

EARLY STAGES OF DEVELOPMENT SHED LIGHT ON FRUIT EVOLUTION IN ALLOPOLYPLOID SPECIES OF *GEUM* (ROSACEAE)

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Molecular phylogenetic studies have shown that the evolutionary history of *Geum* L. has been strongly influenced by allopolyploidy, and they have indicated widespread homoplasy in morphological traits within the group. This study of gynoecial development in Colurieae shows that fruits with jointed styles develop similarly. In early stages of development, the position of the joint is basal in all lineages where it is present. In the diploids, it remains at the base of the style, but in the tetraploid and the two hexaploid lineages, a beak forms from the distal part of the ovary, causing the joint to be brought upward as it elongates. In the two lineages of the hexaploid clade, a hook is subsequently developed in the distal part of the beak (the “fishhook fruit”). One of these also undergoes a final stage when the beak is twisted. The similarity of development implies that the joint and the deciduous segment of the style are homologous structures inherited by the polyploids from one of their diploid ancestral lineages, although they are not expressed in all species. Genome-level processes that could affect the expression of morphological traits in groups of allopolyploid origin are discussed.

Keywords: *Geum*, fruit development, character evolution, allopolyploidy.

Introduction

Within Rosoideae Arn. there is a large variation in functional fruits, that is, structures that facilitate dispersal. In this group we find, for example, rose hips, raspberries, and strawberries, as well as the minute nutlets of *Potentilla* L., the follicle-like fruits of *Filipendula* Mill., and the wind-dispersed fruits with long featherlike styles of some *Geum* species. The true fruits are, however, monocarpellate and apocarpous in all of these cases, and, with the exception of the drupelets in *Rubus* L., the individual fruits within Rosoideae are all achenes.

Phylogenetic relationships within Rosoideae are not completely understood. *Filipendula* has been shown to be sister to the remainder of the group (Morgan et al. 1994; Eriksson et al. 1998), but regarding the relationships among the other three lineages, *Rubus*, Colurieae Rydb. (to which *Geum* belongs), and Roperculina (the clade consisting of *Rosa* L., *Sanguisorbeae* DC., and *Potentilleae* Sweet; Eriksson et al. 2003), different analyses have so far given inconclusive or conflicting results. All three possible relationships among these clades have been supported by different phylogenetic analyses: *Rubus* and Roperculina as sister groups (Morgan et al. 1994; Smedmark and Eriksson 2002; Eriksson et al. 2003), *Rubus* and Colurieae as sister groups (Eriksson et al. 1998, 2003; Potter et al. 2002), and Colurieae and Roperculina as sister groups (Smedmark et al. 2005).

Colurieae is unique in Rosoideae in having styles that do not wither at maturity. Instead, they elongate into long vil-

lous plumes or stiff beaks, the latter usually equipped with some means of adhesion. These fruits, with their apical styles, are strikingly similar to the individual achenes of closely related groups, such as *Rubus* and *Rosa*, especially in early developmental stages. In those, however, the styles do not elongate but remain short. *Potentilleae*, on the other hand, are characterized by having subapical or lateral styles. The gynoecia of Colurieae usually contain numerous carpels, but the number varies from one to ca. 250, and the mature achenes often form a dense head on the elongate receptacle. In some species, the style persists on the fruit and is either relatively short and stiff with a hooked apex (fig. 1, *Geum leiospermum*) or straight, and then often becoming greatly elongated (up to 60 mm) and villous (fig. 1, e.g., *Geum glaciale*). In other species, the style has a joint, that is, an abscission layer where the distal part of the style, or in some cases the entire style, falls off after pollination. The segment remaining on the fruit forms a beak, a stiff projection that may be up to 10 mm long, usually with some feature facilitating adhesion. In the fishhook fruit type (Iltis 1913), for example, the distal part of the beak forms a hook (fig. 1, e.g., *Geum rivale*), whereas in the harpoon fruit type (Iltis 1913), retrorse bristles cover the region proximal to the joint (fig. 1, *Geum heterocarpum*).

Wind-dispersed fruits have been shown to be plesiomorphic within Colurieae (Smedmark and Eriksson 2002). A considerable amount of homoplasy is seen within Colurieae when fruit characters are mapped onto a bifurcating phylogenetic tree. According to DNA sequence data from the ITS and *trnL-trnF* regions, the joint either evolved in four different lineages or evolved once and subsequently reversed to different types of nonarticulate styles several times (Smedmark and Eriksson 2002). However, evidence from the nuclear

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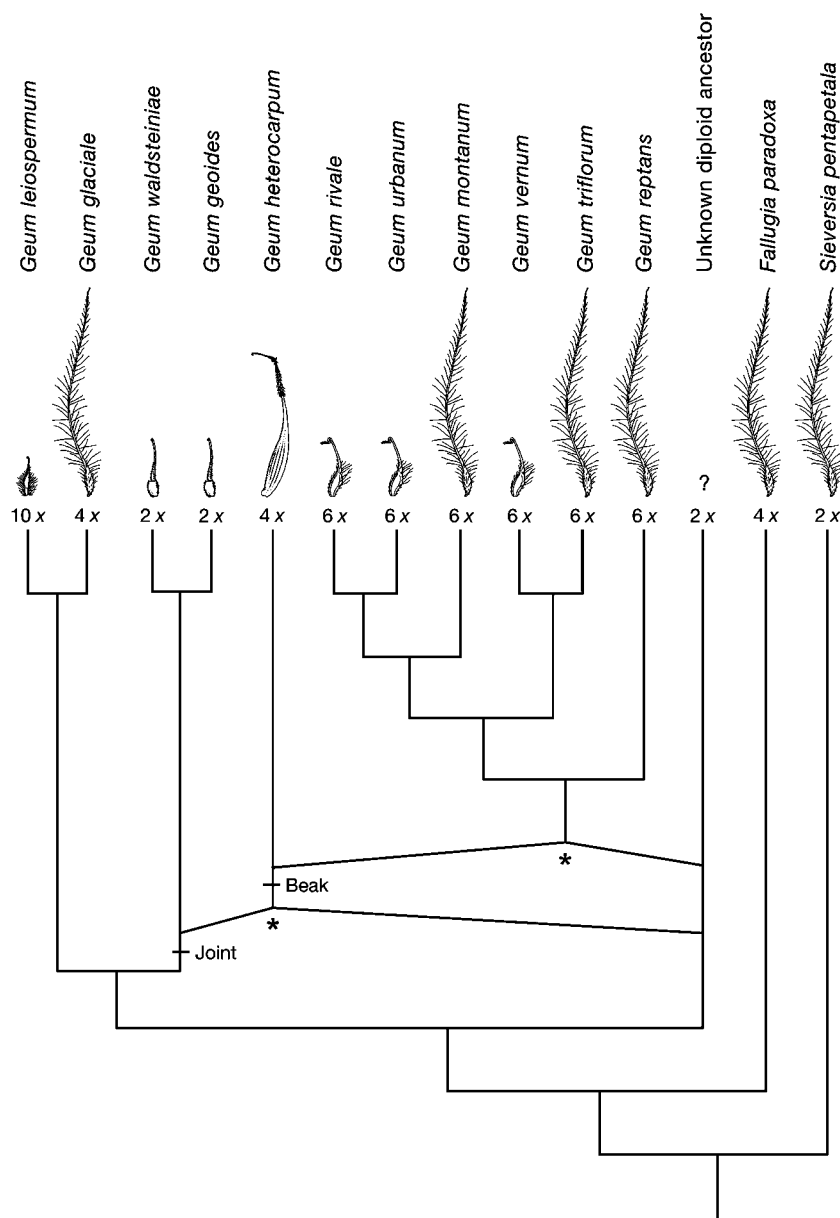


