Chapter 6
Pollination and Fertilization

Summary All conifers rely on wind to move pollen to ovule but form matters as much as chance; pollination is more akin to coordination and synchrony than it resembles a stochastic process. During female strobilus receptivity, ovules exude a localized pollination drop at night. By early morning, the pollination drop retracts, pulling its captured grains inside the micropylar arms, closer to the spongy nucellus. Hydrated by the pollination drop, the pollen grain now germinates into the spongy nucellar tissue. The pollen tube then halts its growth midway through the nucellus during the lengthy interval between pollination and fertilization. During this interval, the female gametophyte completes its development, slowly expanding to its maximum size and forming multiple archegonia. The duration of the pollination-fertilization interval is taxon-specific, lasting many months. The pollen grain resumes its growth a few days before fertilization then delivers one or two male gametes to the egg cell. The close synchrony between male and female reproduction is a sharp contrast to heterospory-induced divergence.

The nightly appearance – and disappearance – of the pollination drop is a mystery. This fluid extends beyond the micropylar arms of its ovule, picking up any deposited pollen grains and then retracts by early morning. The retracting drop deposits its hydrated pollen cache close to the spongy nucellus (Photo 6.1). What physical or chemical cues trigger the drop's withdrawal? Presumably its cue recognition system is localized because the drop is exuded by ovular tissues (O'Leary and von Aderkas 2006).

Contrary to popular opinion, the pollination drop is neither a water droplet nor a product of guttation in the adult tree (O'Leary and von Aderkas 2006). It is a localized phenomenon originating from the ovule's own sporophyte or gametophyte tissues. The drop is aqueous yet protein-rich. Its cues for cessation are thought to require particle size recognition, a chemical interaction or perhaps both. Pollen itself is thought to be the sole stimulus, not mechanical forces or evaporation (Tomlinson et al. 1997). These and other hypotheses have been further tested using *Juniperus communis*, a member of the Cupressaceae (Mugnaini et al. 2007).

C.G. Williams, *Conifer Reproductive Biology*,

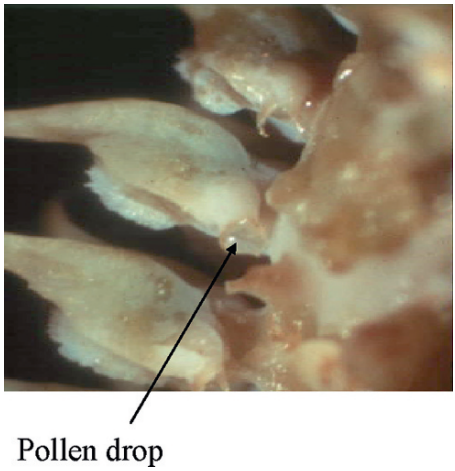


Photo 6.1 *Pinus taeda* pollination drop from an ovule at the base of the cone scale (arrow) (Photograph taken by Floyd Bridgwater, USDA Forest Service. Permission granted)

Juniperus communis provides an elegant *ex situ* system. The pollination period for this species is unusually long, lasting about as long as a month. A single female strobilus has three ovules and the diameter of each ovule's micropyle is 70 µm. Pollination drop emergence is not synchronous on each strobilus. Each drop can appear up to four times before an ovule's ability to form a drop is lost.

The experiments were conducted using branches with receptive female strobili collected on the previous evening. Short sprigs bearing female strobili were inserted into water-filled vials and kept under controlled conditions of 15°C with 52% relative humidity and the drop emerged. Drop volume was measured using a microcapillary tube. Particles were applied using a single human eyelash glued to a wooden stick with paraffin.

Particle applications to the pollination drop included desiccating silica particles in two sizes, small (10–15 µm) and large (63–200 µm), pollen from *Juniperus communis*, pollen from another conifer *Pinus canariensis*, pollen from an angiosperm *Pyrus communis* and live and heat-killed conspecific pollen (20 µm) (Mugnaini et al. 2007).

Experimental findings from this novel system offer new insights into the cuing mechanisms. First, particles are required for cuing; application of the eyelash itself, free of particles, did not alter pollination drop characteristics. Next, live *Juniperus communis* pollen triggered pollination drop withdrawal within 30 min, as expected. Third, the large silica particles raised the drop volume but did not cue its withdrawal. All other particles, including several types of pollen and small silica particles, triggered only a partial reduction in the drop volume. The authors reported that the partial drop withdrawal appears to be the drop's non-specific mechanical response to any small particle. Hydration was not ruled out as another explanation.

Total drop withdrawal, caused only by live conspecific pollen, is thought to be a two-part response to mechanical and molecular cues (Mugnaini et al. 2007).

To fully understand these experimental findings, it is necessary to take a more comprehensive view of pollination biology. Female strobilus receptivity is a logical starting place because this is where its coordination with the ovule and pollination drop begins.

