Maternal-filial transfer structures in endosperm: a nexus of nutritional dynamics and seed development

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Abstract

Although the ultimate purpose of a seed is successful establishment of the next generation, seed development involves more than just embryo growth. In angiosperms, seed development requires the intimate coordination of three distinct entities – maternal tissue and two offspring, embryo and embryo-nourishing endosperm. Although seeds are cornerstones of many terrestrial ecosystems and human diets, we are only beginning to understand the interactions among seed tissues and the molecular processes and genes that determine them. Recent studies of gene expression and function in distantly related angiosperms, combined with over 100 years of angiosperm embryological research, have repeatedly highlighted the endosperm associated with maternal-filial boundaries as a central point in developmental dynamics within seeds. In this review, we highlight evidence that links this zone with nutritional dynamics, developmental signaling, and imprinted gene expression. We suggest that the underappreciated diversity of this specialized endosperm across angiosperms deserves further study from developmental, molecular, and genetic perspectives.

Keywords: endosperm, haustoria, chalazal endosperm, seed development, endosperm transfer region, developmental signaling, gene imprinting

Highlights:

Endosperm, a biparental, nutritive seed tissue, differentiates into specialized zones.

Transfer-specialized endosperm is found at maternal-filial boundaries in many species.

Transfer endosperm functions in nutrient dynamics, developmental signaling, and gene imprinting.

29 Distinct and diverse endosperm transfer structures (including haustoria) are found across angiosperms.

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Introduction

At the inception of each flowering plant (angiosperm) seed, two fertilization events produce two offspring with distinct fates: the embryo, which represents the next sporophyte generation, and the endosperm, an altruistic embryo-nourishing tissue. These fertilization events occur within an ovule, an organ derived from the maternal sporophyte tissue that houses the gamete-producing female gametophyte. The offspring therefore develop completely enclosed within the ovule: ovule integuments become the seed coat, gametophyte-surrounding nucellus tissue may be incorporated into the seed structure, and all maternally-supplied resources must be channeled to the offspring through ovule vasculature [1] (Fig. 1A). From the site where maternal phloem terminates and nutrients are unloaded, resources and developmental signals are passed on to the endosperm and embryo (Fig. 1A).

The apparent lack of symplastic connections between the embryo, endosperm, and maternal tissues [2] begs the question: how do resources and developmental signals travel between the mother and offspring tissues? The only direct embryo-maternal sporophyte connection is the embryo-derived suspensor, the appearance and function of which varies greatly across flowering plants [3]. Rather, the endosperm separates the embryo from the maternal sporophyte throughout seed development. Indeed, endosperm is recognized as an important mediator of the developmental and nutritional relationships between a mother and her embryo in all but two angiosperm lineages (the endosperm-less Orchidaeae and Podostemaceae). Endosperm can perform a suite of functions related to nutrient dynamics within the seed, including some combination of nutrient acquisition, processing, storage, mobilization, and transfer to the embryo. In addition, the endosperm develops precociously relative to the embryo, expanding the seed to allow for embryo growth and participating in cross-talk that coordinates developmental cues with the embryo [4,5] and surrounding seed coat [6,7]. The space occupied by endosperm is at least partially supplanted by the growing embryo, yet endosperm persists

through seed maturity and germination, and plays an active role in controlling embryo sheath formation [8] and germination [9].

While endosperm is an ephemeral tissue, it nonetheless differentiates into distinct zones or tissues. One such zone occurs at the maternal-filial boundary, adjacent to maternal vascular tissue or near the site of vascular strand termination (Fig. 1). The endosperm adjacent to this boundary often assumes distinct morphologies associated with transfer cells and tissues. This zone, which we refer to as *transfer endosperm at maternal-filial boundaries*, is best studied in *Arabidopsis thaliana* and cereal crops and displays a remarkable diversity across angiosperms.

