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# DEVELOPMENTAL BASIS OF AN ANATOMICAL NOVELTY: HETEROARTHROCARPY IN *CAKILE LANCEOLATA* AND *ERUCARIA ERUCARIOIDES* (BRASSICACEAE)

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To understand the developmental basis of a novel anatomical feature, we present a comparative developmental study of an ecologically significant novelty in fruit morphology. Most members of the tribe Brassiceae have heteroarthrocarpic fruits, in contrast to the unsegmented fruits of many Brassicaceae. Heteroarthrocarpy is characterized by a joint that bisects fruits into heteromorphic segments and by partial or complete indehiscence. In order to better understand the development of heteroarthrocarpic characteristics and their relationships to typical siliques, we studied carpel and fruit development in two closely related species of the Brassiceae, *Erucaria erucarioides* and *Cakile lanceolata*. Our results indicate that proximal segments of heteroarthrocarpic fruits correspond to valves of typical siliques, regardless of whether these segments are dehiscent. Indehiscent distal segments are composed of both stylar and ovary elements, although the ovary wall of this segment does not differentiate into valve tissue. The joint itself comprises the distal extent of the valve margin and an internal proliferation of the mesocarp. Additionally, *Cakile* fruits form a transverse dehiscence zone through the joint, allowing the segments to separate. Heteroarthrocarpy entails modifications of lignification patterns and alterations of relationships between valve, style, ovary, and mesocarp. Possible genetic mechanisms underlying these modifications are discussed in reference to what is known about silique development in *Arabidopsis*.

*Keywords:* fruit morphology, silique, heteroarthrocarpy, Brassiceae, dehiscence, indehiscence.

## Introduction

Traits that show a high degree of evolutionary lability, varying even among closely related taxa, offer special opportunities to study morphological evolution. This is largely due to the fact that such changes are typically of recent derivation and can easily be considered in isolation. Furthermore, highly labile traits facilitate the analysis of the genetic basis for morphological evolution by reducing the level of genetic differentiation between comparison taxa. Across the angiosperms, a number of traits are highly variable, including fruit morphology. Recent molecular-based phylogenies indicate that fruit morphology is very evolutionarily labile in numerous families (e.g., Rubiaceae: Bremer and Eriksson 1992; Bremer et al. 1995; Rosaceae: Morgan et al. 1994; Melastomataceae: Clausing et al. 2000; Solanaceae: Knapp 2002; Capparaceae: Hall et al. 2002). In order to better understand this type of variability, a key first step is to characterize the development and homology of the structures in question, especially when novel features are involved. To these ends, we have undertaken an analysis of the morphology and development of fruits in two closely related representatives of the family Brassicaceae that exhibit different forms of the same anatomical novelty.

Fruits in the family Brassicaceae are extraordinarily diverse in terms of shape, structure, and size (Rollins 1993; Appel and Al-Shehbaz 2003; Koch et al. 2003), in contrast to the relatively uniform floral ground plan. Most members have a fully dehiscent silique: a two-valved capsule with a persistent placenta (replum) often connected by a thin membranous septum that separates the ovary into two chambers (Rollins 1993). Hereafter, this more common fruit morphology of Brassicaceae will be referred to as a typical silique. At maturity, the valves detach from the replum along a separation layer (SL) only a few cells wide, allowing the seeds to disperse. The wide array of variations on this typical silique has been a source of diagnostic and taxonomic characters for many classifications of the family (Rollins 1993; Appel and Al-Shehbaz 2003; Koch et al. 2003). However, molecular phylogenetic analyses have demonstrated that homoplasy is encountered in all aspects of fruit morphology such that characters previously used as taxonomic indicators do not accurately predict phylogenetic relationships (Appel and Al-Shehbaz 2003; Koch et al. 2003).

The tribe Brassiceae is one of the few traditionally recognized tribes that is monophyletic based on molecular evidence (Warwick and Black 1997; Appel 1999; Koch et al. 2003; Warwick and Sauder 2005). The 50 genera (ca. 180 species) of the Brassiceae are characterized by heteroarthrocarpic fruits and/or conduplicate cotyledons. Heteroarthrocarpic fruits are modified siliques with a "joint" that divides the fruits laterally into two heteromorphic segments (fig. 1; table 1). We will distinguish between the joint itself, which has been defined as the articulating surface between the two fruit segments (Al-Shehbaz 1985; table 1), and the "joint region," which includes the internal constriction that bisects the ovary into two chambers (table 1). This lateral bisection of the ovary often includes an abscission zone that allows the distal

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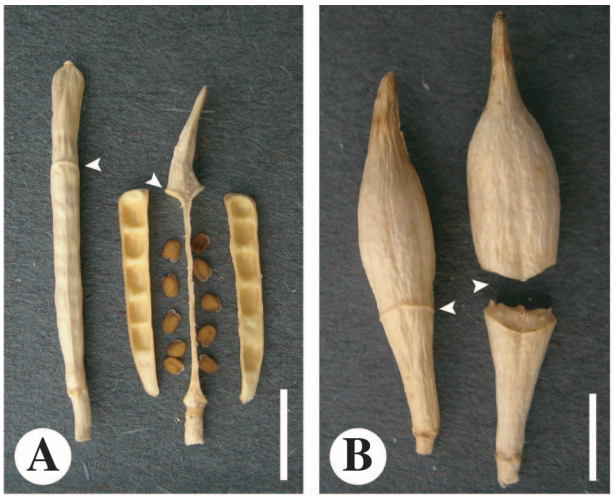


**Fig. 1** Mature heteroarthrocarpic fruits. A, *Erucaria erucarioides* fruit in lateral view before dehiscence (left) and medial view (right) after dehiscence of valves. Arrowheads indicate joint region. *B*, *Cakile lanceolata* fruit in lateral view before (left) and medial view after (right) abscission at the joint region (arrowheads). Scale bars = 0.5 cm.

segment to be dispersed as a subunit independent of the proximal segment (fig. 1*B*). In the Brassiceae, therefore, the joint is an evolutionary innovation that enables an ecologically significant shift in seed dispersal mechanism (Rodman 1974; Donohue 1998). When the joint occurs, the distal segment is always indehiscent, while the proximal segment may be indehiscent or dehiscent (Appel 1999). Across the entire family, heteroarthrocarpy is found only in the Brassiceae, although it is important to note that 20 of the 50 genera in the tribe have typical siliques (Gómez-Campo 1980, 1999). Given this fact, it has been debated whether heteroarthrocarpy represents an ancestral (Appel 1999) or derived (Gómez-Campo 1980) state in the Brassiceae. However, on the basis of molecular studies, it is likely that this novelty is derived and evolved multiple times in the tribe (J. C. Hall, T. E. Tisdale, K. Donohue, A. Wheeler, C. Gómez-Campo, and E. Kramer, in preparation). Thus, there are two significant, combinatorial alterations in heteroarthrocarpic fruits when compared with typical siliques: (1) indehiscence, either partial or throughout the entire fruit, and (2) the joint, which may or may not abscise.

Considerable confusion exists regarding the developmental origins and homology of heteroarthrocarpic fruits, especially with respect to the distal segment and the joint. To a large extent, this may be due to the wide range of fruit morphologies found throughout the tribe. The variation in fruit morphology observed encompasses fully dehiscent typical siliques; segmented fruits with enlarged persistent, sterile styles (referred to as "seedless beaks"); and heteroarthrocarpic fruits (Zohary 1948; Gómez-Campo 1980, 1999; Al-Shehbaz 1985). Much discussion of the evolutionary relationships between these fruit forms exists in the literature. Issues regarding the nature of heteromorphic fruits have been conflated because of inconsistencies in terminology and, as a result, in the identification of which taxa even have heteroarthrocarpic fruits (Appel 1999). However, all authors appear to agree that the proximal segment is valvular and, by implication, homologous to the valve of a typical silique (Zohary 1948; Appel 1999). Zohary (1948) specifically proposed that a reduction in the apical extent of the valves of a typical silique, without corresponding reduction in the ovary, leads to heteromorphic fruits in which the proximal segment is valvular. Questions remain, however, regarding the nature of the distal segment. Some authors refer to the distal segment as "substylar," a term meant to imply a stylar origin (Gómez-Campo 1999). This hypothesis suggests that the distal segment represents a fertile variation of the sterile "beaks" present in some members of the tribe. A stylar derivation of the distal segment further implies that when both segments are indehiscent, there

**Table 1**

**Terminology for Various Components of Siliques and Heteroarthrocarpic Fruits**

| Term | Definition |
|---|---|
| Segmented silique | Fruit with morphologically distinct sections, which may or may not be fertile. |
| Heteroarthrocarpic silique | Specialized type of segmented silique with two segments, each of which contains ovules or rudimentary ovules. |
| Distal segment | Upper, seed- or rudimentary ovule-bearing portion of the heteroarthrocarpic fruit that never dehisces at fruit maturation. |
| Proximal segment | Lower, seed- or rudimentary ovule-bearing portion of the heteroarthrocarpic fruit that may or may not dehisce at fruit maturation. |
| Valve | Portion of the ovary wall that separates from the replum during fruit dehiscence and is bounded by a morphologically distinct valve margin. |
| Ovary wall | Pericarp surrounding developing ovules regardless of whether this tissue differentiates into valve. |
| Valve margin | Region between the valve and replum in dehiscent siliques that is composed of two layers: (1) separation layer and (2) lignified layer. |
| Replum | Persistent placental tissue characteristic of siliques. |
| Septum | Partition that separates the ovary into two locules and is formed as outgrowths from the carpel margins (reviewed in Bowman and Smyth 1999; see also Skinner et al. 2004). |
| Placental tissue | Tissue from which the ovules are initiated. |
| Joint | Articulating surface between the two fruit segments, which may or may not abscise during development. |
| Joint region | Region of the fruit where the two segments meet. There are three components: (1) the joint, which disintegrates when the two segments separate at maturity; (2) adjacent cells of the proximal segment; and (3) adjacent cells of the distal segment. |

should be different developmental mechanisms controlling indehiscence in the distal and proximal segments. In contrast, Appel (1999) emphasized that the distal segment is not equivalent to the style, but he did not propose an alternative hypothesis of homology. An alternative to the stylar origin theory is that a constriction in the ovary of a typical silique has given rise to two heteromorphic fruit segments, each composed of replum, septum, and valve. The presence of septal tissue in both segments has been observed in some heteroarthrocarpic fruits (Rodman 1974) and is consistent with a nonstylar origin of the distal segment. Under this interpretation, indehiscence in the two segments may or may not be due to the same developmental alterations. If constriction occurred, then the joint is a novel feature, whereas the other alternatives (stylar origin of the distal segment or a simple reduction in the valves) imply some relationship between the joint and the apex of the valve margin. There has been no explicit hypothesis on the developmental origin of the joint.

To examine the development of heteroarthrocarpy, we have focused on a subset of the tribe Brassiceae. The *Cakile* clade is supported as monophyletic on the basis of molecular evidence (Warwick and Black 1997; Warwick and Sauder 2005), reducing the possibility of multiple origins of heteroarthrocarpy between our study species. The four genera that comprise this clade exhibit variation in fruit morphology that reflects the range in morphologies observed across the tribe. Although many species in the clade have fully indehiscent, heteroarthrocarpic fruits that separate at maturity (*Cakile*, *Didesmus*, *Erucaria microcarpa*, *Erucaria pinnata*), the proximal segments of other species dehisce (*Erucaria erucarioides*, *Erucaria hispanica*) or are obsolete (*Crambella*). We have investigated the development and maturation of two representatives of this clade, *Cakile lanceolata* and *E. erucarioides*, that vary with regard to abscission of the joint and dehiscence of the proximal segment. Thus, our studies include taxa with distinct forms of heteroarthrocarpy. However, because of the close relationship of *Erucaria* and *Cakile*, their joints presumably share the same evolutionary and developmental origin. Moreover, preliminary molecular phylogenetic data indicate that the partially dehiscent *Erucaria* is basal to the completely indehiscent *Cakile* and may represent a transitional form (J. C. Hall, T. E. Tisdale, K. Donohue, A. Wheeler, C. Gómez-Campo, and E. Kramer, in preparation).

