

Extra-gynoecial pollen-tube growth in apocarpous angiosperms is phylogenetically widespread and probably adaptive

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Summary

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- Fusion of floral carpels (syncarpy) in angiosperms is thought to have allowed for significant improvements in offspring quantity and quality in syncarpous species over gymnosperms and apocarpous (free-carpelled) angiosperms. Given the disadvantages of apocarpy, it remains an evolutionary puzzle why many angiosperm lineages with free carpels (apocarpy) have been so successful and why some lineages show reversals to apocarpy.
- To investigate whether some advantages of syncarpy may accrue in other ways to apocarpous species, we reviewed previous studies of pollen-tube growth in apocarpous species and also documented pollen-tube growth in nine additional apocarpous species in six families.
- Anatomical studies of a scattering of apocarpous paleodicots, monocots, and eudicots show that, after transiting the style, 'extra' pollen tubes exit fully fertilized carpels and grow to other carpels with unfertilized ovules. In many species this occurs via openings in the simple carpels, as we report here for *Sagittaria potamogetifolia*, *Sagittaria pygmaea*, *Sedum lineare*, and *Schisandra sphenanthera*.
- The finding that extra-gynoecial pollen-tube growth is widespread in apocarpous species eliminates the possibility of a major fitness cost of apocarpy relative to syncarpy and may help to explain the persistence of, and multiple reversals to, apocarpy in the evolutionary history of angiosperms.

Introduction

Flowering plants (angiosperms) are today the most diverse and ecologically important group of land plants world-wide. They achieved global dominance over gymnosperms during the Cretaceous and early Tertiary, and have formed the foundation of nearly all terrestrial ecosystems ever since. In considering features responsible for angiosperm success, carpel closure (and concomitant germination of pollen on stigmas rather than ovule micropyles) and carpel fusion (syncarpy) are commonly invoked as among the most important key innovations (Stebbins, 1974; Mulcahy, 1979; Endress, 2001). These features, in combination with animal pollination, are thought to be partly responsible for angiosperms gradually displacing gymnosperms as the dominant photosynthetic organisms on land (Regal, 1977; Mulcahy, 1979; Carr & Carr, 1961; Stebbins, 1974; Endress, 1982, 2001; Armbruster *et al.*, 2002; but see Bond, 1989; Berendse & Scheffer, 2009 for additional hypotheses).

Adaptive explanations for the frequency of transitions to syncarpy and their contribution to angiosperm success have focused on enhanced physical protection of the ovules and economy of ovary construction (Stebbins, 1974), greater floral precision in pollination (Armbruster *et al.*, 2002, 2009), improved dispersal

capacity (Stebbins, 1974; Endress, 1982), intensified pollen competition (Endress, 1982; Williams *et al.*, 1993; Armbruster *et al.*, 2002), and fertilization of a larger proportion of the ovules ('pollen-tube dispersion'; Carr & Carr, 1961; Endress, 1982; Armbruster *et al.*, 2002). Recent research has suggested that improvements in the number and quality of offspring through pollen-tube dispersion and intensified pollen competition are probably the most important factors (Endress, 1982; Armbruster *et al.*, 2002). However, all analyses of the evolutionary trends in, and adaptive significance of, syncarpy are based on the assumption that movement of pollen tubes between separate carpels is impossible when carpels are physically isolated (apocarpy), except when there is obvious adhesion of stigmas or secretions between them ('extra-gynoecial compita'; Endress, 1982; Endress *et al.*, 1983). New research calls this assumption into question and may force us to change our ideas about the evolution of carpel fusion in angiosperms.

One question to arise from the adaptive analysis of syncarpy is that, if syncarpy is so advantageous, why do so many angiosperms (*c.* 20%; Soltis *et al.*, 2005) lack syncarpy and why have there been multiple reversals to apocarpy (Endress, 2001, 2011; Armbruster *et al.*, 2002; Endress & Doyle, 2009; Rudall *et al.*, 2011)? Further, if a major advantage of angiosperms over

gymnosperms accrued through syncarpy (Mulcahy, 1979; Endress, 1982), why did apocarpous angiosperms diversify, possibly at the expense of the gymnosperm? We suggest that the advantages of increased fertilization and pollen competition normally associated with syncarpy may accrue also to many apocarpous plants by virtue of having cryptic routes of pollen-tube communication between carpels (extra-gynoecial pollen-tube growth (EGPG)). More specifically, infra-stylar EGPG has been largely overlooked and presents adaptive advantages not seen in the more widely observed supra-stylar EGPG.

