SEXUAL REPRODUCTION OF INTERIOR SPRUCE (PINACEAE). II. FERTILIZATION TO EARLY EMBRYO FORMATION

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Stages of normal sexual reproduction between pollen tube penetration of the archegonium and early embryo formation are described for interior spruce. These pre- and postzygotic stages were studied by light and electron microscopy in more detail than was possible in previous studies, and new observations have been made. The pollen tube tip penetrates between the neck cells toward the deteriorating ventral canal cell, and sperm (male gametes) and accompanying cytoplasm are released into the egg. A carbohydrate plug forms around the remnant cell walls of the pollen tube and ventral canal cell and appears to seal the archegonium against penetration by later arriving pollen tubes or from release of egg cytoplasm. One sperm fuses with the centrally located egg nucleus, and the other remains in the peripheral cytoplasm of the egg. Modified plastids of the egg deteriorate, and mitochondria of the egg accumulate around the zygote nucleus. Male plastids and mitochondria cluster near the zygote nucleus. Successive mitoses result in a four-nucleate proembryo. The nuclei and surrounding neocytoplasm migrate to form a single chalazal tier of proembryo nuclei. All plastids in the proembryo appear paternal in origin, and all mitochondria appear maternal. Cell divisions and cell wall formations result in a four-tiered proembryo. Elongation of the suspensor tier forces the early embryo into the megagametophyte.

Keywords: development, *Picea*, spruce, conifer, sexual reproduction, proembryo, gamete.

Introduction

Development within the pollen tube and ovule of *Picea* from pollination to archegonial maturation were described in the first article in this series (Runions and Owens 1999, in this issue). The fertilization period covered here for interior spruce includes development from the time pollen tubes contact the mature archegonium until the early embryos are formed. Previous studies of sexual reproduction in *Picea* (Merget al. 1965; Owens and Molder 1979, 1984; Singh and Owens 1981) were done using paraffin-embedded tissue and did not allow detailed description of fertilization. Many observations are included here for the first time.

The earliest transmission electron microscopical (TEM) studies of conifer megagametophytes were done by Camefort (e.g., 1959), Willemsen (1968), and Chesnoy and Thomas (1971). Camefort described the nature of the egg cytoplasm, mechanisms of cytoplasmic inheritance, and formation of proembryos in *Pinus* and *Larix*. More recently, cytoplasmic inheritance and proembryo development have been studied in *Pseudotsuga* (Owens and Morris 1991; Owens et al. 1991), *Larix* (Owens et al. 1994), and in the Araucariaceae (*Agathis*, Owens et al. 1995).

Singh (1978) describes the events of fertilization and proembryo formation in Pinaceae as summarized in figure 1. Karyogamy occurs soon after pollen tube entrance into the egg, and as a result, many authors describe the difficulty of finding this stage in embedded specimens. The zygote nucleus undergoes mitosis shortly after karyogamy. Synchronous mitoses of these nuclei result in four free proembryo nuclei surrounded by neocytoplasm within the archegonial cytoplasm.

Free nuclei of the pinaceous coenocytic proembryo migrate, while surrounded by neocytoplasm, to the chalazal end of the archegonium (Owens and Morris 1991; Bruns 1993) and settle into a single tier. Development of the proembryo continues until it comprises four tiers of four cells each in all Pinaceae studied thus far except *Pseudotsuga*, in which the late proembryo has only three tiers (Allen and Owens 1972; Owens and Morris 1991). Singh’s (1978) description of proembryo tier formation was derived from many sources and is characteristic. The late proembryo consists of four cell tiers: (from the chalazal pole) embryonal, suspensor, nonfunctional suspensor, and open. Owens and Molder (1984) called these tiers “apical,” “suspensor,” “rosette,” and “open,” respectively.

Unlike most angiosperms, in which cytoplasmic organelles are maternally inherited, chloroplast DNA is inherited from the paternal parent and mitochondrial DNA from the maternal parent in Pinaceae (Neale and Sederoff 1989; Mogensen 1996). Genomic analysis (Stine et al. 1989; David and Keathley 1996) has allowed confirmation of this pattern of cytoplasmic inheritance in *Picea*, but the process has not been observed cytologically. Neither the mechanism of cytoplasmic inheritance nor proembryo formation in *Picea* have been subjects of ultrastructural study.