Distinct endosperm at maternal-filial boundaries in *Arabidopsis* and cereal crops

In many angiosperms endosperm is initially free-nuclear, developing as a multi-nucleate coenocyte with nuclear-cytoplasmic domains differentiating along the micropylar-chalazal axis of the seed (Fig. 1A). In *Arabidopsis thaliana*, endosperm at the chalazal pole forms transcriptionally distinct nodules and a cyst [10**]; both are cytoplasmically dense and contain multiple nuclei, with cyst nuclei dividing infrequently. The cyst interfaces with nucellar lysate and the chalazal proliferative tissue (CPT), a nucellus-derived tissue adjacent to where ovule vasculature terminates [11,12]. Unlike the rest of endosperm, the chalazal endosperm never fully cellularizes, but is rather characterized by dense, organelle-rich cytoplasm [11,12] and multiple, polyploid nuclei [13]. The chalazal cyst also displays transfer-cell characteristics: an absence of cuticle (which is otherwise prominent along endosperm-maternal sporophyte boundary), projections into the CPT, and elaborated cell wall morphology [11,12]. Deterioration of cells within the nucellus/CPT is important for chalazal endosperm development and function, suggesting some form of communication between the tissues [14]. The chalazal endosperm cyst, meanwhile, persists throughout most of seed maturation [12,15].

Cereal crop endosperm makes up a larger portion of the seed throughout development and is the primary site of nutrient storage. Accordingly, there are also more distinct regions of the endosperm in these species, such as the aluerone (a protein- or lipid-rich peripheral layer linked to nutrient processing) and starchy/chalky central endosperm (which functions in nutrient storage). The concept of a chalazal pole is generally less applied to cereal crop seeds, perhaps due to the longer contact zone between ovule vasculature, nucellus and other maternal tissues, and endosperm (Figure 1B). Some cereals have a vascular strand that runs along the length of the seed, near persistent nucellar tissues that can project into endosperm cavity space [16,17,18]. The endosperm cells adjacent to the persistent nucellar tissue, called endosperm transfer cells (ETCs), have been compared to chalazal endosperm of *Arabidopsis* [16]. The ETC region in maize is called the basal endosperm transfer layer (BETL) and is relatively restricted (Fig. 1B). BETL cells are distinctly elongated and exhibit transfer cell characters such as prominent wall ingrowths [19]. In contrast to the chalazal endosperm of *Arabidopsis*, maize BETL and ETCs in other cereals are fully cellularized. Another distinct feature of many cereals is the extensive degeneration of maternal or endosperm cells near vascular-adjacent regions of the seed to create fluid- or gel-filled cavities [20,21]. In maize, maternal cells in the placental-chalazal region (P-C) next to the BETL undergo programmed cell death [19], which may be instigated by the BETL [22]. In wheat and barley, a fluid- or gel-filled cavity occupies the P-C region of the endosperm and separate ETCs from the nucellar projection [19,21]; this cavity in barley is formed by programmed cell death in the nucellus [23*]. Proper development of ETCs and the nucellar cavity impact seed development, hydration, and filling [20,23*].

Gene activity associated with nutrient transfer and processing at the maternal-filial interface

In addition to morphology, gene expression profiling supports a nutrient transfer function for endosperm at the maternal-filial boundary [10**,24,25]. In early Arabidopsis seed development, the cyst is enriched for GO terms relating to phloem sucrose unloading and protein catabolism, while the chalazal nodules are enriched for genes involved in a key step of one-carbon metabolism [10**]. Localization of a SWEET sugar transporter to the CPT further suggests that apoplastic loading of fructose and glucose are key to import by the chalazal endosperm [26**], which itself shows high expression of sugar transporters and invertases during early seed development [27]. A SWEET gene in maize, *ZmSWEET4c*, is expressed in the BETL cells, and both *ZmSWEET4c* and a rice ortholog are required for proper nutrient accumulation in endosperm [28]. Indeed, the BETL transcriptome in maize is generally enriched for transmembrane transport, ion transport, and sucrose transport [29] and transport proteins are enriched at the BETL plasma membrane [1]. Transcriptomic information from ETCs of barley before, during, and after endosperm cellularization show signatures of a range of cellular process, including methionine and C:N metabolism and nutrient trafficking [16].

Maternal-filial transfer structures as developmental regulators

Transfer-specialized endosperm regions at maternal-filial boundaries also appear to act as hubs of developmental regulation for seeds as a whole. Expression and activity of genes involved in hormone dynamics, cross-talk between maternal tissue and endosperm, and regulation of developmental events in other endosperm zones have been documented in both Arabidopsis and cereal crops.