The primary aim of this study was to determine when and where developmental changes that lead to the morphologies associated with heteroarthrocarpy occur. Specifically, we examined (1) how the carpel develops and differentiates into two morphologically distinct segments, (2) the anatomical differences between dehiscent and indehiscent segments both within and between species, and (3) the development of the joint. To our knowledge, no detailed developmental studies of heteroarthrocarpy have been conducted. Our results lead us to propose a model for the developmental basis of heteroarthrocarpy within the *Cakile* clade and reevaluate hypotheses regarding the evolution of heteroarthrocarpy. We also briefly discuss possible genetic mechanisms underlying heteroarthrocarpy, using the model plant *Arabidopsis thaliana* as a reference.

## Material and Methods

### Material

The two study species differ in their fruit morphology. In fruits of *Erucaria erucarioides*, only the distal segment is indehiscent, and at maturity, the two segments do not separate along the joint (fig. 1*A*). In contrast, both segments of *Cakile lanceolata* are indehiscent and separate via the joint at maturity (fig. 1*B*). Like *Cakile*, both segments of *Erucaria* bear seeds. Thus, these two species represent distinct variants of heteroarthrocarpy that differ in two critical features: (1) dehiscence in the proximal segment and (2) abscission along the joint. Russell Reardon (U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Florida Keys National Marine Sanctuary) kindly provided seeds of *C. lanceolata* from Dry Tortugas National Park, Garden Key, Florida. Seeds of *E. erucarioides* were obtained from C. Gómez-Campo's seed collection (a germ plasm collection of crucifers), with original collections from west Algeria, near Béchar.

### Methods

All plants were grown from seed in the Harvard University greenhouses. Flowers were pollinated by hand to ensure seed set, although no effort was made to prevent selfing. For SEM studies, inflorescences including flowers just past anthesis were harvested and fixed under vacuum in FAA (50% ethanol, 4% formalin, and 5% glacial acetic acid). Inflorescences were then transferred to 70% EtOH for storage. Inflorescences were dehydrated through an ethyl alcohol series and critical-point dried with $CO_2$ in a Tousimis/Autosamdri-815 dryer. Material was dissected, mounted on aluminum stubs with carbon conductive adhesive tabs (Electron Microscopy Sciences, Ft. Washington, PA), sputter-coated with gold palladium in a Denton/Desk II, and studied in a model Quanta 200 SEM (FEI, Hillsboro, OR) at 5–20 kV.

For histological studies, we collected inflorescences and fruits at varying stages ranging from young buds to mature fruits. At later stages in fruit development, distal and proximal segments were separated before fixation. Younger fruits were fixed under vacuum in FAA, dehydrated in an ethyl alcohol series, embedded in paraplast, and stored at 4°C until use. More mature fruits were fixed under vacuum in FAA, infiltrated with TBA (*tert*-butyl alcohol) as described (Ruzin 1999), embedded in paraplast, and stored at 4°C until use. Samples were sectioned to 8 $\mu$m with a disposable steel blade on a Reichert-Jung microtome. Serial slides were stained with either (1) 0.025% alcian blue 8GX and 0.01% safranin O in 0.1 M acetate buffer (pH 5.0), following Roeder et al. (2003), or (2) lignin-specific phloroglucinol (as per Ruzin 1999) such that we obtained both types of staining on comparable sections. Sections were recorded digitally using a Leica Leitz DMRD microscope equipped with a Retiga EXi imaging system (Harvard Imaging Center).

## Results

The flowers of *Erucaria* and *Cakile* are similar to most species of Brassicaceae. Four sepals alternate with four free

petals. The androecium consists of six stamens, four medial and two lateral, with the lateral stamens shorter than the four medial. The gynoecia of both study species are formed from two congenitally fused carpels that are positioned transversely. The two carpels meet medially at the replum (fig. 2; table 1), which is persistent placental tissue demarcated internally by prominent vascular bundles and externally by longitudinal rows of rectangular-shaped cells. In each species, the replum is connected internally by the septum throughout the entire length of the ovary. By anthesis, the ovary is bisected laterally into proximal and distal segments by the joint. The joint is distinguished both internally and externally by two to three cell layers of smaller, densely packed cells. The pericarp of both proximal and distal segments contains three major tissue types: a single cell layer of outer epidermis (exocarp), multiple cell layers of central mesocarp, and the internal endocarp (fig. 2). The endocarp is further composed of two cell layers: a thin layer of longitudinally organized cells (en*a*) and the inner epidermis (en*b*). The stigmatic papillae are sessile on the distal segment such that the gynoecia lack externally discernible styles.

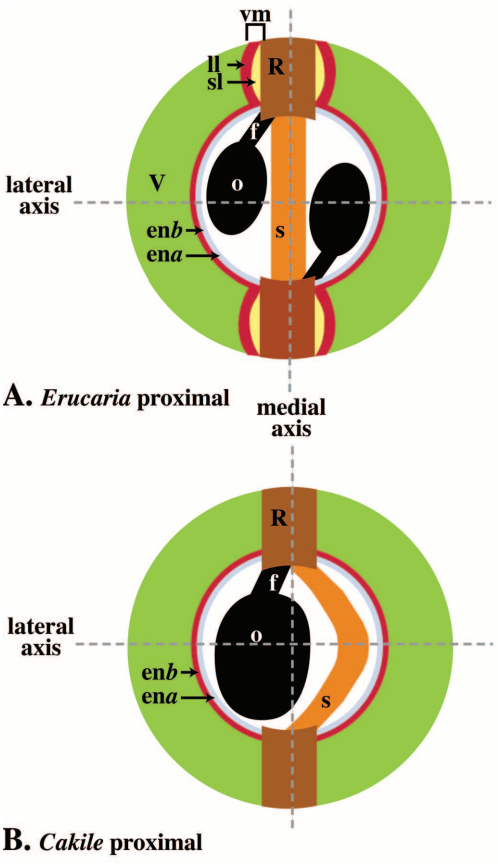


**Fig. 2** Diagrammatic representation of transverse sections through mature fruits showing cell types and medial and lateral axes. *A*, Proximal segment of *Erucaria erucarioides*. *B*, Proximal segment of *Cakile lanceolata*. *R* = replum; *vm* = valve margin; *ll* = lignified layer; *sl* = separation layer; *f* = funiculus; *o* = ovule; *V* = valve; *s* = septum; *en*b = endocarp *b*; *en*a = endocarp *a*.

## Erucaria erucarioides

*Morphology of anthetic carpel.* The proximal and distal segments are approximately the same length and are separated by the joint (fig. 3*A*, 3*C*). The two segments are unified by the replum, which extends longitudinally from base to stigma (fig. 3*A*–3*D*). Internally, the two repla are connected by septal tissue that is present throughout the entire ovary (fig. 3*E*–3*H*). The proximal segment is distinguished from the distal segment by a number of features including, most notably, the presence of distinct valves that are bounded by the small cells of the valve margin (fig. 3*A*–3*C*). Externally, the valves are composed of morphologically distinct, large, interlocking cells interspersed with closed stomata (fig. 3*B*). The replum of the proximal segment is slightly raised and clearly demarcated by the furrows of the valve margins (fig. 3*A*–3*C*). Internally, the proximal replum is distinguished by large medial vascular bundles and bounded by a valve margin (figs. 2*A*, 3*F*). In contrast, the epidermal cells of the distal segment are more homogenous than those of the proximal segment, with only subtle differentiation between the cells of the replum and the ovary wall (fig. 3*D*). However, repla can still be discerned externally as prominent ridges and internally by medial vascular bundles that are surrounded by smaller, tightly packed cells (fig. 3*H*). The distal segment is terete near the joint and becomes flattened in the medial plane approaching the stigma (figs. 1*A*, 3*G*–3*I*). Internally, stylar tissue can be discerned in the apical region of the distal segment where the mesocarp surrounds transmitting tract tissue, which becomes continuous with the septum in the ovary below (fig. 3*I*). The internal composition of the distal ovary wall appears to be similar to that of the valves (fig. 3*E*, 3*F*, 3*H*). The joint region itself is internally defined by a constriction of the locule that is due to a localized expansion of mesocarp cells both above and below the apex of the valves (fig. 3*E*, 3*G*).

*Development.* The gynoecium primordium arises as an oval dome of cells in the center of the floral meristem (fig. 4*A*). After a period of initial growth, an invagination forms in the center of the dome, which later becomes a deepening cleft (fig. 4*B*) such that continued growth leads to an open oval tube (fig. 4*C*, 4*D*). As the thecae form, the gynoecial tube grows vertically (fig. 4*D*, 4*E*), and the apical region of the rim becomes extended in the medial position (fig. 4*C*, 4*E*). Cells along the gynoecial tube are maintained in rows as the tube continues to elongate (fig. 4*D*–4*F*), and the septum starts to form (fig. 4*G*). The apical region of the gynoecial tube begins to close after the locules of the stamens are fully developed and the petals are approximately equal to half the length of the ovary (fig. 4*F*). As the stigmatic papillae initiate and the ovules develop (fig. 4*H*), the pericarp below the apex expands outward in the medial plane (fig. 4*F*, 4*I*). The continued outward expansion of the medial subapical cells leads to an external distinction between the proximal and distal segments of the carpel (fig. 4*J*, 4*L*, 4*M*), although the outer epidermal morphology remains homogeneous (fig. 4*I*, 4*J*), and the locule of the ovary is continuous through both segments (fig. 4*K*, 4*N*).

By the time the stigmatic papillae have formed, the gynoecium is approximately equal in length to the petals but half