6.1 Female Strobilus Receptivity

Female strobili become receptive to pollen entry when cone scales separate (Photos 6.1-6.2). The ovuliferous scales attached to the cone axis at an angle and the angle of the scales change with the stage of receptivity. If open, the angle favors pollen grains reaching the ovules.

As shown in Chapter 4, strobilus morphology has been divided into five stages (Pattison et al. 1969) but a six-stage classification system is more widely used (Bramlett and O'Gwynn 1980); both systems are based on degree of budbreak, strobilus elongation, size and distance between ovule-bearing scales.

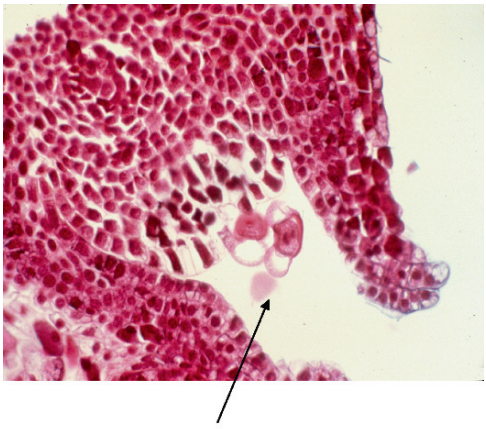
Windborne pollen sifts between the open scales and some will land on the ovule's micropylar arms. Each pair of *Pinus taeda* ovules is located at the base of each fertile cone scale (Photo 6.1). Recall that the ovule has an inverted orienta-

Photo 6.2 A receptive *Pinus taeda* female strobilus from Bramlett and O'Gwynn (1980). Scales are starting to flex so that pollen can reach the pair of ovules located at base of each scale (Photographs by Floyd Bridgwater, USDA Forest Service. Permission granted)

tion so its micropylar arms hang down towards the cone axis. After the pollination drop emerges at night (Photo 6.1), it will retract, pulling pollen into the micropylar chamber (Photo 6.3A) where the hydrated pollen will germinate into the nucellar tissue.

After pollen capture, the drop will no longer emerge. The ovule closes its micropylar opening (Photo 6.3B). Ovuliferous scales of the female strobilus swell, sealing the entry to the ovules. This occurs even if the pollen grains do not germinate. This was the case for the ovule shown in Photo 6.3.

A *Pinus taeda* ovular opening is sealed at 4 weeks after pollination. The micropylar arms are sealed above the sharp outline of the nucellus even though the pollen grains did not germinate (Photograph by author)



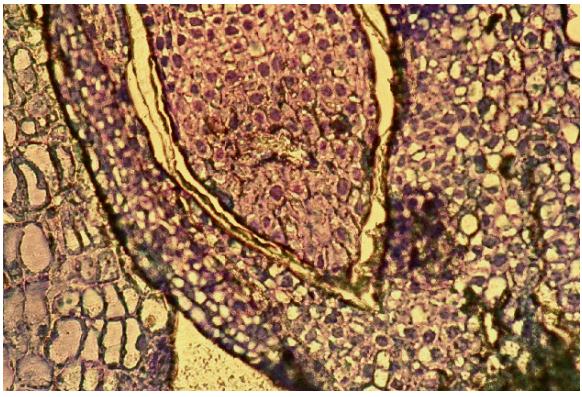


Photo 6.3A *Pinus taeda* pollen grains inside the micropylar chamber (arrow) just before germinating into the nucellar tissue of the ovule (Photograph taken by Floyd Bridgwater. Permission granted)

Photo 6.3B *Pinus taeda* ovule has sealed closed after pollination (Photograph taken by the author)

6.2 Pollination Drop: Localized Exudation from Each Ovule

The pollination drop is secreted by ovular tissues (McWilliam 1958). It could be a product of nucellar tissues although the integument and female gametophyte have also been suggested. Such definitive experiments on pollination drop origin have not yet been reported (see review by Gelbart and von Aderkas 2002). It is clear that the exudation is localized in ovular tissues. Recent experiments have clearly refuted the influences of guttation or xylem water potential changes in the adult sporophyte or high humidity in the atmosphere (O'Leary and von Aderkas 2006). The localized nature of the pollination drop is consistent with the lack of vascularized connections to the ovule (Singh 1978).

In *Pinus*, pollen capture is accomplished by means of a pollination drop exuded at night by the apex of the nucellus, filling the micropylar opening (Doyle and O'Leary 1935; Lee 1955; Tomlinson 1994). Secretion of the pollination drop starts at nightfall, reaching maximum exudation around 2 A.M. then recedes before daybreak. This emergence of the pollination drop is precise. Odlly, the drop is not secreted during daylight hours (Doyle and O'Leary 1935) nor in the presence of rain (Greenwood 1986; Brown and Bridgwater 1987).

When the pollination drop is reabsorbed, the pollen floats upwards into the ovule (Runions and Owens 1999) and transported to the surface of the nucellus (Brown and Bridgwater 1987). The pollination drop provides liquid for pollen hydration then deposits pollen at the nucellus to start tube growth through the maternal sporophyte tissue.