Previous studies have shown unusual pollen-tube growth in a few aquatic plants, for example, pollen tubes growing into receptacle, pedicel, or even stem tissues (Philbrick, 1984; Wang *et al.*, 2002, 2006; Huang, 2003). Other studies have shown unusual pollen-tube growth patterns in cleistogamous flowers, where pollen germinates in undehisced anthers and tubes grow down the filaments and receptacle to the carpels (Anderson, 1980; Márquez-Guzmán *et al.*, 1993). One study of a basal angiosperm showed pollen-tube communication (EGPG) between free carpels via the 'apical residuum' or receptacle (Williams *et al.*, 1993). Finally, many additional angiosperms, especially in the basal paleodictots, have EGPG, where tubes grow between carpels before, or without, transiting the style (see Endress & Igersheim, 2000). We call this supra-stylar and extra-stylar EGPG, respectively. Such unexpected pollen-tube behavior has been largely regarded as exceptional, solving special fertilization problems (but see Endress & Igersheim, 1997, 1999, 2000). However, if pollen tubes commonly surmount physical barriers to pass between free carpels, we would have to re-evaluate not only major evolutionary trends in angiosperms (see Endress & Doyle, 2009), but also the adaptive significance of the numerous independent origins (and reversals) of syncarpy and the role of syncarpy as a key innovation in the diversification and success of angiosperms.

Here we examined nine apocarpous species in six families to assess whether apocarpy generally precludes pollen-tube growth to unfertilized ovules in other carpels or whether, instead, EGPG is common and widespread. We were particularly interested in whether pollen tubes passed between carpels (EGPG) *before* or *after* travelling down the styles. We therefore investigated in detail the routes of pollen-tube growth and examined carpel anatomy in these species to explore why pollen tubes can grow between carpels in some species but not in others. Our survey reveals that infra-stylar EGPG is widespread, with about half of the apocarpous species surveyed showing this pattern of intercarpellary fertilization. This observation, in combination with previous published work, forces us to re-evaluate the commonly cited advantages of morphological syncarpy and may provide an explanation for the frequency of reversals from syncarpy to apocarpy in flowering plants.

Materials and Methods

Study material

To examine the possibility of extra-gynoecial growth of pollen tubes in apocarpous species generally, we surveyed pollen-tube

growth pathway and carpel anatomy in nine species in the Alismataceae (*Sagittaria potamogetifolia* Merr. and *Sagittaria pygmaea* Miq.), Crassulaceae (*Sedum lineare* Thunb.), Cabombaceae (*Cabomba caroliniana* L.), Ranunculaceae (*Ranunculus ternatus* Thunb. and *Ranunculus sieboldii* Miq.), Rosaceae (*Duchesnea indica* (Andr.) Focke and *Rosa multiflora* var. *cathayensis*), and Schisandraceae (*Schisandra sphenanthera* Rehd. et Wils.).

Using the same methods (see the 'Observations of pollen-tube growth' section below) as in the survey, we also examined one species, *S. potamogetifolia*, in greater detail. This species is an herbaceous, aquatic monocot, endemic to southeast China (Chen, 1989). It is monoecious, with female (pistillate) flowers in a basal whorl of racemes blooming earlier and numerous male flowers blooming later. As in other *Sagittaria* (arrowheads), each whorl generally has three flowers, each bearing three white petals. The gynoecia of the female flowers comprise *c.* 160 separate carpels on a globose receptacle, and the androecia of male flowers comprise *c.* 20 stamens (Wang *et al.*, 2002). Carpels are uniovulate and flattened, with short, papillate, stigmatic crests. This species is summer-flowering and insect-pollinated, and single flowers last 1 d. Forty individuals were collected from a population in a marsh in Chaling County, Hunan Province, China (26°50'N, 113°40'E) and established in a glasshouse at Wuhan University, Wuhan.

Observations of pollen-tube growth

We used the aniline-blue fluorescence method (see Williams *et al.*, 1993) to observe the routes of pollen-tube growth. We bagged single inflorescences before the female flowers opened. When petals of the female flowers opened and the gynoecia were exposed, we hand-pollinated a subset of stigmas using fresh pollen from another plant. The mix of pollinated and unpollinated carpels in a single flower allowed us to assess the potential for intercarpellary growth of pollen tubes (EGPG; i.e. detect those tubes coming from pollen on stigmas of other carpels). Gynoecia were collected 5 h after pollination and were fixed in formalin-acetic acid-alcohol (FAA), and then rinsed with water, cleared and stained with aniline blue. In addition to making cross-sections by hand to observe (under an epifluorescence microscope) pollen-tube growth within the apocarpous gynoecia (Wang *et al.*, 2002), we also carefully removed the pistils from the receptacle and made thin sections of the receptacle in order to observe pollen-tube growth between separate carpels.

