Chapter 8
The Dynamic Wind-Pollinated Mating System

Summary The dynamic wind-pollinated mating system in conifers is more than a random game of pitch and catch; orderly forces work towards maximizing chances of pollen capture while minimizing selfing and interspecific hybridization. Aerodynamics of moving branches, leaves and female strobili favor pollen movement into ovules while more cryptic molecular mechanisms influence paternal parent choice from pollination onward to seed maturity. Outcrossing is the general outcome for most conifers but a few interesting exceptions include mixed mating systems, selfing, hybridization, reproductive sterility and the singular case of paternal apomixis. Self-pollination occurs at moderate rates yet few selfed seed are recovered in some of the Pinaceae; most selfed embryos die before reaching maturity so this is known as the embryo lethal system. Hybrid matings can be blocked by a few pre-zygotic barriers but more often matings between close relatives produce viable, fertile F1 offspring without a change in ploidy. Conifer reproduction is often abundant to the point of nuisance; it is not unusual for a conifer's wind-pollinated mating system to have a genetic footprint extending tens or even hundreds of kilometres from adult trees. At the other extreme, rare cases of reproductive sterility are reported for both the Pinaceae and the Cupressaceae.

Conifers have complex wind-pollinated mating systems. Although they lack colorful flowers and the means to attract bees, birds or butterflies as pollinators, their mating systems are no less intriguing. These wind-pollination systems are closer to an exercise in aerodynamics efficiency between donor and recipient than hit-and-miss capture. Release pollen too soon and the seed will have the same parent as mother and father. Catch pollen originating too far away and the seed might be a hybrid between different species. If synchrony in time and space fails then safeguards select against pollen or the offspring of the unwanted pollen parent. Mate choice is increasingly nonrandom after pollination. But little is known about how selective mechanisms operate in conifers.

Pollen capture occurs within the confines of a local aerodynamic environment created by the female strobilus, needles and branches, all moving with gusting wind currents (Niklas 1982; Niklas 1984). This is known as the turbine model because the female strobilus resembles a turbine. Wind tunnel studies show that

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

the female strobili of *Pinus taeda* generates its own micro-turbulence pattern suited to capture of conspecific pollen (Niklas 1984). Needles create tiny airflow eddies around the female strobilus which act to trap pollen in between flexed cone scales (Niklas 1985).

The aerodynamics of pollination extend beyond the female strobili; in real-time field studies, the branches subtending the female strobilus enhance the local aerodynamic environment in favor of pollen capture. Gusting causes the branches to oscillate such that they sweep in circular arcs. This circular arc alters the inclination angle of the female strobili at the tops of the branches, tilting at a 45-degree angle with strobili tips pointing downwind which favors pollen capture (Niklas 1985). The turbine model is elegant but it is also possible that pollen accumulation occurs only by simple impaction (Cresswell et al. 2007). The aerodynamics of pollination are not yet fully resolved.

Subsequent events leading up to pollen capture are not random either. These are modulated by various recognition systems. One such system exists for the pollination drop which can distinguish a pollen grain from other small particles. Another system signals for pollen tube arrest if non-specific pollen germinates inside the ovule; this has been reported only for pollen from distantly related species or other subgenera (McWilliam 1959; Hagman 1975).

The signal for renewed pollen tube growth just prior to fertilization is also part of a recognition system (Takaso et al. 1996). But perhaps better described is the embryo lethal system. the self-exclusion system operative after zygote formation (Koski 1971). Selfed embryos usually die between the proembryo and late embryogeny stages (Koski 1971). All of these recognition systems, yet to be fully elucidated at the molecular level, show that pollination and mate choice in conifers are orderly events.

8.1 Wind-Pollinated Mating System: Outcrossing or Mixed Selfing/Outcrossing

Nearly all mating systems of conifers are predominantly outcrossing or mixed selfing/outcrossing (Table 8.1). As a general rule, conifers and particularly the Pinaceae, are outcrossing. Predominantly outcrossing species are defined as having less than 5% selfed seed (Brown 1990). Selfing is measured by s where s = 0.05 in this case and its complementary portion (t = 1–s) measures outcrossing. Outcrossing among conifers (Table 8.1) has been well-documented. This can be seen from Table 8.1. which includes a part of the larger meta-analysis study of 52 conifers (O’Connell 2003).