Few pollen grains reach the ovule although heavy quantities of pollen are released. Such an abundance of pollen leads to the presumption of allergies but conifer pollen rarely causes allergies. The unfortunate exceptions are a few members from the Cupressaceae family (Box 6.2).

6.3 Pollen Capture and the Role of the Micropyle

Most extant conifers have a pollination drop (Tomlinson et al. 1997; Owens et al. 1998; Gelbart and von Aderkas 2002). The notable exceptions include all *Abies* species and some *Tsuga* species. Some taxa rely on pollination drops exuded by the nucellus (Doyle and O'Leary 1935), others rely on pollination drops although rainwater also is an effective substitute (Greenwood 1986; Brown and Bridgwater 1987). Of the conifers, only members of the Araucariaceae completely lack a pollination drop (Gelbert and von Aderkas 2002).

The presence or absence of the pollination drop is only one character in a suite of correlated pollination characters among conifers (Tomlinson et al. 1997). Its absence correlates with germination of pollen outside the nucellus, defined as extended siphonogamy, found in *Tsuga* species and all members of the Araucariaceae. But it

Box 6.1 Zooidogamy and the pollination drop

The pollination drop was required for sperm delivery in the system of zooidogamy but now captures pollen (Labandeira et al. 2007). The drop once served the watery transport for motile antherozooids in the absence of a pollen tube (Fig. 6.1).

Only one living gymnosperm, *Gingko biloba*, has flagellated, motile sperm cells as well as the pollination drop – and a pollen tube (Fig. 6.1, stage b). Its pollen tube is branching and serves a haustorial function. The tube grows into the nucellar tissue like fungal hyphae then extracts nutrients for the gametophytic cells at the tube's growing end (Gifford and Foster 1989, p. 333).

The *Gingko biloba* pollen tube delivers two free-swimming sperm (Lee 1955) so the pollination drop is not required for sperm delivery. Each spermatozoid, including its ciliated tail, measures roughly 50–80 μm in length at release (Lee 1955). Upon their arrival, the egg cell first forms a small opening or beak at the top of its archegonium. A liquid forms then the first of the two sperm swim into the liquid. As soon as the sperm attaches to the egg, the beak of the egg retreats, making a path for only the head of the sperm so that most of the sperm body is left outside the archegonium. The egg forms a rigid membrane to prevent the entrance of the second sperm. The pollination drop, once so central to the prepollen delivery system, now provides the role of pollen scavenger.

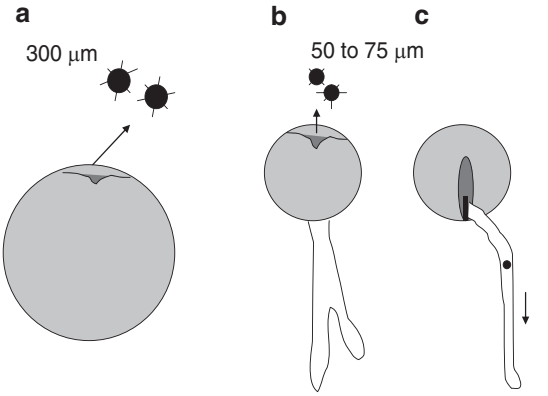


Fig. 6.1 The proposed evolutionary transition from prepollen to pollen transition, is redrawn from Poorts et al. (1996). Drawings represent prepollen and pollen without outer covering or exine: (**a**) male gametes by the late Paleozoic was large (300 μm) and zooidogamous, releasing motile spertherozoids through a proximal aperture, (**b**) from the late Paleozoic to early Mesozoic, a transitional form of zooidogamous prepollen is proposed which still releases motile antheroids through the proximal aperture but now has a haustorial pollen tube for nutritive functions only and (**c**) from Mesozoic to present-day, the siphonogamous pollen grain (50–75 μm) has a pollen tube which delivers immobile sperm nuclei (Pinaceae) or sperm cells (Cupressaceae) to the archegonium (From Poort et al. 1996. Copyright (1996) National Academy of Sciences, USA) Copyright permission granted

also correlates with pollen germinating inside the pollen chamber as in the case of *Larix* spp. and *Pseudotsuga* spp. (Tomlinson et al. 1997).

Its presence is the more common condition. This character correlates with (1) either saccate or non-saccate pollen and (2) bursting of pollen upon hydration (Tomlinson et al. 1997). The classification of correlated pollination characters was further expanded to five pollination types (Owens et al. 1998) based on a larger suite of traits including ovular morphology and orientation. It is interesting to note that one or more members of the Pinaceae are found in four of the five classification types, attesting to the considerable variation within a single family.

Another character, the delayed pollination drop, has now been included in this suite of characters (Gelbart and von Aderkas 2002). *Larix* spp. and *Pseudotsuga* spp, two genera in the Pinaceae, have delayed secretion where the drop appears weeks after pollination. In the case of *Pseudotsuga menziesii*, a secretion fills the micropylar canal about 7 weeks after pollination until fertilization (von Aderkas and Leary 1999). This phenomenon is better defined as a prefertilization drop rather than a pollination drop (Gelbart and von Aderkas 2002).