Carpel anatomy

The standard paraffin-embedding method was used to obtain longitudinal sections of the pistils to show the transmitting tissue of the carpel, and the route of pollen-tube growth between carpels. Serial sections of thickness 10 µm were taken and stained with safranin-fast green and observed under a light microscope. Studies have suggested that the extracellular matrix (ECM) of transmitting tissue in carpels is associated with intercarpellary growth of pollen tubes (Lyew *et al.*, 2007). To detect ECM through the carpels, we used a semi-thin sectioning method to observe transverse sections from the six positions of the mature

gynoecia of *S. potamogetifolia*. Opened flowers from the bagged inflorescences were randomly picked and fixed, dehydrated and embedded. The embedded samples were sectioned with a glass knife to a thickness of 1 μm . The sections were stained with 0.5% toluidine blue (Wang *et al.*, 2006).

Comparative analyses

The literature on EGPG was compiled and the type of EGPG classified and tabulated (see reviews in Armbruster *et al.*, 2002; Huang, 2003; Endress & Doyle, 2009 and original studies cited in Table 3). Intra- and extra-gynoecial pollen-tube growth and the recognized types of EGPG were optimized onto a pruned angiosperm phylogeny based on the representative angiosperm molecular trees published in Soltis *et al.* (2005) and combined molecular-morphological trees published in Endress & Doyle (2009).

Results

Pollen-tube growth in *Sagittaria potamogetifolia*

Virtually all pollen grains deposited on a stigma germinated, and the pollen tubes grew through the transmitting tissue to the ovule. When numerous pollen tubes arrived at the basal part of one ovary, one or two pollen tubes could be seen growing toward the ovule, but only one pollen tube tip entered the embryo sac through the micropyle (Fig. 1a). Other pollen tubes were observed growing through the ovary base or otherwise failing to orient to the ovule and were seen wandering near the ovule. Some tubes were observed to pass into and across the receptacle to enter ovules in nearby ovaries (if these ovules were not already occupied by pollen tubes). Typically, the tubes entered the surface layer of

receptacle tissue, turned to the basal part of another carpel, and entered the ovule (Fig. 1b). Observing the receptacle from which pistils had been removed, we found a network of pollen tubes stretching across the surface of the receptacle. Pollen tubes grew between carpel attachment points, with some twisting on the surface of the receptacle and some successfully entering the carpels (Fig. 1c), and (seen in other preparations) the embryo sac. This behavior results in fertilization of otherwise unfertilized ovules and should thus increase seed set.

Structure of the gynoecium in *Sagittaria potamogetifolia*

Longitudinal sections of the carpel showed that there is a long, narrow canal in the style and an obvious opening at the base of each ovary (Fig. 1d). This opening corresponds exactly to the point at which the pollen tubes grew from the ovary toward the receptacle. This opening also corresponds to the position at which pollen tubes entered the base of other carpels to access the embryo sac. Fig. 1(b) summarizes diagrammatically our observations of the pollen-tube pathway.

Transverse sections of a mature pistil of *S. potamogetifolia* indicated that the stigmatic crests were lip-like (Fig. 1e), the styles were hollow (Fig. 1f,g), and the extracellular matrix was distributed continuously down the styler canal, and across the inner surface of the ovary wall (Fig. 1h) and the outer surface of the funiculus to the opening of the ovary (Fig. 1i), leading to the surface of the receptacle (Fig. 1j). Pollen tubes were strictly limited to this tract (Fig. 1b).

Family survey

Similar extra-gynoecial growth of pollen tubes was observed in species from Alismataceae, Crassulaceae and Schisandraceae

