Inbreeding Depression in Conifers: Implications for Breeding Strategy

Claire G. Williams and Outi Savolainen

ABSTRACT. Use of selfing as a breeding tool for conifers is controversial; this topic is addressed with a review of genetic models, theory, and experimental results based on a wide range of plants and animals. Some supporting evidence is available from conifer studies.

For most conifers, selfing will not be the best method for reducing inbreeding depression in small subpopulations or elite lines of deleterious alleles; sib- or random-mating is a better option in the early generations of conifer domestication. Possible exceptions are conifer species that have few lethal alleles. Few organisms have been studied which have more lethal equivalents than conifers, so slower rates of inbreeding than selfing are needed initially to prevent large losses to low offspring survival and adult fecundity.

Inbred breeding populations will also require large numbers of replicate lines and progeny per replicate because the probability of extinction for each line is expected to be high. Like maize, few valuable lines will result from selfing in the initial generations. If inbreeding depression is based on deleterious mutations then it is hypothesized to decline with stringent selection against deleterious alleles (purging). After the initial purging phase, selfing would be efficient. Advantages of selfing include perfect assortative mating, increased selection efficacy among lines and increased uniformity within lines.

Theoretical predictions for inbreeding depression in conifers have outpaced experimentation. Operational breeding programs will not provide needed data on changes in inbreeding depression, but the inbreeding assumptions for breeding strategies must be tested experimentally. We advocate using experimental inbred populations to study direct use of inbreeding depression as a breeding method. It provides first-hand results and lends confidence to long-term population management decisions. The greatest value will be to reveal unforeseen problems, preventing irreversible mistakes. As an example, we outline a plan for a rapidly cycled experimental inbred population for *Pinus taeda* L. which combines early selection, rapid screening for adult fecundity, and traditional genetic testing.

Inbreeding depression research is central to the success of long-term population management. It has become more powerful with integrated classical genetics-molecular approaches, accelerated breeding techniques, and computer simulation models. For. Sci. 42(1): 102–117.
Additional Key Words: Selfing, experimental inbred populations, genetics, plant breeding, deleterious alleles

The question of using selfing rather than milder forms of inbreeding or even random mating in elite breeding lines is relevant and timely. Advanced-generation conifer breeding strategies now rely on subdivided breeding populations (e.g., Lowe and van Buijtenen 1981, Barnes 1984, Cotterill et al. 1988, Dean 1990, Namkoong and Kang 1990, McKeand and Bridgwater 1993, Williams and Hamrick 1996). Inbreeding is controlled through subdivision

of breeding populations (see review by Lindgren and Gregorius 1976) but has not been used directly as a breeding method. To a lesser extent, the new option of control-pollinated seed on a commercial scale in many programs has indirectly stimulated interest in inbred lines. This production orchard design makes it possible to use as few as two unrelated selections and still produce outcrossed seed for reforestation (see review in Williams and Askew 1993). In all cases, seeds for plantations

Claire Williams is with Texas A&M University, Department of Forest Science, College Station, Texas 77843. claire_williams@tamu.edu. Outi Savolainen is with the Department of Genetics, University of Oulu, SF 90570 Oulu Finland.

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are assumed to be outcrossed, the topic of inbreeding here is relevant only within breeding populations.

Selfing as a breeding tool for forest trees was first advocated 3 decades ago, using the experience with maize as a justification (Righter 1960). Righter suggested development of selfed lines for conifers and production of hybrid seed for plantation forestry, although he also noted that delayed flowering and loss of reproductive capacity were potential impediments. His ideas were not implemented because conifer breeding programs were in their infancy, and the few inbreeding studies at that time reported high rates of selfed seed mortality. Early conifer programs worldwide were managed for inbreeding avoidance and relied upon open-pollinated production seed orchards. A single, large breeding population was preferred then, although today population subdivision strategies are more commonly used (see review in White 1993, Williams and Hamrick 1995).