Hormone biosynthesis genes, including those for gibberellic acid, abscisic acid, and cytokinin, are highly expressed throughout Arabidopsis and *Brassica napus* chalazal endosperm development, as are genes related to auxin signaling [24,25,30**]. Similar signatures of hormone activity have been found in cereals, including links between ETCs and auxin in wheat [31] and barley [16,32]. In rice, the importance

of auxin synthesis in the dorsal aleurone is borne out by the localization of auxin biosynthesis gene OsIAA29 specifically in this region of the endosperm, between the vascular-adjacent nucellus and the rest of the offspring tissues [33]. Furthermore, hormones like auxin, ethylene, and/or cytokinins are important for proper differentiation of the BETL in maize and ETCs in other cereals [1,16,34]. While the role of hormones in seed development are as varied as they are across other aspects of plant biology, it appears that endosperm transfer tissues as maternal-filial boundaries are a central point of hormone signaling.

The role of transfer endosperm in developmental signaling is not limited to hormones. In Arabidopsis, chalazal endosperm is enriched for defensins, a class of small signaling peptides that function beyond plant immunity [35]. Transcriptional profiles of ETCs in barley cells show expression of almost all two-component signaling system elements annotated in the barley genome [16] and the BETL-specific gene expression module in maize includes multiple classes of small cysteine-rich proteins [29], suggesting that cereal ETCs actively participate in signaling pathways. Furthermore, transport of signal proteins from BETL cells to the adjacent maternal tissue has been documented [36]. Thus, ETCs at maternal-filial boundaries are not only importing resources, but are also capable of generating and exporting signaling molecules. Such signaling may underlie crosstalk between the maternal tissues and chalazal endosperm that affect spatial tradeoffs during development and nutrient partitioning, such as degeneration of CPT or P-C regions, which subsequently impact seed development as a whole [14].

Transfer endosperm at the maternal-filial boundaries also appears to impact differentiation of other areas of the endosperm. The chalazal endosperm of Arabidopsis generates TFL1 protein, which subsequently moves to peripheral endosperm and interacts with an ABA-sensitive mechanism to control timing of peripheral endosperm cellularization [37**]. Timing of endosperm cellularization in Arabidopsis and cereals is an important determinant of endosperm size and the ability to import/process nutrients, which in turn affects final seed size [38,39]. Thus, the chalazal endosperm may

also influence nutrient dynamics in seeds indirectly as a regulator of overall endosperm developmental timing.

Imprinted gene expression and parental genome dosage sensitivity in maternal-filial transfer structures

A gene is described as imprinted when expression of an allele depends on whether it was maternally or paternally inherited. Imprinted gene expression has been linked to parent-of-origin effects on seed development and the different strategies that mothers and fathers use to maximize their own fitness in the context of nutrient investment during reproduction. According to interparental conflict theory, genetic conflict over distribution of maternally-supplied resources to asymmetrically related offspring should manifest during nutrient transfer between maternal tissue and biparental offspring [40,41]. Maternal control over maternal resources can act on both sides of the maternal-filial interface. Paternal control is meanwhile limited to expression in tissues with paternal genetic contribution, such as endosperm, and could be expected to manifest most strongly in structures and processes related to nutrient allocation to offspring.

In Arabidopsis, several lines of evidence suggest that chalazal endosperm function is sensitive to gene imprinting and parental gene/genome dosage. The MADS box transcription factor gene *PHE1* is a paternally expressed imprinted gene (PEG) that is specifically expressed in the chalazal endosperm [42] and has been shown to regulate expression of genes related to endosperm cellularization, including other imprinted genes [43]. Chalazal-specific expression during some stages of seed development has also been documented for the Polycomb Repressive Complex 2 (PRC2) members, FIS2 and MEA, which contributes to epigenetically marking the silenced maternal alleles of PEGs [10**,24,44]; *fis2* mutants exhibit enlarged chalazal endosperm and altered cellularization patterns across the rest of the

endosperm [45]. More broadly, single-nuclei analyses of imprinted gene expression in Arabidopsis endosperm demonstrated that about half of PEGs are most highly expressed in chalazal endosperm compared to other endosperm regions, and that this is due to increased expression specifically from the paternal allele [10**]. Studies from crosses between plants of different ploidies further highlight the importance of paternal gene dosage in chalazal endosperm development. Endosperm with excess paternal genome dosage develop enlarged cysts and show enrichment for gene expression programs associated with chalazal endosperm identity [46,47].