6.4 Composition and Function of the Pollination Drop

Pollination drops capture pollen but they might also provide pathogen protection. The pollen drop is rich in pathogenesis-related (PR) proteins including glucan-β-1,3-glucosidases (PR-2), chitinases (PR-3) and thaumatin-like proteins (PR-5) which degrade fungal cell walls and deter fungal growth (Wagner et al. 2007). Pollination drop may serve to retard fungal activity.

Box 6.2 Pollen allergens from the Cupressaceae family: a case of mistaken germination

Rarely does conifer pollen trigger allergies in humans but the notable exceptions are a few species within the Cupressaceae family. The well-documented species on the list includes *Juniperus ashei* in the southern central United States, *Cupressus sempervirens* in Italy, *Cupressus arizonica* in Spain and *Cryptomeria japonica* in Japan. Pollen from these species triggers an allergic response known as cedar fever. The pollen is inhaled then its epitopes enter the human blood stream and trigger an immune response. Certain air pollutants may heighten allergen expression; cedar fever affects nearly 10% of the exposed population in Japan yet nearly 20% of city dwellers are affected.

(continued)

Box 6.2 (continued)

The first question: what is the pollen biology behind these events? The pollen particles are small, ranging 15–35 µm in diameter, nonsaccate and star-shaped (Tomlinson 1994). The pollen grain enters the nose or mouth where it is hydrated by the moist mucosal lining, saliva or nasal fluids. The pollen grain starts to germinate into the mucosal lining. It bursts out of its thin exine within seconds then its thicker intine swells until the pollen grain becomes round rather than star-shaped. Now prepared to germinate, the pollen tube emerges from its aperture (Tomlinson 1994; Suarez-Cervera et al. 2003).

The second question: where is the allergen protein located? For *Cupressus sempervirens*, allergen proteins are highly concentrated on the pollen wall (including the intine) in addition to the central capsule's cytoplasm (Suarez-Cervera et al. 2003). When the pollen grain lands in the upper respiratory tract, its intine swells into a round shape bringing allergen proteins into contact with the human respiratory tract. Often, the pollen grain bursts then its rupture coats the allergen-rich cytoplasm contents on mucosal surfaces (Canini et al. 2004; Suarez-Cevera et al. 2003).

The third question: what is the identity and function of the candidate allergens? Oddly, the allergens are pathogen response (PR) proteins similar to those found in the pollination drop. Candidate *Jun a* 3 is a thaumatin-like protein (TLP) was isolated in *Juniperus ashei* (Midoro-Horiuti et al. 1999) and then used as a trans-specific probe to identify similar proteins in other conifers (Midoro-Horiuti et al. 2001; Suarez-Cervera et al. 2003; Cortegano et al. 2004).

As long suspected, air pollution enhances allergen expression (Cortegano et al. 2004) so humans living in urban settings will suffer cedar fever to a greater degree than those living in rural areas. It is not yet clear whether the carbohydrate moiety in *Jun a 3* glycoproteins also contributes to their immunoglobulin (Ig)E-binding capacity and ability to elicit IgE-mediated allergic symptoms (Breiteneder 2004). These and other similar questions are important to developing therapeutic relief from cedar fever.

Another candidate allergen isolated from *Juniperus ashei* is *Jun a 1*, a pectate lyase which degrades the pectin-rich intine, ensuring pollen tube germination (Suarez-Cervera et al. 2003). It has been characterized as a 40 kD glycoprotein (Midoro-Horiuti et al. 1999) with a full-length transcript of 1,101 nucleotides. Close relative *Juniperus virginiana* possesses an interesting mutation which reduces allergen response (Midoro-Horiuti et al. 2001).

Pollen from other conifers does not seem to induce these respiratory allergies but the reasons for this anomaly have not been explored. While it is true that *Pinus* spp. pollen does not burst nor shed its exine upon hydration, its PR-related allergens have not been localized nor characterized. This is also the case for other taxa which share this non-bursting or intact hydrated pollen character: *Larix*, *Pseudotsuga*, *Tsuga* as well as some of the Podocarpaceae (Tomlinson 1994).

(continued)

Box 6.2 (continued)

Pine pollen does not cause allergies because "it is too heavy to travel or that it falls beneath its source and therefore does not make its way up into the human nose." Long-distance dispersal of pine pollen has been well-established for over 100 years. Neither dispersal distance nor particle size determine whether conifer pollen triggers human allergies. A comparative analyses of candidate genes or gene products would be a better approach than relying on a medical myth.

6.5 Pollen Germination into the Nucellus

Once the pollen grain hydrates, its tube emerges. Immediate germination is the case for *Picea* and *Pinus* whether the pollen lands on agar, water, sugared water or nucellar tissue (Box 6.3). Other genera in the Pinaceae delay pollen germination by 3 weeks (*Pseudotsuga*), 3 months (*Keteleeria evelyniana*) or even as long as 9 months (*Cedrus* spp.) (Konar and Oberoi 1969).