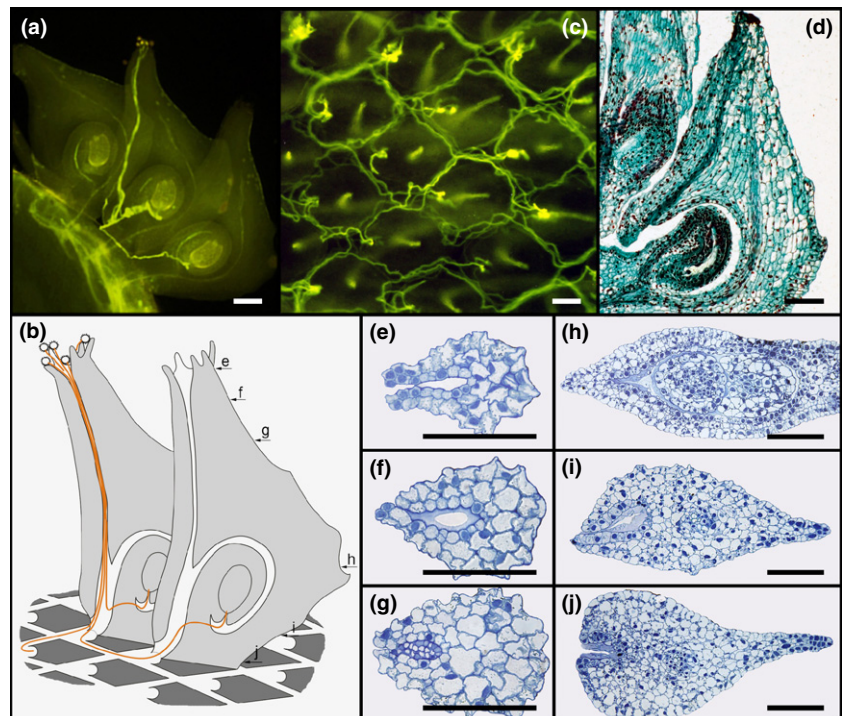


Fig. 1 Carpel structure and pathway of extra-gynoecial pollen-tube growth in an apocarpous monocot *Sagittaria potamogetifolia*. (a) Longitudinal view of the pathway of pollen tubes passing from one carpel to an unfertilized ovule in another carpel (fluorescence microscopy). (b) Diagram of the path taken by pollen tubes leaving one carpel and entering another carpel. (c) Transverse view of pollen tubes within a receptacle from which carpels have been removed, showing the network of pollen tubes growing freely from one ovary to another on the surface of the receptacle. (d) Longitudinal view of pollen-tube pathways between individual carpels. (e–j) Semi-thin transverse sections of carpels of *S. potamogetifolia*, with sectioning: (e) at the stigma, (f) at the middle of the pistil, (g) at the styler canal with pollen tubes inside, (h) at the inner surface of the ovary wall, (i) near the micropyle, and (j) at the base of the carpel (see labels in b). Bar, 0.25 mm.

Table 1 Distribution of extra-gynoecial pollen-tube growth (EGPG) across apocarpous angiosperms

Taxon	Clade: family	Extra-gynoecial pollen-tube route	Advantage accrued	Source
Supra-stylar EGPG				
<i>Amborella</i>	Amborellaceae	Pollen-tube growth between stigmas	Increased seed set	Endress & Igersheim (2000)
<i>Nymphaea</i> , <i>Nuphar</i>	Nymphaeaceae (aquatics)	Pollen-tube growth between carpels through intercarpellary secretion	Increased seed set	Endress & Igersheim (2000)
<i>Galbulimima</i> , <i>Eupomatia</i> , Annonaceae 7 taxa	Magnoliales			Igersheim & Endress (1997)
Menispermaceae, Lardizabalaceae	Laurales Eudicot: Ranunculanae	Wet stigmas forming an external compitum	Increased seed set	Endress & Igersheim (1997) Endress (1995)
Infra-stylar EGPG				
<i>Illicium floridanum</i>	Austrobaileyales: Illiciaceae	The apical residuum and associated unfused carpel margins	Increased seed set, enhanced pollen competition	Williams <i>et al.</i> (1993)
<i>Kadsura longipedunculata</i>	Austrobaileyales: Schisandraceae	Between unfused carpels after passage down pseudostyle (stigmatic crest) and across extragynoecial compitum	Increased seed set, enhanced pollen competition	Lyew <i>et al.</i> (2007)
<i>Schisandra sphenanthera</i>	Schisandraceae	Open stylar canal in the margin of the carpel	Increased seed set, enhanced pollen competition	Igersheim & Endress (1997); This study
<i>Sedum lineare</i>	Eudicot: Crassulaceae	Open stylar canal in the margin of the carpel	Increased seed set, enhanced pollen competition	This study
<i>Sagittaria potamogetifolia</i>	Monocot: Alismataceae (aquatics)	Receptacle	Increased seed set, enhanced pollen competition	Wang <i>et al.</i> (2002); Huang (2003); this study
<i>Sagittaria guyanensis</i> ssp. <i>lappula</i>	Monocot: Alismataceae	Receptacle	Increased seed set	Wang <i>et al.</i> (2006); this study
<i>Sagittaria pygmaea</i> <i>Ranalisma rostratum</i>	Monocot: Alismataceae Monocot: Alismataceae (aquatics)	Receptacle Open stylar canal in the margin of the carpel	Increased seed set Increased seed set	This study Wang <i>et al.</i> (2006)
Extra-stylar EGPG				
<i>Aspicarpa</i> , <i>Camarea</i> , <i>Gaudichaudia</i> , <i>Janusia</i> (cleistogamous flowers only) <i>Callitriche heterophylla</i> , <i>C. palustris</i>	Eudicot: Malpighiaceae Eudicot: Callitricaceae (aquatics)	From indehiscent anther to carpel via filament, receptacle From anther of staminate flower to carpel of pistillate flower via filament, staminate receptacle, stem, peduncle, pistillate receptacle	Autogamy; enhance pollen competition? Underwater fertilization	Anderson (1980) Philbrick (1984)
<i>Lacandonia schismatica</i>	Monocot: Pandanales: Triuridaceae or Lacandoniaceae	From indehiscent anther through the receptacle to carpel	Autogamy	Márquez-Guzmán <i>et al.</i> (1993)
EGPG not detected				
<i>Cabomba caroliniana</i>	Cabombaceae (aquatics)	None	–	This study
<i>Ranunculus ternatus</i>	Eudicot: Ranunculaceae	None	–	This study
<i>Ranunculus sieboldii</i>	Ranunculaceae	None	–	This study
<i>Duchesnea indica</i>	Eudicot: Rosaceae	None	–	This study
<i>Rosa multiflora</i> var. <i>cathayensis</i>	Rosaceae	None	–	This study