In interior spruce, plastids of the egg cytoplasm distort before egg maturation. Distorted plastids historically are called...
Pollen tubes contact the megaspore membrane (top). Pollen tubes penetrate archegonia and sperm are released into egg cells. The leading sperm, which moves into the egg cytoplasm with the other pollen-tube contents. In either case, vacuolation in the micropylar end of the egg. These may form de novo, but they appear similar to the vacuoles of the pollen tube-cell cytoplasm and likely move into the egg cytoplasm with the other pollen-tube contents. In either case, vacuolation in the micropylar cytoplasm of the egg was the earliest sign of the change in egg appearance that accompanies fertilization.

Sperm are nuclei formed by mitosis of the pollen body cell and are never surrounded by a cell wall. They are the first pollen-tube component to move into the egg cytoplasm. In most cases, no large vacuole forms at the site of sperm entry until after the sperm have moved farther into the cytoplasm (fig. 2B). Coalescence of smaller vacuoles, on occasion, lead to formation of a larger vacuole in this region after the sperm had passed. Sperm become spherical in the egg and are ca. 50 μm in diameter. The leading sperm has an extension toward the egg nucleus as it moves toward it (fig. 2B). Within 24 h, the leading sperm reaches the egg nucleus in the central part of the egg cytoplasm and karyogamy occurs (figs. 2E, F, and 3). Paternal plastids, which moved into the egg cytoplasm with the sperm, retain the morphology of body-cell plastids (fig. 2G). Paternal mitochondria are larger and rounder than maternal ones, which are distorted and contain electron dense and translucent areas. Supernumerary nuclei (pollen tube-cell nucleus and stalk-cell nucleus) and other pollen-tube contents, including the trailing sperm, are confined to the site of entry or the peripheral egg cytoplasm (fig. 2F).

As karyogamy proceeds, supernumerary nuclei are enveloped by membranes and eventually deteriorate. Small inclusions of the egg cytoplasm surround the zygote nucleus except where they have been displaced by passage of the sperm (fig. 2E). Contact between the sperm and egg nucleus results in formation of a depression in the latter into which the sperm settle. In the contact zone, nuclear membrane fusion and segregation events described were from samples collected May 23–27, 1993, 16–20 d after pollination.

Results

Fertilization

It takes ca. 16 d from pollination for pollen tubes to grow through the nucellus and contact the megaspore wall. They then grow expansively over the apex of the megagametophyte until neck cells of an archegonium are encountered. A thin pollen tube process then extends through the megaspore wall and between the neck cells (fig. 2A). Neck cells contain deteriorated cytoplasm by this stage, and pollen tube penetration between them occurs via separation of their middle lamellae. Each archegonium is penetrated by only one pollen tube. The pollen-tube tip grows through the ventral canal cell wall. Opening of the pollen-tube tip and dissolution of part of the common cell wall between ventral canal cell and egg happen concurrently (fig. 2B). The tube tip is much smaller in diameter than the sperm. Pollen-tube contents are released into the egg cytoplasm. A dense deposit, which stains positively for polysaccharides, appears around the remnants of pollen tube and ruptured ventral canal cell wall within the egg cytoplasm (fig. 2B–D). This deposit is located so that the site of pollen tube entry into the egg appears sealed after fertilization. Many small vacuoles appear in the region of common cytoplasm in the micropylar end of the egg. These may form de novo, but they appear similar to the vacuoles of the pollen tube-cell cytoplasm and likely move into the egg cytoplasm with the other pollen-tube contents. In either case, vacuolation in the micropylar cytoplasm of the egg was the earliest sign of the change in egg appearance that accompanies fertilization.

Abnormal developments during the prezygotic stage of sexual reproduction that preclude fertilization will be described in a later article.