A few exceptions to outcrossing shown in Table 8.1 deserve brief mention. These include *Larix laricina* (Pinaceae) and *Thuja occidentalis* (Cupressaceae) which have population outcrossing rates as low as t = 0.53 (Knowles et al. 1987) and t = 0.51 (Perry and Knowles 1990), respectively. These two species are considered

Table 8.1 Outcrossing rates for a few members of the Pinaceae family (Table modified from O'Connell 2003 and Mitton and Williams 2006)

Species	t	Reference
Picea chihuahuana	0.076	Ledig et al. 1997
Larix laricina	0.729	Knowles et al. 1987
Pinus albicaulis	0.736	Krakowski et al. 2003
Pseudotsuga menziesii	0.752	Stauffer and Adams 1993
Picea glauca	0.730	Innes and Ringius 1990
Abies alba	0.890	Schoeder 1989
Pinus sibirica	0.894	Krutovskii et al. 1995
Pinus taeda	0.994	Friedman and Adams 1985
*Picea abie*s	0.956	Morgante et al. 1991
Pinus sylvestris	0.940	Muona and Harju 1989
Picea mariana	0.924	Boyle and Morgenstern 1986
Pinus koraiensis	0.974	Krutovskii et al. 1995
Pinus ponderosa	0.960	Mitton et al. 1977, 1981
Pinus flexilis	0.980	Schuster and Mitton 2000
Abies procera	0.940	Siegismund and Kjaer 1997
Pinus radiate	0.900	Moran et al. 1980
Picea omorika	1.00	Kuitteinen and Savolainen 1992

to be mixed mating systems rather than predominantly outcrossing mating systems (Brown 1990; Mitton 1992). Such a mixed mating system is a likely explanation for the high degree of population differentiation of fragmented conifer species such as *Cathaya argophylla* (Ge et al. 1998) which is not shown in Table 8.1.

Perhaps the most peculiar case shown in Table 8.1 is *Picea chihuahuana*, a species occurring in small, isolated populations in northwest Mexico. It has population outcrossing rates as low as t = 0.076 so it is defined as predominantly selfing (Ledig et al. 1997). It is not yet clear whether this is the lone exception among conifers; all mating systems are not been classified for conifers worldwide.

In the following sections, other exceptions to the outcrossing rule are addressed. These include selfing, interspecific hybridization, reproductive sterility and the unusual case of paternal apomixis (Chapter 2) where unreduced diploid pollen grows into embryos inside its surrogate maternal parent (Pichot et al. 2001).

8.2 Selfing

Selfing, only possible for monoecious conifers, can only be geitonogamous because conifers are monosporangiate. Geitonogamy refers to the case where male and female strobili occur on the same plant but not in a single strobilus (Richards 1997). Conifers do not have autogamy.

8.2.1 Selfing Avoidance

Selfing can be avoided in part by spatial and temporal separation of male and female strobili (Erickson and Adams 1989). Female *Pinus taeda* strobili mostly emerge on the upper branches of the crown, far above the male strobili in older trees (Greenwood 1980); this temporal separation is defined as dichogamy. Younger trees tend to have a prevalence of female or male strobili throughout the crown (Chapter 2). Older *Pinus ponderosa* trees produce more cones and less pollen. Young *Pinus ponderosa* trees are the opposite, serving as pollen donors to older trees loaded with female strobili (Mitton 1992; Mitton and Williams 2006). Proportions of male versus female strobili are also another factor in selfing rates.

Selfing is also avoided if female strobili reach peak receptivity before male strobili on the same tree release pollen (Greenwood 1986). Even so, these are incomplete barriers. Selfing rates fluctuate from year to year with vagaries of weather, wind speeds and age of the tree (Mitton 1992). External factors such as wind speed, wind direction or even stand density determine how much pollen from non-self trees reach receptive female strobili (Mitton 1992; Dyer and Sork 2001). Proportions of selfed pollinations are subject to chance; they vary widely from tree to tree and from year to year. But from pollination onward, mate choice in conifers becomes increasingly nonrandom.

8.2.2 Selfed Embryo Deaths During Development

Direct estimates of selfing are difficult to obtain. Estimated proportions of selfing range from 10% to 25% in *Pinus sylvestris* (Sarvas 1962; Koski 1971). Self-pollination rates are higher than selfed seed recovery (Chapter 9).

The self-incompatibility systems, acting before fertilization, are well characterized for angiosperms but none have not been reported for any conifers yet. Many members of the Pinaceae family exclude selfed embryos via the embryo lethal system. This steep post-fertilization barrier to selfed embryos is attributed to inbreeding depression due to abundant deleterious mutations. More concerted death peaks also occur during embryo development (Williams 2008) but in either case, few viable seeds are recovered from self-pollinations.