Supra-stylar EGPG, extra-gynoecial pollen-tube growth to the stigma such that pollen-tubes do not compete for access to ovules in different carpels. Infra-stylar EGPG, extra-gynoecial pollen-tube growth to other carpels after passage down the style, which permits pollen competition for access to ovules in different ovules. Extra-stylar EGPG, extra-gynoecial pollen-tube growth to other carpels, where pollen tubes do not pass down styles. This should also permit pollen competition for access to ovules in different ovules.

(Table 1; Fig. 2). Examination of carpel anatomy indicated that the entry of pollen tubes into ovules of neighboring carpels was associated with gynoecial architecture similar to that observed in *S. potamogetifolia*. Extra-gynoecial growth of pollen tubes in *S. pygmaea* and *S. guyanensis* was the same as in *S. potamogetifolia*, in which pollen-tube growth was restricted to the stylar canal of one carpel but could escape the canal to nearby ovaries via the surface of the receptacle. In *Sedum lineare* and *Schisandra sphenanthera*, pollen tubes could grow to nearby carpels via a passage permitting pollen-tube exit and entry (Fig. 2e–g). In these species, some pollen tubes grew down the surface of the style and continued to the ovary, while other tubes could cross to and enter nearby ovaries from the middle of the style on which the pollen was deposited. The pollen-tube pathway was on the sealing edge of the carpel. EGPG was not observed in the five species sampled from the Cabombaceae, Rosaceae, and Ranunculaceae. For example, we observed that numerous pollen tubes reached positions near the base of ovules, but no tubes could leave the ovary to access other ovules in *Ranunculus rostratum* (Fig. 2a). Correspondingly, longitudinal sections of the carpel and continuous transverse sections suggested that the carpels lack an outlet for pollen tubes (Fig. 2b–d).

A review of previous studies indicates similar patterns of supra-, extra-, or infra-stylar EGPG across taxa in a total of 14 families. This information is summarized in Table 1.

Phylogeny of EGPG

When we add our observations of EGPG to those published in the literature and map this information onto a well-supported skeleton angiosperm phylogeny, we find that EGPG ('extra-gynoecial compita' in the terminology of Endress & Doyle (2009) is both phylogenetically widespread and probably the basal condition in angiosperms (Fig. 3; see also Endress & Doyle, 2009). Distinguishing between supra-stylar, extra-stylar, and

infra-stylar EGPG is important because only the last consistently promotes pollen competition (although extra-stylar EGPG may do so sometimes, depending on the details of the pollen-tube route). The basal angiosperms have supra-stylar EGPG (reconstructed as the basal state; Fig. 3), with one origin of infra-stylar EGPG in a supra-stylar lineage (Austrobaileales; Fig. 3). There were at least three additional independent origins of infra-stylar EGPG, two origins of extra-stylar EGPG, and three origins of supra-stylar EGPG (Fig. 3).

Discussion

Our study of extra-stylar pollen-tube growth (EGPG) leads us to distinguish among three types of EGPG: supra-stylar, extra-stylar, and infra-stylar. Supra-stylar EGPG depends largely on appressed stigmas and/or secretion of fluids through which pollen tubes can grow to reach stigmas and styles of carpels other than those on which the pollen originally landed. Extra-stylar EGPG is found in species in which pollen tubes do not penetrate the stigmas, and is seen in only a few specialized situations (e.g. cleistogamous flowers). In contrast, infra-stylar EGPG involves pollen tubes growing down the stigma and style on which they landed and exiting the ovary to reach unfertilized ovules in other carpels. Our investigation of nine species showed that infra-stylar EGPG occurs in species with carpels that have openings through which pollen tubes can exit, but appears to be absent in species with carpels that are completely sealed. This suggests that infra-stylar EGPG is influenced by carpel morphology.

