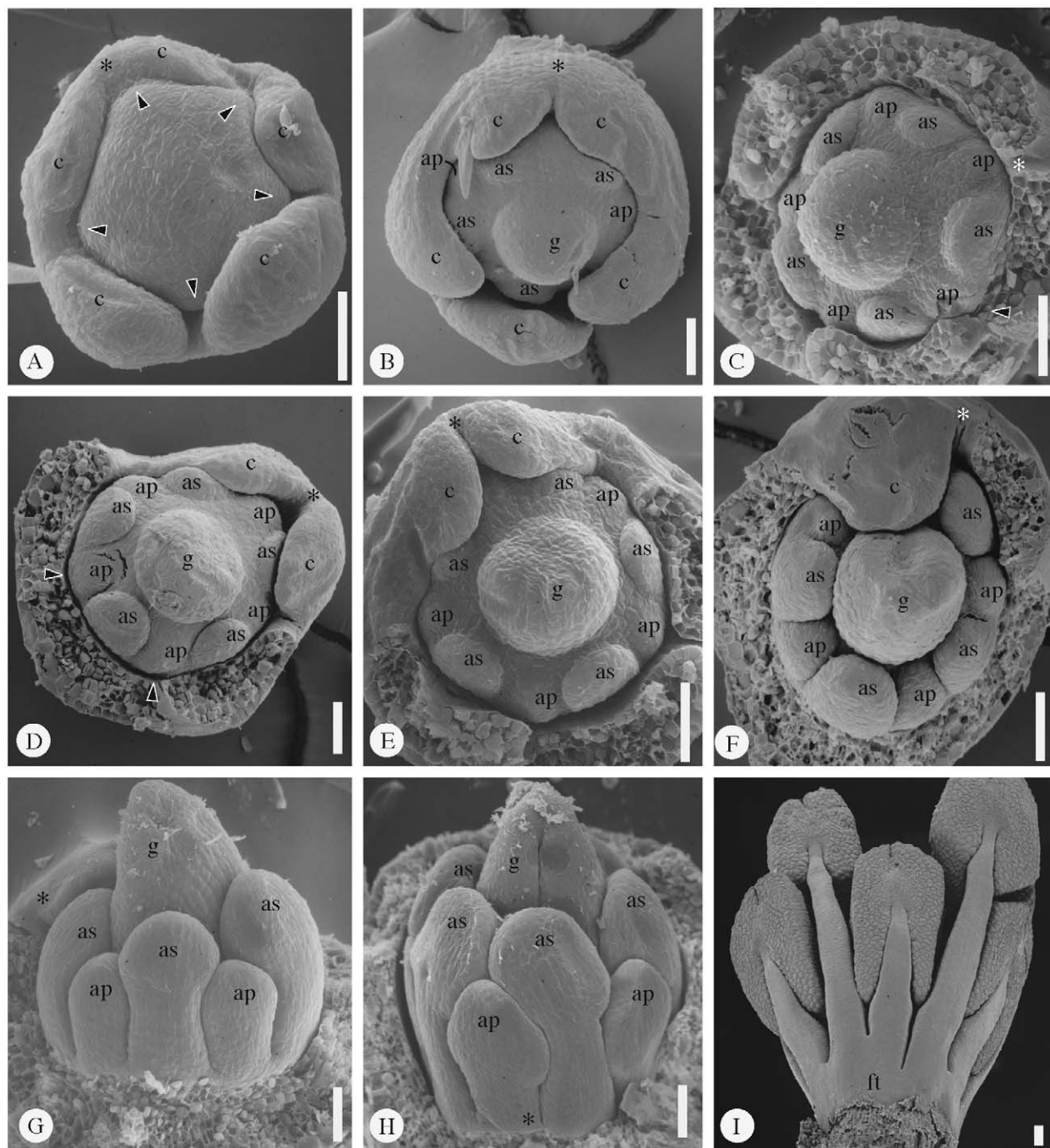
Fig. 7 Floral ontogeny of *Parryella filifolia*; calyx removed in *C–I*. Adaxial indicated by an asterisk in *A–H*. *A*, Polar view of floral meristem on which five sepals have initiated. The remaining meristem is pentagonal; the corners of the meristem (arrowheads) correspond to the locations of petal initiation in other taxa (cf. fig. 1*A*). *B*, Polar view of floral meristem on which sepals cover remaining primordia; the carpel, four antesepalous stamen, and three antepetalous stamen primordia are visible. *C*, Polar view of meristem on which all 10 stamens have initiated. The antepetalous stamens initiate in positions corresponding to the corners of the earlier pentagonal meristem (*A*). No additional primordia are visible where petal primordia are expected (arrowhead). *D*, Oblique polar view of an additional bud showing no visible petal primordia. The adaxial antepetalous stamen is not in the median plane (also seen in *C*). *E*, Polar view of bud after all stamens initiated, showing no apparent petal primordia. *F*, Polar view of bud in which the stamen primordia have elongated. *G*, Lateral view of bud with stamen thecae beginning to form. *H*, Adaxial view of bud showing closure of carpel, stamen thecae forming, and adaxial antepetalous stamen not on the principal axis (asterisk). *I*, Lateral view, showing midstage bud in which the filament tube has formed. Scale bars = 50 μm in *A–H*; 100 μm in *I*. *ap* = antepetalous stamen; *as* = antesepalous stamen; *c* = calyx lobe; *ft* = filament tube; *g* = gynoecium.
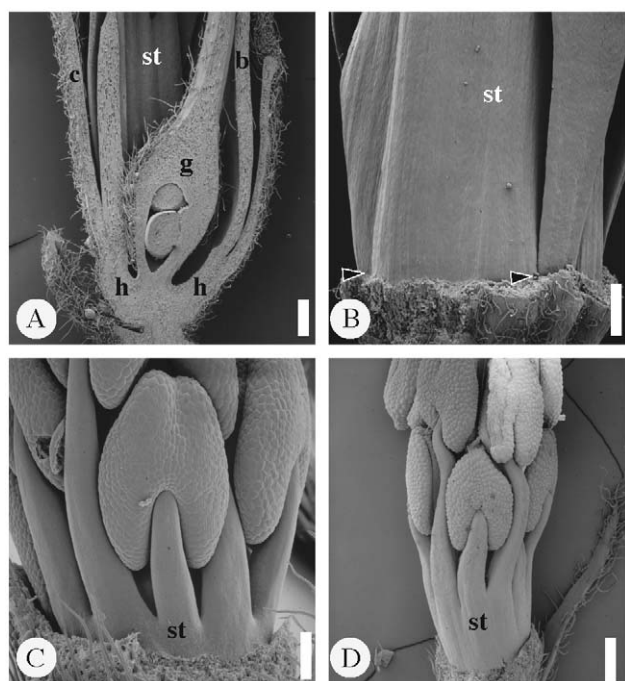
Fig. 8 Morphology of *Errazurizia benthami* and *Errazurizia rotundata*; calyx removed in all. *A*, *E. benthami*, bisection through anthetic flower, showing hypanthium. *B*, *E. benthami*, lateral view of proximal region of flower, showing petals inserted on the rim of the hypanthium (arrowheads). *C*, *E. rotundata*, lateral view of midstage bud, showing short stamen tube. *D*, *E. rotundata*, abaxial view of bud, showing elongated stamen tube and no petals. Scale bars = 0.5 mm in *A*, *B*, *D*; 0.2 mm in *C*. *b* = banner petal; *c* = calyx lobe; *g* = gynoecium; *h* = hypanthium; *st* = stamen tube.

