CHAPTER 16

MAGNITUDE AND IMPLICATIONS OF GENE FLOW IN GENE CONSERVATION RESERVES

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SUMMARY

A practical means of long-term genetic conservation in forest trees is to establish natural (*in situ*) populations (i.e. gene resource management units, GRMUs) or *ex situ* plantings as gene conservation reserves. Results from pollen contamination studies in conifer seed orchards, however, indicate that gene flow in such reserves could be extensive. Although gene flow can be beneficial in terms of introducing new genetic variants, immigration of genes from domesticated populations is likely to reduce total genetic diversity within reserves and potentially lower their fitness. The prudent position on gene flow in reserves, therefore, is to limit it as much as possible. Pollen gene flow can be eliminated in *ex situ* plantings by controlled mating. If controlled mating is not feasible, applying pollen management techniques to increase pollen production within the plantings relative to external sources can minimise gene flow. Strategies for reducing gene flow in GRMUs are to make reserves as large as possible, include buffer zones around reserves (perhaps planted with an exotic species), and to assure that natural regeneration, or seed collection for artificial regeneration, occurs in heavy seed-crop years. Gene flow in forest trees is still poorly understood and the effectiveness of various approaches to limit gene flow in reserves, especially in GRMUs, cannot be quantified with any accuracy. Thus, research on gene flow in forest trees should receive high priority.

16.1 Introduction

Establishment of gene resource management units (GRMUs) is often proposed as a primary means of preserving genetic diversity in forest trees (Ledig 1988; Millar & Libby 1991). GRMUs are defined as parcels of land chosen to include a representative sample of the genetic diversity of the target species in a region, and designated for long-term genetic management. *In situ* reserves are superior to *ex situ* methods of genetic conservation because target species can continue to evolve in their native habitats, and because entire ecosystems are conserved, including other targeted and non-targeted species. In addition, as long as regeneration by local, native, seed sources is assured, timber harvest within GRMUs is compatible with the primary goal of preserving genetic resources.

In intensely managed species it is expected that GRMUs will eventually be surrounded by plantations of improved varieties. A question then arises as to the extent to which gene flow (i.e. immigration of genes via pollen or seeds from surrounding plantations) will influence the integrity of GRMUs. Because the size of GRMUs is central to their acceptance by managers, much has been written about minimum population numbers required to prevent loss of genetic diversity or adaptive potential due to inbreeding and genetic drift in small populations (National Research Council 1991; Frankel *et al.* 1995). Little attention, however, has been directed to potential negative effects of gene flow (Ellstrand 1992a; Ellstrand & Elam 1993). Given large differences in the genetic composition of GRMUs and surrounding plantations, even small amounts of gene flow in GRMUs could influence profoundly their genetic composition and adaptation potential. The magnitude of gene flow and factors influencing this magnitude, therefore, are of much interest.

A problem in evaluating potential effects of gene flow in GRMUs is the paucity of information on the magnitude of gene flow in native populations. Studies of patterns of genetic diversity among and within populations of forest trees using isozyme genetic markers (allozymes), indicate that gene flow has been strong enough in most cases to prevent genetic divergence among populations within regions (see Chapter 6; Ellstrand 1992b; Hamrick *et al.* 1992). Nevertheless, since only a small amount of gene flow is needed to arrest divergence among populations, it is impossible to discern from these data what the magnitude of gene flow might be in any one generation (Frankel *et al.* 1995).

One source of information on the potential magnitude of pollen gene flow in GRMUs are studies of pollen contamination in forest tree seed orchards. Seed orchards are important in forestry because they are the primary source of genetically improved seeds used in reforestation (Zobel & Talbert 1984). Orchards consist of either clones (i.e. grafted cuttings) or offspring (families) of parent trees selected for desirable characteristics (e.g. fast growth, disease resistance, favourable wood quality) in breeding programs. The number of clones (or families) typically ranges from 50 to several hundred, each replicated many times in the orchard. For efficient management, orchard sites often contain more than one orchard block, with the parents of each block derived from a separate geographical region. Because orchard blocks rely primarily on open (i.e. wind-mediated or animal-mediated) pollination, the potential for fertilisation by pollen sources outside blocks (i.e. pollen contamination) is always possible. Pollen contamination is detrimental because it reduces the genetic gains achieved by breeding, and if contaminant pollen comes from trees not adapted to the intended planting region, the adaptability of orchard seed is also negatively affected.