Material and Methods

Material and methods were as described in the first article in this series (Runions and Owens 1999, in this issue). Fertilization events described were from samples collected May 23–27, 1993, 16–20 d after pollination.
Fig. 2  A–C, Pollen tube (pt) penetration of the egg cell (ec).  
A, Pollen tubes grow to the megaspore wall. They grow directly to a neck or turn and grow over the apex of the megagametophyte until a neck is encountered (dashed line indicates pollen tube pathway into egg). LM. Bar = 50 μm.  
B, Pollen tubes push between the neck cells into the ventral canal cell, which deteriorates. Usually the pollen tube extends into the egg cytoplasm and then the male gametes are released. The second male gamete (male symbol ii), which will not effect fertilization, remains in the apical part of the egg. LM. Bar = 25 μm.  
C, After pollen tube penetration of the egg cell, a thick polysaccharide coating, which has staining properties similar to primary cell wall, accumulates around the remnants of the pollen tube and ventral canal cell wall (arrowheads). LM. Bar = 25 μm.  
D (as in C), Polysaccharide coating (arrowheads) on the postfertilization ventral canal cell wall (cw) becomes very thick and appears to plug the rupture caused by pollen tube entry (aj = archegonial jacket). TEM. Bar = 2 μm.  
E, Leading male gamete nucleus (male symbol) fuses with the egg nucleus (female symbol). The pathway of the male gamete can be seen because small inclusions have been displaced (above and to the right of the male gamete nucleus, arrow). LM. Bar = 20 μm.  
F, Trailing male gamete nucleus (male symbol) remains in the apical archegonial cytoplasm. DIC. Bar = 25 μm.  
G, Pollen body cell organelles accompany male gamete nuclei into the egg cell. Paternal plastids (p) are elongate and electron dense. TEM. Bar = 1 μm.
Fig. 3  As in fig. 2G. Nuclear membranes between the male gamete nucleus (male symbol) and the egg nucleus (female symbol) fuse (arrowhead), and channels of nucleoplasm span the gaps that form. Maternal mitochondria remain in several discreet clusters (female symbol m) around the fusion nucleus. Paternal organelles lag far behind the fertilizing male gamete nucleus. Paternal mitochondria (male symbol m) are clustered into large groups and are clearly distinguishable from the maternal mitochondria. Paternal plastids (male symbol p) are rarely seen. They were isolated like the one shown here or occur in groups of five to 10. TEM. Bar = 10 μm.

Segregation results in nucleoplasmic bridges between the nuclei (fig. 3). Neocytoplasm containing maternal and paternal organelles is not well defined at the zygote stage. Maternal mitochondria continue to surround the zygote nucleus in several small groups as they had done in the perinuclear zone. Paternal plastids and mitochondria remain in small clusters at a distance from the zygote nucleus (fig. 3).

Mitosis of the zygote nucleus occurs within 24 h after karyogamy. The zygote nuclear membrane disappears and chromosomes condense within an organelle-free zone of nucleoplasm (fig. 4A). During mitosis the neocytoplasm becomes distinct. By telophase, plastids and mitochondria surround each of the proembryo nuclei. Plastids are paternal in origin, and all mitochondria appear to be the maternal form. Mitochondria are numerous around each nucleus, but plastids are sparsely distributed. The second mitosis proceeds immediately and synchronously within the neocytoplasm in the center of the archegonium resulting in a proembryo with four free nuclei. Microtubule arrays of the spindle fibers are evident during mitosis (fig. 4B), but these mitoses were observed only infre-
quently so the orientation of cell division remains unclear. Single or small groups of two to three microtubules were seen occasionally near sperm and proembryo nuclei.

At karyogamy, the cytoplasm surrounding the neocytoplasm undergoes a significant change in appearance. This change proceeds in a wave from the chalazal end as the archegonial cytoplasm deteriorates (fig. 4C). As a result, fertilized and unfertilized archegonia become distinct in appearance (fig. 4D). Change in the nature of small inclusions accounts for most of the contrast. In fertilized eggs, small inclusions become spherical and vacuolated as the cytoplasm within is reduced in volume and stains more darkly. Modified plastids within the fertilized egg and, within the archegonial jacket cells, become convoluted and deteriorate (fig. 4E).

One special case of fertilization observed is worthy of description. Two normally developed, apparently mature archegonia were within an ovule that had been pollinated by a single pollen grain. The pollen tube grew into the neck of one archegonium, out through the side of the neck, and through the megagametophyte toward the other archegonium. Upon encountering the jacket cells of the second archegonium, the pollen tube turned and grew through them acropetally around the archegonium near the ventral canal cell. Fertilization was then effected by pollen tube penetration through the egg-cell wall near the ventral canal cell (fig. 4F), and a normal appearing proembryo was produced.