Links between imprinting and ETCs at the maternal-filial boundary are also seen in cereal crops. *Meg1* is a maternally expressed imprinted gene (MEG) in maize that impacts BETL differentiation and function in a dosage-dependent manner [48]. Yet in an intriguing parallel to chalazal endosperm in *Arabidopsis*, the gene expression module associated with the maize BETL significantly overlaps with a subset of maize PEGs [29]. The only other endosperm regions similarly enriched in PEGs are the BETL-adjacent region and another area of endosperm specialized for nutrient transfer, the embryo-surrounding region [29]. Maize MEGs, meanwhile, significantly overlapped with expression modules of other endosperm regions. Similar to the effect of increased paternal genome dosage in Arabidopsis, paternal-excess crosses in maize show expansion of BETL identity [1,49]. Mutations in a copy of OsEMF2, a component of the PRC2 complex in rice, share phenotypes with PRC2 mutants in Arabidopsis, including changes to timing of endosperm cellularization [50*,51*]. Effects on ETCs, however, have not been specifically analyzed. Intriguingly, far more rice PEGs were shown to be targets of OsEMF2 activity than rice MEGs, which is consistent with the connection between PRC2, PEG regulation, and chalazal endosperm in Arabidopsis and the BETL in maize. Altogether, the apparent enrichment of imprinted genes, and in particular PEGs, in endosperm transfer cells at the maternal-filial boundary suggests that this region does indeed function as a checkpoint in nutrient dynamics during interparental conflict.

Exploring Chazal/Transfer Endosperm Diversity to Understand Seed Nutritional and Developmental Dynamics

Transfer cell activity, hormonal response and regulation, and genetic imprinting are associated with chalazal endosperm in *Arabidopsis* and ETCs in cereals, despite differences in development and morphology. While this suggests these processes may be a fundamental part of the interface between endosperm and the maternal sporophyte, and may even date back to the origin of endosperm ~140 million years ago, such a hypothesis remains to be tested across the breadth of angiosperm seed biology. Indeed, there are already many records of diverse endosperm ontogenies scattered across over 100 years of embryological literature. Endosperm outgrowths called haustoria, which can invade surrounding maternal tissues, occur across angiosperm phylogeny – including multiple clades of parasitic plants and economically important groups like Cucurbitaceae and legumes [3,52]. These structures are *de facto* associated with nutrient transfer and exhibit distinct morphologies, such as complex branching (e.g. *Jodina*) or growth as a single ceonocyte over 19 mm long (e.g. *Cucumis*)[52] (Fig. 2). Haustoria can also occur in micropylar endosperm [3,52,53], raising the question of whether the documented associations between chalazal endosperm and developmental signaling or PEGs could occur in other regions of the endosperm. In addition, endosperm haustoria appear at some of the earliest divergences in angiosperm evolution: several members of the Nymphaeales are characterized by enlarged, unicellular chalazal domains that extend into maternal seed storage tissues (*Nuphar* [54]; *Nymphaea*[55]). Indeed, haustorial-like appearance of chalazal female gametophyte tissue has been documented in endosperm-less gymnosperms like *Gnetum* [56]. This raises the question of whether such chalazal female gametophyte differentiation represents independent evolution of haustorial function, or whether transfer endosperm in angiosperms may have co-opted a pre-existing developmental program. Studying species from lineages whose origin predate the divergence of

monocots and eudicots, ~136 MYA [57], can determine which aspects of endosperm development at maternal-filial boundaries may have been associated with the very origin of endosperm itself.

Future Directions

So far our views on the diverse functions of transfer-specialized endosperm at maternal-filial boundaries have been restricted to a relatively small number species and seed types. Indeed, the diversity of endosperm transfer structures is rarely discussed outside of the context of being structural oddities, and seed development of most angiosperms remains undocumented. We propose that in order to advance our understanding of maternal and offspring tissue interactions, including economically important processes like seed filling, we must first continue exploring seed development across angiosperm diversity. We can then take advantage of emerging technologies that allow for tissue-specific characterization of gene activity and metabolomic processes in species with few genetic or technical resources. While the enclosed, complex, and internally delicate nature of seeds make them difficult to study, combining traditional histological techniques with advances in confocal microscopy, non-light based 3-D imaging such as micro-CT [58*-61], and metabolite-sensitive imaging [62*] could trigger a renaissance in seed plant embryology. Meanwhile, technologies like single-nuclei sequencing or other low-input sequencing allow for gene expression profiling specifically in tissues at maternal-filial interfaces [10**,16,24,29], and are becoming increasingly accessible and affordable. Even in the absence of stable transformation techniques, applying these technologies to species with diverse structures at maternal-filial interfaces holds much promise for uncovering novel strategies that mothers and offspring use to negotiate resource allocation into seeds.