Fig. 1 Phylogenetic hypothesis of Colurieae based on GBSSI-1 gene sequences. The tree is redrawn from Smedmark et al. (2005). The hypothesis involves two allopolyploidization events, each marked with an asterisk. The hypothesized origins of two morphological characters of the pistil, the joint, and the beak are also indicated on the tree. Ploidy level and fruit type are marked for each species. The fruit of *Geum leiospermum* has a style that is hooked at the apex (drawing based on *Geum cockaynei*). *Geum glaciale*, *Geum montanum*, *Geum triflorum*, *Geum reptans*, *Fallugia paradoxa*, and *Sieversia pentapetala* have fruits with plumose styles (drawing based on *G. montanum*). *Geum waldsteiniae* and *Geum geoides* have styles that are entirely deciduous (drawing based on *G. waldsteiniae*). *Geum heterocarpum* has harpoon fruits (Iltis 1913) with a partly deciduous style and a beak with retrorse bristles. *Geum rivale*, *Geum urbanum*, and *Geum vernum* have fishhook fruits (Iltis 1913) with a partly deciduous style and a hooked beak (drawing based on *G. urbanum*).

low-copy gene GBSSI (Smedmark et al. 2003) indicates that a reticulate representation may better illustrate the historical relationships within the group (fig. 1). According to this interpretation, the joint first arose in the ancestral lineage of *Geum geoides* and *Geum waldsteiniae* (previously classified in *Coluria* and *Waldsteinia*, respectively; Smedmark, forthcoming). This lineage hybridized with another diploid species, giving rise to the tetraploid lineage to which *G. het-*

erocarpum belongs. This tetraploid inherited the joint and later passed this character on to a hexaploid lineage, in another allopolyploidization. Today, this hexaploid lineage constitutes a clade of about 40 species, most of which have articulate styles (e.g., *Geum urbanum*) and some of which do not (e.g., *Geum reptans*).

In this article we study the gynoecial ontogeny using scanning electron microscopy (SEM) for a selection of species in

Colurieae representing different fruit types and different evolutionary lineages identified in earlier phylogenetic work (Smedmark and Eriksson 2002; Smedmark et al. 2003). Previous studies of floral development in Rosaceae have dealt with androecial ontogeny (Lindenhofer and Weber 1999a, 1999b, 2000) and with floral ontogeny of groups outside Rosoideae (Evans and Dickinson 1996, 1999a, 1999b). There are four main aims of this study.

1. *To examine whether the joint is evident in earlier stages of gynoecial development in species belonging to the allohexaploid clade.* In some cases, the ontogeny of an organism may reveal characters that were present in its ancestors. For example, in angiosperm groups where complex leaves are plesiomorphic, species with simple leaves have complex leaf primordia that develop into simple leaves (Bharathan et al. 2002). As mentioned above, a nuclear low-copy gene phylogeny (Smedmark et al. 2003) implied that the tetraploid parental lineage of the hexaploids had jointed styles. However, this character is lacking in fully developed fruits of many species in the hexaploid lineage. Still, these taxa seem to carry the genetic basis for the jointed style, since it reappears in two separate descendant lineages, namely, the two groups with the fishhook fruit (fig. 1). We want to check whether the joint is perhaps present in earlier stages of development in species of the hexaploid clade even though it is not evident in mature fruits.

2. *To test a hypothesis (Gajewski 1957) about homology of different parts of the style of the fishhook fruit of the allohexaploids with the styles of the ancestral lineages.* Based on cytogenetic data, Gajewski (1957) suggested that the hexaploids with the fishhook type of fruit (e.g., *G. urbanum*) are of allopolyploid origin. He proposed that one of the parents had been a diploid ancestor of *G. geoides* or *G. waldesteiniae*, with a style that is articulate at the base and shed in its entirety. The other parent was suggested to have been *Geum montanum*, which he believed to be tetraploid and which has an elongate villous style. He also found morphological evidence in support of this hypothesis. There are differences between the epidermal cells of the deciduous stigmatic part of the fishhook fruit and those of the persistent proximal part of the style, the beak (Iltis 1913). The deciduous part is characterized by numerous stomata and chloroplasts, closely resembling the entirely deciduous styles of *G. geoides* and *G. waldesteiniae*. The beak, on the other hand, lacks stomata and chloroplasts, hence resembling the long and villous style of *G. montanum* (Iltis 1913). Gajewski (1957) concluded that the deciduous stigmatic part of the fishhook fruit of the hexaploids is homologous with the style of one of the hypothesized parents, with an entirely deciduous style, while the beak is homologous with the style of the other parent, hypothesized to have been persistent and plumose.

Molecular phylogenetic studies have later supported the hypothesis of a hybrid origin of the hexaploids, but they have indicated somewhat different and more complicated ancestry. The current hypothesis (Smedmark et al. 2003) includes an initial allopolyploidization involving parental lineages with the morphological traits suggested by Gajewski (fig. 1). One of the parental lineages indeed seems to have been a representative of the ancestral lineage of *G. geoides* and *G. waldesteiniae* (Smedmark et al. 2003). The other one, however, was shown not to have been *G. montanum*. So far, no extant representa-

tive of this parental lineage has been identified, but the polyploids have homoeologous paralogues of the low-copy gene GBSSI that have originated outside the herbaceous perennial clade *Geum* (Smedmark et al. 2005). This indicates that the other parent belongs outside this clade (fig. 1). The results also show that it is not identical to either of the two known Colurieae lineages outside *Geum*, namely, *Fallugia* and *Sieversia*. These two taxa both have long, villous styles. Optimization of fruit characters on a chloroplast *trnL/F* phylogeny (Smedmark and Eriksson 2002) indicates that the common ancestor of *Geum* also had a persistent style, making it likely that this second ancestral lineage had a long, villous style, too. This first polyploid event is hypothesized to have given rise to a tetraploid lineage including the extant *G. heterocarpum* (Smedmark et al. 2003). This species has a type of fruit that is similar to the fishhook fruit: the harpoon fruit (Iltis 1913), which has a beak, but without a hook at its apex. According to the GBSSI hypothesis about reticulate relationships, this lineage, together with a representative of one of the original diploid parental lineages, was involved in a second hybridization and polyploidization that resulted in the hexaploid lineage where the species with the fishhook fruits belong (fig. 1).

Thus, currently available data provide evidence for an allopolyploid origin of taxa with beaked fruits with terminal deciduous stylar segments involving ancestral lineages with the same fruit morphology that Gajewski (1957) suggested. In this study we examine the epidermal cells of the styles for a number of species to test his hypothesis. We examine whether there is any difference in the structure of the epidermis of the style between *G. waldesteiniae* and *G. geoides* as compared with *Fallugia* and whether the beak is different compared with the deciduous segment in fishhook and harpoon fruits. In that event, is the deciduous part more like the style of *G. waldesteiniae* and *G. geoides* and the beak more like the style of *Fallugia*? We also investigate whether ontogenetic data give any indication of homology between the different regions.

3. *To investigate the presence of a joint on the styles of the species Geum triflorum.* Sometimes the very long and villous style of this species has been interpreted as being geniculate and jointed close to the tip, at the point where it changes from hairy to glabrous (Bolte 1933; Cronquist et al. 1997). Other workers (e.g., Gajewski 1957; Hutchinson 1964) do not find that this is the case.

4. *To determine whether the fishhook fruits, inferred to have evolved twice, differ in development.* Phylogenetic analysis of molecular data has rejected homology of the fishhook fruits of *Geum vernum* with those of other *Geum* species (Smedmark and Eriksson 2002; Smedmark et al. 2003). Although the fruits of these two lineages look remarkably similar morphologically, at maturity their ontogeny may show that they are formed in different ways, and this might confirm the molecular phylogenetic results.

Material and Methods

Collections of the species studied were made mainly from plants growing in the Bergius Botanic Garden but also from wild populations. Origin and voucher information are listed in table 1. The selection of taxa was based on earlier results from molecular phylogenies and access to living material.

Buds of different developmental stages, as well as flowers and mature fruits, were fixed in formalin–acetic acid–alcohol for at least 2 wk. The plant material was then transferred to 70% ethanol for storage. Gynoecia for SEM were dissected in 70% alcohol and dehydrated in an increasing alcohol series (80%, 85%, 95%, 99.6%), each wash lasting 24 h. After two final 1-h washes in 99.6% ethanol, the material was critical-point dried using liquid CO₂ in a Bal-Tec CPD 030, mounted on aluminium stubs, and sputtered with gold in an EMITECH K 550. The preparations were then viewed and photographed in a JEOL JSM 6300 at 6 kV.