**Proembryo and Early Embryo**

The four free nuclei of the proembryo are ca. 40 μm in diameter as they migrate toward the chalazal end of the archegonium (fig. 4G). The nuclei do not necessarily remain near each other. Each nucleus is surrounded by a separate zone of neocytoplasm (fig. 4H) that contains numerous mitochondria and some plastids. Small inclusions of the egg cell are completely excluded from neocytoplasmic zones. As the nuclei move closer together in the tapering chalazal end of the archegonium, neocytoplasmic mix and the nuclei become irregular in shape (fig. 4I). The neocytoplasm at this stage contains many small lipid bodies. Once nuclei settle into a single tier, they become spherical and are surrounded by neocytoplasm, which is densely packed with mitochondria and contains sparsely distributed plastids (fig. 5A). Rough endoplasmic reticulum is plentiful around the proembryo nuclei (fig. 5B), and areas of cytoplasm at the periphery of the neocytoplasm become vacuolate.

The four free nuclei undergo synchronous mitoses (fig. 5C). Nuclear divisions are oriented perpendicular to the long axis of the archegonium to produce a two-tiered proembryo with eight nuclei. Before this mitosis, all plastids had migrated into a chalazal position relative to the nuclei so that they are included in only one chalazal-most tier of cells once cell wall formation occurs. Transverse walls form first between the daughter nuclei in each tier (fig. 5D), followed by longitudinal walls. Cells at the micropylar end remain open to the archegonial cytoplasm. Subsequent mitoses are synchronous within tiers and asynchronous between tiers. Primary open tier nuclei divide perpendicularly to the long axis of the archegonium and cell walls form (fig. 5E) before mitosis starts in the primary embryonal tier at the chalazal end (fig. 5F). The first sign of cell wall synthesis is an accumulation of membranes and small vesicles at the cell plate (fig. 6A). At a slightly later stage, thin cell walls and plasmalemma are visible (fig. 6B). Cytoplasm of cells in the embryonal tier is dense with ribosomes, endoplasmic reticulum, dictyosomes, lipid bodies, and protein crystals in the vicinity of the forming cell wall. Some mitochondria, which remain spherical until this stage, elongate during cell wall synthesis. All proembryonic cells except those in the open tier are surrounded by cell walls and even open tier cells were occasionally observed to be separated from the archegonium cytoplasm by partial and complete cross walls (fig. 6C).

A fully formed proembryo of interior spruce consists of four tiers of four cells each (fig. 6D). In order from the chalazal end, these are called the (1) embryonal tier, (2) suspensor tier, (3) nonfunctional suspensor tier, and (4) open tier. Embryonal cells are small and densely cytoplasmic with plastids and small nuclei. Cells of the suspensor tier are very vacuolated and lack plastids (fig. 6E). Cells are larger with larger nuclei and are more vacuolated toward the open tier. Deterioration of cytoplasm in peripheral regions of embryonal tier cells adjacent to the suspensor tier is coincident with the appearance of lipid bodies near the site of breakdown (fig. 6F). Embryonal tier cells have a very thin, enclosing cell wall that is distinct from the common wall between the former egg, and archegonial...
Fig. 5  A, Free nuclei (fn) of the proembryo become spherical after settling into a single tier at the chalazal pole of the archegonium. The neocytoplasm loses the thin zone that had been free of organelles and, particularly on the chalazal side of the nuclei, mitochondria (m) congregate against the nuclear membrane. Plastids (p) are sparsely distributed around the nuclei (aj = archegonial jacket). TEM. Bar = 5 µm. B, Rough endoplasmic reticulum (er) occurs in abundance around the free nuclei of the proembryo once they settle into a single tier. TEM. Bar = 1 µm. C–F, Cell divisions and cell wall formation in the proembryo. LM. Bars = 50 µm.  C, Synchronous mitoses occur in all four proembryo nuclei. All plastids that surrounded the free nuclei move to the chalazal pole (*) before mitosis. D, Cell walls form between products of the previous mitosis resulting in a two-tiered proembryo with four nuclei in each tier. All of the proembryo plastids (*) remain in the primary embryonal tier (pE), and mitochondria segregate into both tiers. The primary open tier (pO) remains open to the archegonial cytoplasm. E, After cell walls form between cells within each tier, the primary open tier cells divide synchronously to form the nonfunctional suspensor tier (nS) and the open tier (O). The primary embryonal tier (pE) has not divided (arrowhead = cell plate formation). F, Mitosis in the primary embryonal tier (pE) to produce the embryonal and suspensor tiers occurs once cell walls have fully formed in the two other tiers.
jacket cells in the chalazal-most region where the embryo will penetrate into the megagametophyte (fig. 6G, also see fig. 5A).