Estimates of pollen contamination (i.e. proportion of offspring sired by contaminant pollen) have been obtained recently for a variety of species and orchard management conditions with the aid of allozymes. In this chapter, we summarise the results of pollen contamination studies and evaluate their relevance to predicting levels of gene flow in GRMUs. We then discuss implications of gene flow in terms of effects on the integrity and fitness of GRMUs, and ways in which gene flow might be limited. *Ex situ* plantings of genetic resources (e.g. clone banks, arboreta, provenance trials) are also important in forest genetic conservation programs (National Research Council 1991; Rogers & Ledig 1996), and may even be more susceptible to gene flow. Thus, we also discuss implications of gene flow in *ex situ* plantings. We refer to GRMUs and *ex situ* gene conservation plantings collectively in this chapter as 'gene conservation reserves'.

16.2 Pollen contamination in seed orchards

Because pollen contamination (m) is the proportion of orchard seeds resulting from fertilisation by background stands (versus fertilisation by pollen produced within the orchard), it ranges in magnitude

Box 16.1 Statistical estimation of pollen contamination

Several procedures are used to estimate pollen contamination from genotypes observed in the offspring of mother trees (Smith & Adams 1983; Friedman & Adams 1985; El-Kassaby & Ritland 1986a; Devlin & Ellstrand 1990; Xie *et al.* 1991; Stewart 1994; Adams *et al.* 1997). The most commonly used approach is based on simple paternity exclusion (Smith & Adams 1983; Devlin & Ellstrand 1990). The first step is to determine the multilocus genotypes of all parents in the recipient population. Multilocus genotypes of seed offspring are then compared to parental genotypes and the proportion of offspring that could not have been sired by males in the recipient population are determined (detected immigrants). The proportion of detected immigrants (b) provides only a minimum estimate of pollen contamination because some immigrants are likely to have multilocus genotypes that are indistinguishable from those that can be produced by parents within the recipient population. To estimate the true proportion of immigrants (m), b must be adjusted by the probability that an immigrant offspring has a detectable genotype. This process is relatively straightforward in conifers where the genotype of a pollen gamete can be directly inferred by comparing the genotype of the megagametophyte (equivalent to the female gamete) to that of the embryo in the same seed. The detection probability, d, can then be estimated from allele frequencies in surrounding stands, such that:

$$m = \frac{b}{d} \quad (1)$$

In angiosperms, estimating m by paternity exclusion is more complicated because genotypes in pollen gametes rarely can be determined directly and detection probabilities vary depending upon the genotype of the mother (Devlin & Ellstrand 1990). As an example of estimating m, 16 out of 200 seeds sampled from an orchard crop had pollen gametes with multilocus genotypes that could not have been produced by parents in the orchard. Thus, a minimum estimation of pollen contamination is $b = 16/200 = 0.08$. The probability that stands surrounding this orchard produce pollen gametes with genotypes different from those produced within the orchard is $d = 0.19$. The estimate of m is, therefore, $0.08/0.19 = 0.42$.

from 0 (no contamination) to 1 (total contamination). We list pollen contamination estimates (m, Box 16.1) for seed orchards of six conifer species, but include only cases where orchards have not been subjected to special management conditions to limit contamination (Table 16.1). The estimates, which cover a wide range of orchard sizes, ages and isolation (distance to nearest stands of the same species), range widely (0.01–0.91), but on average are quite high (mean $m = 0.45$). In some cases, only a minimum estimate of m, the proportion of detected contaminants (b), was available (Box 16.1). Typically only 0.25 to 0.5 of contaminated seeds are detected genetically using allozymes (see references in Table 16.1); thus, these minimum estimates probably underestimate true m two-fold to four-fold, supporting the high levels of pollen contamination observed in the other orchards.