Results

Comparative Ontogeny of Gynoecia

Fallugia paradoxa. Initiation of ca. 50 gynoecial primordia occurs acropetally on the conoidal floral apex. They first appear as rounded projections on the receptacle but soon become bifacial with a crease along the adaxial surface (fig. 2A). This furrow will later develop into the ovary locule. As the hypanthium widens, it carries the stamens and the perianth upward and outward, in relation to the gynoecium. It also becomes villous on the inside. Each primordium then becomes slightly tubular at the base and differentiates into a distinct ovary and style. While still in bud, the carpels reach a length of <1 mm, and hairs start to appear in the proximal region of the style (fig. 2B). The gynoecium remains at this developmental stage throughout flowering. During this period, a villous stipe begins to develop underneath each carpel. After flowering, the style elongates intensively, reaching a final length of ca. 40 mm when it is fully developed. During this process, the cells of the epidermis become long and narrow, and the few stomata become widely dispersed. The entire achene, except for the 1 mm immediately below the

stigma, becomes covered by long, soft, spreading hairs, which tend to be twisted. The glandular stigma is punctate but stretches out somewhat toward the adaxial furrow. The fusiform ovary is 2.5 mm long when fully developed and inserted on a villous stipe on the conoidal gynoecial receptacle. The suture is clearly visible throughout development (fig. 2C). Although appearing to be open, it is in fact closed by appression of the margins of the carpel (Juel 1918). The hypanthium is obconical and villous inside.

Geum leiospermum. The earliest stages of gynoecial development have not been studied for this species. There are ca. 20 carpels per flower, and once these have differentiated into ovary and style, the distal part of the style begins to re-curve (fig. 3A). Although the suture is visible along the adaxial side of the carpel, it appears to be closed. While the carpel is still very small, the style elongates to nearly its full length. The epidermal cells of the style become straight and elongate, while those of the ovary are small and rounded. Long hairs develop on the gynoecial receptacle. As the ovary enlarges the epidermal cells become irregular (fig. 3B). Stomata develop on the ovary but not on the style. When the carpel is fully developed, the tip of the style forms a distinct hook (fig. 3C), terminated by the punctate, glandular stigma. The completely glabrous achenes are sessile on the conoidal, villous gynoecial receptacle. They reach a total length of 2 mm, of which the style is no more than 0.3–0.4 mm. The hypanthium is obconical and glabrous inside.

Geum geoides. Ca. 20 gynoecial primordia are initiated on the rounded floral apex, and soon a furrow forms in their adaxial side (fig. 4A). The glabrous hypanthium widens to a cuplike shape. Each carpel differentiates into ovary and style and becomes raised on a glabrous stipe (fig. 4B). At this point the lower part of the carpel wall has become tubular. The style elongates further, and a constriction at its base marks the initiation of a joint (fig. 4B). Hairs begin to appear in the proximal part of the style. When the achene is fully developed, the style reaches a length of ca. 8 mm, and its proximal part becomes covered by hairs, regular as well as glandular and stellate hairs. The stigma is punctiform. The ovary is 2 mm long and covered by conspicuous papillae (fig. 4C), except for a smooth liplike border around the suture. Each achene is inserted on a rounded projection of the sparsely villous receptacle. The hypanthium is narrowly obconical and glabrous.

Geum waldsteiniae. One to four gynoecial primordia are initiated on the flattened floral apex, each of which soon develops an adaxial furrow. The hypanthium widens so that the gynoecium is situated below the stamens. The individual primordia acquire a clearly defined ovary and style (fig. 5A). They get a three-lobed shape by two additional furrows running from the base of the ovary to the punctiform stigma. This may be caused by thickening of the carpel wall around the three vascular bundles present in the carpel wall (Juel 1918), one abaxial and two adaxial. A glabrous gynophore is formed that raises the carpels from the hypanthium (fig. 5B). If there are several carpels, each one is positioned on a ramification of the gynophore. A joint begins to form at the base of the style (fig. 5B). Hairs appear on the ovary and in the proximal part of the style (fig. 5C, 5D). The joint becomes more pronounced as the carpel grows. The style lengthens to ca. 7 mm, its proximal part becoming hispid and with

Table 1

Species Included in the Study, with Information about Voucher Specimen and Origin

Species	Voucher
<i>Fallugia paradoxa</i> (D. Don) Endl.	T. Eriksson No. 796 (SBT)
<i>Geum geoides</i> (Pall.) Smedmark	J. E. E. Smedmark No. 2 (SBT); cult. Bergius Botanic Garden
<i>Geum heterocarpum</i> Boiss.	J. E. E. Smedmark No. 5 (SBT); cult. Bergius Botanic Garden
<i>Geum leiospermum</i> Petrie	M. Chase; cult. Royal Botanic Gardens Kew
<i>Geum rivale</i> L.	J. E. E. Smedmark No. 10 (S)
<i>Geum triflorum</i> Pursh	J. E. E. Smedmark No. 8 (SBT); cult. Bergius Botanic Garden
<i>Geum urbanum</i> L.	J. E. E. Smedmark No. 3 (S)
<i>Geum vernum</i> (Raf.) Torr. & Gray	J. E. E. Smedmark No. 6 (SBT); cult. Bergius Botanic Garden
<i>Geum waldsteiniae</i> Smedmark	J. E. E. Smedmark No. 4 (SBT); cult. Stockholm University

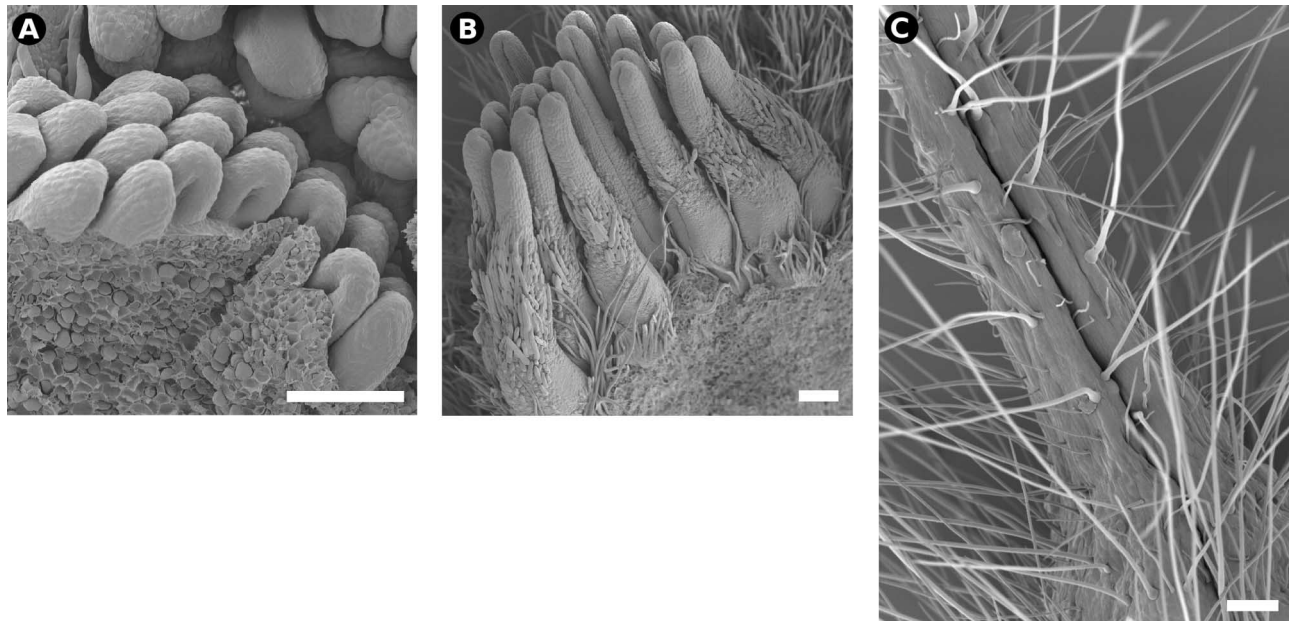


Fig. 2 *Fallugia paradoxa*. Development of gynoecium. **A**, Gynoecial primordia with adaxial furrow on floral apex. **B**, Portion of gynoecium with young carpels. The gynoecium will remain at this developmental stage from bud until after flowering. **C**, Partial view of adaxial side of mature achene. Scale bars = 100 μm .

numerous stomata (fig. 5E), while its distal part remains glabrous. The ovary becomes tomentose and reaches a length of 3 mm. The achenes are inserted on the ramified, glabrous 2-mm-long gynophore and enclosed by the obconical and glabrous hypanthium.