(Gould 1977)? That is, do the mature amorphoid petals resemble petals at earlier stages of development in *P. scoparius*? The petal of *P. scoparius* appears not to pass through a stage that resembles the mature *E. megacarpa* petal; as the distal portion is expanding, it does so asymmetrically and abruptly above a claw. Therefore, pending further developmental study of near outgroups, we conclude that the ontogeny of the amorphoid petal is novel and is not due to heterochrony in the form of paedomorphosis.

Evolution of Corollas in the Amorphoids

The ancestral floral form for the amorphoid clade is papilionaceous (McMahon and Hufford 2004). When we used parsimony to infer evolutionary changes on the maximum likelihood tree obtained from the 22-taxon data set of ITS and *trnK* combined, we found that few steps were needed to explain the results from our developmental morphology analysis (fig. 11). On this tree, we reconstructed one evolutionary transformation from three types of petals to two types (i.e., petal dedifferentiation) and one loss of an abruptly delimited petal claw. These changes could have been at the base of the amorphoids, as shown (fig. 11), or loss of lateral petal differentiation could be limited to those taxa that have lateral petals. Likewise, we reconstructed a single change to suppres-

sion of four petals, shown for *A. canescens* and *A. fruticosa* and inferred for the rest, at the base of *Amorpha*, but this change could have happened before the split with the zero-petal taxa. On this tree, we found a single loss of the fifth petal and a single loss of lateral petal primordia.