Among factors which influence the magnitude of pollen contamination in seed orchards are:

(a) degree of isolation from background stands

(b) orchard size

(c) pollen production within the orchard

(d) synchrony of flowering in the orchard with flowering in background stands (although not botanically correct, in this chapter we refer to mating structures in conifers as flowers).

In cases where isolation distances are not reported, it is likely that there is no separation between the orchard and stands of the same species. Thus, cases of more than nominal isolation of orchards in Table 16.1 are few. Nevertheless, it is clear from the estimates of m, that isolation of less than a few hundred metres affords little protection from pollen contamination. The lowest m among all reported was for a *Picea glauca* (Moench) Voss (white spruce) orchard with isolation of 1000 m (Stewart 1994). In addition, the lowest

TABLE 16.1 **Estimates of proportion of detectable immigrants (*b*) and pollen contamination (*m*) in conifer seed orchards**

		Orchard					
Species	Location	Size (ha)	Isolation (m)[A]	Age	*b*	*m* (s.e.)	Reference
Larix decidua	Slovakia	—[B]	—	—	0.05	—	Paule & Gomory (1992)
Picea abies	Sweden	—	—	—	0.10	—	Paule *et al.* (1991)
	Sweden	—	—	—	0.17		Paule *et al.* (1991)
Picea glauca	Canada	—	1000	11–12	—	0.01 (0.01)	Stewart (1994)
Pinus sylvestris	Germany	—	—	—	0.02	—	Müller-Starck (1982)
	Finland	3.0	—	27	—	0.33 (0.03)	Harju & Muona (1989)
	Finland	3.2	—	29–33	—	0.26[C] (0.02)	Harju & Muona (1989)
	Finland	22.9	2000	20–23	—	0.48 (0.06)	Harju & Nikkanen (1996)
	Finland	22.7	—	31–33	0.18	0.67[D]	Pakkanen & Pulkkinen (1991)
	Finland	13.7	—	20–22	0.06	0.49[D]	Pakkanen & Pulkkinen (1991)
	Finland	6.0	—	26	—	0.53 (0.10)	Pakkanen *et al.* (1991)
	Poland	3.0	1000	16–18	0.15[D]	—	Burczyk (1992)
	Slovakia	—	—	11–12	0.11	—	Paule & Gomory (1992)
	Sweden	6.0	500	18–25	0.38	—	Nagasaka & Szmidt (1985)
	Sweden	16	—	14–18	0.21	—	El-Kassaby *et al.* (1989b)
	Sweden	12.5	—	17–18	0.36	—	El-Kassaby *et al.* (1989b)
	Sweden	13.8	—	18–21	0.35[E]	—	Paule (1991)
	Sweden	12.5	100+	17–18	—	0.72[F]	Yazdani & Lindgren (1991)
	Sweden	16	100	29–31	0.29	0.56	Lindgren (1991)
	Sweden	16	100	25–27	0.30	0.55	Wang *et al.* (1991)
Pinus taeda	S. Carolina	2.0	100	15–17	—	0.36[G] (0.03)	Friedman & Adams (1985)
	Texas	—	—	—	—	0.51 (0.05)	Wiselogel (1986)
Pseudotsuga menziesii	Oregon	1.8	None	14	—	0.52[H] (0.06)	Smith & Adams (1983)
	Oregon	—	None	20	—	0.29	Smith & Adams (1983)
	Oregon	3.3	None	8–9	—	0.91 (0.08)	Adams & Birkes (1989)
	Oregon	2.0	None	14–24	—	0.49[I] (0.05)	Adams *et al.* (1997)
	Washington	20	500	15	—	0.11	Wheeler & Jech (1986)
	B.C. Canada	—	None	11	—	0.34	Xie *et al.* (1991)

[A]Distance to nearest stand of the same species. [B]A dash means no information or estimate available. [C]Mean over four crop years. [D]Mean over three crop years. [E]Mean for two orchard blocks in one crop year. [F]Mean for three orchard blocks in two crop years. [G]Mean over two orchard blocks in three crop years. [H]Mean for 10 orchard blocks in one crop year. [I]Mean for one orchard block in five crop years.