The success of maize (*Zea mays* L.) hybrid production is a poor justification for selfing in conifers. Maize hybrid production began when Jones (1917) first demonstrated hybrid vigor between selfed lines of maize. By the 1920s, intensive maize selfing programs were begun by the U.S. Department of Agriculture using this cross-pollinated species that had been under domestication for several thousands of years. This program required very large breeding populations because loss of reproductive capacity and poor yields resulted in only a fraction of acceptable selections (Lindstrom 1939). It is estimated that more than 100,000 lines were actually screened prior to 1939 and that 97% of these were lost after 1 to 3 generations of selfing. Of the lines retained, all were defective for one or more traits including yield (Lindstrom 1939, Hallauer and Miranda 1981, p. 321). Loss of fertility in maize was the chief barrier, even though there was strong directional selection for seed production. Selfing prevails in U.S. maize programs because it requires less time to develop a true-breeding line than sib-mating (Stringfield 1974, see also Figure 1) It is not the most efficient means, improvement of exotic or wild relatives of maize rely on sib-mating prior to selfing. Otherwise fitness is reduced substantially if selfing is used initially (Goodman 1992).

The maize success does not translate directly to conifers. For maize, grain yield is both a fitness trait and a trait of direct economic value and thus selection for grain yield is selection for continued reproduction. For conifers, reproductive capacity and survival are also fitness traits, but they do not have direct economic value. Conifer traits of economic value include wood quality and growth. For conifers, use of inbreeding will mean a temporary shift in emphasis to selection for fitness traits such as reproductive capacity and survival, perhaps at the expense of traits of direct economic value.

Genetic Models Underlying Inbreeding Depression

The deleterious effects of inbreeding have long been observed in plants (e.g., Darwin 1876). These observations have been accounted for by various genetic models (see review by Charlesworth and Charlesworth 1987). Examination of these genetic models is important because the predictions for long-term consequences of inbreeding differ widely among the models.

Measuring Inbreeding

Measuring inbreeding is the first step toward quantifying its effect at the phenotypic level. The main consequence of inbreeding is an increase in homozygosity and a general decrease in heterozygosity across the genome. However, inbreeding does not by itself cause changes in allelic frequency. Matings that result in inbreeding include at the extreme selfing, mating between full-sibs, matings between parents and offspring, and a virtual continuum of matings between other kinds of relatives. As a consequence of in-
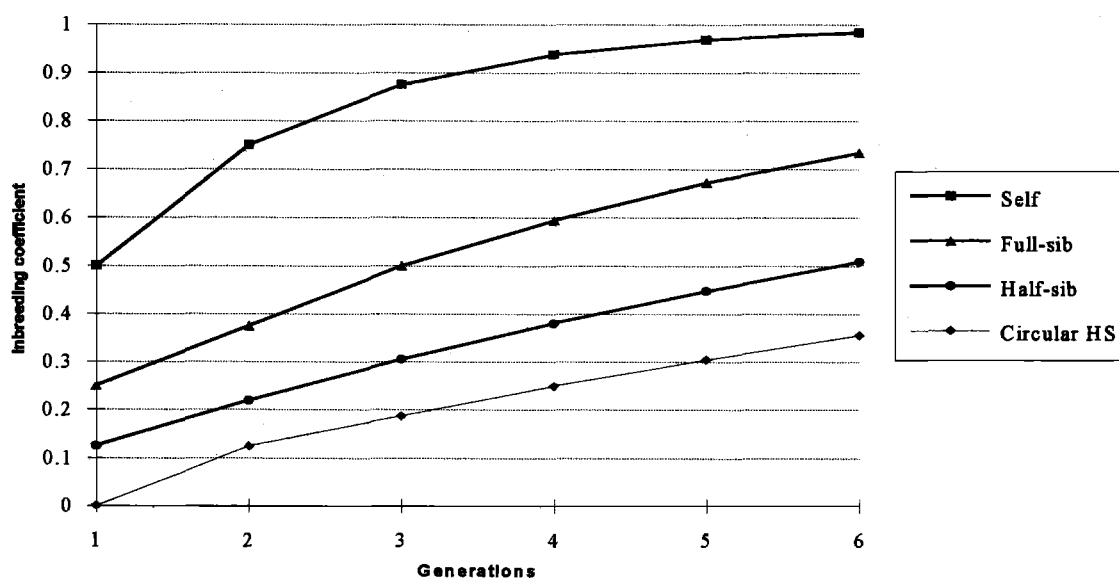
Figure 1. Change in inbreeding coefficient *(F)* across generations for selfing, repeated full-sib mating, repeated half-sib mating, and for random mating within a population based on recurrence equations (Falconer 1981, p. 83–84, Crow and Kimura 1970).