***Geum heterocarpum*.** The seven to 12 gynoecial primordia are initiated on the hemispherical floral apex (fig. 6A). A cylindrical gynophore differentiates, raising the gynoecium from the developing hypanthium. The gynoecial pri-

mordia, clustered on the gynophore, first become bifacial and then conduplicate with an adaxial furrow (fig. 6B). The distal portion of each carpel elongates to a neck that develops into the style. The ovary is smooth, while the epidermal cells of the style become somewhat swollen. A constriction, where the joint will develop, appears at the base of the style (fig. 6C). Hairs first emerge on the style, distal to the developing joint, and later on the ovary. At this point, the base of the ovary has become slightly tubular, while the distal part has begun

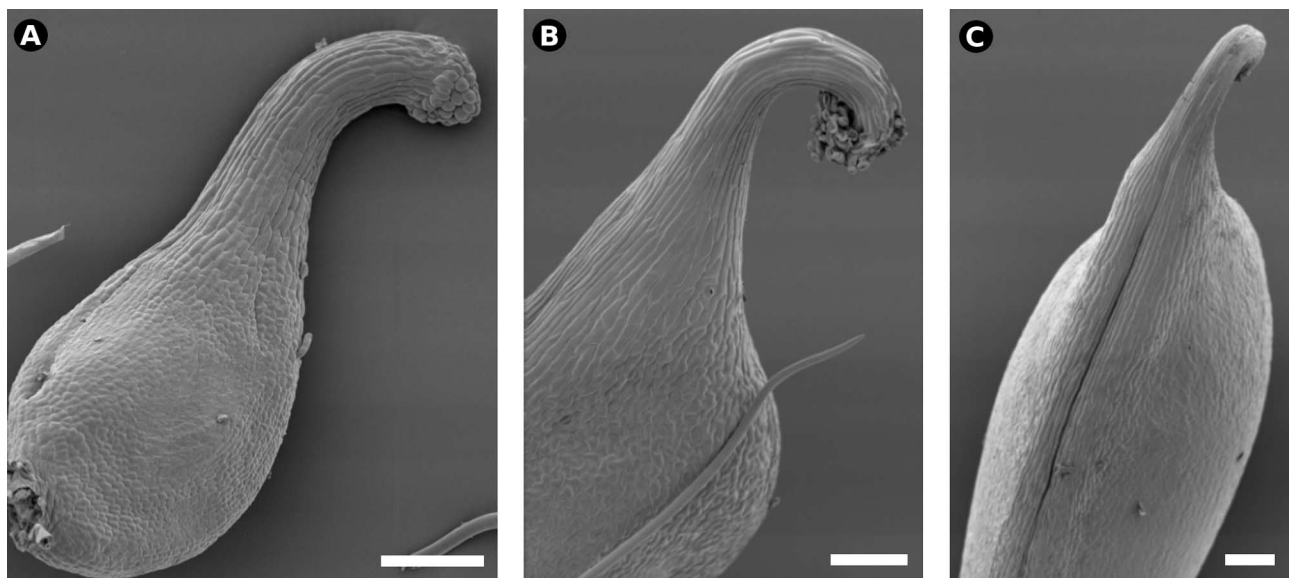


Fig. 3 *Geum leiospermum*. Development of gynoecium. **A**, Lateral view of young carpel at initiation of recurvation of stigmatic region. **B**, Lateral view of fully developed hook at apex of style. **C**, Adaxial view of mature achene. Scale bars = 100 μm .

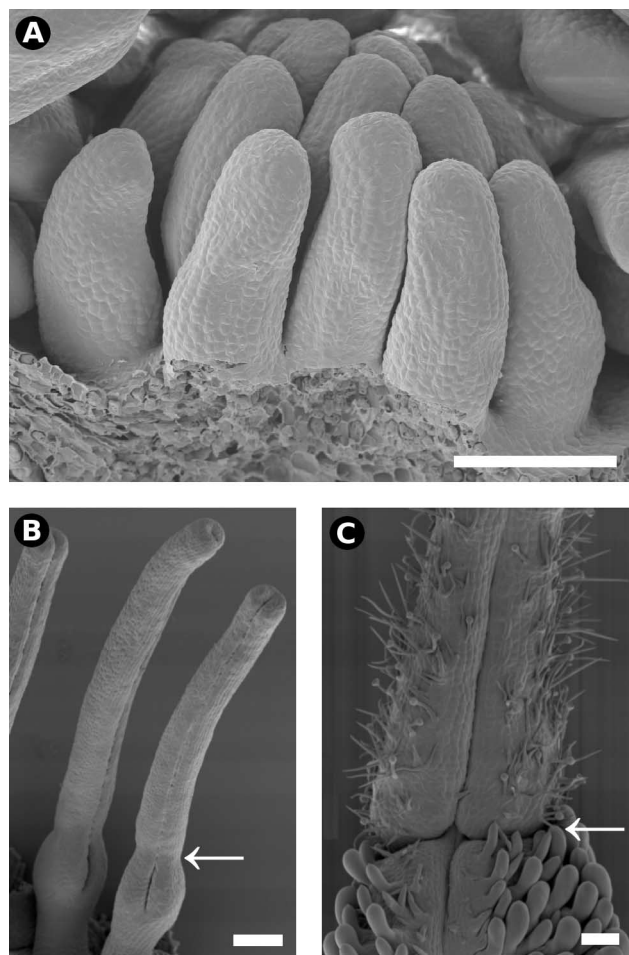


Fig. 4 *Geum geoides*. Development of gynoecium. A, Young gynoecium with conduplicate gynoecial primordia. B, Adaxial view of two pistils at initiation of joint. C, Adaxial view of mature achene showing the proximal part of the style, the joint, and the papillate ovary. Scale bars = 100 μ m. Arrows indicate the position of the joint.

to elongate, forming a beak (fig. 6D). The deciduous segment is the same length as the rest of the carpel. Then hairs start to form on the distal part of the beak (fig. 6E). Before anthesis, when the carpel is <4 mm long, the deciduous segment has reached its full length of ca. 1.7 mm. The beak elongates extensively after anthesis, reaching a final length of 7 mm. It becomes stiff, with a pointed, straight apex covered with retrorse bristles (fig. 6F). The fully developed ovary is fusiform, 7 mm long, and covered with short, soft, appressed hairs. All over the achene, glandular hairs occur together with nonglandular hairs. The achenes are sessile on the villous gynophore that elongates to about 7 mm, pushing the fruits out of the funnel-shaped hypanthium. Curiously, one carpel always remains inserted at the base of the gynophore.