among several *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) estimates (m = 0.11), was for a Washington orchard with isolation of 500 m (Wheeler & Jech 1986). A Finnish *Pinus sylvestris* L. (Scots pine) orchard was separated by 2000 m from the nearest stands of the same species, yet m was very high (0.48, Harju & Nikkanen 1996). Some individual *Pinus sylvestris* trees, however, were scattered in the *Picea abies* (L.) H. Karst. (Norway spruce) stands immediately surrounding the orchard. The value of isolation in limiting pollen contamination in seed orchards is unclear. Isolation distances of at least 500 m to 1000 m appear necessary for at least some protection. Nevertheless, large amounts of pollen can be dispersed into seed orchards from stands 50 km to 60 km away (Di-Giovanni *et al.* 1996). If this far-distant source of pollen is an important component of contamination, isolation zones within the natural range of species may be ineffectual.

Although gene flow is expected to decrease as recipient populations become larger (Ellstrand 1992a; Ellstrand & Elam 1993), no relationship between pollen contamination and orchard size is evident in Table 16.1. A complicating factor is that the magnitude of orchard flowering can vary widely from year to year, and estimates of orchard pollen production are often not available. Fertilisation and stem girdling were applied in a 15-year-old *Pseudotsuga menziesii* orchard to increase flowering (Wheeler & Jech 1986). As pollen production within orchard blocks increased, m decreased dramatically. Levels of pollen contamination, however, did not vary significantly over six crop years in another *Pseudotsuga menziesii* orchard where pollen production ranged six-fold (Adams *et al.* 1997). Likewise, there was no relation between the magnitude of pollen crops within orchards and contamination over three years in two *Pinus sylvestris* seed orchards (Pakkanen & Pulkkinen 1991). The influence of within-orchard flowering on contamination cannot be completely assessed without information on levels of pollen produced by background sources, because it is the relative concentration of orchard versus background pollen that largely determines the success of contaminant pollen (Adams 1992). By placing pollen traps inside the orchard to measure the sum of pollen produced by orchard and background sources, and in open fields nearby to measure background pollen levels, pollen contamination can be estimated as the ratio of background to orchard pollen cloud densities (Greenwood & Rucker 1985; Webber & Painter 1996). Estimates of pollen contamination based on relative pollen cloud densities have been found to roughly approximate m derived from genetic markers (Greenwood & Rucker 1985; Wheeler & Jech 1986).

The degree of synchrony in floral phenology between the orchard and background stands is another factor influencing relative fertilisation success of background versus orchard pollen. Normally, if an orchard is located in the same region as its parents there is large overlap in floral phenology with background stands. Flowering within *Pseudotsuga menziesii* orchards, however, can be delayed, relative to background stands, by slowing flower development in late winter and spring with cooling water mists. This technique, called 'bloom delay' can be very successful in reducing pollen contamination in this species. For example, in a 15-year-old orchard in the State of Washington, m in one orchard block was reduced by more than 50% from 1983 (m = 0.56), when there was no bloom delay, to 1985 (m = 0.26), when bloom delay was applied (Wheeler & Jech 1986). Pollen density within this block increased three-fold during this period, suggesting that the reduction in m may also be due to an increased concentration of within-block pollen. Nevertheless, in an adjacent orchard block with a similar increase in pollen production from 1983 to 1985, but where bloom delay was not applied, m decreased only from 0.43 to 0.33. Bloom delay also appears to have had a dramatic effect on pollen contamination in a British Columbia seed orchard. In a year when bloom delay was applied, m was nearly zero (0.002) (El-Kassaby & Ritland 1986a). In the following year, with no bloom delay (but, with poorer within-orchard flowering), m = 0.12 (El-Kassaby & Ritland 1986b). Although a promising tool, application of bloom delay requires very special circumstances (e.g. overhead irrigation systems, good soil drainage) and may not work in all cases (Webber & Painter 1996). Indeed, few *Pseudotsuga menziesii* orchards use this methodology, either because they have no overhead irrigation system, or because trees have become too large, making misting impractical.