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Similar to [51*], this paper characterizes aspects of seed development in rice mutants of *OsEMF2a*, which is homologous to the Polycomb Repressive Complex 2 member *FIS2* in Arabidopsis. Timing of endosperm cellularization is altered in *OsEMF2a* mutant seeds in a manner similar to *fis2* mutants in Arabidopsis. This paper furthermore characterizes H3K27me3 patterning in *OsEMF2a* mutant endosperm, thereby identifying PRC2 targets in rice.

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Similar to [50*], this paper characterizes aspects of seed development in rice mutants of *OsEMF2a*, which is homologous to the Polycomb Repressive Complex 2 member *FIS2* in Arabidopsis. Similar to *fis2* mutants, endosperm cellularization and gene imprinting is altered in *OsEMF2a* mutants. Intriguingly, while these phenotypes were associated with altered cytokinin production, they were not linked with over-production of auxin (as is the case in Arabidopsis mutants that show similar changes to endosperm cellularization). These results show that hormones are broadly, if variably, important regulators of endosperm development.

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This study presents improvements to micro-computed tomography, by demonstrating single-distance X-ray phase-contrast imaging on complex samples. Seeds of *Lepidium* are used as an example of a complex biological sample; resulting phase tomograms display differentiated tissues with cellular resolution throughout whole seeds.

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In order to characterize the spatial patterns for metabolism of a cyanogenic glucoside in germinating sorghum seeds, the authors combine combined targeted metabolite profiling with matrix-assisted laser desorption/ionization-mass spectrometry. Furthermore, the authors show tissue-specific distribution patterns of key sugar and protein metabolites and amino acids across whole seeds. This study demonstrates the utility of metabolic-sensitive imaging techniques to address nutrient dynamics and metabolism in seeds.