Germination begins when the exine splits (Singh 1978, p. 132). In conifer taxa with sacci, the exine splits between the sacci at the distal end, along the suture or leptoma. Here, the hydrated pollen grain swells to the point that its sacci separate, exposing the leptoma. In *Pinus* spp. the pollen tube slowly emerges from the leptoma and begins its movement through the nucellus or spongy diploid ovular tissue. By contrast, germination starts on the cone (Tomlinson 1994). Other taxa germinate the pollen tube outside the ovule. Some pollen types in the Cupressacceae burst upon hydration to release the pollen tube (Box 6.2).

At this point, the tube nucleus migrates into the growing pollen tube and the generative cell divides equally to form two more cells: a fertile body cell and sterile stalk cell. The pollen tube begins its growth through the nucellus in various ways (Singh 1978, p. 137).

The simplest case is described for the Pinaceae. Intercellular signalling occurs between the growing pollen tube and the nucellus or other parts of the maternal sporophyte (Owens et al. 1990). This is consistent with experimental findings where angiosperm pollen tube growth depends on a calcium-mediated signal cascade as well as cues from haploid cells and diploid ovular tissue (Wilhelmi and Preuss 1999). In *Pseudotsuga menziesii*, signaling appears to start as early as 8 weeks after pollination (Takaso and Owens 1996).

The pollen tube elongates between nucellar cells. At this stage, the female gametophyte does not yet exist; only the megaspore mother cell (MMC) is present. The pollen tube exudes secretions which cause cell collapse including pectinase, cellulose, hydrolase, acid phosphatase, esterase, amylase, proteases and other degradative enzymes (Owens and Morris 1990). The pollen tube elongates through its own milieu of hydrolytic enzymes and the degenerating nucellar tissues.

Inside the elongating pollen tube of *Picea abies*, a well-organized network of microfilaments that extends the length of the tube (Terasaka and Niitsu 1994; Lazarro 1996, 1998). The microfilament network is orderly, forming two distinct zones within the elongating pollen grain. These zones partition plastids from mitochondria (Justus et al. 2004). Microfilaments in *Picea abies* pollen move in a fountain pattern, a pattern that is reversed in angiosperm pollen (Justus et al. 2004).

Box 6.3 Protocol for *Pinus taeda* pollen germination

This agar-based pollen viability assay (Goddard and Matthews 1981) is widely used for testing pollen prior to controlled pollinations.

1. Add 0.625 g agar to 125 ml distilled water to obtain a solution of 0.5% wt./vol. using Difco Bacto agar.
2. Sterilize agar in autoclave for 20 min.
3. Pour melted agar into petri dishes, filling sterile Petri dishes only ¼ full. Minimize exposure of agar plates at all stages to prevent microbial contamination.
4. After agar has solidified, dust re-hydrated pollen lightly on surface using a camel hair art brush. Use a different, clean art brush for each pollen sample.
5. Incubate dishes at 29°C for 48 h.
6. Using a dissecting microscope, tally at least 200 pollen grains per Petri dish. Only pollen grains with tubes equal to or exceeding the width of the grain are viable. Pollen germination above 80% germination is considered very good for stored pollen.

6.6 Pollen Tube Dormancy

The *Pinus taeda* pollen tube ceases growth partway through its germination into the nucellus. The pollen tube appears to be dormant. Dormancy of the pollen tube dormancy coincides with female meiosis, continues through the formation of the female gametophyte and does not break until a few days before fertilization. The pollen tube revives in response to some unknown cues. Candidates for cues include ovular secretions (Takaso and Owens 1996) or rapid female gametophyte growth (Gifford and Foster 1989). Other authors have suggested that this could be a stage suited to gametophytic selection (Takaso et al. 1995) but such evidence has yet to be recovered (Williams 2008). Similarly, archegonial development can be ruled out as the stimulus for resumption of pollen tube growth. In some conifers, pollen tube growth proceeds independently whether archegonia are dead or alive (Dumont-BeBoux et al. 1998).

6.7 Female Gametophyte Development After Pollination

All female reproductive cycles in conifers, regardless of duration, share a common feature: the female gametophyte develops *after* pollination in conifers. Its highly conserved development proceeds through three major stages: (1) a free nuclear phase, (2) a cellularization phase and (3) a cellular growth phase (Singh 1978; Konar and Moitra 1980; Friedman and Carmichael 1998). The breadth of variation among conifers and other gymnosperms has been reviewed in depth by Konar and Moitra (1980).

6.8 Fertilization

Once the pollen tube resumes elongation, it penetrates the megaspore wall in order to reach an archegonium (Pettit 1985). About a week before fertilization, its body cell divides to form two male nuclei which are unequal in size. The male gametes are nuclei or cells formed by the mitosis of the body cell.