Early embryo penetration of the megagametophyte is by elongation of the suspensor tier cells (fig. 6H). Nonfunctional suspensor tier and open tier cells of the proembryo do not divide or elongate.

Discussion

Pollen tube growth through the nucellus of interior spruce coincides with maturation of the archegonia so that no delay was observed between the time pollen tubes reached the megagametophyte and fertilization. Pollination to fertilization requires 15–20 d. Time from pollination to fertilization is variable among genera of Pinaceae. No delay occurs in Picea (Owens and Molder 1979; Singh and Owens 1981), whereas, in genera such as Pseudotsuga, Larix, Tsuga, and Abies, pollen germination is delayed for several days after pollination as the megagametophyte matures (e.g., Takaso and Owens 1994), in Pinus, winter dormancy occurs between pollen germination and fertilization (Owens et al. 1982). Seed development from the time of fertilization is roughly the same, requiring ca. 3 mo, for all temperate Pinaceae (Singh 1978).

Pollen Tube Penetration and the Ventral Canal Cell

Pollen tubes grow with little or no deviation through the nucellus until they contact the megaspore wall. Tubes then splay over the apex of the megagametophyte until an archegonial chamber is encountered above the neck of an archegonium. If a chemical or physical signal exists that causes pollen tube growth toward an archegonium (e.g., the lipid accumulation in the archegonial chamber that was described in Runions and Owens 1999, in this issue), it is not strong enough to result in tube growth through the nucellus directly toward an archegonial neck. Instead, by splaying over the megagametophyte apex, pollen tubes encounter archegonia. A similar description for Engelmann spruce was provided by Singh and Owens (1981). Deterioration of the neck cells begins before pollen tube arrival. This may reduce their turgidity and makes a conduit for the pollen tube. In one anomalous case, the pollen tube ignored the neck cells and grew through the side of an egg cell. In all other cases, neck cells separated at their middle lamellae as the cells deteriorated and pollen tubes pushed between them. Opening of the pollen tube occurs as it grew into the ventral canal cell and, in some cases, the opened pollen tube projected well into the micropylar part of the egg cytoplasm. A substance that accumulates at the site of pollen tube entry into the egg has the same staining characteristics with toluidine blue as the primary cell wall. This substance is probably pectinaceous or some other α(1-4)-linked polysaccharide (O’Brien and McCully 1981). At high magnification, no fibrils were observed in the substance, indicating that it may not be cellulose. Functionally, the substance forms a plug at the site of pollen tube entry and may prevent penetration by other pollen tubes or release of egg cytoplasm.

Fertilization and Cytoplasmic Inheritance

Literature on conifer reproduction usually describes a large vacuole, the receptive vacuole (Singh 1978), forming in the egg cytoplasm and into which the sperm are delivered. In interior spruce, sperm (which are free nuclei in Picea) and surrounding body cell cytoplasm are delivered into the egg cytoplasm, and vacuolation occurs afterward as the vacuolated contents of the pollen tube cell enter the egg.

Both sperm enter the egg. The leading one moves directly toward the egg nucleus, while the trailing one remains in the micropylar or peripheral region of the egg. Within Pinaceae, paternal plastids and some mitochondria usually accompany the fertilizing sperm or follow it closely in a large cluster (Camelfort 1968; Chesney 1987; Bruns and Owens 1989; Owens and Morris 1991; Mogensen 1996). In cases where karyogamy was observed by TEM in this study, paternal plastids and mitochondria were observed clustered but trailing the fertilizing sperm at some distance. Paternal plastids eventually move into the neocytoplasm of the proembryo. Paternal and maternal mitochondria are distinguishable, and all mitochondria in the neocytoplasm have maternal morphology. Camelfort (1966) did not find paternal mitochondria in the neocytoplasm of Picea. Results of genetic-probe-based studies (Sutton et al. 1991; David and Keathley 1996) indicate that mitochondrial inheritance is strictly maternal in Picea. The observations of this study support this and show that Picea differs from Pinus (Bruns and Owens 1989; Wagner et al. 1992) and Pseudotsuga (Owens and Morris 1991), in which ca. 10% of mitochondria may be paternally inherited.