***Geum urbanum*.** Up to ca. 100 gynoecial primordia are initiated acropetally on the hemispherical floral apex (fig. 7A, 7B). These develop into conduplicate carpels, each with an adaxial furrow. The carpels soon become slightly tubular at the base (fig. 7C, 7D). Then the distal part forms a neck that differentiates into the style, so that the carpel becomes flask

shaped with a distinct ovary and style. The style is narrowest at the base, becoming gradually thicker toward the apex (fig. 7D). The cells of the style have a swollen appearance, while the ovary is smooth. Then the region between the ovary and style begins to elongate, initiating formation of the beak (fig. 7E). Hairs appear first on the gynoecial receptacle and then on the ovary and the distal segment of the style. Below the middle of the style, there is a displacement of its proximal part in the adaxial direction (fig. 7F). Distal to the resulting curve, the style is sparingly tomentose, the epidermal cells are irregular, and stomata are quite numerous, while proximally, the style is glabrous and the cells long and straight, and stomata are lacking. The initiation of the joint becomes evident as a region of small rounded cells on the segment where the style is being bent adaxially (fig. 7G). The adaxial furrow is now closed along the beak but still evident on the deciduous segment. The deciduous part of the style reaches its final length of ca. 1 mm when the flower is still in bud. The beak is then less than half its mature length and does not elongate to its full length of 5 mm until after anthesis. The region below the joint continues to curve downward until a well-defined hook is formed (fig. 7H). The ovary is ca. 5 mm long when fully developed and hispid mainly in the upper part. The achenes are inserted directly onto the cylindrical, tomentose gynoecial receptacle, without a stipe. The hypanthium is shallowly concave and glabrous.

***Geum rivale*.** The fruit development follows the same pattern as that of *G. urbanum*, differing only in quantitative aspects. In contrast to *G. urbanum*, a prominent gynophore develops in the flowers of *G. rivale*.

***Geum triflorum*.** The earliest stages of gynoecial development have not been studied for this species. There are ca. 70 carpels inserted on the hemispherical floral apex. When they have reached the flasklike stage, the style is completely straight, and the adaxial furrow still appears to be open (fig. 8A). The base of the carpel becomes tubular, and hairs begin to appear in the proximal part of the style (fig. 8B). The stigma gets a splayed, almost fanlike shape (fig. 8B). A stipe supporting each carpel is formed (fig. 8C). The style elongates, mainly in its distal part. At anthesis the entire carpel is <10 mm long, and the proximal quarter of the style is densely villous and glandular, while the distal three-quarters is glabrous (fig. 8C). Later, the proximal part of style undergoes extensive elongation. Finally, the style may be up to 60 mm long and villous, except for the 5 mm below the stigma that remains glabrous. The ovary is ca. 3 mm long when fully developed and is inserted on a stipe on the 2-mm-long pubescent, conoidal gynoecial receptacle. The hypanthium is cup shaped and glabrous. There is no evidence of a joint during any phase of the development.

***Geum vernum*.** Ca. 60 rounded gynoecial primordia develop acropetally on the hemispherical floral apex (fig. 9A). The receptacle soon differentiates into a hypanthium and a gynophore, the latter carrying the gynoecium. The carpels become bifacial, with a furrow forming on their adaxial side. Their distal portion extends, forming a neck that will become the style. The base of the carpel becomes tubular, bringing the lower end of the suture up to the upper part of the developing ovary (fig. 9B). Hairs start to appear on the distal part of the style, and the epidermis of this region becomes

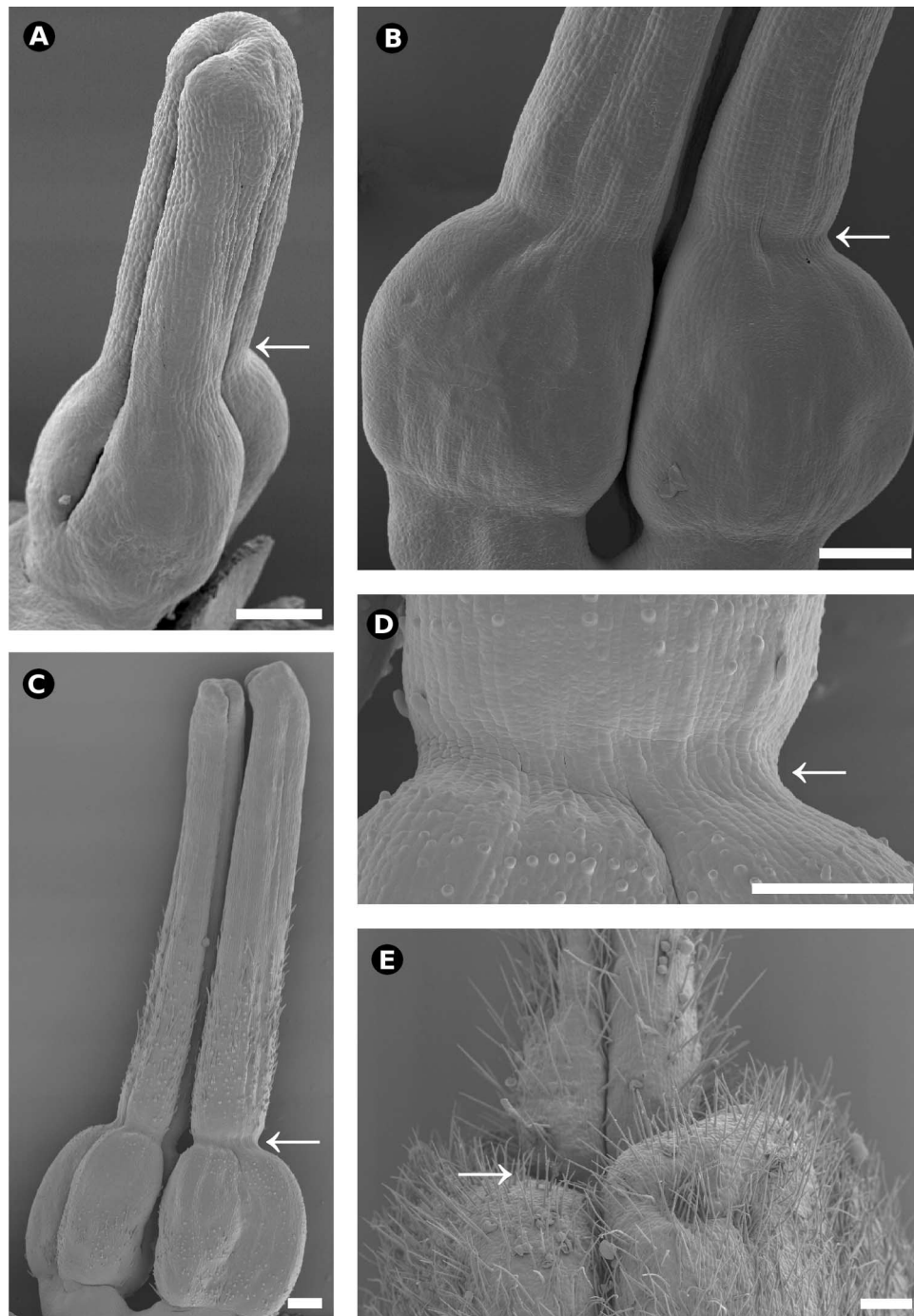


Fig. 5 *Geum waldsteimiae*. Development of gynoecium. *A*, Adaxial view of young carpel. *B*, Lateral view of proximal portion of two carpels at initiation of joint. *C*, Lateral view of two slightly older carpels on ramified gynophore. *D*, Detail of single carpel with developing joint. *E*, Adaxial view of mature achene showing proximal portion of style. Scale bars = 100 μm . Arrows indicate the position of the joint.

different from that of the proximal part: the cells become irregular, more swollen, and interspersed with stomata. The distal part of the style is also thicker than the proximal part, especially toward the apex (fig. 9*B*). Hairs, both glandular and nonglandular, begin to cover the ovary. Below the middle, the style begins to bend toward the adaxial side, and a joint is initiated as a region of small rounded cells (fig. 9*C*).