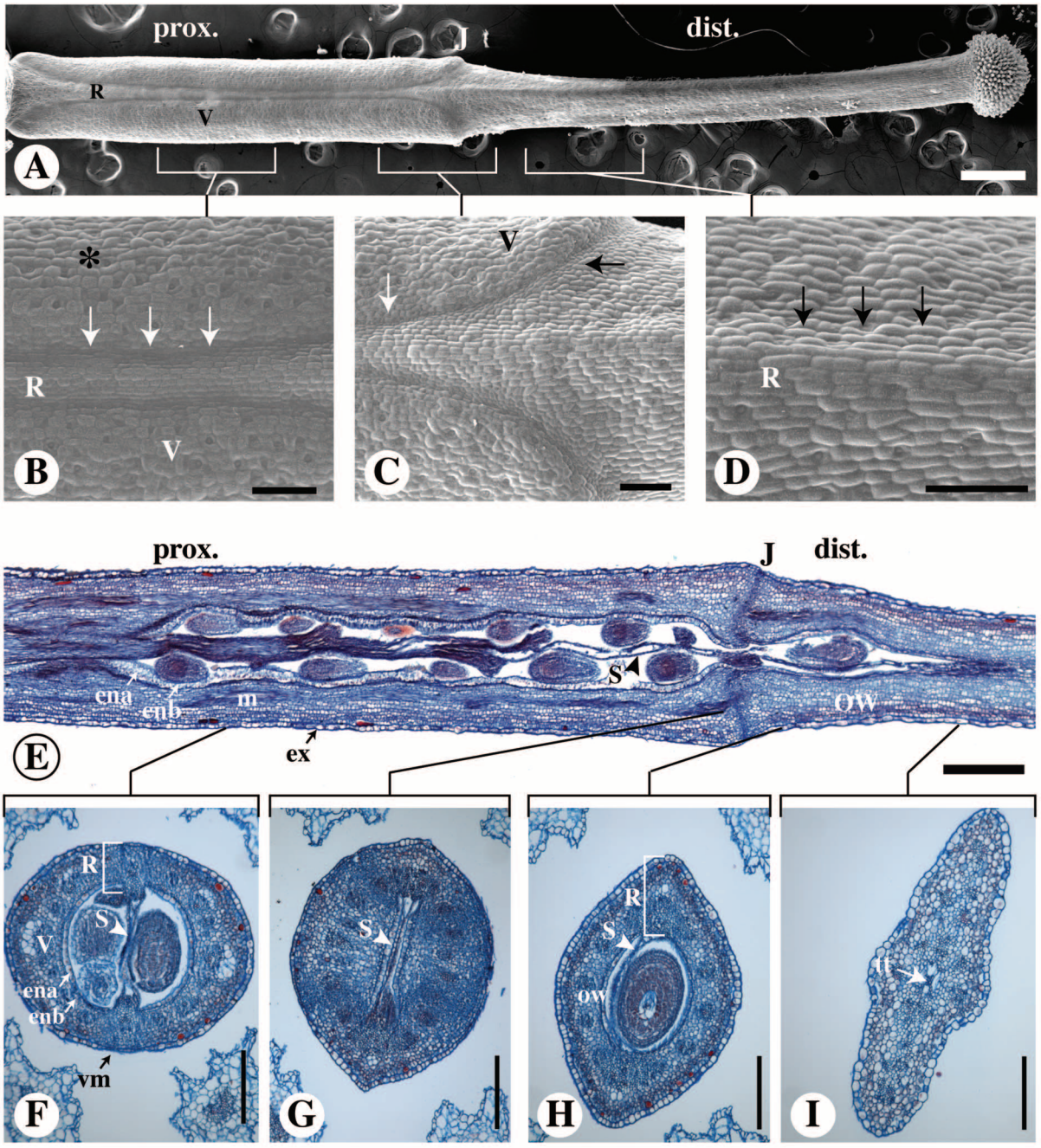


**Fig. 3** Anthetic carpel of *Erucaria erucarioides*. Scanning electron micrographs with selected organs removed to expose carpel and alcian blue/safranin O–stained sections. *A*, Medial view of carpel showing proximal and distal segments separated by the joint region. *B*, Magnified medial view of proximal segment, showing thin lines of small cells constituting the valve/replum boundary (white arrows). Note the presence of open stomata on the valve surfaces (asterisk). *C*, Magnified medial view of the joint region, showing thin lines of cells positioned between the valve and replum in the proximal segment (white arrow) and between the distal portion of the valve margin and distal segment (black arrow). *D*, Magnified medial view of distal segment showing the raised replum (black arrows). *E*, Longitudinal section of carpel with ovules in both proximal and distal segments. The septum is continuous from the locule of the proximal segment through the joint region into the locule of the distal segment. *F–I*, Serial transverse sections of anthetic carpel through the proximal segment (*F*), joint region (*G*), distal ovule (*H*), and distal segment near stigmatic papillae (*I*), with cell layers indicated. Scale bars = 500 $\mu$m (*A*), 100 $\mu$m (*B–D*), 2.5 mm (*E–I*). *R* = replum; *V* = valve; *J* = joint region; *S* = septum; *OW* = ovary wall; *ena* = endocarp *a*; *enb* = endocarp *b*; *m* = mesocarp; *ex* = exocarp; *vm* = valve margin; *tt* = transmitting tract.

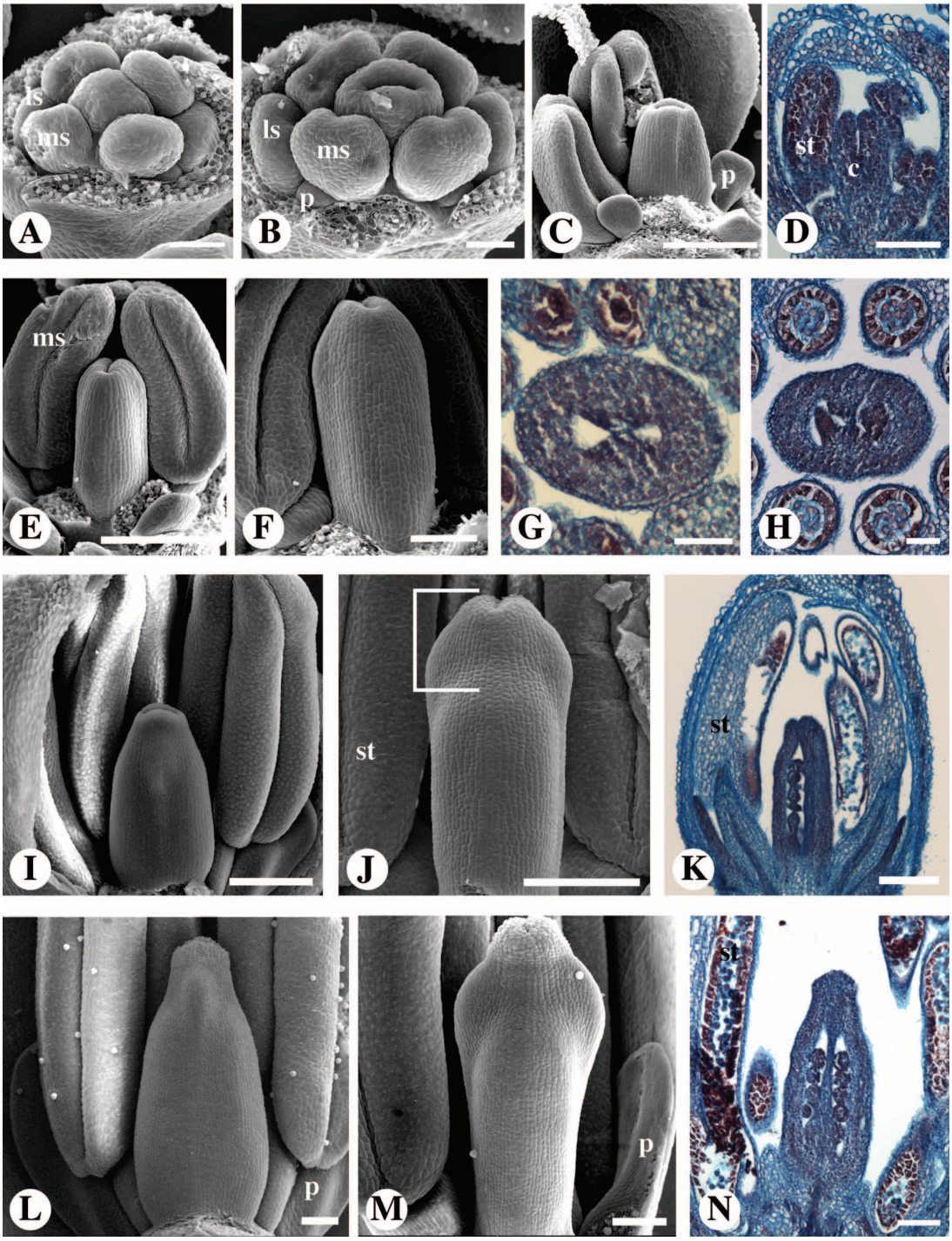


**Fig. 4** Early development of *Erucaria erucarioides*. Scanning electron micrographs with selected organs removed to expose carpel and alcian blue/safranin O–stained sections. *A*, Polar-medial view of floral meristem after initiation of carpel and petals. *B*, Polar-medial view of floral meristem. An invagination has formed on the elongated gynoecial dome. *C*, Medial view of gynoecial cylinder. *D*, Longitudinal section of bud at same stage as *C*, showing tubular nature of the carpel. *E*, Lateral view of carpel showing relative stamen and petal size. *F*, Oblique-medial view of carpel during initiation of expansion in the medial plane. *G*, Transverse section of bud at same stage as *E*, showing initiation of septum formation. *H*, Transverse section of bud at same stage as *I* and *J*, showing initiation of ovule formation. *I*, Medial view of carpel showing relative stamen and petal size. *J*, Lateral view of carpel at same stage as *I*, showing external distinction between proximal and distal (bracket) segments. *K*, Longitudinal section through bud at same stage as *I* in oblique lateral orientation. Locule is present in both segments. *L*, Medial view of carpel during initiation of stigmatic papillae. *M*, Lateral view of carpel at same stage as *L*. *N*, Longitudinal section through bud at same stage as *L* in medial orientation. Scale bars = 50 $\mu$m (*A*, *B*, *G*, *H*), 200 $\mu$m (*C*, *E*, *F*, *I*–*N*), 100 $\mu$m (*D*). *ms* = medial stamen; *ls* = lateral stamen; *st* = stamen; *p* = petal; *c* = carpel.

the length of the stamens (fig. 5*A*, 5*B*). There are few morphological differences in the epidermal cells between the style, distal segment, and proximal segment (fig. 5*A*–5*C*). The distal portion continues to expand in the medial plane, and the proximal segment lengthens longitudinally (fig. 5*A*–5*C*). Internally, the distinct layers of the mesocarp and the valve margin start to differentiate, as does the valve margin in proximal segment (fig. 5*H*, 5*I*). Once the carpel reaches the length of the lateral stamens, the proximal and distal segments are roughly equal in length (fig. 5*D*, 5*E*). At this stage, it is becoming more externally evident where the joint will form because of the shape of the presumptive joint, although the cells are relatively homogenous (fig. 5*F*). Within the presumptive joint region, the locule remains relatively open at this stage, although it is clear where the joint is starting to form because of the smaller, densely stained cells (fig. 5*G*). As the carpel continues to develop, it lengthens to the point where the stigmatic surface attains the height of the medial stamens (fig. 5*L*). At this stage, differences are more apparent between the epidermal cell types, most notably the cells of the valve margin, which are no longer expanding and are smaller than valve or replum cells (fig. 5*M*). Internally, cell types in the pericarp are fully differentiated (fig. 5*J*, 5*K*), and there are clear differences in the replum region between the proximal and distal segments. In particular, there is a distinct valve margin in the proximal segment, while the distal segment completely lacks this differentiation (fig. 5*J*, 5*K*). As the flower reaches anthesis, the internal constriction marking the joint region becomes pronounced because of proliferation and expansion of mesocarp cells immediately below and above the apex of the valves (fig. 5*N*–5*P*). The positioning of this constriction is highly consistent and always coincides with the apex of the valve margin, which internally corresponds to a position immediately below the most apical ovule within the ovary (fig. 5*N*, 5*P*). Interestingly, this apical ovule shows an erect orientation that is inverted relative to the others (fig. 5*G*, 5*N*, 5*O*). Proliferation of the joint region continues until the constriction tightly appresses the septum.

*Fruit maturation.* It takes ca. 24 d postfertilization for the fruits to mature, at which stage they are ca. 1.5 cm long. During this process, the proximal segment continues to increase in length to reach about two-thirds the total fruit length (fig. 1*A*), and the boundary defined by the valve margin becomes more distinct both externally and internally (figs. 1*A*, 6*A*). Throughout maturation, increased lignification of multiple cell types is observed. Lignification occurs first in the cells surrounding the vasculature, in the lignified layers (LLs) of the valve margin of proximal fruit segments, and in the en*b* layer of both segments (fig. 6*A*, 6*B*, 6*D*). Later, mesocarp cells adjacent to the en*b* become lignified until there is a continuous lignified band connecting the vascular bundles (fig. 6*C*, 6*F*). The patterns of lignification in the repla differ between the proximal and distal segments. In the proximal segment, a central core of lignification surrounds the vascular bundle and comprises the replum proper. Flanking this core on either side are two to three rows of SL cells, which remain unlignified throughout fruit maturation (fig. 6*C*). Adjacent to the SL cells along the borders of the valves, there are additional bands of lignified cells that represent the LLs and extend from the endocarp outward toward the exocarp (fig. 6*B*, 6*C*). The replum of the distal segment also becomes lignified, but its morphology is distinct from that of the proximal segment. Corresponding with the medial expansion observed in the distal segment (fig. 6*C*, 6*G*), the inner portion of the mesocarp underlying the distal replum has undergone proliferation and expansion (fig. 6*D*–6*F*, 6*J*). The region is further thickened by two wide bands of heavy lignification that lie internal and to either side of the medial vascular bundles (fig. 6*D*–6*F*). In contrast, the rows of cells immediately underlying the medial vasculature do not become lignified (fig. 6*E*, 6*F*). Although this organization has some similarity to a dehiscent zone (DZ), no SL forms in this region. Another distinction in the lignification patterns between the proximal and distal segments is that the en*a* layer becomes lignified in the distal but not in the proximal segment (fig. 6*H*, 6*I*). The outer layers of the mesocarp and the exocarp of both segments remain unlignified throughout fruit maturation (fig. 6*C*, 6*F*), with the exception of the cells in the LLs of the valve margins (fig. 6*H*, 6*I*). The morphology of the apical valve margin is notable since it corresponds with the joint region (fig. 6*H*). As described above, the mesocarp below and above the apex of the valve proliferates to form a constriction in the ovary. Therefore, in this region the DZ of the valve margin must propagate internally across the entire thickness of the constriction, with LL cells forming on both sides of the SL (fig. 6*H*, 6*I*). The formation of the DZ is limited to the valve margin, however, and reinforcement of the replum and septum through this region allows the distal segment to remain connected to the maternal plant when the valves dehisce (fig. 1*A*). That is, the DZ does not transverse the replum or septum (fig. 6*K*, 6*L*).

