Evolution and patterning of the ovule in seed plants

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ABSTRACT

The ovule and its developmental successor, the seed, together represent a highly characteristic feature of seed plants that has strongly enhanced the reproductive and dispersal potential of this diverse group of taxa. Ovules encompass multiple tissues that perform various roles within a highly constrained space, requiring a complex cascade of genes that generate localized cell proliferation and programmed cell death during different developmental stages. Many heritable morphological differences among lineages reflect relative displacement of these tissues, but others, such as the second (outer) integuments of angiosperms and Gnetales, represent novel and apparently profound and independent innovations. Recent studies, mostly on model taxa, have considerably enhanced our understanding of gene expression in the ovule. However, understanding its evolutionary history requires a comparative and phylogenetic approach that is problematic when comparing extant angiosperms not only with phylogenetically distant extant gymnosperms but also with taxa known only from fossils. This paper reviews ovule characters across a phylogenetically broad range of seed plants in a dynamic developmental context. It discusses both well-established and recent theories of ovule and seed evolution and highlights potential gaps in comparative data that will usefully enhance our understanding of evolutionary transitions and developmental mechanisms.

Key words: chalaza, character evolution, integument homologies, nucellus, ovule patterning, programmed cell death, seed-plant evolution

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The ovule and its developmental successor, the seed, together represent arguably the most significant innovation of seed plants because they represent a developmentally dynamic structure that facilitates an efficient means of reproduction and dispersal in a variety of habitats. In contrast with animals, in which the term ovule is used as an occasional synonym for the ovum (the egg cell), the ovule of seed plants is a complex assembly of tissues that variously contribute to a range of functional roles throughout development. Ovules operate in pollination biology to attract pollen tubes for fertilization, a complex role that is typically undertaken by both the micropylar region of the nucellus and an integument (for Glossary see Table 1). Both ovules and seeds have a reproductive role, in nurturing the developing megagametophyte and embryo respectively. Ultimately, the seed often has a critical role in dispersal, a process sometimes associated with a period of dormancy.

The evolution of an organ complex with such a broad functional range required structural innovations such as the integument(s), micropyle and nucellus that characterize the seed-plant ovule. By contrast, in other vascular plant lineages, outer envelopes (integuments) are mostly lacking, and nutrients are accumulated and stored prior to gametophyte development. Most inferences on the early evolution of the ovule are drawn from Late Devonian and Carboniferous seed-fern fossils that are anatomically preserved, allowing clear identification of the various tissue regions (Rothwell, 1986; Nishida, 1994; Hilton & Bateman, 2006; Seyfullah et al., 2010). Based on such comparisons, the origin and early evolutionary history of the ovule are commonly viewed as a series of evolutionary transitions involving traits associated with heterospory, pollen capture (e.g. elaboration of the nucellar apex into a pollen chamber) and protection (e.g. fusion of telomes to form an integument) (Smith, 1964; Long, 1966, 1975, 1977; Bateman & DiMichele, 1994; Herr, 1995; Doyle, 1998; Taylor, Taylor & Krings, 2009).

Seed size is widely regarded as a key trait that is partly correlated with genome size and also with several aspects of plant ecology and evolution, including seed dormancy and seed dispersal, especially the co-evolution of angiosperms with biotic seed-dispersal agents such as dinosaurs and mammals (Linkies et al., 2010; Eriksson, 2016; Liu et al., 2017). Small seeds characterize most early-divergent angiosperm lineages, but seed size diversified significantly from the late Cretaceous [ca. 80 million years ago (Mya)] until the early Eocene, resulting in the extreme ranges that occur within some derived monocot and eudicot clades (Sims, 2012; Eriksson, 2016). Non-angiospermous seed-plant lineages also display considerable diversity in relative seed sizes. Among extant species, the largest seeds occur in cycads, whereas relatively small seeds characterize Gnetales and conifers (Sims, 2012; Rudall & Bateman, 2019). Zonation of the nucellus has undergone a range of evolutionary transformations that contribute to modifications in relative volume. Focusing on grasses, Wilkinson et al. (2019) suggested that nucellus volume and associated ovule size impact grain development and crop yield by influencing the timing of maternal resource allocation to the fertilized embryo sac.

A critical re-examination of the ovule is required to unravel structural and developmental aspects of traits such as seed size. Different seed-plant lineages contrast widely in the relative proportions of tissues and the timing of ovule tissue patterning. One outstanding question is how this diversity is genetically and/or epigenetically regulated. Previous reviews have described ovule diversity across angiosperms (e.g. Bouman, 1984; Endress, 2011a) but comparative data on gymnosperms are sparse, and homology evaluations between extant angiosperms and gymnosperms are highly problematic, especially given a contentious phylogenetic context for the various seed-plant lineages (Fig. 1). Furthermore, few studies have thus far attempted to integrate

Table 1. Glossary of terms relating to ovules

| Term                  | Definition                                                                 |
|-----------------------|-----------------------------------------------------------------------------|
| Anatropy             | Condition in which the ovule is recurved in the longitudinal plane          |
| Bitegmy              | Possession of two distinct integuments                                       |
| Chalaza              | Region that gives rise to the integument(s)                                 |
| Cupule                | Structure that bears one or more ovules (in extinct seed plants)            |
| Funicle (funiculus)  | Stalk connecting the ovule or seed with the ovary placenta                  |
| Hypodermal layer     | Cell layer immediately below the epidermis                                  |
| Integument           | Ring-like or hood-like structure that envelopes the nucellus                |
| Micropyle            | Opening in integument(s) close to apical region of nucellus                 |
| Nucellus (megasporangium) | Complex structure that produces and encloses the megagametophyte (embryo sac) |
| Orthotropy           | Condition in which the ovule is not recurved in the longitudinal plane       |
| Perisperm            | Storage tissue derived from the nucellus                                    |
| Pollen chamber       | Degenerated region of nucellar cap cells where pollen germinates            |
| Raphe                | Vascularized region linking funicule and chalaza in longitudinally curved (anatropous or campylotropous) ovules |
| Siphonogamy          | Delivery of non-motile male gametes via a pollen tube                       |
| Unitegmy             | Possession of only a single integment                                       |

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I. INTRODUCTION
morphological and developmental diversity with comparative gene expression data in an explicit phylogenetic context. Recent developmental–genetic studies, primarily using the model angiosperm species *Arabidopsis* (a small annual eudicot), have identified some of the active genes involved in ovule development and localized their regions of activity. This review aims to break out of the confines of *Arabidopsis* by addressing the evolution and development of ovular patterning in a broad range of living seed-plant taxa.