In *Pinus*, the tip of a pollen tube forces itself between the neck cells of an archegonium and then ruptures, discharging the two male gametes, the tube nucleus and the sterile cell into the cytoplasm of the egg (Gifford and Foster 1989, p. 438; Owens and Morris 1990). The larger, leading sperm reaches the egg nucleus first and fertilization occurs (Runions and Owens 1999). The smaller sperm nucleus, the tube cell and the sterile cell now degenerate. In *Pseudotsuga menziesii*, microtubules associated with paternal organelles migrate with the leading sperm as it moves toward the egg nucleus (Owens and Morris 1990).

The members of the Cupressaceae have equally-sized male cells, rather than nuclei, which form much later when the pollen tube enters the archegonial chamber (Singh 1978). The number of sperm cells varies among these taxa although two sperm cells (defined as diplospermy) is common. An exceptional case has been reported for *Cupressus arizonica* which is reported to have 12–14 sperm cells (Doak 1932).

Conifers do have a few rare cases of multiple fertilization. A single *Callistris* pollen tube can deliver multiple sperm to more than one archegonium (Baird 1953; Willson and Burley 1983). On rare occasions, two separate sperm fertilize an egg nucleus and a second cell nucleus within the same archegonium (Friedman 1992).

6.9 Different Female Reproductive Cycles

Conifer reproduction is synchronous with seasonal change in temperate zones. Reproductive development slows to a halt during winter then resumes each spring. The cycle, punctuated by seasonal change, can take 1, 2 and even 3 years from pollination to seed maturation.

Conifers are classified as 1-, 2- or 3-year reproductive cycles (Singh 1978). The cycle refers to the completion of female strobilus development from initiation to seed maturation. By comparison, male strobilus development is completed in a single year (Singh 1978) regardless of the duration of its respective female reproductive cycle.

All three types of reproductive cycles have a lengthy gap between pollination and fertilization, another feature that distinguishes gymnosperms from angiosperms (Fernando et al. 2001). As described in the following section, 3-year reproductive cycle is a heterogeneous grouping.

6.9.1 One-Year Reproductive Cycle

The genera in this group include *Abies*, *Picea*, *Cedrus*, *Pseudotsuga*, *Tsuga*, *Keteleeria* (Pinaceae) and *Cupressus*, *Thuja*, *Cryptomeria*, *Cunninghamia* and *Sequoia* (Cupressaceae). As an example, female strobili are initiated in late summer or fall of 2000 then they overwinter. Female strobili emerge followed by pollination in spring 2001. Fertilization takes place in summer of 2001, only 3–4 months after pollination (Singh 1978, pp. 245–246). Cones mature and seeds are then shed by the end of 2001. Pollination and fertilization occurs within the same year in a single growing season. The pollination-fertilization interval for the 1-year cycle is measured in months, not years.

6.9.2 Two-Year Reproductive Cycle

The genera included here are *Widdringtonia*, *Sequoiadendron* (Cupressaceae) and most species of *Pinus*. Female strobilus initials are formed in late summer or fall of 2000 then overwinter. In spring 2001, female strobili emerge, receive pollen in the first spring 2001 and become conelets. The conelet goes through another winter rest and in spring 2002, archegonia form in the conelet. Fertilization of the archegonia occurs by early summer of 2002 so the pollination-fertilization interval exceeds a year. After fertilization, the conelet is considered an immature cone. Maturation occurs by autumn 2002 at which time seeds are shed. Note that in this case, the 1-year and the 2-year cycles differ mainly in the duration of the pollination-fertilization interval (Singh 1978, pp. 246–247).

6.9.3 Three-Year Reproductive Cycle

Very few conifer species have a 3-year cycle but even so, all of these species do not share the same pollination-fertilization interval. Three of these are pine species (*Pinus pinea*, *Pinus leiophylla*, *Pinus torreyana*) which have pollination and

fertilization events separated by a 2-year interval. As an example, female strobili initiated during late summer or autumn 2000 overwinter until spring 2001. Female strobili emerge then pollination occurs in spring 2001 then the pollinated strobili become conelets in 2001. The female gametophytes in the conelet develop so slowly that the megaspore does not go through free-nuclear divisions until autumn 2002. The conelet then overwinters again in the free-nuclear female gametophyte stage. Fertilization takes place by early summer 2003 and seeds mature in the cones by autumn 2003 (Singh 1978, p. 249). By contrast, the pollination-fertilization the interval varies for other species in this group. For example, *Juniperus communis* takes only one year (Ottley 1909) but for *Callitris robusta*, the pollination-fertilization interval takes 18 months (Baird 1953).

6.10 Closing

The mystery of the nocturnal pollination drop has yet to be completely solved but recent experiments have contributed substantially. Pollination can be seen as the convergence of opposing selective forces: (1) heterospory-induced divergence for male and female reproductive morphology versus (2) a precisely coordinated synchrony between female and male reproductive development as both move towards the singular goal of fertilization. The best example of this convergence is the pollination drop itself. It emerges at this critical female-male juncture, the coinciding of female strobilus receptivity and pollen shed. The drop captures pollen, hydrates pollen then positions pollen next to the spongy nucellus. Finding the pollination drop's cues opens an interesting research topic.