breeding, the offspring may inherit from both its parents alleles that are identical by descent (Malécot 1948). These alleles are essentially copies of a sequence of DNA from an earlier generation. The probability of an individual getting two copies of a gene that are identical by descent (IBD) is defined as the coefficient of inbreeding (F). This is a measure of the level of inbreeding.

An equivalent way of defining the inbreeding coefficient is as the coefficient of correlation between two uniting gametes (Wright 1922). A third measure of inbreeding is the coefficient of parentage between I and J which is the probability that a pair of genes drawn from I and J is identical by descent. Thus the coefficient of parentage between two individuals equals the inbreeding coefficient of their offspring. Crow and Kimura (1970, p. 61–68) review various ways of measuring inbreeding. For offspring of random mating in an infinite population, the inbreeding coefficient is zero. For an offspring of a selfed mating, the probability that it gets copies of the same allele is 0.25 for each allele of the parent, thus the inbreeding coefficient is 0.50. Similarly, for mating between full-sibs, $F = 0.25$, for half-sibs the inbreeding coefficient is $1/8 = 0.125$, and for first cousins $1/16 = 0.0625$. The probability of identity by descent has to be defined with respect to a reference generation, and any earlier inbreeding is ignored. For example, typical adult conifer populations seem to contain no selfs and they can become parents, and they can be considered to be almost ideal reference populations (Yadzani et al. 1985).

All of the above descriptions measure inbreeding levels for a pedigree. Inbreeding can also be quantified at the population level. If the inbreeding coefficient of all individuals is known, then the population inbreeding coefficient is merely their average. Recurrent systems of partial inbreeding lead to an equilibrium level of inbreeding in the population. Mixed partial selfing and random outcrossing, without any selection, lead to an equilibrium $F = (1 - t)/(1 + t)$, where t is the proportion of outcrossing at the zygote stage (e.g., Hedrick 1983).

Inbreeding can also arise in a small population or breeding line, even if mating is apparently random. A small population of size N must have been produced by $2N$ gamete genotypes. In a population capable of selfing, the probability that two gametes unite that are identical by descent is $1/2N$. Thus, some inbreeding will accumulate merely due to the finite size of the population.

The effects of continuing inbreeding accumulate over generations. If the same mating system prevails, the inbreeding coefficient increases regularly, such that $F(t) = 1 - (1 - \Delta F)^t$, where $F(t)$ is the inbreeding coefficient in generation t and ΔF is the change in inbreeding coefficient in one generation (Falconer 1981, p. 83–87). The expected cumulative inbreeding levels, given no selection, show that essentially complete homozygosity for selfing is achieved after 6 to 10 generations (Figure 1).

The increase in homozygosity of a population can be quantified using the inbreeding coefficient. In a random mating population, the single-locus genotypes AA, Aa, and aa will be in Hardy-Weinberg proportions, p^2, $2pq$ and q^2

where p and q are the frequencies of alternative alleles A and a, respectively. In a population with average inbreeding coefficient F, the proportions will be $p^2 + Fpq$, $2pq(1 - F)$, and $q^2 + Fpq$. The higher the inbreeding level is, the more the genotypic proportions deviate from Hardy-Weinberg proportions. While inbreeding effects accumulate over generations, a single generation of random mating will restore Hardy-Weinberg proportions at individual loci.