II. SEED PLANTS, EXTINCT AND EXTANT

Spermatophytes (seed plants) are represented by a range of diverse and ancient lineages, several of which are known only from fossils (Fig. 1). Resolving the relationships among these lineages remains highly problematic because it necessarily involves either morphological analyses that are species-rich but relatively data-poor (e.g. Doyle, 2006; Hilton & Bateman, 2006), molecular analyses that are data-rich but species-poor and lacking crucial taxa (e.g. Graham & Iles, 2009; Ran *et al*., 2018), or a combined and constrained approach that is potentially illuminating but at the expense of emphasizing a particular character suite (e.g. Doyle, 2008). All of these methods are suboptimal in different ways, making character optimization throughout the seed plants notoriously challenging (Bateman, Hilton & Rudall, 2006; Rudall & Bateman, 2007, 2019). Gene expression studies are mostly focused on model angiosperms, although the sparse recent comparative studies on gymnosperms permit speculative evolutionary–developmental inferences.

Several distinct forms of well-preserved Carboniferous fossilized ovules have been identified and assigned to a series of lineages, notably the hydraspermans (e.g. *Lagenostoma*), medullosans (e.g. *Trigonocarpus*) and callistophytaleans (*Callospermarion*) (Rothwell, 1986; Nishida, 1994; Serbert & Rothwell, 1995; Hilton & Bateman, 2006; Seyfullah *et al*., 2010). Among the living gymnosperms, the cycad and *Ginkgo* lineages both date from the late Palaeozoic Era and diversified during the Mesozoic but later succumbed to extensive extinctions (Fig. 1). Cycads subsequently experienced more recent diversification to establish ca. 330 living

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**Fig 1.** Relationships between some of the major extant and extinct lineages of seed plants. Tree diagram based on morphological analysis in Hilton & Bateman (2006). Black lines and triangles indicate extant lineages, grey lines and blue font represent extinct lineages known only from fossils. Diagrams of ovules coloured to illustrate different tissue regions as indicated in key. (A) Palaeozoic seed fern *Genomosperma latens* (extinct Palaeozoic seed fern, from Long, 1960). (B) *Bowenia spectabilis* (extant cycad, Kershaw, 1912). (C) *Pinus banksiana* (extant conifer, from Chamberlain, 1935). (D) *Caytonia thomasi* (extinct Mesozoic seed fern, from Harris, 1933). (E) Generalized bitegmic angiosperm shown in Fig. 2.
III. OVULE PATTERNING AND DEVELOPMENT

(1) Functional patterning of the ovule

The ovule consists of a megasporangium (nucellus) protected by one or more sterile outer envelopes (integuments), which are initiated as annular outgrowths and ultimately extend upwards around the nucellus to delimit an apical opening, the micropyle (Figs 2, 3; Table 1). These sterile ovule tissues display some overall similarities in zonation, patterning and development among the different groups of seed plants, allowing evaluation of their homologies.

The nucellus is a complex structure whose cells retain the potential for differentiation, including both sterile and fertile tissues (Ranganath, 2004). The micropylar (apical) region of the nucellus represents the primary interface with the outer surface and hence with the male gametophytes during pollination. In angiosperms, conifers and Gnetales, the sperm nuclei are relatively small and non-motile; they are transported to the megagametophyte by cytoplasmic streaming within a siphonogamous pollen tube (Van Went & Willemse, 1984; Johri, 1992; Rudall & Bateman, 2007; Van Hautegem et al., 2015). Angiosperm pollen typically germinates on the stigma, but in most extant conifers and Gnetales, the pollen grain germinates in an open pollination drop (in many conifers) or within a small pollen chamber (in Gnetales) and forms a short pollen tube that grows between the neck cells of the archegonium (e.g. in Ephedra; Land, 1907). Cycads and Ginkgo represent the only living gymnosperms in which the male gametophytes are large, flagellate and self-propelling (motile) and are released within the pollen chamber. Pollen tubes are formed in these taxa, but they are non-siphonogamous; they penetrate the nucellus and form a branching haustorium that derives nutrients directly from the nucellar tissue. Subsequently, a swollen non-invasive branch (close to the microspore) ruptures to release two motile sperm within the pollen chamber (Friedman, 1987; Johri, 1992; Rudall & Bateman, 2007).

In angiosperms, conifers and Gnetales, the primary role of the pollen tube is siphogamy and the haustorial role is at best secondary. However, branched pollen tubes can occur even in these taxa, especially conifers (Owens, Takaso &
Pollen tubes are highly dynamic structures that require sustenance, and in some angiosperms (e.g. spinach), branches of the pollen tube seek nutrients by invading the integumentary and nucellar tissues (Johri, 1992). To achieve delivery of the male gametes to the egg cell, siphonogamous pollen tubes typically enter the ovule via the micropyle, although entry via the chalaza (chalazogamy) occurs in some angiosperms in which ovules are immature at pollination (Endress, 2011a). The region where the integuments emerge from the nucellus is termed the chalaza, which constitutes the integument-forming meristematic region in the early developing ovule. The chalaza is essentially part of the nucellus, and precise tissue demarcation between the two regions of tissue is frequently lacking. Fagerlind (1971, p. 315) referred to an “illusory nucellus/chalaza border” at the junction between the top of the ring-like inner integument and the nucellus.

The ovule is linked to the ovary placenta by a vascularized stalk (funicle, syn. funiculus), although a funicle is short or absent in most gymnosperms, both living and extinct. In angiosperm ovules, vasculature often extends into the chalaza and integuments, but rarely into the nucellus. In many angiosperms, the ovule is longitudinally curved (anatropous or campylotropous) so that upon ovule maturity the micropyle is located close to the point of attachment of the funicle to the placenta (Bouman, 1984; Endress, 2011a). Orthotropous ovules, which lack such curvature, characterize some angiosperms and most gymnosperms.