8.3 Interspecific Hybridization

Conifers, particularly members of the Pinaceae, do hybridize naturally with sympatric relatives. If two parental species are not closely related then pollen tube arrest or other aberrant signs appear (McWilliam 1959). The degree of incompatibility ranges from slight to strong for many interspecific crosses made between distantly related *Pinus* species or distant related *Picea* species, as shown by Hagman (1975). As one would expect, crosses between soft and hard pines produced the strongest signs of incompatibility between pollen and nucellar tissues; this was described as a pathogen invading a host plant (Hagman 1975).

But other matings between closely related species do produce viable F1 offspring which often prove to be fertile adults. Examples include hybrid complexes for pines documented in the paleobotanical record (Mason 1949) as well as present-day examples found in major centers of species diversity ranging from the southeastern United States (Edwards-Burke et al. 1997), China (Wang et al. 2001; Ma et al. 2006) or the highlands of Mexico (Matos and Schaal 2000). Divergent environmental factors favor stable hybrid complexes; this is the case for the naturally occurring hybrid between Coulter and Jeffrey pine which parallels major physiographic gradients (Zobel 1951). Hybridization is an open-ended, reticulating event; such hybrids not only freely introgress with one another but they can mate with either parental species or even a third sympatric relative.

But the fate of a hybrid event is not always a new species (Table 8.2). The outcome of hybridization in conifers varies widely but it does seem to depend on three factors: (a) hybrid vigor, (b) reproductive isolation and (c) ecological divergence. Rather, a conifer hybrid swarm can reach a state of equilibrium or continue to differentiate into a new species. This has been elegantly shown using a series of phylogenetic reconstructions for two *Pinus* species and their hybrid in Asia (Wang et al. 2001; Ma et al. 2006).

Artificial matings between closely related species tend to produce fertile offspring without a change in ploidy. This was established by early taxonomy studies based on huge, systematic crossability studies (i.e. Righter and Duffield 1951). Successful hybridizations were limited to a subsection or rarely between two closely related subsections. Artificial hybrids have even been made between divergent North American and Asian soft pines within the same subsection and these hybrids become fertile F1 adults (Stone and Duffield 1950). One such hybrid was

Table 8.2 Fate of hybrid complexes for different seed plants. A hybrid complex refers to a group of species in which natural hybridization has occurred. Hybrid complexes are classified according to their fate or stabilization mode of reproduction in the natural hybrids or hybrid progeny (Grant 1981)

Type	Stabilization mode	Examples
Homogamic	Sexual reproducing diploids with normal meiosis	*Pinus* spp. (Pinaceae) *Gilia* spp. (Polemoniaceae) *Eucalyptus* spp. (Myrtaceae)
Clonal complex	Vegetative reproduction	*Opuntia* spp. (Cactaceae)
Agamic	Apomixis; unfertilized seeds	*Citrus* spp. (Rutaceae)
Heterogamic	Permanent translocation heterozygosity; permanent odd polyploidy	*Rosa canina* (Rosaceae) *Oenethera biennis* (Onagraceae)
Polyploid	Sexual reproducing polyploids	*Sanicula* spp. (Umbelliferae) *Asplenium* spp. (Polypodiaceae)

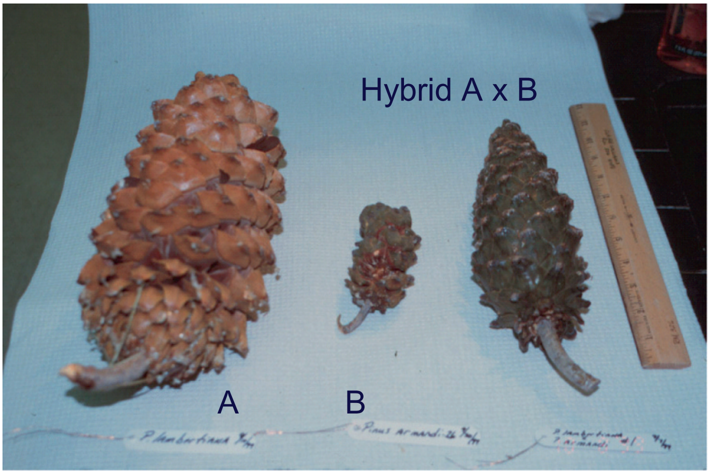

Photo 8.1 A comparison of cone size between a hybrid and its two parental species, a North American soft pine, *Pinus lambertiana* (Parent A) and an Asian soft pine, *Pinus armandii* (Parent B). Their hybrid, planted in the Arnold Arboretum, has a cone size intermediate between the two parental species

known as *Pinus x schwerinii*, came from a cross between Asian soft pine *Pinus wallichiana* x North American soft pine *Pinus strobus* although both parents belong to the same subsection *Strobi* (Price et al. 1998). The hybrid has intermediate reproductive characters such as the cone size comparison (see Photo 8.1).