16.3 Expected magnitudes of gene flow in gene conservation reserves

How relevant are estimates of pollen gene flow in conifer seed orchards to predicting gene flow in gene conservation reserves? Levels of pollen contamination

in *ex situ* gene conservation plantings of conifers probably will be similar to those observed for seed orchards. *Ex situ* plantings resemble orchards in terms of containing a single species, and having limited size (2–20 ha), uniform spacing of individuals and intensive management (e.g. competition control, fertilisation). Evidence that pollen gene flow can also be large in small populations of insect-pollinated angiosperm trees comes from several studies of effective pollen dispersal in natural populations (see following paragraphs).

GRMUs, however, could differ from seed orchards in several ways. First, one might expect GRMUs to be much larger than seed orchards. GRMUs must be large enough to ensure with reasonable probability that the reserve will survive and evolve in perpetuity (Ledig 1986; Millar & Libby 1991; National Research Council 1991; Rogers & Ledig 1996). The minimum number of individuals required for this purpose is called the minimum viable population (MVP) size. MVP size depends on several demographic, genetic and environmental factors that are likely to vary unpredictably over time (National Research Council 1991; Frankel *et al.* 1995). The MVP size most often suggested as a minimum necessary to maintain evolutionary potential (i.e. genetic diversity) is 500 (Frankel *et al.* 1995), but Lande (1995) argues that this number is an order of magnitude too small. Furthermore, MVP sizes refer to effective population size (N_e). Frankel *et al.* (1995) suggest that N_e is 20% to 10% of the actual census number (N) in forest trees. Thus, actual population sizes that are needed lie somewhere between 2500 and 50 000. Orchards cited in Table 16.1 range from a few hundred individuals to over 8000, so numbers in seed orchards are at the lower end of recommended minimum population sizes for GRMUs. Because trees often show interpopulation variation within regions, several small GRMUs may conserve total genetic diversity more effectively than a single large reserve (National Research Council 1991). This is basically the approach taken by the Washington Department of Natural Resources in establishing, perhaps, the only extensive GRMU system for a forest tree in North America (Wilson 1990). They have designated over 100 reserves for *Pseudotsuga menziesii* in western Washington—each about 10 ha and containing more than 400 dominant or codominant trees. Certainly the size of these GRMUs are well within population sizes typical for seed orchards.

Stand structure is likely to be much more complex in GRMUs, with unevenness in spacing and tree size, and presence of multiple tree and shrub species. The influence of complex stand structure on pollen gene flow is unknown, but the few estimates of effective pollen dispersal in natural populations of both conifers and angiosperm tree species suggest that the levels of gene flow observed in seed orchards are representative of what occurs in similarly sized natural stands (Hamrick & Murawski 1990; Adams 1992; Boshier *et al.* 1995a; Schnabel & Hamrick 1995; Burczyk *et al.* 1996; Chase *et al.* 1996b; Dawson *et al.* 1997). For example, minimum estimates of pollen gene flow (i.e. proportion of detected pollen immigrants) in two shelterwood stands of *Pseudotsuga menziesii*, each 2.4 ha and containing 36 to 43 old growth (> 200 years) trees, were 20% and 27%, respectively (Adams 1992). Minimum estimates of pollen gene flow in two stands of the dioecious, insect-pollinated *Gleditsia triacanthos* L. (honeylocust) (each stand about 3 ha and containing 60 males), were 17% to 19% in good seed years and 28% to 30% in poor seed years (Schnabel & Hamrick 1995). In both these examples, the studied populations were not isolated spatially from background sources of pollen.

Only pollen gene flow is of concern in seed orchards, but gene flow by seed (or fruits or vegetative propagules) is an additional possibility in GRMUs, where seedlings can become established naturally and ultimately interact genetically with the population. Seed dispersal is usually more restricted than that of pollen (Levin & Kerster 1974; Ellstrand 1992a), but gene flow by seed, especially from nearby populations, can be substantial (Adams 1992; Dow & Ashley 1996). Because seed immigrants carry twice the number of genes than pollen gametes, they have twice the effect on gene flow.

Based on evidence from pollen contamination studies in seed orchards, and pollen and seed gene flow in natural stands, gene flow in *ex situ* plantations and GRMUs could be extensive, even when reserves are relatively large. Until further data become available, it is prudent to expect significant gene flow in all situations, except when gene conservation reserves are exceptionally well isolated (i.e. by several thousand metres) from populations of the same species.