The megagametophyte and its interface with the ovular tissues

Although fertile ovule tissues are not the primary topic of this review, relevant factors include the location of the megagametophyte relative to the sterile tissues and its shift during development. A megasporocyte (megaspore mother cell) is formed from archesporial tissue (or a single archesporial cell) within the nucellus. In many extant seed plants, the procreative region is subsequently shifted inwards by periclinal divisions in the outer layers. For example, in some gymnosperms, cell proliferation and enlargement in the apical region ultimately push the developing megagametophyte down towards the chalazal region (e.g. in Ginkgo: Douglas, Stevenson & Little, 2007). Typically, the megasporocyte undergoes megasporogenesis to form a tetrad of megaspores, of which the chalazal megaspore is functional and the remaining three degenerate (e.g. Willemse & Van Went, 1984). The functional megaspore undergoes megagametogenesis to produce a megagametophyte (the embryo sac). Most angiosperms possess a single megagametophyte that is most commonly (although arguably not ancestrally) eight-nucleate (Willemse & Van Went, 1984; Friedman & Williams, 2003; Lersten, 2004; Rudall, 2006). Within the megagametophyte, the egg cell is
located closest to the micropyle; its fertilization results in a zygote that subsequently forms the embryo in the developing seed. In most land plants, the egg cells are produced within archegonia that develop within the megagametophyte, but in angiosperms the megagametophyte is so reduced that an archegonium is either absent or imperceptible, resulting in contrasting theories about the possible derivation of the angiosperm megagametophyte from an archegoniate structure (Favre-Duchartre, 1984; Rudall, 2006; Sokoloff & Remizova, 2020).

Given the existence of both sterile and fertile tissues within highly constrained confines, the ovule is prone to development of an epithelial layer; a modified cell layer that provides an interface between two distinct organs or tissues, thus assuming a nutritive or transfer role. Epithelial cells become enlarged and are often endopolyploid and secretory. The epithelium readily provides a ‘safe’ interface between the tissues of two distinct individuals: the host sporophytic tissue and the developing gametophytic/sporophytic tissues of the embryo sac and embryo (Ranganath, 2003). One example of an epithelium is the abaxial epidermis of the embryo scutellum in grass seeds; this layer interfaces with the endosperm, secreting enzymes that help to mobilize food reserves across the two organs (Loreti et al., 2001). Ovule epithelia can occur either in the integument or the nucellus.

IV. GENES THAT INFLUENCE OVULE DEVELOPMENT

Developmental–genetic studies of Arabidopsis and other eudicots (e.g. Impatiens, Prunus, Nicotiana, Solanum) have identified several interacting genes that influence ovule and integument development. However, comparative gene expression studies in ovules of gymnosperms and early-divergent angiosperms remain relatively sparse, making broad-scale evolutionary–developmental inferences undesirably speculative.

Genes that regulate integument development include the YABBY gene INNER NO OUTER (INO) (Villanueva et al., 1999; McAbee, Kuzoff & Gasser, 2005; Lora et al., 2011; Lora, Hormaza & Herrero, 2015; Skinner et al., 2016) and the KANADI gene ABERRANT TESTA SHAPE (ATS or KAN) (McAbee et al., 2006; Gasser & Skinner, 2019), which are expressed in the outer and inner integument respectively in bitemgic angiosperms (summarized by Arnault et al., 2018). The gene ETITIN/ETT forms a protein complex with ATS (Kelley et al., 2012; Lora et al., 2013). Several other genes that encode transcription factors are also implicated in early ovule development and integument initiation in Arabidopsis, notably WUSCHEL (WUS), BELLI (BEL1) and AINTEGUMENTA (ANT) (Gross-Hardt, Lenhard & Laux, 2002; Galbiati et al., 2013; Yamada et al., 2019). WUSCHEL functions in shoot apical meristems to organize central zone activity. In the ovule, WUSCHEL is expressed in the developing nucellus, where it establishes endochalazal development and promotes integument growth in a manner comparable to the initiation of lateral organs in shoots (e.g. Gross-Hardt et al., 2002). Both ANT and BEL1 are mostly expressed in the chalazal region in Arabidopsis, although their precise roles remain enigmatic. Becker et al. (2002a) suggested that BEL1 controls the entire integument-forming meristematic region (i.e. the chalaza). The BEL1 (BEL1) gene was one of the first ovule regulatory genes to be identified. In Arabidopsis, BEL1 negatively regulates the MADS-box gene AGAMOUS (AG) to establish ovule rather than carpel identity (Modrusan et al., 1994; Cucinotta, Colombo & Roig-Villanova, 2014). In some mutations of BEL1, only a single integument forms and then develops abnormally, sometimes being ultimately transformed into a carpel-like structure.

Precise structural differentiation is influenced by antagonistic interactions between auxin, which promotes cellular proliferation, and cytokinin, which has an inhibitory effect (Chettoto & Evans, 2015). Thus, given the restricted available space in the ovule, relatively minor physiological shifts during evolution could have a disproportionate effect on its patterning and biology. Overall, the proximal–distal patterning of the nucellus is maintained by a distal auxin maximum (e.g. Lora, Yang & Tucker, 2019b). Concentration of the AUXIN RESPONSE FACTOR 3 (ETT/ARF3) and focuses auxin in the apical cells at this early stage, resulting in distal production of the megaspore mother cell and (at least in some taxa) proximal proliferation of the nucellus and chalaza (Su et al., 2017; Lora et al., 2019b). Subsequent development of both the megagametophyte and sporophytic tissues is promoted by expression of PIN1 orthologues, which help to determine auxin accumulation (Cecatto et al., 2013; Shirley et al., 2019).

Programmed cell death occurs in several regions of the nucellus during mid- to late development of the ovule. It can occur in the apical region to form a pollen chamber in some taxa (Table 2), or in the proximal nucellus or chalaza during late development in other taxa. In Arabidopsis and Oryza (rice), orthologous B-sister MADS-box genes have been shown to regulate eventual degeneration of the nucellus (Domínguez, Moreno & Cejudo, 2001; Van Hautegem et al., 2015; Magnani, 2018). B-sister genes have also been found in gymnosperms and are expressed in the nucellus and inner integument in Gnetum, although not associated with cellular degeneration (Becker et al., 2002b). Few studies have specifically addressed the developmental genetics of pollen-chamber formation in gymnosperms, although Li et al. (2019) found a set of localized and differentially expressed genes involving hormone signalling resulting in nucellar cell death in Gnetum.

V. OVULES OF PALAEOZOIC SPERMATOPHYTES

(1) Extinct Palaeozoic lineages

Ovules of early-divergent Palaeozoic seed-plant lineages (hydraspermans and medullosans) typically possessed ovules.
with a single envelope (Oliver, 1903; Oliver & Scott, 1904; Serbert & Rothwell, 1995; Hilton & Bateman, 2006). Rare exceptions included Camptosperma, with two envelopes (Long, 1966). Palaeozoic seed-plant ovules mostly lacked curvature, although a few hydrasperman ovules were more or less curved longitudinally (e.g. Anasperma and Ecroustosperma: Long, 1966; Bateman & Rothwell, 1990). The single integument of most early seed plants is widely believed to have originated by aggregation of the lobes around a megaasporangium and their subsequent union into a more closed ring-like structure (Long, 1966; Nishida, 1994; Herr, 1995; Gasser & Skinner, 2019). The proto-integument was apically cup-shaped and often lobed, each lobe possessing a single vascular bundle and putatively representing a sterile telome (e.g. Long, 1966; Rothwell, 1986). Hydrasperman ovules varied in the degree of lobing of the integument, ranging from deeply lobed (with 2–10 lobes) to lobing absent or reduced to cusps around the microple (Long, 1966). In many hydrasperman seed ferns, the integument was integrally united with the nucellus for most of its length, diverging from it only in the mid-region, near the level of the pollen chamber. In medulosan seed ferns, the integument was free from the nucellus and both the integument and nucellus were independently vascularized (Serbert & Rothwell, 1995), although such vascularization of the outer part of the nucellus led to an alternative hypothesis that the medulosan integument represents a fused cupule (e.g. Meyen, 1984).