## Cakile lanceolata

*Morphology of anthetic carpel.* At anthesis, the distal segment of the carpel is longer than the proximal (fig. 7*A*). The replum is observed externally throughout the length of the entire carpel, apparently terminating at the stigmatic papillae (fig. 7*A*–7*D*). Internally, the septum is similarly present throughout the entire length of the ovary, transcending the constriction of the joint region (fig. 7*E*, 7*G*). In the proximal segment, the bricklike epidermal cells of the replum can be distinguished from the irregularly shaped cells and closed stomata of the ovary wall (fig. 7*B*). The proximal replum can also be distinguished in cross section by invaginations on the medial surfaces of the exocarp (fig. 7*F*). The bricklike cells that externally define the replum are continuous from the proximal segment into the distal, but the distal replum protrudes and is internally less distinct than that in the proximal segment (fig. 7*D*, 7*H*). Separating the two segments, the joint is composed of a few rows of small cells that are visible both externally (fig. 7*C*) and internally (fig. 7*E*). In the joint region, the mesocarp below and above this band of small cells is greatly expanded internally to form a constriction in the ovary (fig. 7*E*, 7*G*). Although two ovules typically initiate in both segments, one on each side of the septum, at maturity there is usually only one functional ovule in each. Growth of these single ovules causes the septal tissue to be appressed to one side internally (fig. 7*E*, 7*F*, 7*H*). Although the style is not clearly discernible externally, sections through the upper region of the distal segment reveal that only about half of the

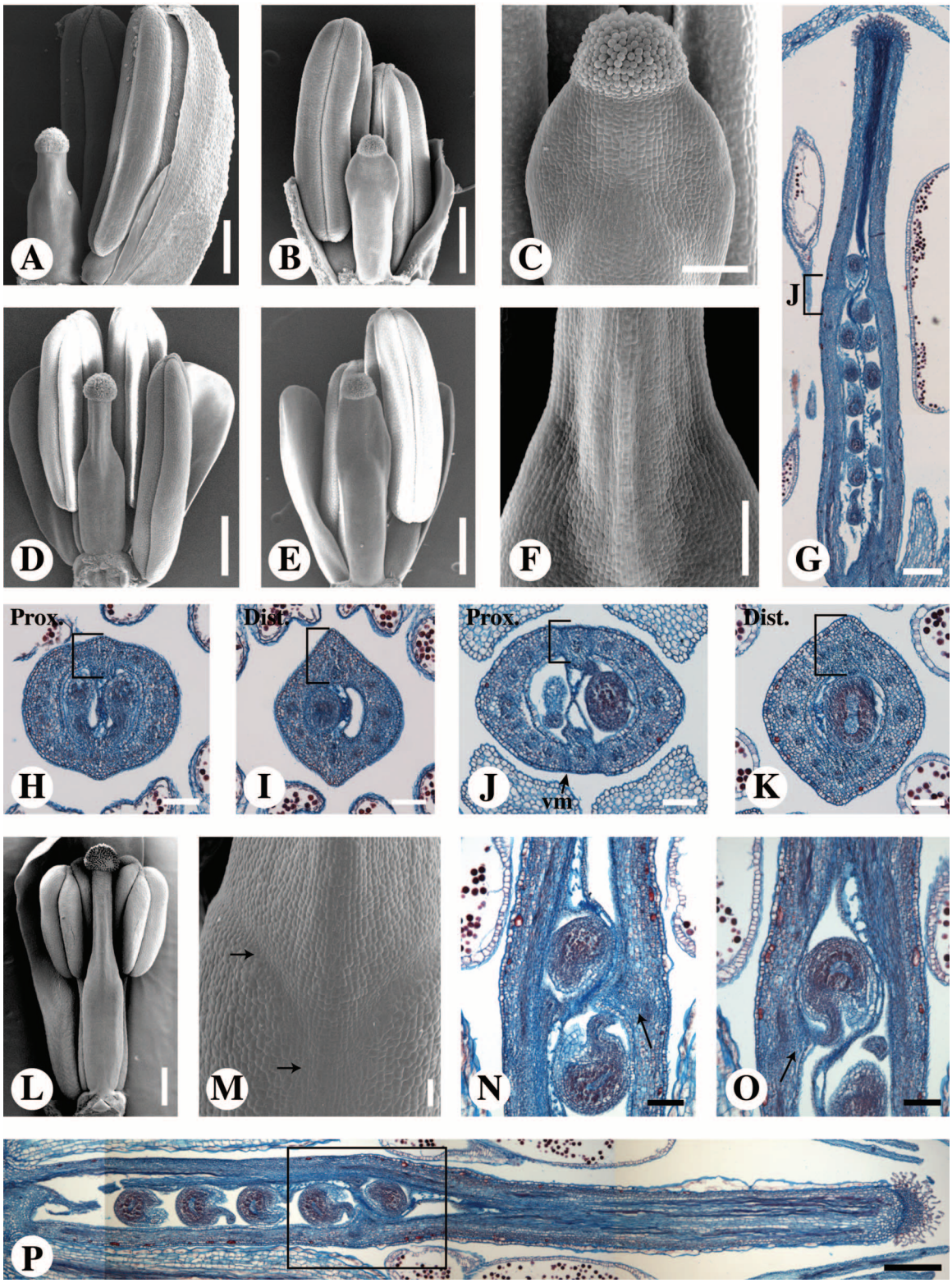


**Fig. 5** Mid-late development of *Erucaria erucarioides*. Scanning electron micrographs with selected organs removed to expose carpel and alcian blue/safranin O–stained sections. *A*, Medial view of carpel after formation of stigmatic papillae, showing relative stamen and petal size. *B*, Lateral view of carpel at same developmental stage as *A*, showing continued differentiation between distal and proximal segments. *C*, Carpel of *B* under increased magnification, showing homogeneity of cellular morphology across segments. *D*, Medial view showing elongation of the carpel relative to petal and stamen height. *E*, Lateral view of carpel at same developmental stage as *D*. *F*, Carpel of *D* under increased magnification, showing dimensionality at the joint region. *G*, Longitudinal section of carpel at same stage as *D*, showing internal initiation of mesocarp

segment is occupied by the ovary, with the rest composed of the transmitting tract embedded in mesocarp (fig. 7*E*, 7*I*).

*Development*. The gynoecium primordium initially appears as a bulge in the center of the floral meristem (fig. 8*A*) that later invaginates and elongates into a tube (fig. 8*B*, 8*C*). The cells at the medial, apical portion become slightly raised during this elongation (fig. 8*D*). By the stage when the stamens are stalked at the base, there is a distinct outward expansion of pericarp cells in the apical, medial portions of the carpel wall (fig. 8*E*, 8*F*). At this time, the septum starts to form internally (fig. 8*G*). As the stigmatic papillae begin to appear and carpel fusion initiates, the outward expansion in the distal segment continues (fig. 8*H*, 8*I*), and the carpel locules remain open throughout (fig. 8*J*). By the stage when the carpel is approximately the same height as the petals, carpel fusion is complete and the stigmatic papillae have fully formed (fig. 8*K*, 8*L*). The medial expansion of the developing carpel leads to a clear external distinction between the two segments, with the distal segment about twice the length of the proximal (fig. 8*K*, 8*L*). Internally, the septa have almost fused; the ovules are well formed; and the mesocarp, endocarp, and exocarp are just starting to differentiate (fig. 8*M*).

Once the petals surpass the length of the stamens and the stigmatic surface reaches the height of the medial stamens, there is further external differentiation of the two segments (fig. 9*A*–9*C*). This includes a slight external constriction of exocarp cells where the joint will form (fig. 9*B*, 9*C*) and the internal initiation of the ovary constriction (fig. 9*E*, 9*G*). All of the cell layers of the pericarp are fully differentiated at this stage (fig. 9*D*–9*F*). There are also slight differences in the replum region between the proximal (fig. 9*D*) and the distal (fig. 9*F*) segments. As the developing carpel nears anthesis, the stigmatic surface surpasses the length of the stamens. At this stage, there is a clear distinction in the external cell morphology of the joint, where the epidermal cells are much smaller (fig. 9*H*–9*J*). The joint region continues to close because of an internal expansion of the mesocarp cells that are adjacent to the funiculi of the developing ovules (fig. 9*L*, 9*M*). The ovules are oriented such that the ovule in the proximal segment is pendulous, whereas the ovule in the distal segment is erect (fig. 9*K*, 9*M*).

*Fruit maturation*. After the remaining floral organs have abscised, it takes just more than 60 d for *Cakile lanceolata* fruits to mature, at which stage the fruit is ca. 1.8 cm long. An increase in lignification occurs through multiple cell types during maturation. Initially, only a few cells surrounding the vasculature are lignified (fig. 10*A*, 10*D*, 10*G*, 10*J*). As the fruit starts to brown, however, there is a rampant increase in lignification of the mesocarp in both segments (fig. 10*B*, 10*C*, 10*E*, 10*F*, 10*H*, 10*I*, 10*K*, 10*L*). During this process, the mesocarp expands and the cells become corky. Because of increased cell wall thickening and lignification, the en*a* and en*b* layers become indistinguishable from one another (fig. 10*B*, 10*C*, 10*E*, 10*F*). In the region of the replum, the cells surrounding the medial vascular bundle are heavily lignified, with very thick cell walls nearing maturation (fig. 10*B*, 10*C*, 10*E*, 10*F*). There is a difference in the pattern of lignification between the repla of the two segments: the proximal segment has an additional "horseshoe"-shaped patch of lignified cells (fig. 10*B*, 10*C*). The "arms" of the horseshoe stretch outward toward the exocarp. Aside from these cells, the outermost layers of the mesocarp remain unlignified throughout maturation (fig. 10*G*–10*I*). Within the joint region, there is broad lignification on both sides of the SL of the abscission zone, which remains unlignified (fig. 10*H*, 10*I*). This abscission zone is present in both lateral (fig. 10*H*, 10*I*) and medial (fig. 10*K*, 10*L*) planes. At maturation, the two segments abscise as the result of cell separation that completely transverses the fruit, including the replum and septum tissue present in the joint (fig. 1*B*).

## Discussion

### *Developmental Origin of Heteroarthrocarpy*

The fruit of Brassicaceae is unique enough to warrant its own name: the silique (Rollins 1993). Although the basic silique is easily recognizable, heteroarthrocarpy is a prime example of the variations of fruit morphology found across the family. Many features of heteroarthrocarpic fruits are the same as in other Brassicaceous fruits: two fused carpels with repla formed from persistent placenta and connected by false septa. Organogenesis and early development of the heteroarthrocarpic carpel are similar to the development of carpels that mature into typical siliques (e.g., the closely related *Brassica napus*: Polowick and Sawhney 1986; Spence et al. 1996). Organ elaboration associated with heteroarthrocarpy becomes apparent later in development through remodeling of the carpel relative to typical siliques.