Box 16.2 An example of the strong force of gene flow

As an illustration of the potential for gene flow to maintain maladapted genes in populations, we plot change in the frequency (p) of an undesirable gene (A_2) in a recipient population, using a migration-selection model (Hartl & Clark 1989) (Figure 1). We assume A_2 is fixed in the donor population, all immigration (m) is by pollen, A_2 has an additive effect on fitness (with selection coefficient s), and all selection occurs in offspring after random mating. Notice that when selection against allele A_2 is of the same magnitude as the rate of immigration (0.05), change in p (Δp) is always positive, such that A_2 will eventually become fixed in the recipient population. Even when selection against allele A_2 is relatively strong (s = 0.10 or 0.20), the limited gene flow we have assumed causes p to be positive whenever the frequency of A_2 is low, such that A_2 is never completely purged from the recipient population.

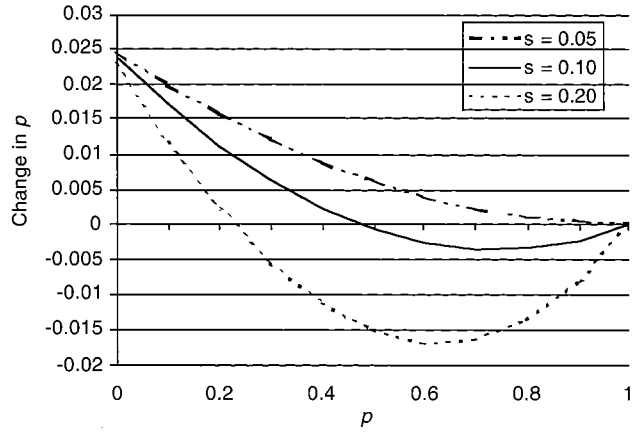

Figure 1 Change in the frequency (p) of an undesirable allele (A_2) under a migration–selection model, where the loss of A_2 due to selection (selection coefficient, s, is 0.05, 0.10 or 0.20) is countered by gene flow (m = 0.05) from a donor population where A_2 is fixed.

16.4 Significance of gene flow in gene conservation reserves

The effect of gene flow depends on the magnitude of immigration and the degree of genetic differentiation between donor and recipient populations (Hartl & Clark 1989). Studies based on allozymes have revealed little differentiation between domesticated populations (e.g. seed orchards, breeding populations) and natural stands in early generations of tree improvement programs (Adams 1981; Szmidt & Muona 1985; Chaisurisri & El-Kassaby 1994; Williams *et al.* 1995; El-Kassaby & Ritland 1995b; Stoehr & El-Kassaby 1997). Thus, gene flow may initially have little influence on the genetic integrity of reserves. These effectively neutral genetic markers, however, may underestimate changes that occur at loci under selection in breeding programs (Williams *et al.* 1995). In addition, genetic differentiation between surrounding stands and reserves is bound to increase in future generations as surrounding stands are replaced by increasingly domesticated varieties.

Gene flow into reserves may be beneficial if the immigrants are well adapted to local environmental conditions, because the immigrants may be a source of new genetic variants (Ellstrand 1992a; Frankel *et al.* 1995). Indeed, in early generations of breeding, genetic variation in domesticated populations may be greater than within local natural stands because seed orchards contain selections from broad areas (i.e. many stands) (Chaisurisri & El-Kassaby 1994; El-Kassaby & Ritland 1995b; Stoehr & El-Kassaby 1997). Nevertheless, as domestication proceeds over generations, genetic variation in domesticated varieties is expected to be reduced to only a fraction of the amount in reserves, such that substantial gene flow from surrounding stands will reduce

the amount of genetic diversity within reserves (Ellstrand & Elam 1993).