The traditional view is to regard natural populations of conifers as random-mating organisms. This is based on observations of germinating seeds, among which there is only a low proportion of actual selfs (Muona 1990). There are no direct estimates of selfing at the zygote stage, i.e., proportion of archegonia fertilized by a tree's own pollen. Estimates based on the pollen cloud surrounding the crown suggest that conifers have a system of mixed self-fertilization and random outcrossing. The proportion of self-fertilization in terms of zygotes produced may range from 10 to 25% in *Pinus sylvestris* (Sarvas 1962, Koski 1971).

Measuring Inbreeding Depression

The immediate interest of plant breeding programs is genetic gain for traits of economic value. However, if inbreeding is used as a tool, it becomes crucial that the plants be fit enough to survive and produce offspring for the next generation. Inbreeding depression for these fitness traits means lack of vigor and perhaps a failure to reproduce which would end recurrent breeding efforts. Inbreeding depression upon selfing, δ, can be described by the fitness disadvantage of selfed offspring relative to outcrossed offspring:

$$\delta = 1 - \left(w_s / w_c\right) \tag{1}$$

where w_s and w_c are measures of fitness for selfed and outcrossed individuals, respectively.

A general approach to measuring inbreeding depression is to estimate a regression line between changes in trait performance and increasing levels of inbreeding. Inbreeding depression is thus described empirically by the regression between the trait's mean phenotype and the inbreeding coefficient, F.

$$Y = a + b(F) + \varepsilon \tag{2}$$

where Y represents the phenotypic value, a the intercept, b the regression line or slope, F the inbreeding coefficient, and ε the residual error term. The phenotypic values can be measured within a single generation or compared across generations. In either case, inbreeding depression is measured by the percentage change in phenotypic values per unit decrease in F. The regression line, b, is usually represented as percentage change per unit of 0.1 F since this value is conventionally used for breeding strategy.

Genetic Models for Inbreeding Depression

Since early in this century, there have been two main competing models to account for inbreeding depression (Table 1), and its counter phenomenon, hybrid vigor or

Table 1 Fitness values for genotypes at a single locus with two alleles (A_1 and A_2) for additive, dominance, and overdominance genetic models. Selection coefficients are denoted as s. Dominance coefficient h measures the loss of fitness in mutant heterozygotes.

	Genotypes and their fitnesses		
Model	A_1A_1	A_1A_2	A_2A_2
Additive	1	$1 - 0.5s$	$1 - s$
Dominance	1	$1 - hs$	$1 - s$
• Complete dominance	1	$1 - hs$ where $h = 0$	$1 - s$
• Partial dominance	1	$1 - hs$ where $0 < h < 0.5$	$1 - s$
• Complete recessivity	1	$1 - hs$ where $h = 1$	$1 - s$
• Partial recessivity	1	$1 - hs$ where $0.5 < h < 1.0$	$1 - s$
Overdominance	$1 - s_1$	1	$1 - s_2$

heterosis (see review in Charlesworth and Charlesworth 1987). When the mating system is altered, as when inbreeding is introduced as a tree breeding tool, the genetic and phenotypic consequences depend on the underlying genetic model for inbreeding depression. Hence, it is important to understand the genetic basis. These alternative models are described for a single locus, A, with two alternative alleles A_1 and A_2. The fitness of the best genotype is 1.0. The selection coefficient for each genotype is s and the degree of dominance of A_1 over A_2 is symbolized by h (Table 1). The degree of dominance, h, describes the expression of the mutant allele in heterozygotes relative to mutant homozygotes.

Overdominance

In this model, heterozygotes have a higher fitness than either homozygote (Table 1, Hayman 1953, Charlesworth and Charlesworth 1987, Ziehe and Roberds 1989) because complementary alleles at a locus are needed for optimum physiological function.