We present a single tree for character analysis but emphasize that the relationships among the basal lineages in the amorphoid clade are far from resolved. The maximum likelihood tree for the combined data set with the reduced set of daleoids gives a conservative estimate of the character changes. If we consider petal number as an ordered character
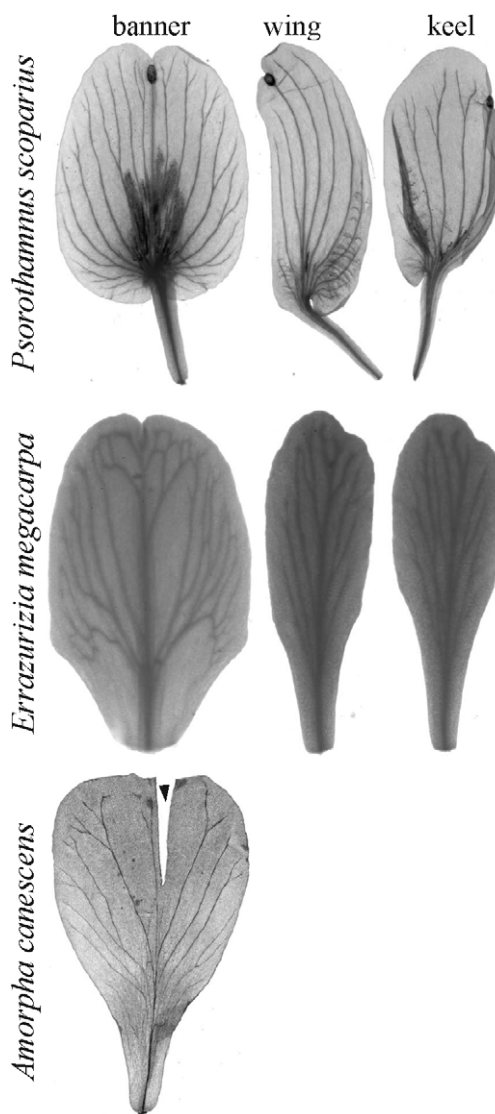