Potentially even more damaging to the genetic integrity of reserves is if domesticated varieties are less well adapted to current or changing local environments. If so, gene flow may disrupt or prevent local adaptation and reduce the fitness of reserves (Millar & Libby 1991; Ellstrand 1992a; Ellstrand & Elam 1993; Frankel *et al.* 1995). Some forest geneticists believe that immigration of maladapted genes into GRMUs is not a problem because with dense regeneration, natural selection will remove unfit individuals (Ledig 1986; Millar & Libby 1991). Even modest gene flow, however, can be a potent force in counteracting relatively strong selection and in maintaining maladapted genotypes in populations (Box 16.2). Immigration of maladapted genes may even be more of a problem in *ex situ* plantings that are regenerated artificially. Some of the strongest selection occurs at early seedling stages in field environments (Campbell 1979), but maladapted genotypes may undergo little selection, or perhaps may even be favoured, when raised under mild nursery conditions (Campbell & Sorensen 1984). Thus, genotypes that otherwise may not have made it beyond the seedling stage could be planted, and perhaps survive to reproduction.

Gene flow can be a severe detriment to the integrity and survival of gene conservation reserves. We agree with Millar & Libby (1991), that the most appropriate strategy in managing these reserves is to do all that is possible to limit immigration of foreign genes.

16.5 Strategies for limiting gene flow in gene conservation reserves

Three general approaches, used independently or combined, can be applied to limit gene flow in reserves:

(a) control the location, size and isolation of reserves

(b) increase the ratio of pollen production within reserves relative to that in surrounding populations

(c) control mating.

16.5.1 Controlling location, size and isolation of reserves

When feasible, reserves should be located where gene flow, when it occurs, will have the least negative effect (Frankel *et al.* 1995). These are areas where surrounding populations have not been domesticated (e.g. locations in, or adjacent to, parks or other natural reserves), or where harvesting is followed by natural regeneration or accomplished artificially using seed from local, native stands. Unfortunately, the areas in greatest need of gene conservation reserves are those where management is the most intense and where populations surrounding reserves are under the most rapid domestication. Thus, optimal conditions for reserve placement often are not available.

Despite ambiguous results from pollen contamination studies in seed orchards, increasing the size of reserve populations should decrease gene flow, whether genes are transported by wind or by pollinators (Ellstrand 1992a; Ellstrand & Elam 1993). Most *ex situ* reserves are not likely to be large, simply because population sizes do not need to be great to capture most of the useful genetic diversity within a region (i.e. N in hundreds, or less, is adequate; Frankel *et al.* 1995). MVP size sets the minimum numbers necessary to ensure long-term evolutionary potential of GRMUs, but because harvesting is compatible with gene conservation, GRMUs much larger than the minimums required may be acceptable to managers (Ledig 1988).

As indicated earlier, spatial separation of reserves from plantations of the same species may need to be substantial for isolation to have a reasonable effect on limiting contamination. Spatial isolation is easiest to achieve in *ex situ* plantings that could be located in areas far removed from pollen sources of the same species. A drawback with this strategy, however, is that if the planting environment differs from the one in the source location, the genetic composition of the reserve population could be altered significantly by selection. Isolation of both *in situ* and *ex situ* reserves is probably best accomplished by surrounding them with buffer zones or wind breaks, to provide a physical barrier to foreign pollen and seeds (Di-Giovanni & Kevan 1991; Millar & Libby 1991). Buffer zones could consist of natural stands, or planted trees of local origin, of the same species in the reserve. An alternative is to plant buffers with an exotic species known to be adapted to the local environment, but not invasive. The advantage of using an exotic is that seed immigrants can be easily identified, and the buffer would absorb, not generate, contaminating pollen. The width of buffer needed for adequate protection is unclear, but will depend on the species, size of the

reserve and meteorological factors (Di-Giovanni & Kevan 1991), and is crucial to the total land area required. For example, with a buffer of 200 m around a square reserve of 100 ha, a total land area (reserve plus buffer) of nearly 200 ha would be required.