Cytoplasmic inheritance in Pinaceae differs from that in the majority of angiosperms in which organelles are maternally inherited. The reason why organelles are usually uni-

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**Fig. 6**  A, B, Cell wall (cw) formation within the embryonal tier. TEM. Bars = 1 μm. A, First indication of cell wall formation in the embryonal tier is an accumulation of membranes and coated vesicles (arrowheads) across the phragmoplast. B, As the cell wall forms, storage product metabolism is evidenced. Lipid bodies (lb) arise in the cytoplasm and move through the plasmalemma to lie against the forming cell wall. Protein crystals (pc), elongate mitochondria, dictyosomes, and endoplasmic reticulum are all concentrated near the site of cell wall formation. C, Occasionally cell walls (arrowhead) form to enclose, or partly enclose, cells in the open tier (O) of the proembryo. Bar = 25 μm. D, Mature proembryo consists of four tiers of four cells each (E = embryonal tier; S = suspensor tier; nSt = nonfunctional suspensor tier; O = open tier). Nuclei are smallest in the embryonal tier and are progressively larger through the tiers toward the micropylar end. Bar = 25 μm. E, Embryonal tier cells contain dense cytoplasm relative to cells in the suspensor tier. Large clusters of mitochondria are scattered throughout suspensor tier cells. TEM. Bar = 2 μm. F, After cell wall formation in the embryonal tier, large vacuoles appear in the cytoplasm toward the micropylar end. Vacuole formation coincides with formation of lipid bodies. TEM. Bar = 1 μm. G, Before embryo penetration of the megagametophyte, the thin cell walls (arrowheads) of the embryonal tier cells thicken relative to the thicker wall of the archegonium (αt) at the chalazal pole. TEM. Bar = 1 μm. H, Suspensor cells (α) elongate and push the early embryo (εn) into the megagametophyte (μg). No elongation occurs in the nonfunctional suspensor tier or the open tier of the proembryo. DIC. Bar = 50 μm.
parental in origin is unclear (Reboud and Zeyl 1994; Mogensen 1996). Perhaps uniparental inheritance provides a selective advantage that would be lost if incompatible organelle genomes co-occurred. As illustration, recombination between mitochondrial genomes can occur (Birky et al. 1982) and may result in disruption of selectively advantageous gene linkages, with subsequent reduced fitness of progeny. Determination of the mechanism by which separate organelle systems are targeted for movement or elimination should be a major priority because it would help elucidate the general mechanism of organelle movement. A recent report by Sutovsky et al. (1998) describes ubiquitin tagging of mitochondria in mammalian sperm cells and indicates that the egg cell may recognize and dispose of tagged mitochondria after fertilization resulting in uniparental inheritance.

The leading sperm elongates toward the highly invaginated egg nucleus. Zygote formation occurs by fusion and separation of the bounding nuclear membranes of sperm and egg nuclei. Mitosis of the zygote occurs within 24 h of karyogamy as estimated by the infrequency with which this stage was observed in embedded samples. By the time chromosomes have condensed at the metaphase plate in the zygote, the perinuclear zone, containing maternal mitochondria and paternal plastids, is well defined and bordered by small inclusions. After zygote mitosis, the perinuclear zone of each proembryo nucleus is defined as neocytoplasm. Parental plastids do not remain in a discrete cluster as described in Pseudotsuga menziesii (Owens and Morris 1991) but surround the free nuclei at low density within the neocytoplasm. A second, synchronous mitosis of the daughter nuclei occurs, resulting in four free nuclei of the coenocytic proembryo, each surrounded by neocytoplasm within the central region of the archegonial cytoplasm.

During fertilization, the appearance of the archegonium cytoplasm changes. This change proceeds in a wave from the chalazal end of the archegonium. Owens and Morris (1991) describe the same change occurring from the micropylar end in P. menziesii. Cytoplasmic areas within the small inclusions shrink, and small vacuoles appear throughout the archegonium cytoplasm while modified plastids disintegrate. In this process, therefore, maternal plastids are eliminated by the time the free nuclear proembryo forms.