*Valve/replum disassociation*. In both *Erucaria* and *Cakile*, how the ovary wall differentiates relative to the replum is altered when compared with typical siliques. In the latter, the valves (which are equivalent to the mature ovary walls) and the replum are specifically defined in relation to each other (*Arabidopsis thaliana*: Bowman and Smyth 1999; Liljegren et al. 2000; *Brassica*: Spence 1996; Spence et al. 1996). This reflects the fact that the edge of the valve is always bounded by the replum and vice versa. The septum is also tightly

expansion at the joint region (bracket). *H*, *I*, Transverse sections of carpel at same stage as *D* through the proximal segment (*H*) and distal segment (*I*), with replum in brackets. Cell types of the pericarp have begun to differentiate. *J*, *K*, Transverse sections of carpel at same stage as *L* through the proximal segment (*J*) and distal segment (*K*), with replum in brackets. Cell types of pericarp are distinct, and cell types of the valve margin have differentiated in the proximal segment. *L*, Medial view of bud nearing anthesis. *M*, Carpel of *L* under increased magnification showing small cells of the valve margin (*black arrows*) in comparison to much larger surrounding cells. *N*, *O*, Serial longitudinal sections of carpel at same stage as *L*, showing expansion of the mesocarp in the joint region and the resulting internal constriction of the ovary. *N*, Distalmost proximal ovule in a pendulous orientation, with funiculus originating near the expand mesocarp (arrow). *O*, Funiculus of the distal ovule, erect in orientation, originates near expanded mesocarp (arrow). *P*, Longitudinal section of carpel near anthesis showing internal constriction of joint region. Boxed region is shown under increased magnification in *N*. Scale bars = 300 $\mu$m (*A*), 400 $\mu$m (*B*, *E*), 100 $\mu$m (*C*, *F*, *H*–*P*), 500 $\mu$m (*D*, *G*). *J* = joint; *vm* = valve margin.

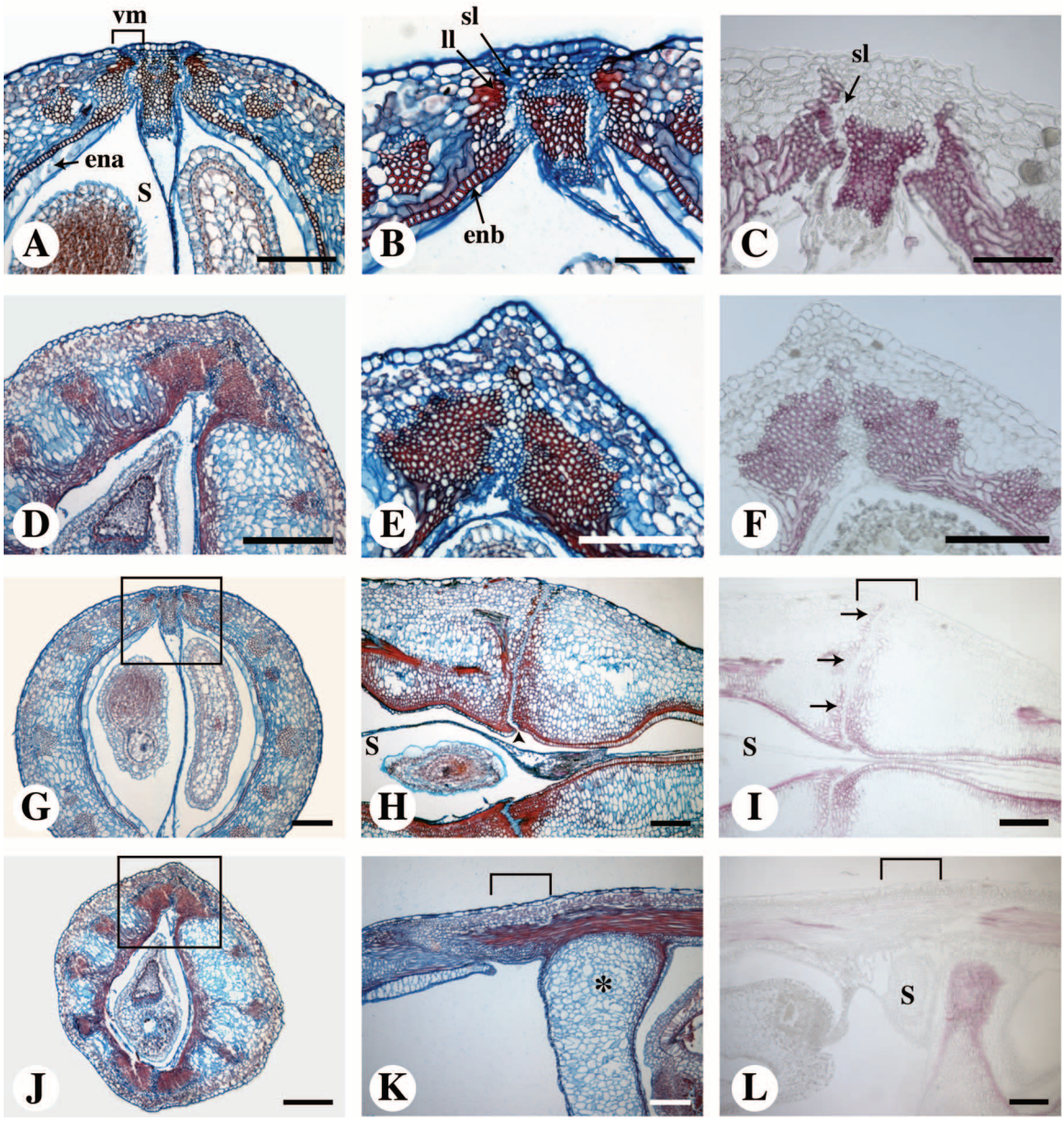


**Fig. 6** Fruit maturation of *Erucaria erucarioides*. Sections stained with alcian blue/safranin O (*A*, *B*, *D*, *E*, *G*, *H*, *J*, *K*). Other sections stained with phloroglucinol (*C*, *F*, *I*, *L*), which colors lignified cells pink. *A*, Replum region of proximal segment of young fruit. The valve margin is comprised of two distinct layers. At this stage in maturation, the en*b* layer has become lignified, as indicated by the red color, as well as the surrounding vasculature. *B*, Replum region of proximal segment in *A* under increased magnification. The two cell layers of the valve margin are clearly discernible; the separation layer is stained blue, and the lignified layer is stained red. *C*, Phloroglucinol-stained transverse section through proximal segment of fruit slightly more mature than *B*. Much of the mesocarp adjacent to the en*b* layer is lignified, while cells of separation layer are not. *D*, Replum region of distal segment of young fruit. The en*b* layer is lignified in addition to surrounding vasculature. *E*, Replum region of distal segment of maturing fruit. *F*, Phloroglucinol-stained section through distal segment serial to *E*, showing extensive lignification around the replum, en*b* layer, and mesocarp adjacent to en*b* layer. *G*, Transverse section through proximal segment of young fruit showing context of the replum region (box), as seen in *A*–C. *H*, Longitudinal section on the lateral axis (perpendicular to the septum) through joint region of mature fruit. Arrowhead indicates separation (abscission) layer. *I*, Phloroglucinol-stained section through the joint region (bracket) serial to that of *H*. Black arrows highlight the lignified cells of the distalmost edge of the valve margin. *J*, Transverse section through distal segment of mature fruit showing context of the replum region (box) as seen in *D*–*F*. *K*, Longitudinal section on the medial axis in parallel with the septum through joint region (bracket) of mature fruit. Note the expanded mesocarp of the joint bisecting the ovary (asterisk) and the absence of separation layer in the persistent replum through the joint region. *L*, Phloroglucinol-stained section of mature fruit of *K*; joint region in bracket. Note that the lignification pattern contrasts with that seen through the lateral axis in *I*. Scale bars = 200 $\mu$m (*A*, *D*–*L*), 100 $\mu$m (*B*, *C*). *S* = septum; *vm* = valve margin; *ena* = endocarp *a*; *enb* = endocarp *b*; *ll* = lignified layer; *sl* = separation layer.

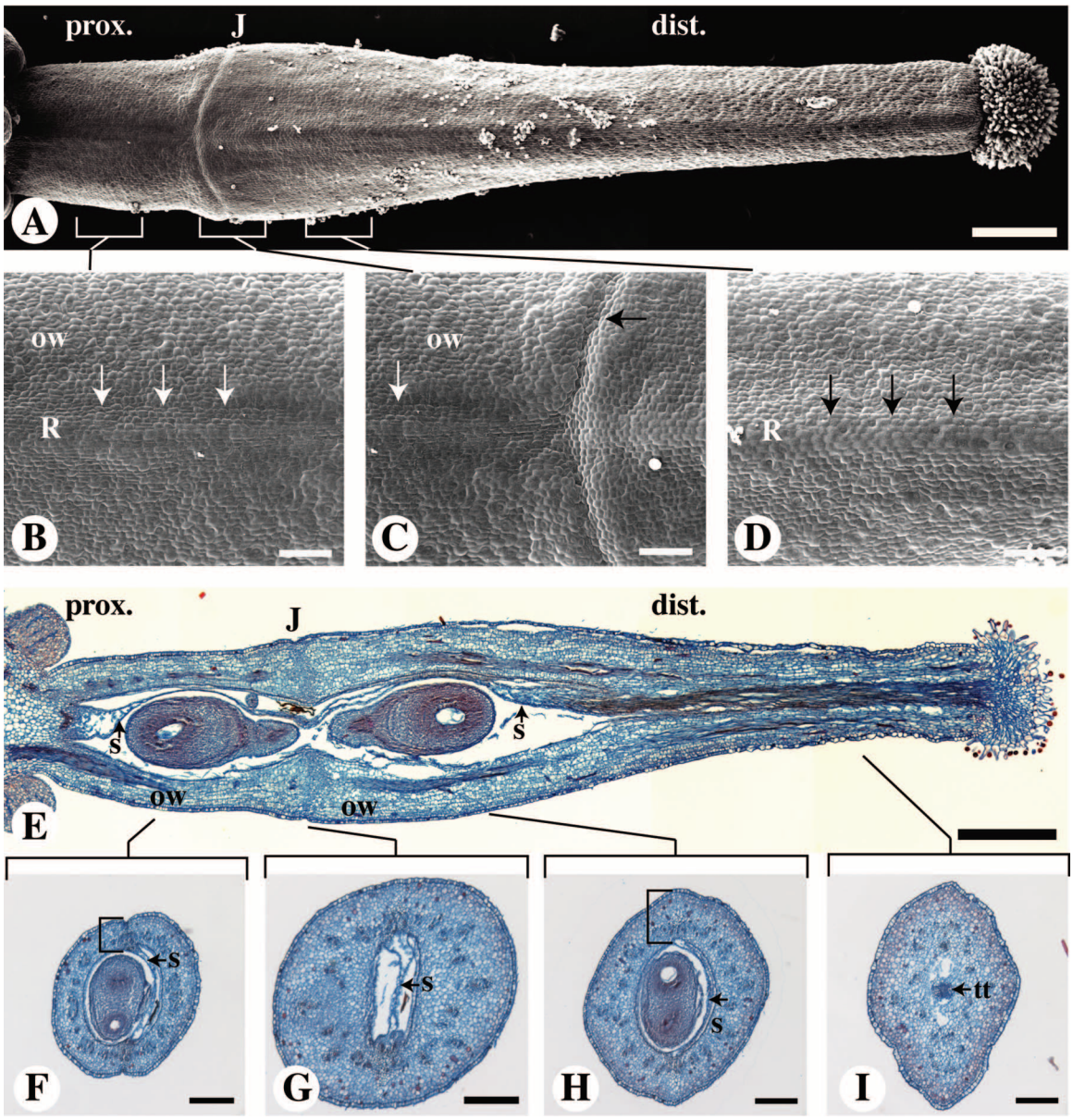


**Fig. 7** Anthetic carpel of *Cakile lanceolata*. Scanning electron micrographs and alcian blue/safranin O–stained sections. *A*, Medial view of carpel showing proximal and distal segments separated by the joint region. *B*, Magnified view of the proximal segment showing thin lines of small cells (white arrows) delineating replum. *C*, Magnified view of the joint region. Note the furrowed layer of cells of the joint (black arrow) that bisects the replum as compared with the cells delineating the replum (white arrow) in the proximal segment. *D*, Magnified view of the distal segment showing the raised replum (black arrows). *E*, Longitudinal section of anthetic carpel. The septum transverses the joint region, and ovules are present in both segments. *F–I*, Transverse sections of anthetic carpel through the proximal segment (*F*), joint region (*G*), distal ovule (*H*), and distal segment near stigmatic papillae, with brackets designating replum regions. Scale bars = 500 μm (*A*, *E*), 100 μm (*B–D*), 200 μm (*F–I*). *J* = joint region; *R* = replum; *ow* = ovary wall; *s* = septum; *tt* = transmitting tract.

associated with the replum since this connection forms as outgrowths from the replum (Skinner et al. 2004). In fact, the presence of septum can be taken as a clear indicator of replum since repla are often present without septa in fruits of closely related species (e.g., Cleomaceae; Judd et al. 1994; Hall et al. 2002), but false septa are never observed in the absence of repla. Thus, in typical siliques, the valve, replum, and septum represent closely associated tissue types whose definitions are largely interdependent.