Fig. 9 Mature petals of three species. *Psorothamnus scoparius* has three types of petals, *Errazurizia megacarpa* has only two types, and *Amorpha canescens* has only one type. In the latter two species, the change from blade to claw is not abrupt. In all petal types of *P. scoparius*, the claw is abruptly delimited. All petals were pressed flat under a glass coverslip. The banner petal of *A. canescens* was sliced distally (arrowhead) to allow flattening.
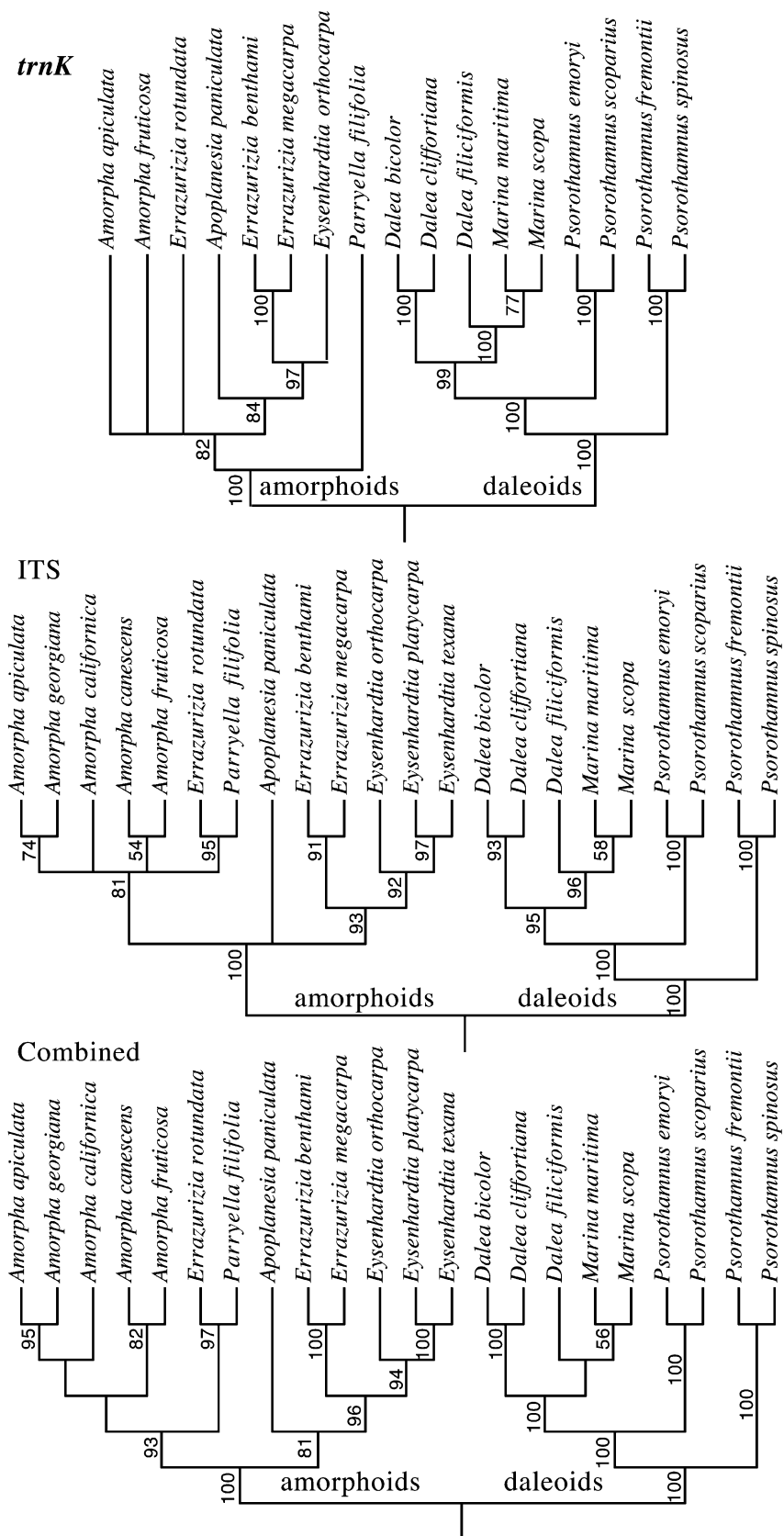
Fig. 10 Results from phylogenetic analyses of *trnK*, ITS, and the combined data sets. Shown are maximum likelihood (ML) trees with ML bootstrap support greater than 50% indicated. Trees were rooted between the daleoids and amorphoids.
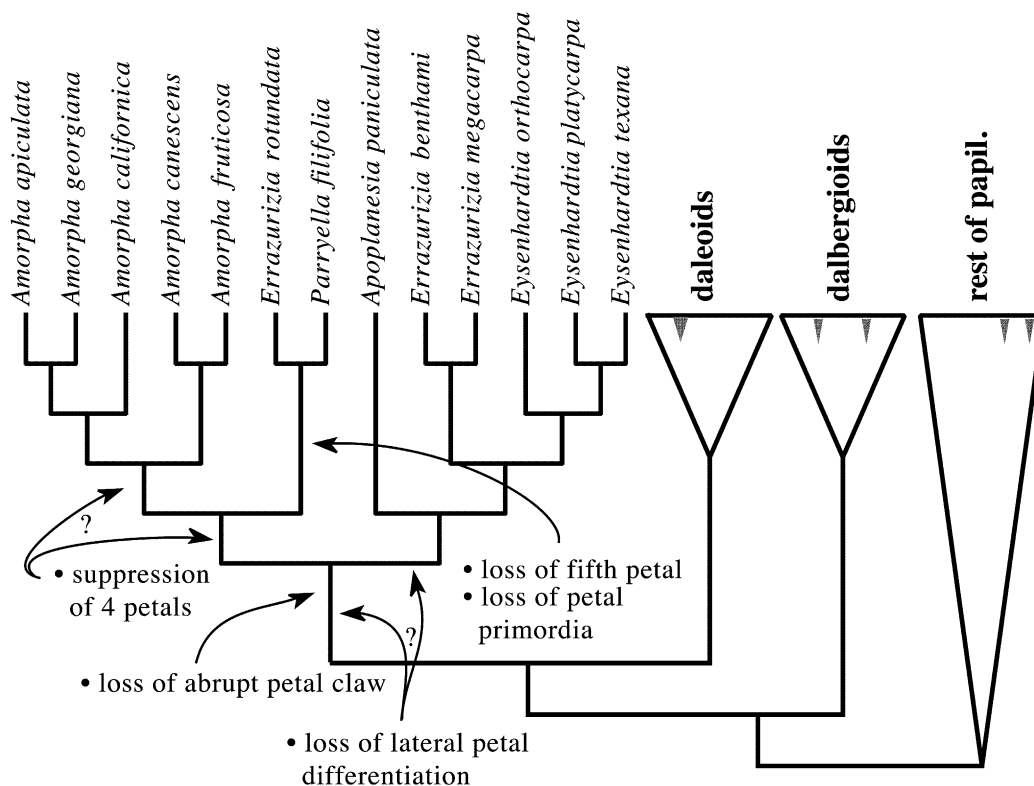
Fig. 11   Reconstruction of five characters using parsimony. On this tree, each character requires a single step. Two of the characters can be reconstructed to change in either of two lineages, indicated by question marks. The phylogenetic tree was inferred using maximum likelihood and combined *trnK* and ITS data (fig. 10), with additional outgroup relationships from previous results (McMahon and Hufford 2004). Shaded triangles in the outgroups indicate other taxa with nonpapilionaceous floral forms.