Overdominance was originally proposed to account for the superiority of hybrid maize (East 1936, Hull 1945). After half a century, the experimental evidence supporting this model is modest. Few biometrical studies support this model (e.g., Berger 1976, Simmons and Crow 1977, Frankel 1983, Charlesworth and Charlesworth 1987). For single loci, there are also few clearcut examples of overdominance. Recent studies of gene action for quantitative trait loci have suggested overdominance, but these results pertain to chromosome segments rather than individual loci, thus pseudo-overdominance due to repulsion linkages cannot be ruled out (Stuber et al. 1992). Overdominance does not appear to be a prevalent cause of inbreeding depression (Charlesworth and Charlesworth 1987, Johnston and Schoen 1995). However, even a small number of overdominant loci could have an important influence on evolutionary dynamics. This will be addressed specifically for conifers in a later section.

Partial Dominance

The model is traditionally called the dominance model; it is based on deleterious or partially recessive alleles (Davenport, 1908, Bruce 1910, Jones 1917). The normal homozygote (A_1A_1) has a fitness value of 1, $w = 1$, and its allele is completely or partially dominant. The deleterious allele (A_2) is recessive ($0 < h < 0.5$, Table 1), and its effect is fully or partially masked in the heterozygous (A_1A_2) state. As an example in Table 1, lethal alleles would have selection coefficient, s, of 1, and all A_2A_2 genotypes die. At $h = 0.2$, the viability (fitness) of heterozygotes $(1 - 0.2)$ is reduced to

80% of the original value. Inbreeding, through leading to an increase in homozygotes, results in a reduction in fitness. Partially dominant deleterious alleles should be efficiently eliminated by selection and thus be quite rare. Heterosis under this model is possible, if different homozygous lines have fixed deleterious alleles at different loci. Upon crossing, the deleterious effects are masked by the normal alleles in the other parental line so that hybrid superiority or heterosis is observed.

There are very few estimates of the degree of dominance for deleterious alleles in plants (Fu and Ritland 1994). Dominance estimates for deleterious alleles ranged from 0 to 0.35 in *Amsinckia* (Johnston and Schoen 1995). In *Drosophila melanogaster*, deleterious alleles were frequent and varied in the degree to which they were recessive. Mildly deleterious alleles tended to have a higher dominance value than lethals ($h = 0.20$) which are almost fully recessive (Simmons and Crow 1977). The viability of heterozygotes for lethal alleles is typically reduced by 1 to 3% (Simmons and Crow 1977).

Apparent Overdominance.—Overdominance results in an excess of heterozygotes in a population. However, such a finding, either in the progeny of crosses or in populations, can be due to several other reasons as well. In pedigrees, pseudo-overdominance could be due to two linked loci with advantageous alleles in repulsion phase (Rumbaugh and Lonnquist 1959, Robinson et al. 1960, Bingham 1983, Stuber et al. 1992). At the population level, the term associative overdominance is often used (see review in Charlesworth 1991) to describe a situation where the selective effects due to deleterious alleles at one locus cause an excess of heterozygotes at another locus. This is possible when the genotypes at two loci are not independent but correlated. Such effects can arise because of gametic disequilibrium between alleles of two loci, often on the same chromosome (e.g., Ohta 1971), due to selection or drift. On the other hand, they can also be due to correlations between two genotypes, when a heterozygote at one locus is likely to be heterozygous at another locus as well (Haldane 1949). This kind of genotypic association arises frequently with inbreeding (Cockerham and Rawlings 1967). If there is partial selfing, genotypes that are homozygous for deleterious alleles due to inbreeding are likely to be homozygous at marker loci also. A positive correlation between marker locus heterozygosity and fitness will result. This can be erroneously interpreted as evidence for overdominance at the marker loci. This explanation may at least in part account for the observed correlations between heterozygosity and growth in some conifers. Many such corre-

lations have been interpreted in terms of overdominance (see Bush and Smouse 1992, Bush et al. 1987).

Epistasis.— The proposed genetic models for inbreeding depression have been based on single-locus interaction. However, there are also interlocus interactions that can also contribute to inbreeding depression (Crow and Kimura 1970, p. 81–83). Only dominance × dominance and additive × dominance interactions between loci give rise to inbreeding depression (Cockerham 1954, Kempthorne 1973, p. 445–447). Epistasis without dominance cannot account for inbreeding depression, but it can act in concert with directional dominance (Falconer 1981, p. 227, Crow and Kimura 1970, p. 81–83). The contribution of these types of epistasis to total genetic variance rises exponentially with inbreeding (Cockerham 1954, Kempthorne 1973, p. 445–447).