16.5.2 Maximising pollen production within reserves relative to surrounding stands

Artificial manipulation of floral phenology and magnitude is probably feasible only in *ex situ* gene conservation plantings where tree location, spacing and size can be controlled. The magnitude of flowering and pollen production in *ex situ* collections can be increased greatly using floral stimulation treatments developed for seed orchards (Wheeler & Jech 1986; Wheeler & Bramlett 1991). Increasing flower production within reserves will also reduce gene flow in tree species with animal-mediated pollination, because pollinators will be encouraged to forage exclusively within reserve populations (Levin & Kerster 1974). Only one stimulated crop should be needed for regenerating an *ex situ* population. But, if by chance, pollen production in surrounding stands is large relative to that within the reserve, seed collection can be deferred to another crop year when flower stimulation produces a more favourable ratio of reserve to background pollen density. Bloom delay could be used to offset timing of peak flowering within *ex situ* plantings relative to surrounding natural stands, but it is unlikely that the necessary irrigation systems would be available in most cases.

Manipulation of the ratio of pollen production within GRMUs relative to surrounding stands can be done silviculturally by controlling harvesting. Timing of harvests to promote natural regeneration (e.g. seed tree or shelterwood cuts) should coincide with heavy seed crops within the reserves. Surrounding stands should be harvested several years before regeneration within reserves is anticipated and adult trees removed (e.g. by clearcutting or by overstory removal after seed tree or shelterwood regeneration) to reduce background pollen (Millar & Libby 1991). If the GRMU is to be regenerated artificially, seed should be collected from scattered trees within the GRMU only in good seed years. In addition, seedlings should be raised under conditions that are least likely to promote artificial selection (e.g. sow at wide spacing in nursery beds or single sow in containers; El-Kassaby & Thomson 1996). Planting in the field, however, should be at relatively high density to promote early competition and natural culling of maladapted genotypes.

16.5.3 Controlling mating

The optimum solution to pollen contamination is to control mating completely by applying pollen artificially to bagged flowers. It is possible to make controlled crosses among trees in GRMUs by climbing the trees on site or by conducting crosses in clone banks on grafted cuttings of the trees (Wilson 1990). It is hard to imagine, however, that these expensive alternatives could be justified except under very special circumstances, such as might occur with highly valued or endangered species. Controlled pollination is most feasible in *ex situ* plantings, but even here, costs may be prohibitive. An alternative to completely controlled crossing is the broadcast application of pollen onto unbagged female flowers (called supplemental mass pollination, SMP, Bridgwater *et al.* 1993). SMP has a lot of promise, but reported success rates have been variable, with often less than 50% of offspring resulting from the applied pollen (Bridgwater *et al.* 1993; Eriksson *et al.* 1994). In concert with other tools, such as isolation and flower stimulation, SMP may be helpful in reducing pollen contamination, but if SMP is to have a primary role for this purpose, more work will be needed to perfect the technique.

16.6 Need for research and monitoring

We have a lot to learn about gene flow in forest trees, its effect on the integrity of gene conservation reserves and the methods by which it can be curtailed or controlled. Much continues to be learnt about pollen management in seed orchards (Bramlett *et al.* 1993; Webber & Painter 1996), and as this technology becomes available, it will be applicable to more intensely managed *ex situ* gene conservation plantings. Control of gene flow in GRMUs may be difficult to achieve, and the relative roles of reserve size, isolation, buffer zones and background versus reserve pollen production, on levels of pollen contamination are nearly impossible to quantify with any accuracy. Research on gene flow in GRMUs, or other natural populations with properties similar to GRMUs, must be given high priority. Methods similar to those employed in measuring pollen contamination in seed orchards can be used, but the reliability of these methods is highly dependent on the ability to

genetically discriminate pollen from local and foreign sources. Application of these methods using allozymes has been possible in seed orchards only because of the relatively small number of genotypes involved (Adams *et al.* 1992a). Estimation of gene flow in larger populations, like GRMUs, requires more polymorphic markers than available with allozymes. Hopefully, hypervariable microsatellites (or simple sequence repeats) will prove more suitable (Frankel *et al.* 1995). Some early applications of these molecular markers to gene dispersal patterns in forest trees are promising (Chase *et al.* 1996b; Dow & Ashley 1996; Dawson *et al.* 1997).

Regardless of the approaches taken to limit gene flow in gene conservation reserves, it will be important to monitor their success in at least a representative sample. Only in this way can the validity of the approaches be confirmed and improvements designed.