Our developmental studies have demonstrated that, compared with many other species of Brassicaceae, the valves of heteroarthrocarpic fruits are disassociated from the repla. While the replum and septum develop from base to tip, defining the functional ovary, only a basal portion (at most) of the ovary wall differentiates as a valve that is bounded by a morphologically distinct margin. In other words, the ovary walls of heteroarthrocarpic fruits are not synonymous with the valves, a condition first proposed by Zohary (1948). This can

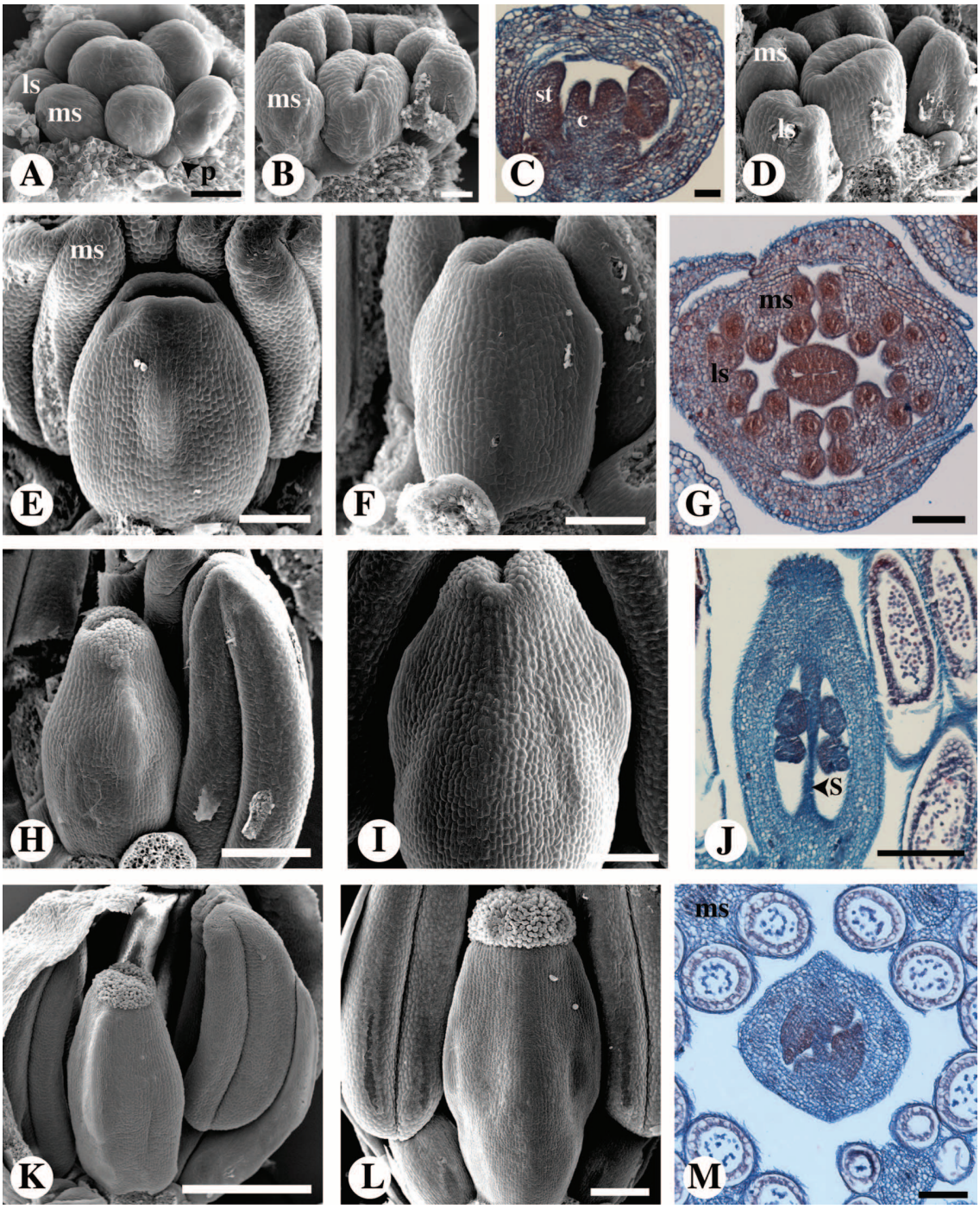


**Fig. 8** Early development of *Cakile lanceolata*. Scanning electron micrographs with selected organs removed to expose carpel and alcian blue/safranin O–stained sections. *A*, Polar-medial view of floral meristem after initiation of carpel, stamen, and petals. The carpel is a distinct mound located centrally in the meristem. *B*, Polar-lateral view of floral meristem in which the gynoecial dome has expanded and an invagination has formed. *C*, Longitudinal section through gynoecial tube of carpel at same stage as *B*. *D*, Polar-medial view of the elongating gynoecial cylinder. *E*, Medial view of carpel showing increased elongation relative to stamen length and circumferential expansion. *F*, Lateral view of carpel at same stage as *E*, showing expansion of the distal pericarp. *G*, Transverse section of bud at same stage as *E*, showing septa starting to form. *H*, Oblique-medial view of carpel in which stigmatic papillae have started to form. *I*, Lateral view of carpel at same stage as *H*, showing lateral expansion in the medial plane of the distal portion. *J*, Longitudinal section of carpel slightly older than *H*. The locules are open throughout the functional ovary. *K*, Oblique-medial view of carpel with formed stigmatic papillae. *L*, Lateral view of carpel at same stage as *K*. Elongation of the carpel results in a distal segment of greater length than the proximal. *M*, Transverse section of bud at same stage as *K*. Layers of the pericarp have started to differentiate. Scale bars = 50 $\mu$m (*A–D*), 100 $\mu$m (*E–G*, *I*, *J*, *M*), 200 $\mu$m (*H*, *L*), 500 $\mu$m (*K*). *ls* = lateral stamen; *ms* = medial stamen; *c* = carpel; *S* = septum.

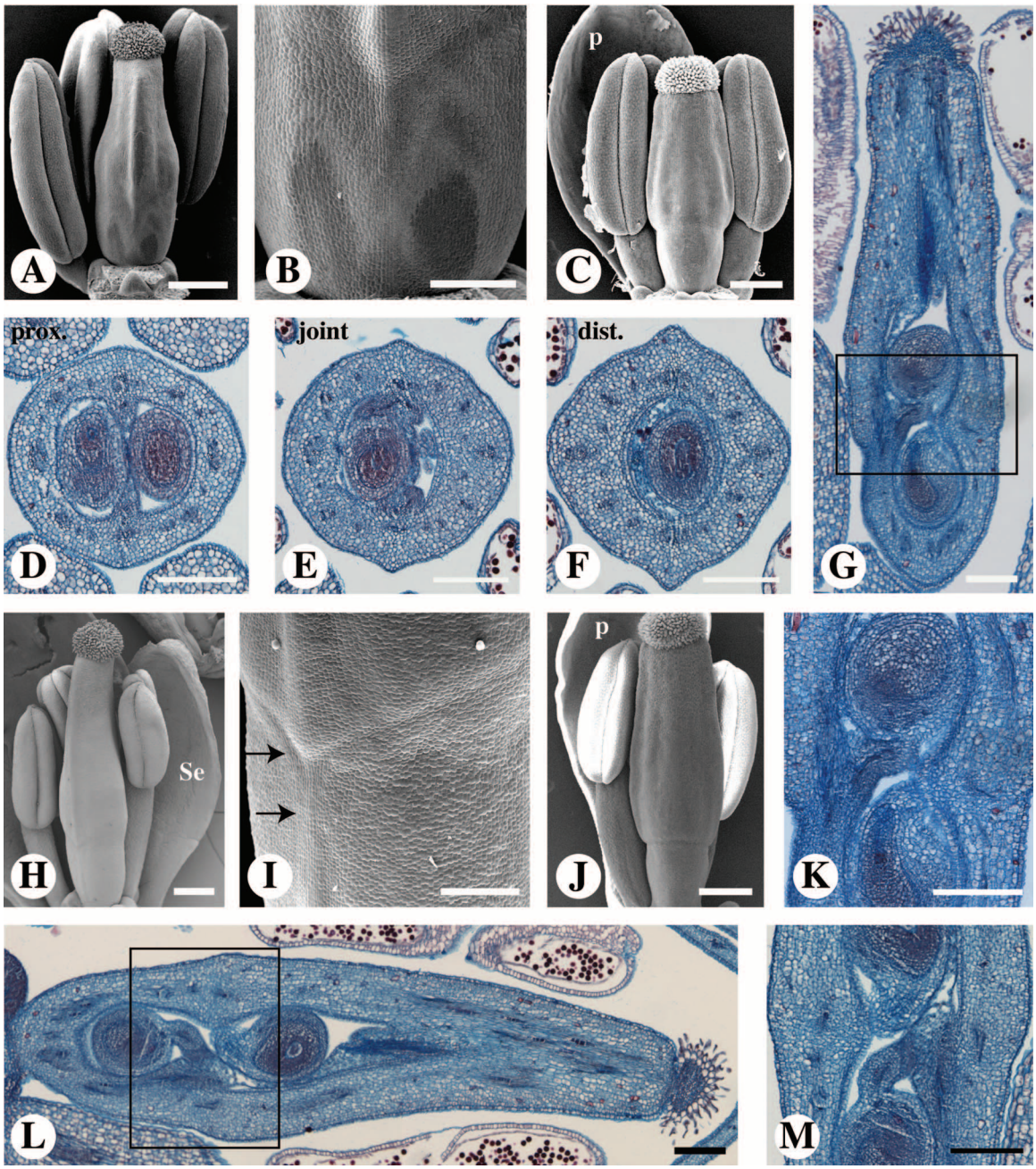


**Fig. 9** Mid-late development of *Cakile lanceolata*. Scanning electron micrographs with selected organs removed to expose carpel and alcian blue/safranin O–stained sections. *A*, Medial view of carpel after elongation such that carpel length has reached tips of lateral stamens. *B*, Magnified view of *A* showing external cell differentiation at the joint and replum regions. *C*, Lateral view of carpel at same stage as *A*. Note the elongation and expansion in the medial plane of the distal segment relative to the proximal. *D–F*, Transverse sections of carpel at same stage as *A* through the proximal segment (*D*), joint region (*E*), and distal segment (*F*). Layers of the pericarp have fully differentiated. *G*, Longitudinal section of carpel at same stage as *A–C*, showing context of the joint region (box), which has started to constrict internally. *H*, Oblique-medial view of the carpel just before anthesis when the elongated carpel has surpassed height of stamens. *I*, Magnified view of *H* showing rows of thin long cells of the replum and furrowing at the joint (black arrows). *J*, Lateral view of carpel at same stage as *H*. *K*, Magnified view of joint region of *G* (box) showing internal expansion of mesocarp where funiculi are attached to placenta. Note the erect orientation of the ovule in the distal ovule. *L*, Longitudinal section of carpel just before anthesis, with joint region in box. *M*, Magnified view of joint region in *L* (box) showing funiculus of proximal ovule originating from the joint region. Scale bars = 500 $\mu$m (*A*, *H*, *J*), 200 $\mu$m (*B*, *D–G*, *I*, *K–M*), 400 $\mu$m (*C*). *Se* = sepal; *p* = petal.