Net epistatic effects have been detected by testing for a curvilinear relationship between the metric trait mean and F across generations (Crow and Kimura 1970, p. 81–83). This is a conservative test for epistasis. Failure to observe a curvilinear relationship can be construed as an absence of epistasis, but other explanations are that positive and negative epistatic effects cancel out, resulting in a linear relationship to inbreeding (Hallauer and Miranda 1981, p. 147). Either could account for the observed linear relationship between grain yield and inbreeding levels across generations (Burton et al. 1978, Cornelius and Dudley 1974, Good and Hallauer 1977). Epistasis has been considered a minor component of inbreeding depression in *Drosophila* (Simmons and Crow 1977) and in *Mimulus guttatus* (Willis 1992).

In forest trees, single-generation related mating studies (Squillace and Kraus 1962, Kurinobu et al. 1991, Matheson et al. 1995) can be used to give approximate estimates of the epistatic component, but this method does not discern which type of inter-locus interaction is occurring. Additive × additive epistatic interactions, which do not contribute to inbreeding depression, have been estimated from a radiata pine selfing study (Wilcox 1983) using a joint covariance approach developed for self-fertilizing plants which typically exhibit complete additivity (Matzinger and Cockerham 1963). The joint covariance approach has the theoretical drawback of confounding all epistatic interactions with estimates of additive × additive interactions and indeed does not fit with the observed data.

Distinguishing Between Models

It is difficult to distinguish between the models based on conifer studies. There is little evidence for overdominance in conifers. Observed correlations between number of heterozygous loci and fitness in conifers have been interpreted as evidence for overdominance for fitness at marker loci (e.g., Bush and Smouse 1992), but others (Strauss 1986, Strauss and Libby 1987) have concluded that these data are best interpreted as support for partial dominance for knobcone pine (*Pinus attenuata*) and radiata pine (*Pinus radiata*). They found no evidence for a relationship between the number of heterozygous marker loci and fitness. In Scots pine (*Pinus sylvestris*), which has a very large continuous population and an absence of inbreds among adults, Savolainen and Hedrick

(1995) found no evidence for relationship between heterozygosity at marker loci and fitness. Partial selfing or mating between relatives (Park et al. 1984, Knowles et al. 1987) could generate these occasional correlations that do not in fact reflect overdominance. More detailed studies are needed which test the mode of gene action.

Predictions for Long-Term Consequences

The long-term consequences of inbreeding will depend on the genetic nature of the inbreeding depression (e.g. Barrett and Charlesworth 1991, Hedrick 1994). If overdominant loci are the cause of inbreeding depression, the average fitness will decrease continuously over generations of inbreeding. If the selection coefficients for the two homozygotes at a locus are symmetrical, polymorphism will persist in the population. If selection is intense and asymmetrical, the population will eventually become monomorphic for the allele conferring higher homozygote fitness (Ziehe and Roberds 1989)

With the partial dominance model, deleterious recessive alleles would cause most of the inbreeding depression. It should be possible to eliminate or purge them by fixing the normal, dominant alleles. Inbreeding depression will decline across generations as purging effectively removes deleterious alleles (Barrett and Charlesworth 1991, Hedrick 1994). Selective pressure must be strong in this case; otherwise genetic drift and weak selection will lead to fixation of some deleterious alleles. A combination of low selection intensity and genetic drift will thus give rise to a long-term decline in fitness.

Experimental Evidence of Inbreeding Depression

Most reports of inbreeding depression in conifers center on embryonic-stage lethals and other deleterious recessives which affect seedlings. Less is known about how inbreeding depression varies over the course of plant development Among adult traits, fecundity has not been studied much, but it could well prove to be the major deterrent to selfing in breeding programs. Conifer studies have emphasized the average inbreeding depression for a pedigree, provenance, or species; the variability of these estimates also bears on the effective use of inbreeding.