We found EGPG to be surprisingly common, even in species whose flowers did not have obvious extra-gynoecial compita (see Endress & Igersheim, 1997, 1999, 2000). For example, in the apocarpous monocot genus *Sagittaria*, we found that 'extra' pollen tubes grew out of the ovary through a basal opening of the incompletely sealed carpel, and across the surface of the receptacle, to other carpels, thus fertilizing virgin ovules. In this species,

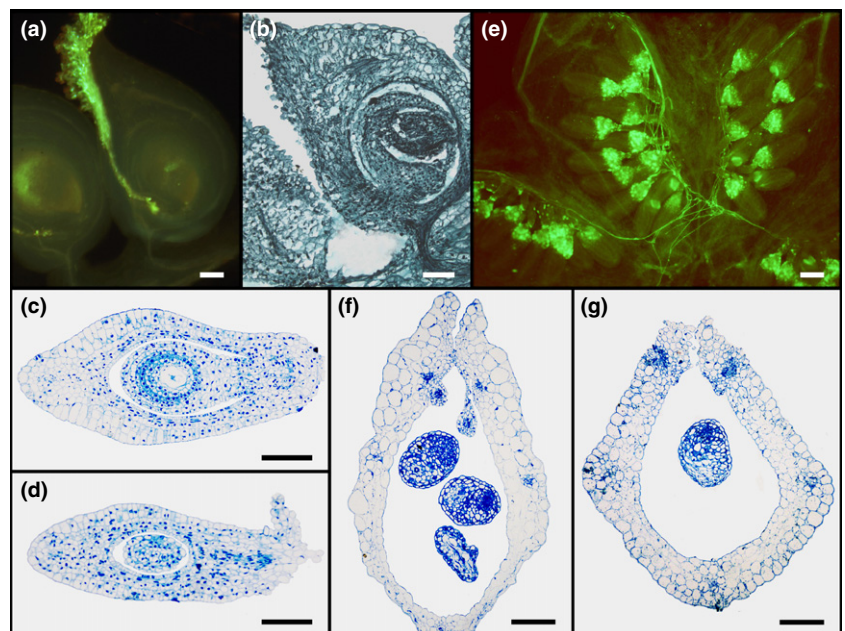


Fig. 2 Comparison of carpel structure and extra-gynoecial pollen-tube growth between two apocarpous species, *Ranunculus rostratum* (Ranunculaceae; a–d) and *Sedum lineare* (Crassulaceae; e–g). No pollen tubes are seen exiting the carpel (a) and no outlet exists in the sealed carpel (b). Transverse sections at the middle (c) and base (d) of the carpel show a complete sealed carpel. Extra-gynoecial pollen-tube growth occurs in *S. lineare* (e). Transverse sections at the middle (f) and base (g) of the carpel show an opening at the base. Bar, 0.25 mm.