The fate of hybridization is a useful means of classification (Table 8.2) (Grant 1981). Conifers belong to the homoploid hybrid complex which has the following properties: newly derived species of hybrid origin is diploid or at least undoubled (homoploid) compared to the original parental species, backcrossing to parental species is possible because F1 hybrids have no reproductive isolation created as a direct consequence of hybridization and recombination rates are comparable between hybrid derivatives and their parental species (Grant 1981). Homogamic species complexes are often composed of multiple interfertile species and their hybrids (Grant 1981) suggesting a species complex acts as a single gene pool (Fig. 8.1).

Conifers loosely meet all three conditions. Adult hybrids have stable diploid genomes (Chapter 3), this is the case for the adult hybrid *Pinus x schwerinii* in Photo 8.1 (Williams et al. 2002). This hybrid is also capable of backcrossing to either parent so the second condition is met. Recombination rates are more difficult to quantify. They are similar for two *Larix* species and their hybrid (Sax 1932); however, it is not clear if this condition full met because other F1 hybrids for *Pinus* spp. show lower recombination rates relative to the parental species (Shepherd and Williams 2008). Meiotic abnormalities have also been reported for other *Pinus* F1 hybrids (Saylor and Smith 1966).

What is known about adult F1 hybrids when they reach reproductive onset? In *Pinus* spp., F1 hybrids have a stable diploid genome and stable ploidy (Williams

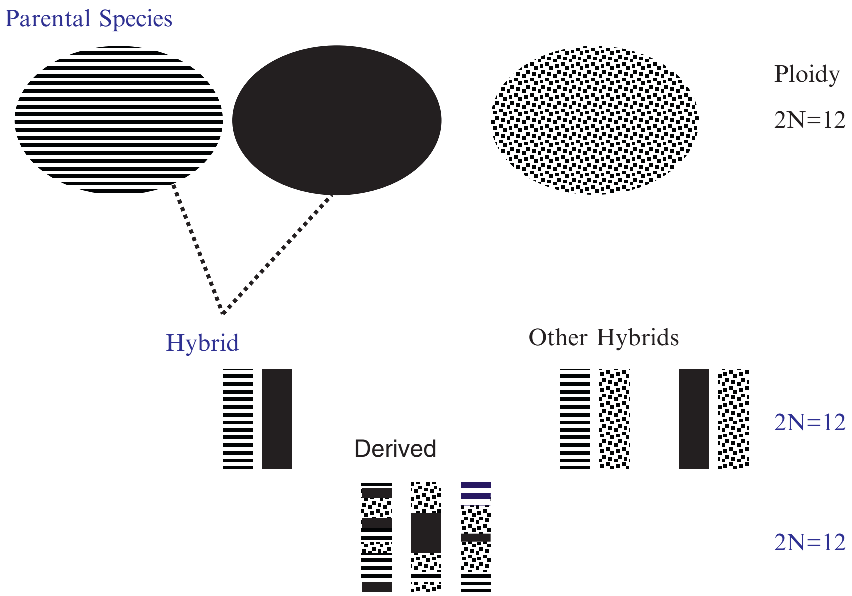

Fig. 8.1 Many conifers mate with sympatric relatives to form homogamic hybrid complexes, as described by Grant (1981) in *Plant Speciation*. Newly derived hybrids are diploid and capable of crossing to their respective parental species. Homogamic hybrid complexes are often composed of multiple, interfertile species and their hybrids

et al. 2002) which adheres to Grant's predictions by the hybrid classification system. No meiotic abnormalities were observed for female meiosis for an adult F1 hybrid soft pine (Sax 1960). Male meiosis for the F1 adult shows more abnormalities such as pollen abortion (Saylor and Smith 1966) and some sterile pollen (Sax 1960). Early stages of male gametophytes sometimes show chromosomal abnormalities and these cannot develop into pollen grains (Saylor and Smith 1966). Still other F1 hybrids have viable pollen grains (Saylor and Smith 1966). No one condition applies to all F1 hybrid adults.