Conifers show a range of interesting variants on the wind-pollination systems. In some taxa, pollen capture is followed by immediate germination while others have germination delays that can last weeks or months. Most pollen germinates inside the ovule but some taxa have pollen that germinates outside the ovule. Other taxa have pollen which floats, sinks or bursts prior to pollen tube emergence. Multiple archegonia located at the micropylar end is the common condition but other taxa form large archegonial complexes and a few form archegonia at the chalazal end. Male gametes can be sperm nuclei, sperm cells or free-swimming sperm.

The female reproductive cycle spans either 1, 2 or 3 years in duration. Its duration is mostly defined by one component, its pollination-fertilization interval. In all cases, female gametophytes slowly develop well after pollination. In all cases, the elongating pollen tube is dormant during the pollination-fertilization interval. Male and female gametophyte development are independent, proceed on different timetables yet converge at the highly integrated, complex event of fertilization.

The role of the sporophyte dominates all aspects of the wind-pollination system. From strobilus initiation to pollination, the adult sporophyte develops the ovular tissues, opens the female strobilus, captures pollen grains on the micropylar arms then provides the nucellar medium for pollen germination. The female gametophyte

has not yet developed up until this point hence it exerts no known functional role (other than perhaps the pollination drop). Just prior to fertilization, the roles of sporophyte and its endosporic female gametophyte suddenly switch. The female gametophyte now becomes dominant, differentiating multiple archegonia and triggering renewed pollen tube growth. Pollination particulars show the intricacy of the monosporangiate system.

References

Baird, A. M. 1953. A life history of *Callitris*. Phytomorphology 3: 258–284.

Bramlett, D. and C. O'Gwynn. 1980. Recognizing developmental stages in southern pine flowers: the key to controlled pollinations, USDA Forest Service Southeastern Experiment Station, 14 pages.

Brown, S. and F. Bridgwater. 1987. Observations on pollination in loblolly pine. Canadian Journal of Forest Research 17: 299–303.

Breiteneder, H. 2004. Thaumatin-like proteins – a new family of pollen and fruit allergens. Allergy 59: 479–481.

Canini, A., J. Giovinazzi, et al. 2004. Localisation of a carbohydrate epitope recognised by human IgE in pollen of Cupressaceae. Journal of Plant Research 117: 147–153.

Cortegano, I., E. Civantos, et al. 2004. Cloning and expression of a major allergen from *Cupressus arizonica* pollen, *Cup a 3*, a PR-5 protein expressed under polluted environment. Allergy 59: 485–490.

Dumont-BeBoux, N., W. Weber, et al. 1998. Intergeneric pollen-megagametophyte relationships of conifers in vitro. Theor. Appl. Genet. 97: 881–887.

Doak, C. 1932. Multiple male cells in *Cupressus arizonica*. Botanical Gazette 94: 168–182.

Doyle, J. and M. O'Leary. 1935. Pollination in *Pinus*. Sci. Proc. Roy. Dublin Soc. 21: 181–190.

Fernando, D., J. Owens, et al. 2001. RNA and protein synthesis during in vitro pollen germination and tube elongation in *Pinus monticola* and other conifers. Sexual Plant Reproduction 13: 259–264.

Friedman, W. 1992. Double fertilization in nonflowering plants and its relevance to the origin of flowering plants. International Review of Cytology 140: 319–355.

Gelbart, G. and P. von Aderkas. 2002. Ovular secretions as part of pollination mechanisms in conifers. Annals of Forest Science 59: 345–357.

Gifford, E. and A. Foster. 1989. *Morphology and Evolution of Vascular Plants*. W.H. Freeman, New York.

Goddard, R. and F. Matthews. 1981. Pollen testing. Editor: E.C. Franklin. In: *Pollen Management Handbook*. Agricultural Handbook 587, Washington, DC, pp. 40–43.

Greenwood, M. 1986. Gene exchange in loblolly pine: the relation between pollination mechanisms, female receptivity and pollen viability. American Journal of Botany 73: 1433–1451.

Justus, C., P. Anderhag, et al. 2004. Microtubules and microfilaments coordinate to direct a fountain streaming pattern in elongating conifer pollen tube tips. Planta 219: 103–109.

Konar, R. and A. Moitra. 1980. Ultrastructure, cyto- and histochemistry of female gametophyte of gymnosperms. Gamete Research 3: 67–97.

Konar, R. and Y. Oberoi. 1969. Recent work on reproductive structures of living conifers and taxads - a review. Botanical Review 35: 89–116.

Labandeira, C., J. Kvacek et al. 2007. Pollination drops, pollen and insect pollination of Mesozoic gymnosperms. Taxon: 56: 663–695.