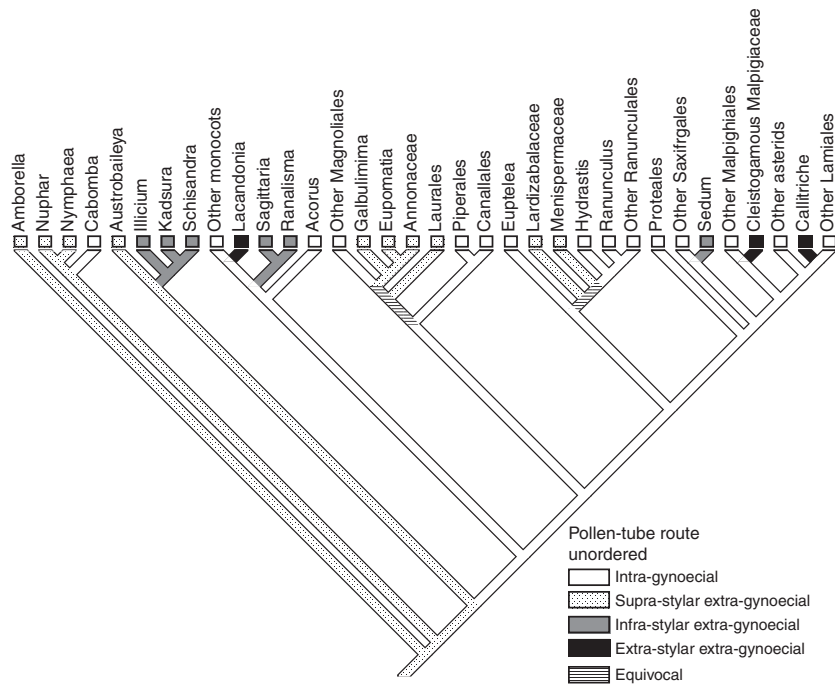


Fig. 3 The distribution of supra-stylar, infra-stylar, and related forms of extra-gynoecial pollen-tube growth (EGPG) across the angiosperm phylogeny. Data on supra-stylar EGPG come from Armbruster *et al.* (2002) and Endress & Doyle (2009). Data on infra-stylar EGPG come from this study, and data on other forms of EGPG come from Huang (2003). The phylogeny is based on the representative angiosperm molecular trees published in Soltis *et al.* (2005) and combined molecular-morphological trees published in Endress & Doyle (2009).

the opening provides a passage for pollen tubes to exit the ovary, allowing them to grow freely across the receptacle to other carpels, and form a network of pollen tubes interconnecting otherwise separate carpels.

The distinction among supra-stylar, extra-stylar, and infra-stylar EGPG has not been previously emphasized (although see Armbruster *et al.*, 2002; Endress, 2011). It is important not only because of the markedly different morphologies and developmental patterns involved, but because of the different adaptive consequences of the three types of EGPG. Supra-stylar and extra-stylar EGPG can improve the seed set of flowers under pollen limitation, as can infra-stylar EGPG. However, only infra-stylar EGPG can consistently increase the intensity of pollen competition and reduce rates of fertilization by genetically inferior male gametophytes (pollen). (Certain types of extra-stylar EGPG can potentially also lead to pollen competition, e.g. cleistogamous Malpighiaceae). The logic of this is the same as expressed for partially syncarpous pistils (Armbruster *et al.*, 2002): pollen tubes that are committed to a single carpel above the style compete only with pollen tubes in their own style. By contrast, pollen tubes that can cross between carpels below the style effectively compete with all pollen tubes in all styles. Because infra-stylar EGPG is more difficult to recognize than morphological mechanisms promoting pollen competition (e.g. morphological compita; Endress, 1982), its frequency and importance have probably been seriously underestimated.

The discovery that infra-stylar, extra-gynoecial pollen-tube growth is phylogenetically widespread (Fig. 3) and perhaps common in apocarpous angiosperms leads us to reassess several aspects of angiosperm evolutionary history. First, redistribution of pollen tubes among carpels and effective pollen competition are not restricted to plants with fused ovaries (syncarpy), as noted by Endress and collaborators (e.g. Endress, 1982, 2011; Endress

& Doyle, 2009). This means that the repeated shifts from apocarpy to syncarpy may not always be driven by selection for the increased quantity and quality of offspring via pollen-tube redistribution, given that one or both of these capacities may already occur in the apocarpous ancestor. This increases the balance of evidence for alternative hypotheses that have been proposed but hitherto thought less important, at least recently, including reduced reproductive investment in ovary walls per seed and/or increased protection of developing ovules for a given investment (Stebbins, 1974), improved seed-dispersal mechanisms (Stebbins, 1974; Endress, 1982), and/or increased floral precision and adaptive accuracy in pollination (Armbruster *et al.*, 2002, 2009). Also, the shifts from syncarpy to apocarpy seen repeatedly in angiosperms may not incur the costs of reduced offspring quantity and quality. This may help explain the occurrence of these otherwise surprising transitions (Soltis *et al.*, 2005; Endress & Doyle, 2009; Endress, 2011; Rudall *et al.*, 2011). For example, if pollen tubes in other apocarpous monocots behave as we observed in *Sagittaria* and *Ranalisma rostratum*, there would be little or no selective cost in terms of pollen redistribution (seed set) and pollen competition (seed quality) in the four or more transitions from syncarpy to apocarpy in monocots (Fig. 3; Endress & Doyle, 2009 (Fig. 10b); Rudall *et al.*, 2011).

Another interesting facet of infra-stylar EGPG is that the average pollen-tube length is increased dramatically for those pollen grains participating in between-carpel fertilizations. This means that the potential intensity and effectiveness of pollen competition are increased (hence potentially increasing mean offspring fitness) relative to both intra-gynoecial and supra-stylar EGPG (Mulcahy, 1983; Armbruster *et al.*, 1995). This feature may be especially important in species such as *Schisandra sphenanthera* and *Sagittaria* spp. that have relatively short styles (Fig. 1).