At first the distal part of style remains upright, but soon it is folding back toward the abaxial side (fig. 9*D*). The curve of the style proximal to the developing joint becomes more pronounced until a hook is formed (fig. 9*E*). In this process, the deciduous segment is brought forward so that the stigma comes to point more or less adaxially. The hook is fully formed when the style is <1 mm long. At this point the deciduous

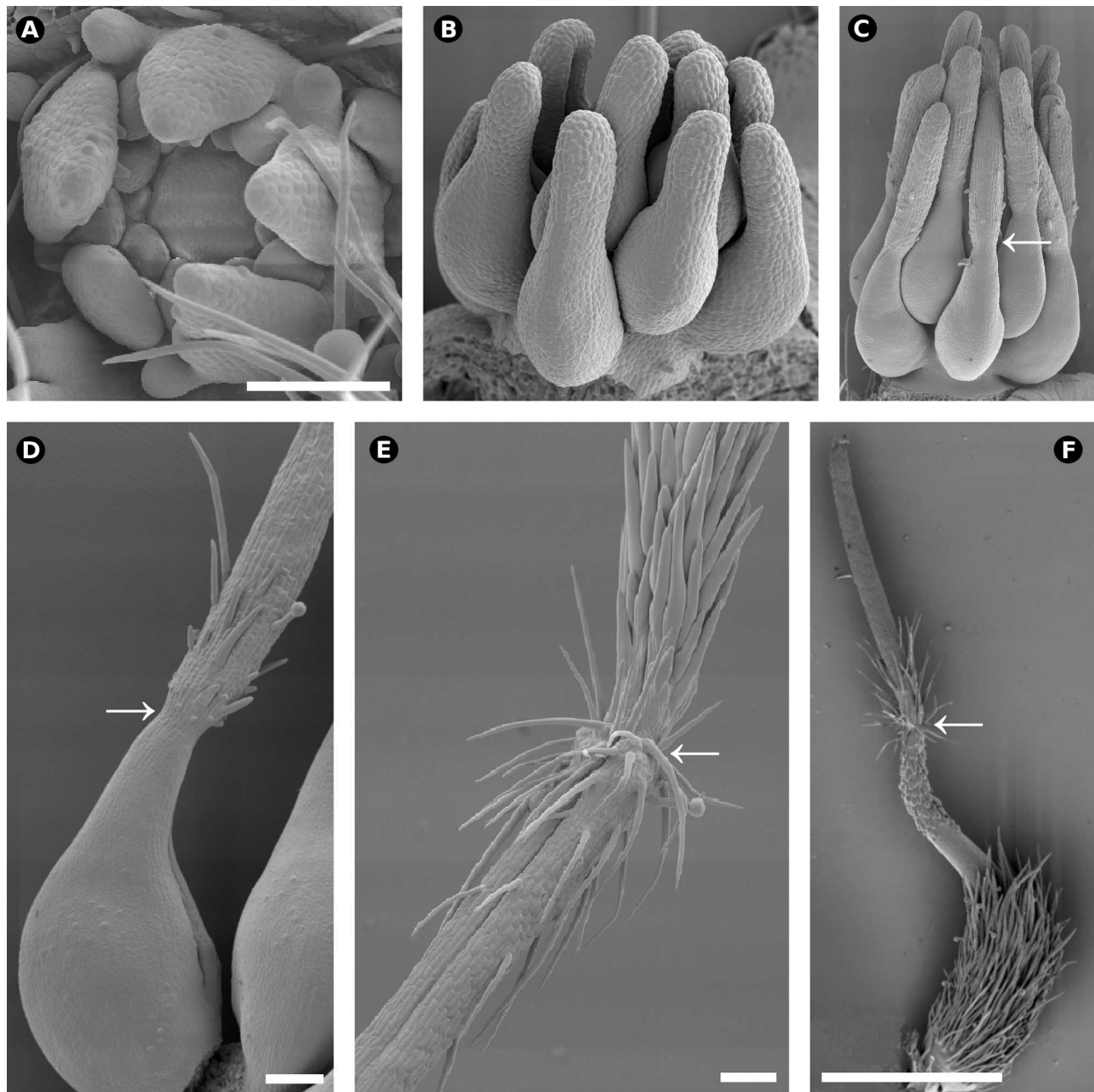


Fig. 6 *Geum heterocarpum*. Development of gynoecium. *A*, Floral primordium with two whorls of sepals, petals, the two outer 5-merous whorls of stamens, and floral apex before gynoecium initiation. *B*, Young gynoecium with conduplicate carpels on gynophore. *C*, Gynoecium at initiation of joint. *D*, Lateral view of single carpel at initiation of beak. *E*, Lateral view of carpel with hairs appearing on beak. *F*, Styler joint with retrorse bristles proximal to the point of abscission. Scale bars = 100 μm (*A-D*, *F*) and 1 mm (*E*). Arrows indicate the position of the joint.

segment has reached its final length of 0.5 mm. As the beak elongates to reach its full length of 3 mm, it becomes twisted (fig. 9*F*, 9*G*), turning the hook around 180°, so that it finally is directed toward the abaxial side of the carpel. The adaxial suture is visible throughout the length of the carpel. The ovary is 2.5 mm long and pilose when it is fully developed, and its point of attachment is shaped like an elliptic, membranous cup. The gynophore is 8 mm long and sparingly pilose with scattered glandular hairs.

***Geum reptans*.** The gynoecial development has not been studied as thoroughly for this species as for the previous ones, but there is no joint on the style in developmental stages when it appears in the species that have articulate styles. The epidermis of the mature style is similar to that of *Fallugia*. The style is ca. 25 mm long and villous, the hairiness decreasing toward the tip. Glandular hairs occur throughout the carpel. The fully developed ovary is cylindrical and pilose and inserted on a stipe.