Chromosomal rearrangements such as tiny paracentric inversions could prove useful for reconstructing past hybridization events in conifer species complexes. As shown in many other eukaryotes, paracentric inversions serve the role of recombination suppression in the hybrid genome by preserving large blocks of co-adapted gene complexes. Although hybrid genomes in pines do have unusually high meiotic and genomic stability, the occasional small-scale paracentric inversion can be detected when the F1 hybrids are crossed back to a parental species (Saylor and Smith 1966; Shepherd and Williams 2008).

The best case study of hybridization and its evolutionary consequences is illustrated by *Pinus densata*, a native of the Tibetan Plateau (Wang et al. 2001). This species originated from hybridization between *Pinus tabuliformis* indigenous to northern to central China and *Pinus yunnanensis* which is limited to southwestern China. Hybridization occurred possibly before the uplift of the Tibetan Plateau over

45 million years ago. Multiple hybridization events must have occurred because populations of *Pinus densata* sampled from different parts of the Tibetan Plateau show reciprocal parentage and thus can be assumed to have independent origins (Ma et al. 2006). These studies of *Pinus densata* show homoploid hybrid speciation. No ploidy change accompanied hybrid speciation. Speciation was favored by two factors: adaptation to an extreme environment and ecological isolation away from both parental species (Wang et al. 2001; Ma et al. 2006).

Chromosomal evidence of these multiple hybridization events is still apparent. The chromosomal landmarks of both parental species can still be identified using molecular cytogenetics (Liu et al. 2003). Novel rearrangements specific to the hybrid are also present (Liu et al. 2003). Such novel, fine-grained rearrangements arose after reproductive isolation rather than as an immediate consequence of hybridization (Liu et al. 2003). Millions of years have passed since this hybrid speciation took place yet its chromosomal signature is still present, attesting to the highly conserved nature of the pine genome.

8.4 Reproductive Sterility

The abundant reproduction of the Pinaceae is often taken for granted or even regarded as a nuisance; a single pine tree can produce millions of offspring and countless pollen grains within its long lifespan. But there are rare cases of conifer sterility reported and this holds growing interest to those who want to curtail unwanted seeds and pollen from transgenic or genetically modified conifers (Williams 2006; Williams et al. 2006).

In the first case, a single *Pinus monticola* tree produced male strobili but no pollen (Wilson and Owens 2003). Closer investigation showed that both male and female gametophyte development was arrested soon after meiosis. Pollen grains had poorly developed pollen walls, reduced cytoplasm and did not release. Male strobili aborted before dehiscence. These changes were mediated in part by a malfunction of the tapetal layer. On the female side, meiosis of the megaspore mother cell provided four megaspores yet no female gametophyte developed from any of the megaspores (Wilson and Owens 2003).

In the second case, sterility was the result of aberrant male and female meiosis in *Cryptomeria japonica* (Cupressaceae) (Hosoo et al. 2005). A few maturing pollen grains were produced but these were uneven in size. The female megaspores were also uneven in size. A few megaspores did survive only to die at the archegonium formation stage (Hosoo et al. 2005). Both cases suggest that isolating or inducing tapetal and meiotic mutants are promising research areas for operational sterility in conifers.

A third case is mimicry of reproductive sterility. A bizarre case of insect parasitism causes the mimicry: the chalcid *Megastigmus spermotrophus* infests a female gametophyte inside an unfertilized ovule then induces the female gametophyte to develop normally as though it has been fertilized and has no embryo (von Aderkas et al. 2005a, b). What are the regulatory cues induced by the chalcid?

8.5 Closing

A close look at the conifer mating system shows the precise synchrony between male and female reproductive development through space and time. Although outcrossing is prevalent within a species, selfing, hybridization and reproductive sterility also occur. For monoecious conifers, self-pollination is avoided through spatial or temporal separation of male and female strobili but when it does occur, the selfed embryos are excluded after zygote formation. Conifers are thought to lack the pre-zygotic mechanism of self-incompatibility typical of many angiosperm taxa but this area of research continues using better genomics-based tools. Naturally occurring hybridization can produce fertile adult F1 individuals which are capable of backcrossing to the parental species or even a third species; this raises the difficult question of actual effective population size. Species complexes for conifers are not unusual; they commonly occur in present-day centers of species diversity as well as in the paleobotanical record.

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