Lazarro, M. 1996. The actin microfilament network within the elongating pollen tubes of the gymnosperm *Picea abies* (Norway spruce). Protoplasma 194: 186–194.

Lazarro, M. 1998. The spermatagenous body cell of the conifer body cell of the conifer *Picea abies* (Norway spruce) contains actin microfilaments. Protoplasma 201: 194–201.

Lee, C. 1955. Fertilization in *Gingko biloba*. Botanical Gazette 117: 79–100.
McWilliam, J. 1958. The role of micropyle in the pollination of *Pinus*. Botanical Gazette 120: 109–117.
Midoro-Horiuti, T., R. Goldblum, et al. 1999. Molecular cloning of the mountain cedar (*Juniperus ashei*) pollen major allergen, *Jun a 1*. Journal of Allergy and Clinical Immunology 104: 613–617.
Midoro-Horiuti, T., R. Goldblum, et al. 2001. Identification of mutations in the genes for the pollen allergens of eastern red cedar (*Juniperus virginiana*). Clinical and Experimental Allergy 31: 771–778.
Mugnaini, S., M. Nepi, et al. 2007. Pollination drop in *Juniperus communis*: response to deposited material. Annals of Botany 100: 1475–1481.
O'Leary, S. and P. von Aderkas. 2006. Postpollination drop production in hybrid larch is not related to the diurnal pattern of xylem water potential. Tree 20: 61–66.
Ottley, A. 1909. The development of the gametophytes and fertilization in *Juniperus communis* and *Juniperus virginiana*. Botanical Gazette 48: 31–46.
Owens, J. and S. Morris. 1990. Cytological basis for cytoplasmic inheritance in *Pseudotsuga menziesii*. I. Pollen tube and archegonial development. American Journal of Botany 77: 433–445.
Owens, J., T. Takaso, et al. 1998. Pollination mechanisms in conifers. Trends in Plant Science 3: 479–485.
Pattison, J., J. Burley, et al. 1969. Development of the ovule strobilus in *Pinus kesiya* Royle ex Gordon (syn. *P. khasya Royle*) in relation to controlled pollination in Zambia. Silvae Genetica 18: 108–111.
Pettit, J. 1985. Pollen tube development and characteristics of the protein emission in conifers. Annals of Botany 56: 379–397.
Poort, R., H. Visscher, et al. 1996. Zoidogamy in fossil gymnosperms: the centenary of a concept, with special reference to prepollen of late Paleozoic conifers. Proceedings National Academy of Sciences USA 93: 11713–11717.
Runions, C. and J. Owens. 1999. Sexual reproduction of interior spruce (Pinaceae). I. Pollen germination to archegonial maturation. Intl. J. Plant Sci. 160: 631–640.
Singh, H. 1978. *Embryology of gymnosperms*. Berlin, Gebruder Borntraeger.
Suarez-Cervera, M., Y. Takahashi, et al. 2003. Immunocytochemical localization of *Cry j 1*, the major allergen of *Cryptomeria japonica* (Taxodiaceae) in *Cupressus arizonica* and *Cupressus sempervirens* (Cupressaceae) pollen grains. Sexual Plant Reproduction 16: 9–15.
Takaso, T. and J. Owens. 1996. Effects of ovular secretions on pollen in *Pseudotsuga menziesii* (*Pinaceae*). American Journal of Botany 81: 504–513.
Takaso, T., P. von Aderkas, et al. 1995. Prefertilization events in ovules of *Pseudotsuga*: ovular secretion and its influence on pollen tubes. Canadian Journal of Botany 74: 1214–1219.
Terasaka, O. and T. Niitsu. 1994. Differential roles of microtubules and actin-myosin cytoskeleton in the growth of *Pinus* pollen tubes. Sexual Plant Reproduction 7: 264–272.
Tomlinson, P. 1994. Functional morphology of saccate pollen in conifers with special reference to the Podocarpaceae. International Journal of Plant Sciences 155: 699–715.
Tomlinson, P., J. Braggins, et al. 1997. Contrasted pollen capture mechanisms in Phyllocladaceae and certain Podocarpaceae (Coniferales). American Journal of Botany 84: 214–223.
von Aderkas, P. and C. Leary. 1999. Micropylar exudates in Douglas fir – timing and volume of production. Sexual Plant Reproduction 11: 354–356.
Wagner, R., S. Mugnaini et al. 2007. Proteomic evaluation of gymnosperm pollination drop proteins indicates highly conserved and complex biological functions. Sexual Plant Reproduction 20: 181–189.
Williams C. 2008. Selfed embryo death in *Pinus taeda*: a phenotypic profile. New Phytologist 178: 210–222.
Willson, M. and N. Burley. 1983. *Mate choice in plants*. Princeton NJ, Princeton University Press.
Wilhelmi, L. and D. Preuss. 1999. The mating game: pollination and fertilization in flowering plants. Current Opinions in Plant Biology 2: 18–22.