Hydrasperman ovules possessed structurally complex pollen chambers with a hemispherical lower chamber with a membranous floor; the chamber narrows to a cylindrical projection at the apex, termed a salpinx or lagenostome (Rothwell, 1986; Hilton, 1998; Hilton & Bateman, 2006). Although the development of the hydrasperman pollen chamber is poorly known, it followed a pathway of programmed cell death in precisely pre-defined regions. The lower chamber was formed by breakdown of a region of nucellar cap cells and the salpinx by lysigenous dissolution of the cells at the nucellus apex, resulting in a pollen drop. After fertilization, a central column of shrivelled hard tissue was pushed upward into the salpinx to close the pollen chamber, simultaneously tearing the membranous floor (Rothwell & Scheckler, 1988; Serbert & Rothwell, 1995). By contrast, the pollen chamber in medulosan ovules was relatively simple (Long, 1966, 1984; Serbert & Rothwell, 1995; Hilton & Bateman, 2006).

There is a correlation between the complexity of the apical region and the mode of delivery of the male gametes into the embryo sac to ensure fertilization (see Section III). Although little direct evidence exists, the more complex pollen chambers of extinct Palaeozoic seed ferns and their similarities with extant cycads have led to the plausible inference that they produced motile gametes (Oliver & Salisbury, 1911; Rothwell, 1986; Serbert & Rothwell, 1995; Hilton & Bateman, 2006), as observed in the extinct Late Permian pteridosperm genus Glossosperus (Nishida, Pigg & Rigby, 2003).

(2) Extant cycads and Ginkgo

As in many Palaeozoic hydraspermans, ovules of some extant taxa, including both cycads (Fig. 1B) and Ginkgo (Fig. 4), lack longitudinal curvature and exhibit profound congenital fusion between the nucellus and the lower part of the single, thick integument, which only diverges close to the distal apex of the ovule (e.g. Lang, 1900; Chamberlain, 1906; Smith, 1910; Kershaw, 1912; Zhang, 2013). In both cycads and Ginkgo, the early nucellar apex rapidly undergoes periclinal divisions to form a nucellar cap (e.g. Takaso, 1980; Douglas, Stevenson & Little, 2007).

To date, early ovule development has been subject to more detailed study in Ginkgo than in cycads. In Ginkgo, the single integument is initiated as a thick ring meristem at the same time as the nucellus, before archesporial tissue is formed; the integument grows to overtop and then envelop
the nucellus apex (Takaso, 1980; Douglas et al., 2007). Lovisetto et al. (2013) reported that in Ginkgo, the B-sister MADS-box gene GORDITA is implicated in early integument development and maintains a strong level of expression throughout the ovule at later developmental stages and during fruit formation.

Both cycads and Ginkgo possess a relatively simple, narrow pollen chamber formed by precisely programmed cell death between the micropyle and the embryo sac (Singh, 1978; Choi & Friedman, 1991). In cycads, the pollen chamber is initiated as a small cavity (the upper pollen chamber) that results from breakdown and apparent resorption of a central band of cells in the nucellar cap (e.g. Lang, 1900; Chamberlain, 1906; Smith, 1910; Kershaw, 1912; Zhang, 2013). Subsequently, further cellular breakdown forms a larger lower pollen chamber, which accommodates the pollen grains. Germinating pollen tubes grow into surrounding tissue, where they obtain nutrients. The region above the upper pollen chamber becomes a nucellar beak of dried hard tissue that forms a plug between the micropyle and lower pollen chamber. Further cell degeneration between the lower pollen chamber and the embryo sac, probably associated with digestion by the growing haustorial pollen tube, allows passage of motile sperm to the embryo sac (Kershaw, 1912; Choi & Friedman, 1991). In Ginkgo, the pollen chamber is surrounded by a distinct region of tissue that is sometimes termed an exothecium due to its transverse cell-wall thickenings that resemble those in the anther (Douglas et al., 2007).

Distal layers of the nucellus contribute to formation of a massive, highly structured chalazal region (pachychalaza) that contains the embryo sac; a well-defined band of flattened cells demarcates the chalaza from the parent tissue (Smith, 1910; Singh, 1978; Douglas et al., 2007). A nucellar epithelium (often termed a tapetum, or sometimes spongy tissue) occurs in the cell layers immediately surrounding the developing embryo sac; the innermost epithelial layer is often multinucleate or endopolyploid (Singh, 1978). Douglas et al. (2007) described the development of a multilayered tapetum in Ginkgo, in which the nucellus adjacent to the embryo sac consists of enlarged densely staining multinucleate cells. This tissue is present even at early stages (Fig. 4), but usually degenerates by the time the embryo sac becomes cellularized.

At maturity, the cycad integument has three distinct tissue layers extending from the apex to the chalaza: a sclerenchymatous layer sandwiched between parenchymatous outer and inner layers. These layers ultimately develop to form the distinctive wall layers of the ripe seed, including a hard protective layer and an outer fleshy layer that attracts seed-dispersal agents, mostly birds and mammals. In Ginkgo, a B-sister gene is involved in growth of the fleshy integument (Lovisetto et al., 2013).

VI. OVULES OF MESOZOIC SPERMATOPHYTES (EXCLUDING ANGIOSPERMS)

(1) Extant conifers
A characteristic feature of living conifers is the profound integration of the ovule with the ovuliferous scale complex (Fig. 1C). In Pinus, the orthologue of the Arabidopsis ANT gene (PtANTL1) is expressed throughout the actively developing regions of the young ovule rather than being largely restricted to the chalazal region as in Arabidopsis (Shigyo & Ito, 2004) – a dispersed pattern that could well have resulted from the extreme congenital fusion in conifers (Yamada et al., 2008).