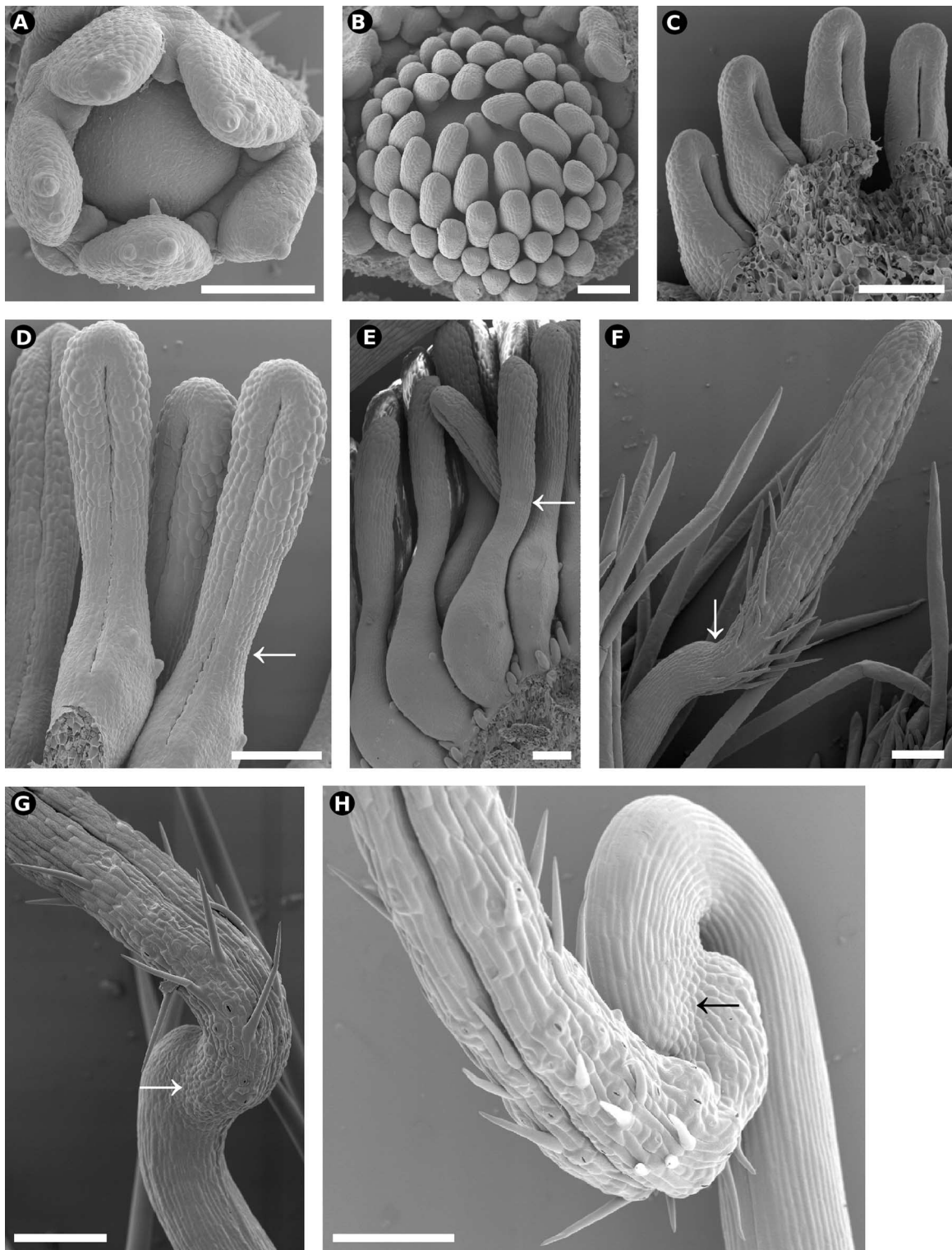


Fig. 7 *Geum urbanum*. Development of gynoecium. *A*, Floral primordium at androecial initiation. *B*, Apical view of floral apex with gynoecial primordia. *C*, Adaxial view of four conduplicate carpels. *D*, Adaxial view of carpels at a later stage. *E*, Lateral view of carpels after initiation of beak. *F*, Partial view of style beginning to bend adaxially. *G*, Style at initiation of joint. *H*, Partial view of style with joint at maturity. Scale bars = 100 μm . Arrows indicate the position of the joint.

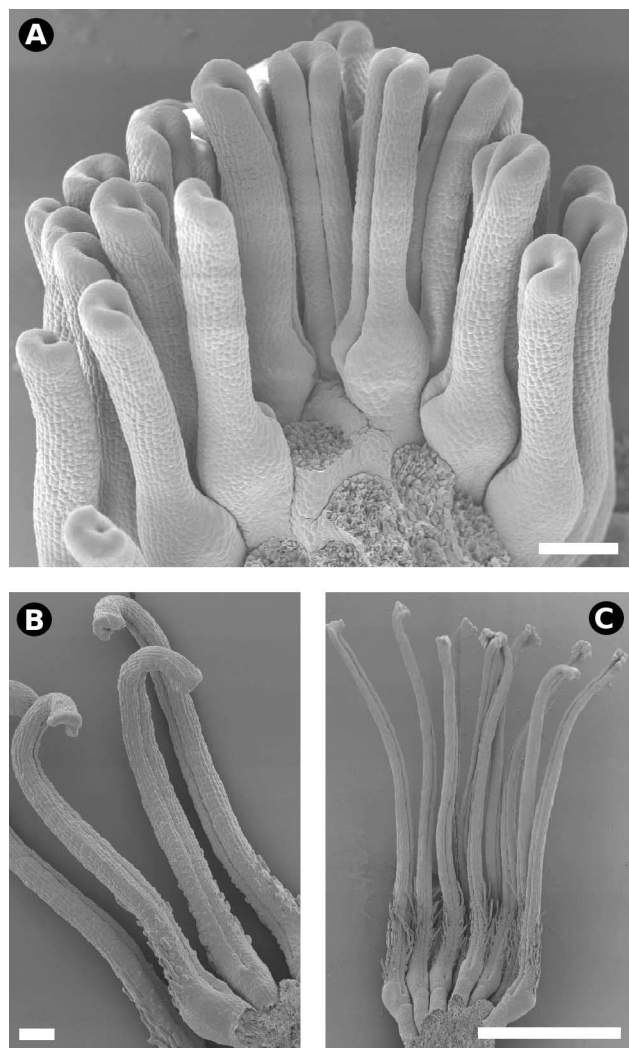


Fig. 8 *Geum triflorum*. Development of gynoecium. A, Portion of gynoecium with young carpels. B, Adaxial view of carpels with hairs appearing in proximal part of style. C, Adaxial view of carpels after initial elongation of style, mainly in distal part. Scale bars = 100 μ m (A, B) and 1 mm (C).

Discussion

The ontogenetic data presented in this study show that early development of the different types of fruits with jointed styles is similar. In the fruits of *Geum heterocarpum*, *Geum vernum*, and *Geum urbanum*, which represent three separate evolutionary origins of the beak, the position of the joint is distal to the ovary, at the base of the style, early in the ontogeny. At this stage, this is the narrowest point of the style, and although the joint is not yet evident as a constricted region of smaller, rounded cells, the continued development makes it clear that this is the position where it will develop. The cells of the style are irregular and more swollen than those of the rest of the carpel at this stage. As the beak begins to elongate, it does so in the region proximal to the position of the joint. The beak is narrower throughout, compared with the

distal deciduous segment of the style, and it has elongate, straight epidermal cells. Thus, the beaked fruits start out like those with basal joints, such as the fruits of *Geum geoides* and *Geum waldsteiniae*, but as the beak develops in the polyploids, the joint is moved upward and away from the ovary. This similarity in early development is perhaps not surprising, since molecular data (Smedmark et al. 2003) have indicated reticulate relationships among these species, implying that the joint evolved in one of their common ancestors. It is, however, not expressed in all of its descendants. We find no indication that the joint is expressed at an early stage of development in the wind-dispersed polyploids *Geum triflorum*, *Geum montanum*, and *Geum reptans* and later reduced.

The similarity in morphology as well as in development between the deciduous segments of the style in the different beaked fruits of the polyploids *G. urbanum*, *G. vernum*, and *G. heterocarpum*, compared with the entirely deciduous style of the diploids *G. waldsteiniae* and *G. geoides*, lends support to part of Gajewski's (1957) hypothesis, which states that there is homology between the deciduous versus persistent parts of the style of the beaked fruits and the different types of styles in their diploid parental lineages. Similarities also exist between the epidermis of the style of *Fallugia* and that of the beak of the fishhook and harpoon fruits. Both regions have long, narrow cells and almost no stomata. However, at the developmental stage when the joint is initiated in the taxa with deciduous styles, before initiation of the beak, *Fallugia* has a miniature version of its fully developed style. If we expect homologous structures to occupy corresponding relative positions in comparable stages of the life histories of two organisms (Jardine 1967), this seems to be evidence for rejecting the hypothesis. If Gajewski's hypothesis were correct, the joint would be expected to be formed some way up the style in the beaked fruit types, thus indicating additivity of the two parental types of style in the allopolyploid. Instead, it forms at the base of the style, and the beak is the result of an elongation of the distal part of the ovary. We interpret the beak as a unique structure evolving in the allopolyploids. It differs from the styles of wind-dispersed species in being stiff and mostly glabrous. According to our interpretation, the similarity of the beak compared to plumose styles is not directly caused by their hybrid origin but seems rather to be the result of similar development. After anthesis, the style of *Fallugia* and other wind-dispersed species elongates rapidly, and hence the cells are stretched. In the beaked fruits, the distal part of the style reaches its final length before anthesis, while the main part of the elongation of the region below the joint takes place after flowering. This rapid elongation of the delimited region below the joint is likely to be the explanation for why few stomata are present in the epidermis of the beak.

It is possible that the division in terms of time, where the distal part of the style elongates first and the proximal part later, is characteristic of the allopolyploids, since the development of *G. triflorum* is similar. To determine whether this is the case, a wider sample of species from this group would have to be studied.