Mechanism of tropism in extra-gynoecial pollen-tube growth

For successful fertilization, pollen must germinate on the stigma, grow tubes, usually through the style, and find and penetrate the ovule micropyle. These processes require growing pollen tubes to undergo numerous changes in growth orientation. The cues in the pistil that guide pollen-tube orientation are believed to be mechanical and chemotropic, but their nature is not well understood (Hülkamp *et al.*, 1995; Johnson & Preuss, 2002; Holdaway-Clarke & Hepler, 2003; Chae & Lord, 2011). The guidance of the pollen tube has been assumed to depend on the architecture and chemical properties of the female reproductive tissues and/or ovules to provide a signal for the target-directed growth of the pollen tube. We observed pollen tubes growing freely but not randomly among carpels and apparently attracted by unfertilized ovules. This observation demonstrates that, if there is a signal to direct pollen tube growth, the signal is probably released only by 'virgin' ovules (Okuda & Higashiyama, 2010).

Significance of extra-gynoecial pollen-tube routes to pollen-tube reallocation

The repeated evolution of syncarpy is one of the dominant features of angiosperm macroevolution. A minimum of 17 independent evolutionary transitions from apocarpy to syncarpy have occurred; about three-quarters of these transitions allowed pollen tubes to cross between carpels and fertilize ovules that would otherwise be left unfertilized (Armbruster *et al.*, 2002). This will generally occur if there is a joint pollen-tube transmission tissue shared by the carpels (the 'compitum'; Carr & Carr, 1961), allowing pollen tubes to cross between carpels (Carr & Carr, 1961; Walker, 1978; Endress, 1982; Williams *et al.*, 1993; van der Schoot *et al.*, 1995). This condition is thought to be the rule in flowers with fully syncarpous ovaries that are unilocular or incompletely multilocular, but occurs also in many flowers with multilocular ovaries and post-genital (after initial formation) fusion of styles or stigmas, forming a compitum (Carr & Carr, 1961; Endress, 1982; Endress *et al.*, 1983).

However, some apocarpous flowers possess extra-gynoecial compita (allowing EGPG). In such cases, pollen tubes can travel on or through a functional (extra-gynoecial) compitum to cross between separate carpels, usually through secretions joining appressed or adjacent ovaries or stigmas (Walker, 1978; Endress, 1982; Endress *et al.*, 1983; Renner *et al.*, 1997). The present study shows the importance of distinguishing between supra- and infra-stylar crossings between carpels, whether by intra-gynoecial compita, obvious extra-gynoecial compita, or other forms of extra-gynoecial pollen-tube growth, such as through the receptacle. Whereas both supra- and infra-stylar crossings of pollen tubes between carpels potentially enhance seed set under pollen limitation, only the latter can enhance offspring quality through intensified pollen competition.

In several apocarpous species, we found long, narrow stylar canals with obvious openings at each end. Pollen tubes could travel along this track and reallocate between carpels by exiting

the base of the canal. This allows apocarpous flowers to function more like syncarpous flowers in terms of the redistribution of pollen tubes between carpels and pollen competition. Our detailed study of *S. potamogetifolia* revealed that inter-carpellary pollen-tube growth can be very extensive (Fig. 1) and may therefore play a major role in increasing both the quantity and the quality of seeds produced by flowers of apocarpous species with infra-stylar EPGP.

Phylogeny of extra-gynoecial pollen-tube growth

Endress and colleagues (Table 1; Endress & Doyle, 2009) have shown that extra-gynoecial pollen-tube growth (EGPG; via extra-gynoecial compita) is widely distributed among basal angiosperms and is probably the basal state (Table 1; Endress & Doyle, 2009). The work presented here adds support to this conclusion. Most basal angiosperms with EGPG have supra-stylar EGPG, but some Austrobaileyales have infra-stylar EGPG (Fig. 3). Repeated evolution of both forms of EGPG in primitively or secondarily apocarpous lineages (Fig. 3) supports the hypothesis that selection for increased offspring quantity and/or quality has promoted this transition.

Future research

Experimental studies are needed to assess whether pollen competition is more intense and offspring quality is improved in plants with infra-stylar extra-gynoecial pollen-tube growth. We anticipate that such experiments will reveal that many apocarpous angiosperms indeed benefit from greater seed set and more intense pollen competition through infra-stylar EGPG, much like in plants with fused carpels and intra-gynoecial compita. Experimental confirmation of these benefits would help explain both 'anomalous' reversals to apocarpy and the early success and radiations of apocarpous angiosperms and their role in replacing gymnosperms as the dominant higher plant life form.

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