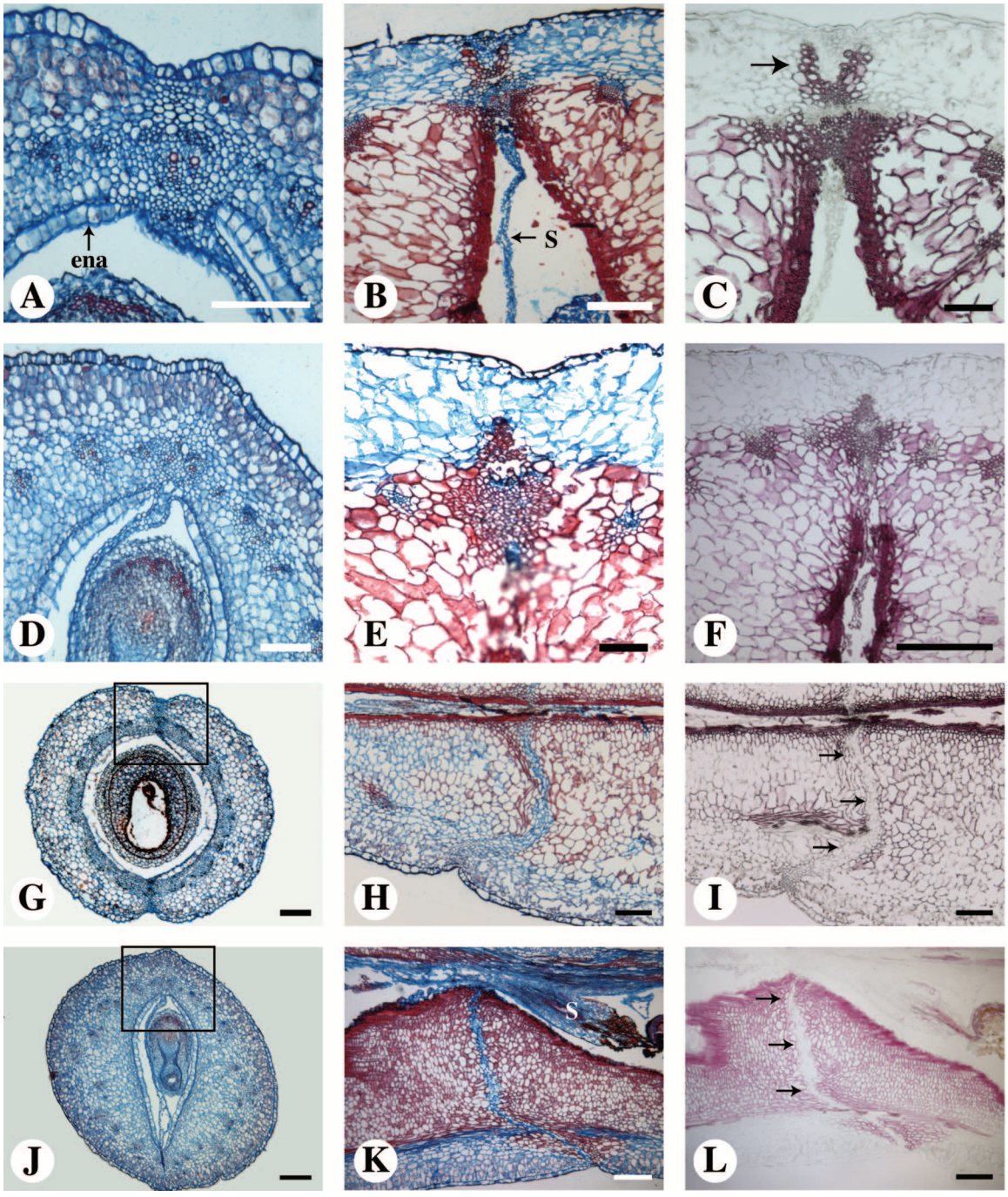


**Fig. 10** Fruit maturation of *Cakile lanceolata*. Sections stained with alcian blue/safranin O (*A*, *B*, *D*, *E*, *G*, *H*, *J*, *K*). Phloroglucinol-stained sections (*C*, *F*, *I*, *L*) coloring lignified cells pink and red. *A*, Replum region of proximal segment of young fruit. *B*, Replum region of maturing fruit. *C*, Phloroglucinol-stained section serial to that of *B*. Note the horseshoe-shaped cluster of lignified cells (arrow) of the replum. The inner mesocarp and endocarp layers are highly lignified. *D*, Replum region of distal segment of young fruit. *E*, Replum region of distal segment of mature fruit. *F*, Phloroglucinol-stained section of distal replum serial to that of *F*, showing extensive lignification in the replum, endocarp, and inner mesocarp. *G*, Transverse section through proximal segment of mature fruit showing context of replum region (box) in *A*–*C*. *H*, Longitudinal section on the lateral axis (perpendicular to septum) through joint region of mature fruit. *I*, Phloroglucinol-stained serial section of *H*. Note the unlignified layer of the joint surrounded by lignified cells (black arrows). *J*, Transverse section through distal segment of mature fruit showing context of replum region (box) in *D*–*F*. *K*, Longitudinal section of joint region on the medial axis parallel to the septum showing lack of lignification in the abscission layer. *L*, Phloroglucinol-stained section of fruit shown in *K*, showing lignification pattern similar to that of *I*. The nonlignified abscission layer (black arrows) bisects the replum at the joint. Scale bars = 100 $\mu$m (*A*–*E*), 200 $\mu$m (*G*–*L*). *ena* = endocarp *a*; *S* = septum.

most clearly be seen in *Erucaria*, where the valve is restricted to the proximal portion of the fruit, encompassing only ca. 90% of the ovary (fig. 1*A*; fig. 3*A*, 3*C*). In the carpels of *Cakile*, no portion of the ovary wall differentiates into a functional valve, although differences between the proximal and distal segments suggest a valvular origin for the proximal segment (see also below). The ovary wall of the distal segment in both *Cakile* and *Erucaria* does not differentiate into valve tissue, despite having septum and replum tissue present in this segment. In this regard, our findings are consistent with those of Rodman (1974), who observed that septum tissue is found in both segments in all seven species of *Cakile*.

*Developmental basis of indehiscence*. In the fruits studied, three instances of indehiscence occur: the distal segments of both species and the proximal segment of *Cakile*. The dehiscent proximal segment of *Erucaria* serves as a useful framework since the valve margin of this segment is similar to that observed in other dehiscent fruits of Brassicaceae (fig. 6*A*–6*C*). Fruit development and dehiscence are perhaps best studied in the model species *A. thaliana* (Spence et al. 1996; Ferrandiz 2002) and crop species of *Brassica* (Meakin and Roberts 1990*a*, 1990*b*; Spence 1996; Spence et al. 1996). Proper dehiscence in typical siliques is dependent on the establishment of morphologically distinct cell types that make up the valves, replum, and valve margin. The valve margin is especially important because it encompasses the DZ. The DZ itself is made up of two main cell types: a thin line of cells adjacent to replum that make up the SL proper and a group of lignified cells adjacent to the valve. The valves detach through the enzymatic breakdown of the middle lamella of cells in the SL (Meakin and Roberts 1990*a*; Ferrandiz 2002; Roberts et al. 2002). Lignification of the valve margin cells and en*b* layer is thought to be required for the production of the proper tensions that enable valve dehiscence (Spence et al. 1996; Rogers and Campbell 2004). Thus, the combination of having one to several rows of thin-walled, nonlignified cells surrounded by lignified tissue is important for proper valve dehiscence (Meakin and Roberts 1990*a*; Rajani and Sundaresan 2001).

The relative distribution of lignified and unlignified cells can be used as a guide to evaluate the developmental basis of dehiscence versus indehiscence in heteroarthrocarpic fruits. As mentioned above, patterns of lignification in the proximal segment of *Erucaria* are consistent with those seen in other dehiscent fruits of Brassicaceae, especially *Arabidopsis* and *Brassica*. In contrast, the pattern of cell lignification associated with the replum differs from traditional siliques in each of the three indehiscent segments studied. Heavy lignification is observed in the inner mesocarp of the replum in the distal segment of *Erucaria*, but this region is entirely morphologically distinct from that of the proximal segment, and there is no functional DZ (fig. 6*E*, 6*F*). The replum of the proximal segment of *Cakile* is different from the other indehiscent segments because of the presence of lignified cells in the outer layers of the replum. Although the horseshoe shape of these lignified cells stretches out toward the exocarp, similar to an LL, they are not associated with a DZ. These differences between the proximal and distal segments of *Cakile* are not evident until after anthesis, appearing during fruit maturation (fig. 10). The absence of lignified cells in the outer layers of the replum observed in the distal segment of *Cakile* is similar to the distal segment of *Erucaria* (fig. 10*E*, 10*F*). In sum, all of the indehiscent segments we examined lack any evidence of the SL and LL combination that is necessary for proper valve dehiscence. Similar alterations in lignification patterns are observed in the replum regions of other indehiscent species of Brassicaceae (Polster 2005). While it is somewhat difficult to directly compare the distal segments of *Cakile* and *Erucaria* because of different patterns of mesocarp proliferation and lignification, their common lack of radial lignified bands may suggest a similar basis for their indehiscence. The presence of this type of lignification in the proximal segment of *Cakile* reflects a different developmental basis for indehiscence in this segment.

*Developmental basis of the joint*. The lateral articulation that separates the fruit into two segments is one of the defining features of heteroarthrocarpic fruits (Appel 1999). External differences between the proximal and distal segments are apparent well before the joint has differentiated internally. In both taxa, the two segments are distinguishable by clear differences in segment shape even before the stigmatic papillae are formed (figs. 4, 8). At the joint itself, the external differentiation of a band of smaller cells is observed by the time the carpels are equal in length to the medial stamens (figs. 5*M*, 9*B*). These similarities in the development of the joint region between the two species are paralleled internally. The constriction associated with the joint region is formed by a proliferation and expansion of mesocarp cells that, interestingly, occurs much later in development than the external differentiation. In both species, the joint region constricts internally by the time the papillae are fully formed, although the constriction is slightly more pronounced in *Cakile* (fig. 9*G*) than in *Erucaria* (fig. 5*N*). The positioning of the constriction is similar in both species, being closely associated with the funiculus of the distalmost ovule. The distal seed is always erect, which is a consistently different orientation than observed in the proximal segments.