An enclosed pollen chamber is absent from most conifers, although a rudimentary pollen chamber reportedly occurs in a few taxa (Arthrotaxis, Cephalotaxus and Taxus: Singh, 1978). Some conifers (e.g. Araucaria) possess a long nucellar beak, resulting from cell proliferation in the nucellar cap, so that its apex protrudes through the micropyle (Favre-Duchartre, 1984).
In most conifers, the ovule possesses a single thick integument that is restricted to the apical region from its inception, so that the embryo sac is embedded in a massive chalaza (e.g. *Torreya*: Kemp, 1959). However, some conifers arguably possess more than one integument. There is a similarity between early ovule development in Podocarpaceae and basal angiosperms; for example, in most podocarps, a structure resembling a second integument (termed an epimatium) encloses the ovule and develops asymmetrically, so that the ovule is inverted and appears anatropous (Tomlinson, 1992; Tomlinson & Takaso, 2002; Tomlinson, 2012). In Taxaceae, the ovule also has a late-developing fleshy outer envelope (sometimes termed an aril) that is either free from the integument (in most genera) or congenitally fused with it (Coulter & Chamberlain, 1917; Takaso, 1981; Stützel & Röwekamp, 1999; Tomlinson & Takaso, 2002; Dörken, Nimsch & Rudall, 2018). However, Englund *et al*. (2011) considered that the fleshy outer envelope in Taxaceae is not homologous with that of Podocarpaceae because they show contrasting expression patterns of their *AGAMOUS* orthologues.

### (2) Extinct Mesozoic lineages

Ovules of the extinct Mesozoic seed-plant *Caytonia* were unitegmic and orthotropous (Thomas, 1925; Harris, 1951, 1958). The nucellus possessed a thick cuticle, as in some angiosperms, although other aspects of *Caytonia* are gymnospermous. The nucellus apex had a short beak and lacked a pollen chamber. The ovulate axes in *Caytonia* each bore two rows of cupules. Each cupule was curved and bore several ovules on the adaxial (inner) surface (Fig. 1D). The *Caytonia* cupule has been analogized either with a carpel or with a single ovule of bitetmic angiosperms (summarized by Doyle, 2006). The former theory would require independent origin of a second (outer) integument. Many authors favour the latter theory, suggesting that the curved cupule could have given rise to the curved outer integument of angiosperms following evolutionary reduction to a single enclosed ovule (Doyle, 2006, 2008; Endress, 2011; Doyle, 2013).

In the extinct order Bennettitales, which was relatively species rich compared with Caytoniales, the ovules were orthotropous and densely clustered on a condensed axis that bore the erect ovules/seeds interspersed among sterile bracts (Crane, 1986; Nishida, 1994; Rothwell *et al*., 2009). The nucellus apex had a beak at the apex but probably lacked a pollen chamber, although contrasting interpretations exist (reviewed by Rothwell *et al*., 2009). The ovules had an unvascularized integument that was free from the nucellus above the chalaza; depending on interpretation, it enclosed either a vascularized nucellus (Rothwell *et al*., 2009) or a second integument that was fused to the nucellus for most of its length (Friis *et al*., 2007; Friis, Pedersen & Crane, 2009).

### (3) Gnetales

Ovules of Gnetales differ in the number of encircling structures that are variously termed envelopes or integuments (here termed integuments). *Ephedra* (Fig. 5) and *Welwitschia* have two integuments, whereas *Gnetum* ovules have three (Martens, 1959; Fagerlind 1971; Singh, 1978; Takaso, 1985; Takaso & Bouman, 1986). In both *Ephedra* and *Gnetum*, the inner integment is initially inserted on the chalaza below the developing embryo sac, but their relative positions are shifted following differential endochalazal cell proliferation of the nucellus, resulting in a more clearly ‘inferior’ embryo sac in the mature ovule. A pollen chamber is present within a thick nucellar cap, especially in *Ephedra* (Fagerlind, 1971; Friedman, 1991), although a small pollen chamber has also been reported in *Welwitschia* and *Gnetum* (Singh, 1978; Carafa *et al*., 1992; Friedman & Carmichael, 1998; Friedman, 2015).

In all three genera of Gnetales, the thin, unvascularized inner integument is the last to be initiated; it grows as a more-or-less radially symmetric ring-like annulus. The middle integument of *Gnetum* is initiated as a ring-like annulus before the inner integument is initiated (Takaso & Bouman, 1986). The thick, vascularized outer integument of *Ephedra* and *Gnetum* is sometimes interpreted as having
evolved from two fused lateral bracts (e.g. Rydin, Khodabaneh & Endress, 2010) because it is initiated from two separate primordia on the lateral sides of the base of the ovule primordium, although it subsequently develops as an entirely closed ring that resembles the inner integument (Fagerlind, 1971). During tissue differentiation in the maturing seed of Gnetum, the outer integument becomes fleshy, the middle sclerenchymatous, and the inner one compressed, thus resembling the fused tissue regions of the cycad seed. In all three genera, the inner integument ultimately extends apically to protrude as a long micropylar tube that receives pollen grains (Fig. 5B).

Some preliminary gene expression data exist for Gnetales (e.g. Yamada et al., 2008; Finet et al., 2016). For example, Yamada et al. (2008) found that the Gnetum gene GpANTL1, an orthologue of ANT, is expressed in developing Gnetum ovules as four distinct rings around the three integument primordia and around the nucellar tip, thus resembling ANT expression in Arabidopsis, which is implicated in early ovule development and integument initiation.

VII. OVULES OF ANGIOSPERMS

(1) Nucleus and chalaza

(a) Nucellar apex

The micropylar region of the nucellus is typically relatively undeveloped in angiosperms (Figs 6, 7), which lack a pollen chamber and rarely possess a nucellar beak – both widely considered to be gymnosperm features (e.g. Rothwell et al., 2009). An exception is the rosid eudicot family Euphorbiaceae, in which cellular proliferation results in a remarkable nucellar beak that extends to the placenta or even into the style in some species (Tilton, 1980a; Kapil & Bhatnagar, 1994).

One aspect of this region that features in systematic studies is the development of the nucellus apex (Bowman, 1984; Endress, 2011a). In crassinucellate ovules, the hypodermal archesporial cell divides periclinally to form an inner megasporocyte and an outer parietal cell that itself sometimes undergoes proliferation. Conversely, in tenuinucellate ovules, a nucellar cap is absent and the archesporial cell functions directly as the megasporocyte. The crassinucellate condition is plesiomorphic in angiosperms, and most closely comparable with the condition in gymnosperms (Endress, 2011a,b). For example, in Amborella – the putative sister species to all other extant angiosperms – the nucellus is crassinucellate (Williams, 2009).