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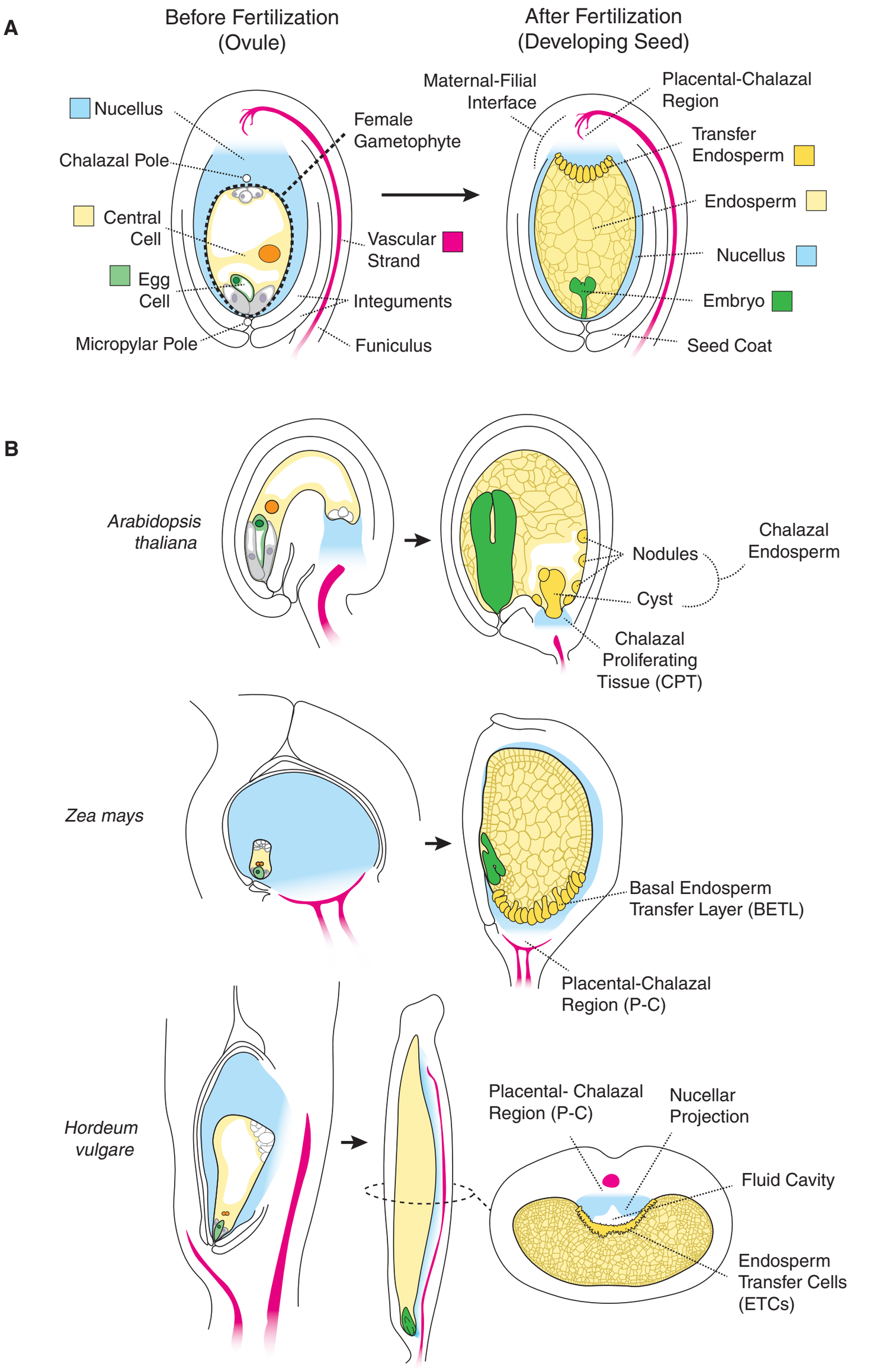
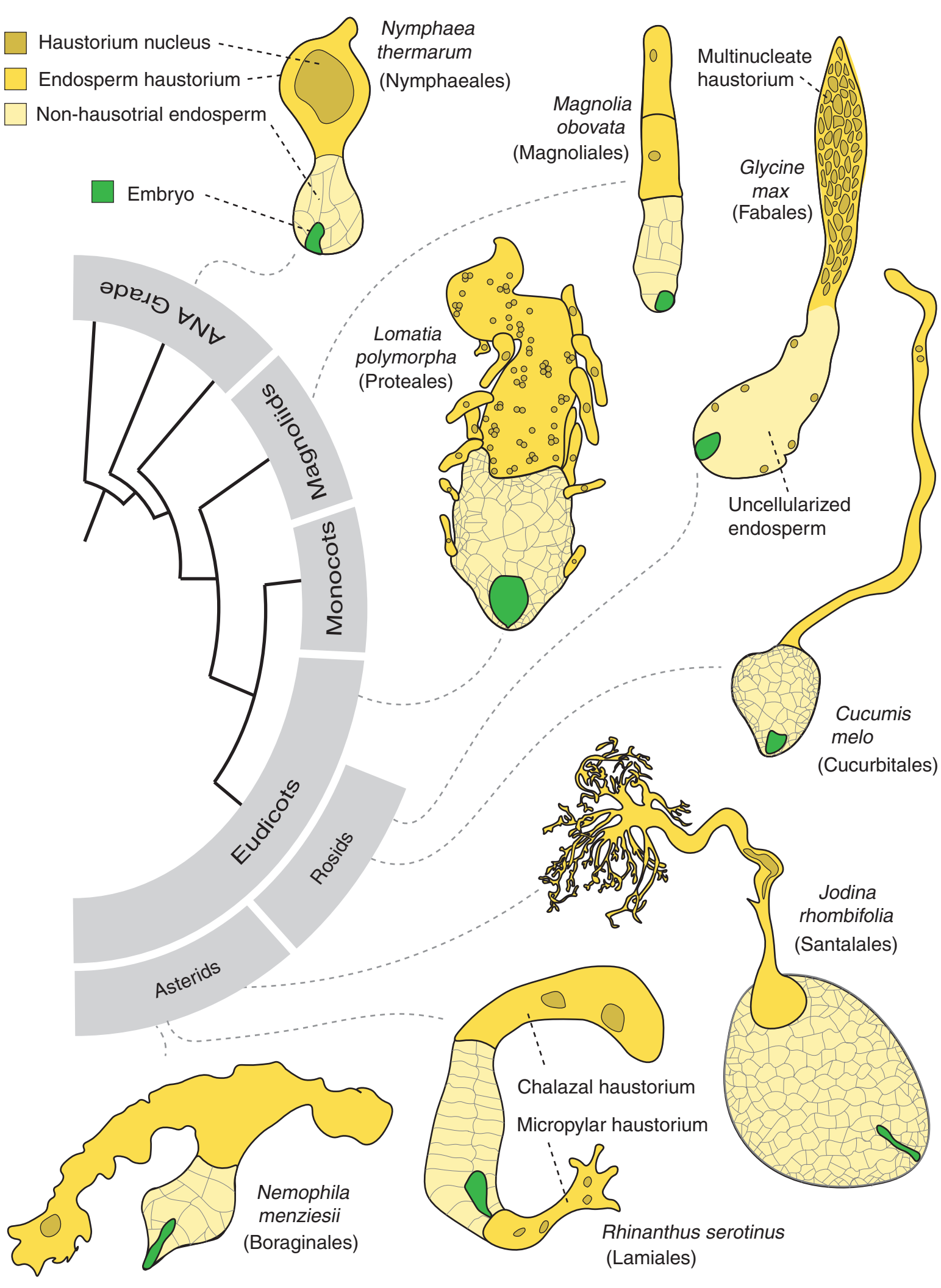
Figure legends