Proembryo and Early Embryo

Migration of the four free nuclei of the coenocytic proembryo to the chalazal end of the archegonium requires 1–2 d. If nuclei are close together, they share a common neocytoplasm; otherwise, each nucleus is surrounded by its own neocytoplasm. As the four free nuclei near the chalazal end of the archegonium, they distort and elongate at their chalazal ends. This distortion does not persist and the nuclei round up before synchronous mitosis. Neocytoplasm is metabolically active before mitosis, as indicated by lipid body accumulation and the large amount of rough endoplasmic reticulum that surrounds each nucleus. Round to elongate mitochondria fill the zone of neocytoplasm in which metabolic activity is highest, but a thin zone of neocytoplasm devoid of mitochondria, described also by Bruns (1993) in Pinus, remains immediately outside each nuclear membrane. Plastids are far fewer in number than mitochondria, and before mitosis they migrate into the chalazal-most neocytoplasm so that it becomes polarized. Huang and Russell (1994) describe synergid and central-cell cytoplasms with polarized organelle distributions of this sort in tobacco. In that study, microfilaments and microtubules were associated with organelle surfaces and may have been involved in establishment of cytoplasmic polarity.

A large body of literature exists on proembryo development and terminology in conifers (see Singh 1978). In general within Pinaceae, a late proembryo consists of four tiers of four cells each. Descriptions of proembryogeny in Pinaceae are provided by Buchholz (1929), Chowdhury (1962), Doyle (1963), Dogra (1967), Mehra and Dogra (1975), and Singh (1978). We will, for the most part, follow Singh’s (1978) terminology for proembryogeny as our observations in interior spruce closely match his description.

Synchronous mitoses of the four free nuclei results in eight free nuclei in two tiers of four. Cell wall formation between the daughter cells results in an enclosed primary embryonal tier at the chalazal end and a primary open tier (Singh’s [1978] primary upper tier). Cell walls then form between nuclei within each tier. Cell wall formation is first evident across the phragmoplast between nuclei. Primary open tier cells remain open to the archegonial cytoplasm, which suggests that cell walls usually form only between nuclei (i.e., no phragmoplast forms distal to an open tier nucleus). Primary embryonal tier cells are densely cytoplasmic and contain all of the plastids of the proembryo in their chalazal cytoplasm.

Cytokinesis perpendicular to the archegonium long axis in the primary open tier results in a three-tiered proembryo and subsequent cytokinesis, in the same orientation, of the primary embryonal tier results in a four-tiered, mature proembryo. Occasionally the open tier cells appeared enclosed by cell walls, although the completeness of closure was not ascertained. Open tier and nonfunctional suspensor tier cells have no apparent function in the mature proembryo other than perhaps isolation of the suspensor and embryonal cells from the rapidly deteriorating cytoplasm of the archegonium. The term “nonfunctional” in the name of the third tier of proembryo cells would seem to refer to its lack of elongation as the suspensor tier cells elongate to push the embryo into the megagametophyte. Division of the primary open tier to form the nonfunctional suspensor tier does not occur in Pseudotsuga (Allen 1943). Various phylogenetic analyses, e.g., Hart (1987), show Pseudotsuga originating later than Picea within Pinaceae. Loss of the cell divisions that produce the nonfunctional suspensor tier probably reflects that tier’s lack of importance for subsequent embryo success.

Suspensor tier cells are vacuolated, which is characteristic of cells undergoing rapid elongation. No delay occurs between proembryo maturation and the division elongation of suspensor cells that forces the embryonal tier into the center of the megagametophyte. Subsequent divisions in the embryo tier produce a compact mass of embryonal cells with subtending embryonal tube cells.

In interior spruce, embryo penetration into the megagametophyte occurs in as little as 3–4 d after pollen tube penetration of the neck cells. Simple polyembryony in interior spruce was not described in this study but, when multiple archegonia were fertilized, the first embryo into the megagametophyte seemed to grow rapidly while others remained small.
or deteriorated. Barring lethal allele combinations then, success may be dependent on slight differences in the time of fertilization or speed of proembryo development and embryo penetration into the megagametophyte. Embryo competition in this sense is probably very real and so rapid development to the early embryo stage might have been selected for in *Picea*.

A subsequent article will describe anomalies of development leading to prezygotic failure of sexual reproduction in interior spruce.

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