The style of *G. triflorum*, which is long and villous in the fully developed fruit, has been interpreted as being geniculate and jointed close to the tip, at the point where it changes

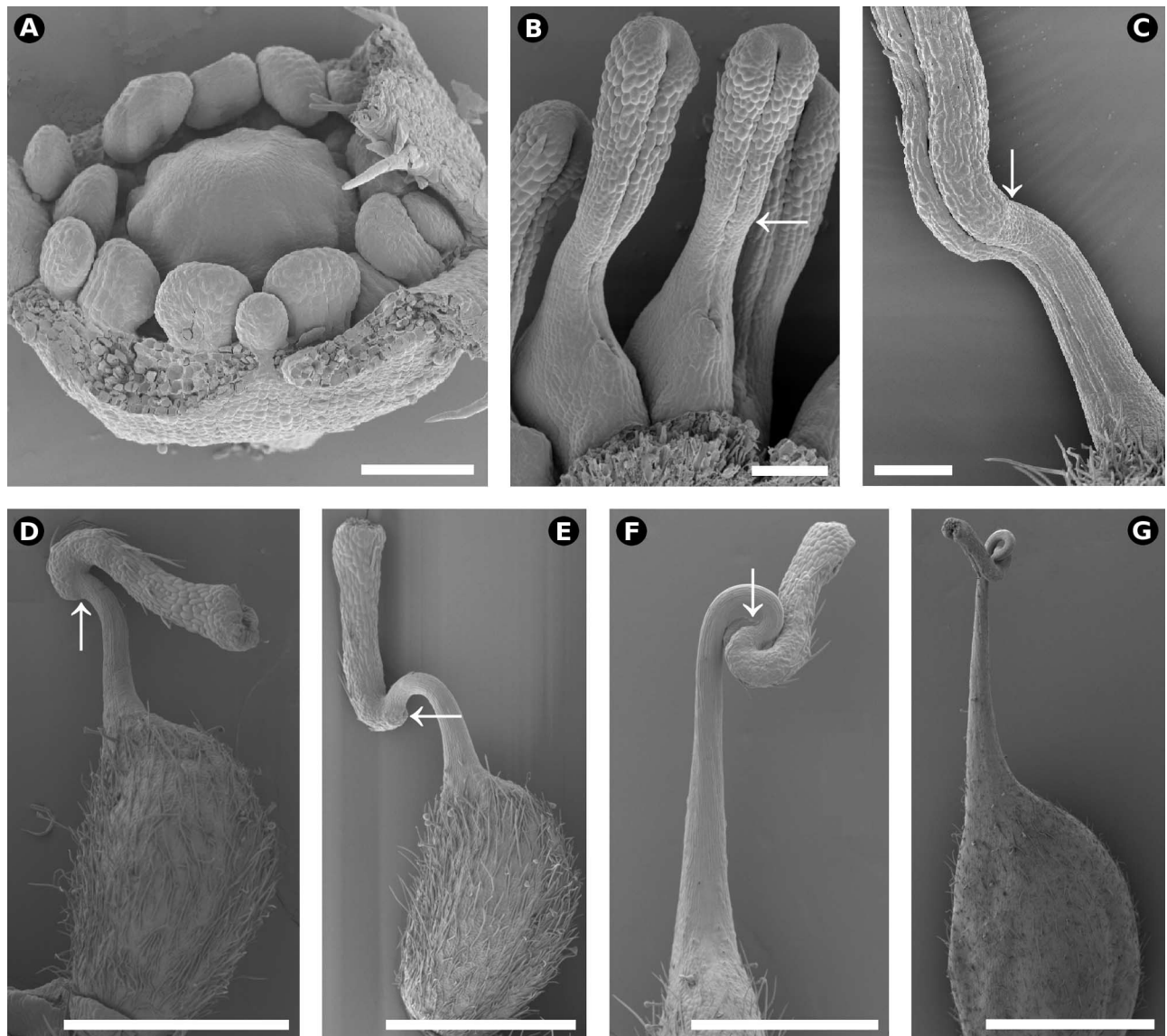


Fig. 9 *Geum vernum*. Development of gynoecium. *A*, Floral primordium at gynoecial initiation. *B*, Adaxial view of young carpels. *C*, Lateral view of carpel at initiation of hook. *D*, Deciduous part of style growing around toward abaxial side. *E*, Lateral view of carpel with apex of beak forming a hook. *F*, Abaxial view of carpel with beak twisted 90°. *G*, Lateral view of nearly mature achene with style twisted almost 180°. Scale bars = 100 μm (*A–C*), 600 μm (*D–F*), and 1.2 mm (*G*). Arrows indicate the position of the joint.

from hairy to glabrous (Bolle 1933; Cronquist et al. 1997). However, we found no evidence of a joint on the style in any developmental stage of this species. The glabrous region immediately below the stigma, which is about 5 mm long when the fruit is fully developed, withers and often finally falls off. This region differs in color from the rest of the style, being yellow instead of reddish. Perhaps this has led to the misconception that there is a joint. The withering stigmatic region is found in other wind-dispersed species as well, such as *G. montanum*, although it is not as long in *G. triflorum*.

The ontogenies of the two parallel occurrences of the fish-hook fruit are essentially the same. They do not differ in any obvious aspects concerning how and in what order structures are formed. However, the fruit of *G. vernum* goes through a

final phase when its beak is twisted 180°, making it different from that of *G. urbanum* and *Geum rivale* at maturity. This yields the effect that the hook points toward the abaxial side of the achene in *G. vernum*, while it points in the adaxial direction in *G. urbanum* and *G. rivale*. From a functional point of view, this is perhaps more efficient. The hooks of *G. vernum* will be directed outward, away from the center of the infructescence, making it more likely that they will catch on to an animal that might disperse the fruits.

In *Geum leiospermum*, a similar function is brought about in a different way. The fruits of this species have styles where a hook forms in the stigmatic region. The hook is directed abaxially, but in this case it is not because of secondary twisting of the style, as in *G. vernum*, but because of initial

recurvation in that direction (fig. 3C). We have looked at mature fruits of one other representative of the southern hemisphere oceanic species that have this type of fruit, *Geum cockaynei*, and they are shaped similarly in this respect.

When considering the ontogenetic data presented here in light of the hypothesis of reticulate relationships within Coluriaceae (fig. 1), we can see a pattern of homoplastic morphological evolution emerging. The joint originally evolved in the common ancestor of *G. geoides* and *G. waldsteiniae*. Judging from extant species, this was coupled with a shortening of the style, which was plumose in its ancestors (Smedmark and Eriksson 2002). Hybridization with a species belonging to the sister lineage of the remainder of *Geum*, thus likely to have had a plumose style, gave rise to the tetraploid *G. heterocarpum* lineage through allopolyploidy (fig. 1). In this lineage the beak first evolved. A second allopolyploidization led to the formation of a hexaploid lineage, whose early representatives seem to have had persistent styles. In this clade, the fishhook fruit, which is in principle a slight modification of the harpoon fruit, evolved independently in two different lineages, both descended from ancestors that are inferred to have had plumose styles. The ontogeny of these two evolutionarily distinct but morphologically very similar structures does not differ considerably. This similarity in development seems to indicate that the same genetic developmental pathways are involved in both cases. It may be that each of two characters, jointed style and beaked fruit, genetically evolved only once and that they are not expressed in all descendants. It is known that allopolyploidy can act as a rich source of phenotypic novelties (Levin 1983; Song et al. 1995; Schranz and Osborn 2000), and recently several studies have addressed the question of what mechanisms may give rise to this variation (Jiang et al. 1998; Comai et al. 2003; Osborn et al. 2003). It has been demonstrated that recently formed allopolyploids

undergo a phase of instability (see Comai 2000 and references therein) when phenotypes not present in their diploid progenitors arise. Studies of synthetic allopolyploids in *Arabidopsis* (Comai et al. 2003) show a partial loss of epigenetic gene regulation that seems to be associated with demethylation of the allopolyploid genome. Comai et al. (2003) found a correlation between changes in methylation and the phenotypic instability and alterations of gene expression that was observed in the first generations following allopolyploidization (Comai et al. 2003). Demethylation may, for example, cause silencing of genes controlling development. Although phenotypic variation and gene expression has been studied in newly synthesized allopolyploids, little is known about the evolution in later generations. We suggest that this type of methylation changes could explain the evolutionary patterns seen in polyploid *Geum*. The genes controlling the development of some morphological traits may be present in all the allohexaploids but have somehow been inactivated in some species, possibly by epigenetic means. If this is the case, methylation changes affecting the expression of regulatory genes do not take place only shortly after an allopolyploidization but may be triggered many generations after polyploid formation. Future studies of these kinds of processes may help to explain why polyploid plant taxa have been so evolutionarily successful.

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