Figure 1: Seed and endosperm structure. A) Generalized diagrams of ovule and seed structure in angiosperms. B) Diagrams of ovule and developing seed structure in *Arabidopsis thaliana* (thale cress), *Zea mays* (maize), and *Hordeum vulgare* (barley). Color scheme is consistent between A and B, in order to facilitate comparisons between different ovule and seed types. Light blue = nucellus; light yellow =

central cell (before fertilization) or endosperm (after fertilization); dark yellow = transfer-specialized endosperm; green = egg cell (before fertilization) or embryo (after fertilization); pink = vascular tissue.

Figure 2: Selection of diverse endosperm haustoria in angiosperms. Offspring tissues are shown, with an emphasis on characters in different endosperm regions: cellularized (ex. *Magnolia obvata*) vs. uncellularized (ex. *Lomatia polymorpha*), unicellular (ex. *Nymphaea thermarum*) vs. multicellular (ex. *Magnolia obvata*), single- (ex. *Nemophila menziesii*) vs. multi-nucleate (ex. *Glycine max*), branched (ex. *Iodina rhombifolia*) vs. unbranched (ex. *Cucumis melo*), and micropylar and/or chalazal haustoria (ex. *Rhinanthus serotinus*). Green = embryo; dark yellow = nucleus; yellow = endosperm haustorium; light yellow = non-haustorial endosperm. Diagrams after: *Cucumis melo* [63], *Glycine max* [64], *Jodina thombifolia* [65], *Lomatia polymorpha* [65], *Magnolia obovata* [66], *Nemophila menziesii* [67], *Nymphaea thermarum* [55], *Rhinanthus serotinus* [68].


A
Before Fertilization
(Ovule)
After Fertilization
(Developing Seed)
Nucellus
Chalazal Pole
Central
Cell
Egg
Cell
Micropylar Pole
Female
Gametophyte
Vascular
Strand
Integuments
Funiculus
Maternal-Filial
Interface
Placental-Chalazal
Region
Transfer
Endosperm
Endosperm
Nucellus
Embryo
Seed Coat
B
Arabidopsis
thaliana
Nodules
Cyst
Chalazal
Endosperm
Chalazal
Proliferating
Tissue (CPT)
Zea mays
Basal Endosperm
Transfer Layer (BETL)
Placental-Chalazal
Region (P-C)
Hordeum
vulgare
Placental- Chalazal
Region (P-C)
Nucellar
Projection
Fluid Cavity
Endosperm
Transfer Cells
(ETCs)


Haustorium nucleus
Endosperm haustorium
Non-hausotrial endosperm
Embryo
Nymphaea thermarum
(Nymphaeales)
Magnolia obovata
(Magnoliales)
Multinucleate haustorium
Glycine max
(Fabales)
Lomatia polymorpha
(Proteales)
Uncellularized endosperm
ANA Grade
Magnoliids
Monocots
Eudicots
Rosids
Asterids
Cucumis melo
(Cucurbitales)
Jodina rhombifolia
(Santalales)
Chalazal haustorium
Micropylar haustorium
Nemophila menziesii
(Boraginales)
Rhinanthus serotinus
(Lamiales)