Such apparently minor differences in the ontogeny of the micropylar nucellus zone can appear trivial when applied solely within the angiosperms. However, early focus on this region highlighted the strong contrast with the condition in gymnosperms, in which the nucellar apex is relatively thick and the developing embryo sac is shifted inwards by multiple periclinal divisions.

(b) Proximal nucellus and chalaza

The proximal region of the nucellus is highly diverse in angiosperms (Fig. 6), extending from the embryo sac to the chalaza. In some species, this region is poorly developed or even ephemeral; it can undergo programmed cell death, as in the eudicot Arabidopsis (Van Hautegem et al., 2015). In both Arabidopsis and the monocot Oryza, orthologous B-sister MADS-box genes have been shown to regulate eventual degeneration of the nucellus (Dominguez et al., 2001; Van Hautegem et al., 2015; Magnani, 2018).

The chalaza is the region where the integuments are initiated in the early developing ovule. In Arabidopsis, expression of the AUXIN RESPONSE FACTOR 3 (ARF3) gene is restricted to the chalaza at early developmental stages (Su et al., 2017; Lora et al., 2019a), indicating a localized role for this region. The homeodomain transcription factor WUSCHEL, primarily known for its expression in the flowering shoot apex (Lenhard et al., 2001), is expressed in the developing nucellus in Arabidopsis ovules (Gross-Hardt et al., 2002; Sieber et al., 2004; Colombo, Battaglia & Kater, 2008; Yamada et al., 2016). WUSCHEL is also expressed in the nucellus in Podostemaceae (Katayama, Koi & Kato, 2010). In Arabidopsis, WUSCHEL appears to establish the chalaza and repress INO expression in the developing inner integument.

In many asterid and rosid eudicots, early degeneration of the nucellus around the embryo sac is followed by formation of an integumentary epithelium (often termed an integumentary tapetum) in the innermost layer of the single integument or inner integument (Kapil & Tiwari, 1978; Bowman, 1984). For example, in Lamiaceae, the cells in the region of the integument epidermis that encloses the embryo sac (and subsequently encloses the chalazal endosperm chamber in the developing seed) form a distinct epithelium that functions in nutrient transfer (Rudall & Clark, 1992).

Conversely, in some monocots and eudicots, the region between the embryo sac and chalaza becomes differentiated during late development into diverse structures that can persist in the developing seed (Tilton, 1980a; Rudall, 1997). A hypostase is a group of cells with thickened cell walls that forms a relatively impermeable barrier between the mature embryo sac and chalaza. A massive hypostase develops in Acorus, the sister taxon to all other monocots (Rudall & Furness, 1997). By contrast, some species of both eudicots (e.g. Drosera) and monocots (e.g. Lomandra) possess a central strand of narrow, axially oriented cells that appear to connect the embryo sac with the vascular supply (Fig. 6J, K). A well-developed proximal nucellus region can accumulate starch and form a nutritive storage tissue, termed a perisperm, which is a common feature of many monocots (Rudall, 1997). In a few angiosperms, some proximal nucellar cells become enlarged and haustorial; they can invade nutritive tissues, including the perisperm and endosperm in the developing seed. In the eudicot family Podostemaceae (Malphighiales), these cells break down to form a nucellar plasmidium (Arekal & Nagendra, 1977; Murgaia-Sánchez et al., 2002).
Fig 6. Angiosperm phylogeny showing distribution of integument number. Tree topology based on Soltis et al. (2011) and APG IV (2016). Optimization performed using Mesquite software (Maddison & Maddison, 2019). White lines/boxes indicate unitegmy and black lines/boxes indicate unitegmy. Ovules of Chloranthaceae and most magnoliids are bitegmic (Endress & Igersheim, 2000; Igersheim et al., 2001; Yamada et al., 2001a,b, 2003a,b,c; Doyle & Endress, 2018). Monocots are almost exclusively bitegmic (Rudall, 1997), although a combination of unitegmy and bitegmy occurs in a few monocot taxa (Amaryllidaceae, Orchidaceae, Poaceae and some Alismatales). For eudicots, data sources include Schnarf (1931), Igersheim & Endress (1997, 1998), Endress & Igersheim (1999). Early-divergent eudicot lineages (Buxales, Gunnerales, Proteales, Trochodendrales and most Ranunculales) are mostly bitegmic, except for some mostly unitegmic Ranunculales: Circaeasteraceae, Sabiaceae, Menispermaceae and Ranunculaceae. In the rosid clade, the earliest-diverging lineages Saxifragales and Vitales are bitegmic. Most fabids are bitegmic, but unitegmy occurs in most Fagales, some Rosales and some Rafflesiaaceae (Malpighiales). In Fagales, unitegmy characterizes Betulaceae, Juglandaceae, Myricaceae and Nothofagaceae, whereas Casuarinaceae and Fagaceae are bitegmic. Within Rosales, both unitegmic and bitegmic species occur in some families (e.g. Rosaceae: Lora et al., 2015). Sapindales are bitegmic except for some unitegmic Anacardiaceae; Myrtales are bitegmic except for some unitegmic Myrtaceae; Brassicales (including Arabidopsis) are bitegmic except for Limnanthaceae. Among early-diverging asterid lineages, Berberidopsidales and Caryophyllales are consistently bitegmic but Santalales and Cornales are unitegmic. The order Ericales is diverse but mostly uniform within families, except for Sarraceniaceae and Styraceae, in which some genera are unitegmic (Schönenberger et al., 2005). Within the early-divergent asterid order Ericales, phylogenetic optimization indicates at least three transitions from bitegmy to unitegmy (Schönenberger et al., 2005). Ovule images were made from the author’s collection of microscope slides at the Royal Botanic Gardens, Kew. All images are oriented with the micropylar pole uppermost. Ovules are

(Figure legend continues on next page.)
(a) Bitegmy and integument homologies

Angiosperms typically possess either one or two (rarely three) integuments that are inserted basally, below the level of the embryo sac (Fig. 2) (Bouman, 1984; Endress, 2011; Sauquet et al., 2017). All extant early-divergent (ANA-grade) angiosperms are bitegmic (Figs 3, 6A), leading to the highly plausible inference that bitegmy is the plesiomorphic (ancestral) condition in angiosperms (e.g. Doyle, 2013; Sauquet et al., 2017). Similarly, ovules of Chloranthaceae and most magnoliids are bitegmic (Fig. 6B, C). The rare unitegmic exceptions among these groups are confined to the magnoliid clade, in occasional species of Hydnoraceae, some Piperaceae and two genera of Monimiaceae (Johnson, 1914; Dastur, 1921; Heilborn, 1931; Kimoto & Tobe, 2003). Siparuna (Monimiaceae) is remarkable in that the ovules possess a single, rather thick integument and a massive nucellus containing multiple (4–6) archesporial cells, an organization reminiscent of gymnosperm ovules (Heilborn, 1931). The taxonomically isolated genus Ceratoiphyllum is unitegmic (Schnarf, 1931; Igersheim & Endress, 1998). Monocots are almost exclusively bitegmic (Fig. 6G–L).