Despite the many similarities in early development of the joint region between these two variants of heteroarthrocarpy, this region is fundamentally different at maturation. Specifically, the two species differ with regard to whether the joint forms a complete abscission zone. Analogous to the DZ of the valve margin, the cells that promote abscission of the joint in *Cakile* are flanked on either side by lignified cells in both the proximal and distal segments (fig. 10*H*; Al-Shehbaz 1985). Thus, it is the contrasting interaction of lignified and nonlignified cells that is important for proper abscission and segment separation. In *Erucaria*, the comparable region is the apex of valve margin, where the valve similarly separates along a DZ that is closely associated with the joint region (fig. 6*H*, 6*I*). However, only the valves separate from the fruit, while the replum, septum, and entire distal segment remain intact (fig. 1*A*). The critical distinction is that in *Cakile*, a DZ forms circumferentially across the entire fruit, traversing the replum (fig. 1*B*). The septum, which is relatively degraded by maturity in *Cakile*, provides no persistent connection between the segments and thereby allows complete separation. In contrast, the replum of *Erucaria* is continuous throughout the proximal and distal segments, and DZ formation is limited to the valve margin. Furthermore, the septum

of *Erucaria* is quite robust and serves to stabilize the connection between the distal segment and the maternal plant. In our judgment, therefore, the joint is a composite structure composed of both the distal extent of the valve margin and a novel internal proliferation of mesocarp. This conclusion is fairly obvious in *Erucaria*, but in *Cakile* there are additional modifications. The longitudinal valve margins have become indehiscent, and a new DZ, possibly still representing the distal extent of the valve margin, has been oriented to bisect the replum (fig. 10*H*, 10*I*, 10*K*, 10*L*).

### *Homology between Siliques and Heteroarthrocarpic Fruits*

Many authors have concluded that the proximal segment of heteroarthrocarpic fruits is valvular in nature (Zohary 1948; Gómez-Campo 1980, 1999; Appel 1999). The relationship of the proximal segment to the valve of a typical silique is readily apparent in the fruits of *Erucaria*, but the valvular origin of the proximal segment of *Cakile* is not so obvious. However, there are both external and internal characteristics associated with the valve margin that are found in the proximal segment of *Cakile* fruits. Externally, there is a furrow between the replum and carpel walls (fig. 7*A*, 7*B*) that is reminiscent of the valve margin of *Erucaria* (fig. 3*A*). This furrow is not observed in the distal segment of either taxon (figs. 3, 7). Internally, there are differences in the replum region between the proximal and distal segments of *Cakile* that are most evident in maturing fruits (fig. 10). Specifically, the replum of the proximal segment contains a pair of radial bands of lignified cells that stretch outward in a manner very similar to the LL cells observed in the proximal segment of *Erucaria* (fig. 10*B*, 10*C*). This pattern of lignification is completely absent from the distal segments of both taxa (fig. 6*E*, 6*F*; fig. 10*E*, 10*F*). Thus, it would appear that the proximal ovary wall of both fruits is in fact valvular in derivation, although there are no functional valves in the indehiscent *Cakile* fruit.

What, then, is the distal segment? Three alternatives exist: (1) the upper portion of a typical silique produced via constriction of the valves, (2) a substylar structure derived from the style (Gómez-Campo 1999), or (3) ovary wall present after the reduction of the valve (Zohary 1948). The morphological differences observed between the distal and proximal segment are consistent only with the latter two interpretations since constriction implies the proximal and distal segments should be similar. In fact, the distal segments of both species are more similar to one another than either is to its respective proximal segment. Moreover, while some indications of presumptive valve are detected in the proximal segment of *Cakile*, there is no internal or external evidence of valvular tissue in the distal segment. With regard to the second hypothesis, the distal segment of both species appears to be composed of both ovary and style. Internally, the ovary component of the distal segment develops in exactly the same manner as the proximal ovary, with continuous replum and septum. Near the stigmatic papillae, the distal segment is internally made up of only mesocarp and transmitting tract (figs. 3*I*, 7*I*), suggesting stylar tissue. It is notable that although the two study species have sessile stigmas, other species in the *Cakile* clade have heteroarthrocarpic fruits with externally distinct styles. This combination of separate styles in closely related species and normal ovary characteristics suggests that the distal segment is not wholly derived from the style. A modification of Zohary's (1948) valve reduction interpretation is therefore most able to reconcile the combination of the lack of valve with the presence of septum/replum found in the distal segment of both species. Under this interpretation, the homology of the distal segment is context dependent. The functional ovary of heteroarthrocarpic fruits in both proximal and distal segments is positionally homologous to the entire ovary of a typical silique. The ovary wall of the distal segment, however, is not valvular and has no identity homology with any part of the ovary wall of a typical silique. This reflects the fact that in a typical silique, the entire ovary wall differentiates into valve, whereas in heteroarthrocarpic fruits, valve tissue differentiates only in the proximal portion of the ovary wall. This hypothesis is consistent with our earlier conclusion that the joint is a composite structure incorporating both the apical portion of the valve margin and an internal proliferation of the mesocarp. While it is possible that the abscission zone of the *Cakile* joint is somehow independently derived, it bears striking similarity to the DZ of the apical valve margin seen in *Erucaria*, especially when viewed in lateral sections (figs. 6*H*, 10*H*).

### *Using Knowledge of* Arabidopsis *Fruit Development to Shed Light on Possible Genetic Mechanisms Underlying Heteroarthrocarpy*

While much of the early work on *A. thaliana* flowers focused on organ identity, recent studies are beginning to elucidate the pathways responsible for the elaboration of fruit morphology, particularly the dehiscence of the silique (Ferrandiz et al. 1999; Ferrandiz 2002; Dinneny and Yanofsky 2005). The alterations that led to heteroarthrocarpy in the *Cakile* clade involved shifts in apical-basal patterning and seed reduction, as well as other changes. However, it is clear there has been major remodeling of the valve/replum boundary and its associated DZ, a developmental genetic pathway that has been well characterized in *Arabidopsis*. There are currently six genes known to play critical roles in patterning of the valve margin layer in *Arabidopsis*. The DZ itself is determined by the combined activities of *SHATTERPROOF1-2* (*SHP1-2*), *INDEHISCENT* (*IND*), and *ALCATRAZ* (*ALC*), which represent a genetic pathway (Liljegren et al. 2004; Dinneny and Yanofsky 2005). *IND* and *ALC* are genetically downstream of *SHP1/2*, although other factors are also involved in their regulation (Liljegren et al. 2004; Dinneny et al. 2005). The other two genes involved in valve margin patterning, *FRUITFULL* (*FUL*) and *REPLUMLESS* (*RPL*), are required to restrict *SHP1/2*, *ALC*, and *IND* to the narrow strip of cells at the valve/replum boundary (Ferrandiz et al. 2000; Roeder et al. 2003; Liljegren et al. 2004). *FUL* is expressed in developing valve tissue (Gu et al. 1998), while *RPL* is expressed in the replum (Roeder et al. 2003). *FUL* and *RPL* appear to play complementary roles by repressing *SHP1/2*, *ALC*, and *IND* expression in the valves and replum, respectively (Ferrandiz et al. 2000; Roeder et al. 2003). Studies in the genus *Brassica*, a member of the Brassiceae with a fairly typical silique, indicate that aspects of this genetic pathway are conserved across the family (Vancanneyt 2003; Liljegren et al. 2004).

Our analyses of *Erucaria* and *Cakile* have revealed morphological parallels between certain *Arabidopsis* mutants and the heteroarthrocarpic fruit of our study taxa. In the dehiscent proximal segment of *Erucaria*, the morphology is very similar to that of wild-type *Arabidopsis* fruits, and the expectation would be that the valve margin genetic pathway should be conserved. The indehiscent proximal segment of *Cakile* is somewhat difficult to interpret, but it is reminiscent of mutant alleles of *alc* or *ind*, which may retain the formation of the LL while losing proper differentiation of the SL (Rajani and Sundaresan 2001; Liljegren et al. 2004). It remains unclear, however, as to whether the lignification observed in the replum of the proximal *Cakile* segment is solely homologous to the replum of a dehiscent fruit or whether it comprises both replum and the LL of the presumptive valve margin. Some insight into this distinction could be provided by gene expression studies. For instance, the extent of *RPL* expression could be used as one indicator for the delimitation of the replum tissue relative to the radial lignification bands. The distal segments of both *Cakile* and *Erucaria* completely lack LLs that cross the mesocarp and, in this regard, resemble *shp1 shp2* mutants or transgenic lines that overexpress *FUL* (Ferrandiz et al. 2000; Liljegren et al. 2000). On the basis of our hypothesis concerning the developmental origin of the distal segment, however, we would predict that this region would lack *SHP1/2* expression in the distal ovary wall not just because it is an indehiscence segment but also because it is fundamentally lacking in valve tissue and, therefore, valve margin. Overall, it should be noted that changes in gene expression or function at multiple hierarchical levels in the pathway could lead to loss of clear SLs and LLs of the valve margin, making it difficult to predict a priori which loci are likely to be modified. Another point to consider is our suggestion that the lateral abscission zone associated with the joint of *Cakile* represents a modification and reorientation of the apical extent of the presumptive valve margin. Such a model would invoke the capacity to differentially regulate valve margin development along the apical/basal axis of the fruit, such that the basal and longitudinal margins became indehiscent while the apical margin retained a DZ. In fact, evidence exists that this is quite possible within the genetic architecture of *Arabidopsis*, and even *shp1 shp2* mutant fruits retain some development of DZ along the apical extent of their valve margins (Liljegren et al. 2000). Determining the expression patterns of the valve margin identity loci in this area would help to test this hypothesis. With regard to the valve/replum dissociation theory, similar morphology has not been observed in *Arabidopsis* loss of function mutants. It has been demonstrated, however, that valve margin positioning is controlled by negative regulators acting on each side of the DZ. Repositioning of the expression domains of these loci could theoretically shift the placement of the DZ, although this has not been tested experimentally.

There are a number of other loci that appear to be important for patterning of the carpel and fruit, some of which may also be involved in the morphological modifications we see in *Cakile* and *Erucaria*. The gene *ETTIN*, for instance, is involved in apical-basal patterning of the carpel in response to auxin (Sessions et al. 1997; Nemhauser et al. 2000) The carpels of *ett* mutants do exhibit reduction of the valve, similar to what is observed in some Brassiceae, but in *ett* plants the ovary is concomitantly reduced, unlike the case in *Cakile* and *Erucaria*. Another gene of interest is the bHLH encoding *SPATULA* (*SPT*; Alvarez and Smyth 1999). *SPT* is very closely related to *ALC* (Savidge et al. 1995; Sessions et al. 1997), although the former appears to play a much broader role in carpel development (Alvarez and Smyth 1998, 1999). Overall, the carpels of *spt* plants exhibit defects in the development of tissues derived from the margins, particularly the septum and transmitting tract. Interestingly, aspects of the dynamic expression pattern of *SPT* suggest that it may play a role in dehiscence, but this function must be redundant with other factors (Heisler et al. 2001), possibly including the closely related *ALC*. There are also several genes that are critical for the final aspects of cell separation, including breakdown of the middle lamella and patterns of programmed cell death (reviewed in Jarvis et al. 2003). However, these loci are unlikely to be directly involved with the evolution of heteroarthrocarpy.

### *Conclusions*

We have found that the heteroarthrocarpic characteristics of *Cakile* and *Erucaria* fruits appear relatively early in development. Furthermore, external differentiation of these traits is present at earlier developmental stages than internal differentiation. Analyses of anthetic and maturing carpels suggest that heteroarthrocarpy involves a disassociation between the repla and the valves, which is consistent with the conclusion that only the proximal segment is valvular in nature. The ovary wall of the distal segment appears to differentiate into a novel tissue type that has no analog in a typical silique. We also conclude that the anatomically novel feature known as the joint is a composite structure that combines the distal extent of the valve margin with an internal proliferation of the mesocarp. Further modifications of the valve margin in *Cakile* have resulted in an indehiscent proximal segment and a joint that abscises. Transitions between different variants of heteroarthrocarpy can be tested via detailed phylogenetic studies and additional comparative developmental studies on putative intermediates. Many of these hypotheses may also be tested through comparative developmental genetic studies in *Cakile* and *Erucaria*.

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