and five as the ancestral state, the most parsimonious scenario is two reductions. This is consistent with the maximum likelihood tree: one reduction (from five petals to one) before the ancestor of [*Amorpha* + (*Parryella* + *E. rotundata*)] and a second reduction (from one to zero) before the ancestor of (*Parryella* + *E. rotundata*). However, we have not excluded other possibilities. For example, if *Parryella* is sister to the rest and *E. rotundata* is nested within *Amorpha*, petal number would have evolved from five to zero, then to one, then to zero. Additional data are required to finalize specific inferences about these relationships and precise inferences of evolutionary changes.

Conclusions

We have inferred that the ancestral floral form for the amorphoid clade was papilionaceous and have shown that the flowers in this clade have undergone multiple evolutionary transformations away from the plesiomorphic form. We found that the ontogeny of petals in our exemplar papilionaceous flower, *Psorothamnus scoparius*, does not pass through a stage that resembles the development of the nonpapilionaceous petals; therefore, these are not likely to be examples of paedomorphosis. Rather, the amorphoid corolla has a novel ontogeny. We also found differences between the ontogenies

of a species lacking a corolla (*Parryella filifolia*) and a species with a reduced number of parts (*Amorpha canescens*). This finding may indicate that they represent independent derivations from the papilionaceous form. Our phylogeny for the group tentatively supports this conclusion.

Floral diversity in Amorpheae has evolved through multiple modifications to ontogenetic trajectories, including losses and the addition of novelties (McMahon and Hufford 2002). We do not yet know a direct cause for this diversification. There may be something unusual about the gene families that control development of the flower. Homologs of genes known to be involved in zygomorphy have been found in legumes (Citerne at al. 2003), although it is not yet clear whether they are directly related to changes in morphology (Citerne et al. 2003; Ree et al. 2004).

Alternatively, or in addition, there may have been something unusual about selection pressures on Amorpheae flowers. Papilionoid flowers are complex, in the sense that they have several different types of parts and the parts have several different interactions (McShea 1996; Westerkamp 1997). Evolutionary changes toward complexity are suggested to be correlated with ecological specialization, and papilionoid flowers are commonly described as specialized for bee pollination (Kalin Arroyo 1981). Simplification of corollas, likewise, has been suggested to be associated with a broadening of the pollinator community (Faegri and van der Pijl 1979;

Pennington et al. 2000). Robertson (1890) observed a variety of visitors to *A. canescens*, including several species of flies, beetles, and bees. In contrast, a nearby legume, *Baptisia leucantha* Torr. & A. Gray, was visited only by a single species of bee (Robertson 1890).

However, we suggest that changes in pollinator community alone may not be sufficient to explain amorphoid flower diversification. Distributed from southern Canada to southern Mexico, members are found in communities with many other papilionoid legumes that have not lost their corollas. Instead, we infer evolutionary release from constraint, likely involving both development and ecology, that led to the current diversity of floral forms in Amorpheae.

Acknowledgments

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