In most bitegmic angiosperms, the outer integument is thicker and more persistent than the inner integument. Ovule curvature leading to anatropy develops by differential growth of the outer region of the outer integument or sometimes of the chalaza. The relative timings of integument initiation vary among taxa. For example, in Arabidopsis the inner integument initiates first (e.g. Sieber et al., 2004), whereas in Amborella the outer integument initiates slightly before the inner one (Yamada et al., 2001b) and in some other early-divergent angiosperms (e.g. Ilicium and many waterlilies), the two integuments are initiated more or less simultaneously (Endress & Igersheim, 1997; Yamada et al., 2001a, 2003b,c; Rudall et al., 2008).

(Figure legend continued from previous page.)
mature except early stages in B, D, E, F, G. (A) Amborella trichopoda (ANA-grade). (B) Chloranthus (Chloranthaceae). (C) Anemopsis californica (magnoliid, Saururaceae). (D) Delphinium mirabilis (early-divergent eudicot, Ranunculales). (E) Lupinus arboreus (resid eudicot). (F) Botanica officinalis (asterid eudicot). (G) Acorus gramineus (monocot, Dioscoreales). (H) Tofieldia calyciflora (monocot, Alismatales). (I) Tricyrtis latifolia (monocot, Liliales). (J) Lomandra longifolia (monocot, Asparagales). (K) Tacca integrifolia (monocot, Dioscoreales). (L) Flagellaria indica (monocot, Poales). Scale bars = 20 μm.
Despite some conflicting data, many studies suggest that the two integuments of bitegmic angiosperms have distinct and somewhat distant evolutionary origins, the inner integument being homologous with the single integument that characterizes most gymnosperms. This hypothesis is based on at least two factors: comparative morphology and development, especially comparisons between angiosperms and both extant Gnetales and extinct gymnosperms. Comparative data exist for the outer integument of bitegmic angiosperms, although comparative data for the outer integument of bitegmic Gnetales are sparse. The YABBY gene INO is specifically expressed only in the outer integument in the bitegmic eudicots Arabidopsis and Impatiens (Villanueva et al., 1999; McAbee et al., 2005) and the bitegmic magnoliid Annona (Lora et al., 2011). Severe loss of INO expression in Arabidopsis can result in complete suppression of the outer integument but does not affect the inner integument (Villanueva et al., 1999; Skinner et al., 2016; Gasser & Skinner, 2019). Furthermore, INO is narrowly expressed in the outer epidermis of the outer integument, suggesting that it specifies abaxial identity, promoting differential growth and hence curvature leading to anatropy. In unitegmic eudicots, INO is expressed in the single integument (McAbee et al., 2005; Lora et al., 2015; Skinner et al., 2016).

In the waterlily Nymphaea, which belongs to the early-divergent angiosperm lineage Nymphaeales, INO orthologues are expressed not only in the outer epidermis of the outer integument but also in the inner integument (Yamada et al., 2003c), suggesting that despite possessing an integument-specific role, INO expression patterns are only partially conserved throughout angiosperms. However, expression of the INO orthologue in the early-divergent angiosperm Amborella closely resembles that of Arabidopsis (Arnault et al., 2016).

In Gnetales, Finet et al. (2016) reported expression of the YABBY gene EdIYABB in the inner ovule integument of Ephedra. This outcome could support the hypothesis of some earlier researchers (e.g. Fagerlind, 1971) that the outer integument of Ephedra could be an independently derived structure formed by fusion of two lateral bracts. More comparative data on INO expression in both angiosperms and gymnosperms would help to test these hypotheses.

(b) Secondary unitegmy

The phylogenetic distribution of unitegmy in angiosperms (Fig. 6) indicates that it represents one or more derived conditions. Possible means of achieving secondary unitegmy include (i) complete suppression of an integument, or (ii) congenital synorganization (‘fusion’) of the inner and outer integuments (Bouman & Calis, 1977; Endress & Igersheim, 2000; Gasser & Skinner, 2019).

Many eudicot families are unitegmic, though bitegmy is also widespread in this large clade. Ceratophyllum, which is placed as sister to eudicots in many (although not all) phylogenetic analyses, is exceptional in possessing a single

**Fig 9.** Ovules of mycoheterotrophs at anthesis, still at megaspore mother cell stage. Integuments poorly developed in A, absent in B. (A) Thesia avene (monocot, Thesiaceae, Dioscoreales). (B) Dactylorhiza incarnata (monocot, Orchidaceae, Asparagales). Scale bars = 20 μm.
integument (Schnarf, 1931; Igersheim & Endress, 1998). Despite this exception, the prevalence of bitegmy among early-divergent eudicot lineages and the ‘core eudicot’ order Dilleniales strongly supports the widely held view that there have been multiple origins of unigeny in eudicots (e.g. Philpson, 1974; Sauquet et al., 2017; Gasser & Skinner, 2019). Bitegmy is plesiomorphic in the two ‘higher’ eudicot clades, asterids and rosids, implying at least five possible transitions to unigeny in rosids (and possible subsequent reversals to bitegmy) and one or more transitions to unigeny in asterids (Fig. 6). By contrast, unigeny predominates among asterids, including all species of the diverse campanulid and lamiid clades (which include model organisms such as Antirrhinum and Solanum).

Evolutionary transition to secondary unigeny could have occurred either by suppression of one integument or by congenital union of both integuments. Suppression of the outer integument is likely to have occurred in many unigenic parastic or mycoheterotrophic lineages, in which the ovules are neutenous; development appears to be halted or truncated around the megasporocyte stage, before an outer integument has been initiated (Fig. 9). These taxa are distributed throughout several major angiosperm clades, not only in eudicots but also in magnoliids and monocots. For example, in the parasitic magnoliid family Hydnoraceae, which has a single integument (unusually among magnoliids), the megasporocyte does not undergo megasporogenesis but operates directly as a functional megaspor (Dastur, 1921). In the monocot families Orchidaceae and Burmanniaceae, which are either partially or fully mycoheterotrophic, the ovules remain poorly developed until after pollination, and in some orchids the outer integument never develops. In the eudicot order Santalales, which includes mostly parasitic plants such as mistletoes and sandalwoods, some species are unigenic or even lack integuments entirely (e.g. Rao, 1942; Brown, Nickrent & Gasser, 2010; Endress, 2010; Gasser & Skinner, 2019).

The second possible evolutionary route from bitegmy to unigeny, through integumentary union (i.e. congenital fusion or synorganization), is widely postulated to have occurred in eudicots, notably in the large asterid eudicot lineage, in which most species have a single thick integument. Congenital union is common in reproductive structures throughout seed plants. For example, in some conifers the ovule is profoundly integrated with the ovuliferous scale complex. Similar boundary losses are implicated in the fusion of floral organs in angiosperms, such as syncarpy, stamen adnation to the petals, and sympatly (often resulting in a floral tube [Erbar, 1991; Endress, 2001]). Indeed, unigeny in eudicots is closely correlated with sympatly (Dahlgren, 1980), and there is potentially a strong correlation between unigeny and sympatly within the asterid eudicot clade.

In terms of morphology, the single integument of unigenic eudicots is occasionally bifid at the tip, suggesting incomplete union in this region. For example, a bifid integument tip occurs in some species of the early-divergent eudicot family Ranunculaceae, leading Bouman & Calis (1977) to infer integumentary fusion in this group. The bifid tip represents probably the sole morphological evidence for integumentary fusion in unigenic angiosperms. However, gene expression could provide a further clue: two genes (INO and ATS/KN1) that are expressed in the outer and inner integuments respectively in bifid species, are expressed in the single integument of some unigenic eudicots (McAbbe et al., 2006; Gasser & Skinner 2019), suggesting congenital union of the integuments in these taxa. Several authors have reported INO gene expression in the single integument of unigenic asterid eudicot species, including Impatiens (Ericales: McAbbe et al., 2005), Solanum (Solanales; Skinner et al., 2016) and Prunus (Rosales: Lora et al., 2015). In Prunus, Lora et al. (2015) demonstrated INO expression in both unigenic and bitergmic species, ATS gene expression in the integument tip of unigenic species, and absence of ETT gene expression in the unigenic species examined.

Thus, congenital union of two ancestral integuments appears to be a plausible pathway to unigeny, at least in some eudicots. Admittedly, neither a bifid tip nor INO gene expression conclusively demonstrates this hypothesis, and alternative explanations exist, such as integument suppression and ectopic expression of INO. Congenital union between the inner and outer integuments in eudicots would imply the loss of a clear boundary between them during development, resulting in a novel phenotype. Several genes have been implicated in floral organ boundary formation, including the LATERAL ORGAN BOUNDARIES (LOB) gene and genes belonging to the NO APICAL MERISTEM/CUP-SHAPED COTYLEDON (NAM/CUC) family of transcription factors (Zhong, Powell & Preston, 2016). In the Arabidopsis ovule, CUC genes are expressed at the base of ovule primordia and the boundary between the nucellus and chalaza (Ishida et al., 2000; Cucinotta et al., 2014; Gonçalves et al., 2015). However, mutant plants reflecting mutations of these genes mostly possess two integuments, albeit sometimes retarded, indicating that they are not involved in integument union but operate at the nucellus/chalaza boundary following integument differentiation. More comparative developmental–genetic studies are needed to examine the potential expression patterns of these genes, especially in asterid species with unigenic ovules such as Solanum, in which CUC gene expression in ovules remains unknown.

VIII. CONCLUSIONS

(1) Ovules are elaborate structures encompassing multiple tissues that perform various roles within a highly constrained space, requiring a complex cascade of gene activity during development. In addition to generating the megaspor and embryo sac, the nucellus and chalaza undergo cell proliferation and programmed cell death at different times during development to facilitate sperm transfer and protect the ovule and resulting seed.
(2) The evolutionary history of the ovule is inevitably obscured by difficulties in making comparisons between living species and those that are known only from fossils, which are themselves often poorly preserved. Despite some overall similarities in ovule structure, which are partly dictated by hormonal clines (distal auxin and proximal cytokinin distribution) that polarize development of ovule primordia, interpretation of homologies of the various organ and tissue regions remains frustratingly problematic. Most morphological differences in ovule structure among lineages reflect heritable changes in differential growth among tissues, often caused by heterochronic shifts in gene expression. For example, relative shifts in cellular proliferation of the chalazal region can result in displacement of the embryo sac and ultimately influence the formation of tissues that nourish the developing seed. By contrast, the second (outer) integument is a novel and apparently profound innovation that has evolved more than once during evolution, at least in angiosperms, Gnetales and some conifers.

(3) The apparent structural similarity between the gymnosperm nucellus apex and a shoot apex has led some researchers to interpret the ovule itself as a transformed shoot apex and the integuments as lateral structures (Singh, 1978). Indeed, whether the integuments and nucellus should be interpreted as organs or tissues remains a problematic question that depends on their evolutionary history. The interesting analogy between an ovule and a shoot apex is reinforced by the discovery that the homeodomain transcription factor WUSCHEL, primarily known for its expression in the flowering shoot apex, is also expressed in the developing nucellus in Arabidopsis ovules (Mathews & Kramer, 2012; Arnault et al., 2018). Furthermore, YABBY transcription factors, which include the integument-specific gene IVO, also influence the growth of lateral organs such as leaves (Yamada et al., 2011; Nakata et al., 2012; Arnault et al., 2018). An analogy with a flower apex was also indirectly evidenced by the observation that the MADS-box gene AGAMOUS, which has a role in floral organ identity, contributes to the establishment of ovule identity (Cucinotta et al., 2014). WUSCHEL expression led Mathews & Kramer (2012) to propose a model in which ovule structure is controlled by a dynamic system of gene expression resulting from co-option of portions of the genetic module of the shoot apex into the developing megasporangium, specifically rejecting traditional notions of homology. This model evokes the earlier ‘babushka doll’ and ‘hierarchical shifts’ models of inter-nested developmental programs among complex branching structures in seed plants, including inflorescences, flowers and ovules (Albert, Gustafsson & DiLaurenzio, 1997; Rudall et al., 2009). It rejects the traditional paradigm of seed-plant evolution as an incremental accumulation of adaptive innovations in favour of a more labile genetic context.

(4) Evaluating these hypotheses in a dynamic developmental and evolutionary context will require not only a greater range of comparative data, especially on localized gene expression, but also more innovative approaches such as ancestral sequence reconstruction (e.g. Groussin et al., 2015) to understand genome evolution in seed plants. Clarifying the homologies and evolution of the ovule will be an important step in understanding seed-plant evolution and the origin of angiosperms.

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