Inbreeding Depression at Different Life Stages

Changes in inbreeding depression with age are important to long-term population management. If inbreeding depression in growth is expressed early, then early selection will be effective. In many plants, inbreeding depression appears severe during seed development, much less severe during growth and then becomes severe again at the onset of reproduction (Husband and Schemske 1996).

Embryonic Lethals: Pollination to Germination

Inbreeding depression during seed development is typically manifested as empty seed (Orr-Ewing 1957). There is no evidence for self-incompatibility in conifers prior to fertilization, and the reduced number of filled seeds is attributed to embryo mortality (Hagman and Mikkola 1963, Sorensen 1982).

In the genus *Pinus*, seed development after pollination takes about 18 months. Fertilization takes place a year after pollination. Unpollinated ovules do not develop into full-sized seeds; they abort as first-year ovules. The seed coat develops in the first year, to be filled by a haploid megagametophyte. The ovule may have multiple archegonia which are fertilized by different pollen grains (archegonial polyembrony). If all of the developing embryos die then an empty seed results (Sarvas 1962). Upon selfing, many of these embryos die because they are homozygous for lethal or deleterious genes which act at the early stages of development. In some genera, notably *Picea*, seeds will not abort if pollination does not occur, so empty seed can occur either as a result of embryo lethals or a lack of pollination. Environmental factors can also cause embryonic death.

The relative fitness of individuals arising from inbreeding and from random mating can be used for estimating the number of lethal equivalents (Morton et al. 1956). Many deleterious genes together can make up one lethal equivalent, if they cause as many selective deaths as one lethal gene. Sorensen (1969) first applied the method of Morton et al. (1956) to conifers. The frequency of empty seeds after self- and cross-pollination can be measured to obtain the relative self-fertility (the ratio of full seeds upon selfing to full seeds upon outcrossing). The number of lethal equivalents was estimated as $-4 (\ln R)$, where R is the relative self-fertility. Later, a model was developed especially for conifers. This model takes archegonial polyembryony into account, assumes that an empty seed results only if all embryos die and that embryo death is due to genetic causes (Bramlett and Popham 1971, Koski 1971).

This model assumes that the lethal genes are completely recessive and that each is fully lethal and acts independently. The model excludes any environmental influence. Other authors have included effects of mortality due to environmental causes at the ovule level (Lindgren 1975a, Bishir and Namkoong 1987). Bishir and Pepper (1977) included a variable number of archegonia per ovule which proved to have only a minor influence on the estimates of embryonic lethals. Savolainen et al. (1992) estimated lethal numbers using a method based on comparing empty seed frequencies after mixed self- and cross-pollination. This resulted in lower estimates of lethal numbers than previous methods. All methods, however, suggest high numbers of lethals per diploid zygote in many conifer species (Table 2). All population estimates for conifers given in Table 2 are based on Sorensen's (1969) method using the average self-fertility for the species. These estimates differ from those based on averaging lethal estimates from individual trees.

Griffin and Lindgren (1985) suggested that the lethals do not act independently, in that the action of several genes may be required for an embryo death. Conversely, Bramlett and Bridgwater (1986) found support for independently acting lethals studying crosses between F1 sibs in Virginia pine (*Pinus virginiana*). The co-lethal versus independent models differ in their predictions for the empty seed frequencies in the second generation of selfing. If lethals are acting independently, second-generation selfed seed (S2 seedlots) should have less empty seed than the first generation selfs (S1). Griffin and Lindgren (1985) did not observe a decline for radiata pine (*Pinus radiata*) in Australia. However, if the S1 parents yielded empty seed because they are poor maternal parents (Sniezko 1984), then inbreeding depression for embryonic lethals in the second generation may have been overestimated. Multiple-generation studies are needed to further test these hypotheses.

These early-acting genes are probably deleterious or lethal genes with near-recessive inheritance. The number of loci which can cause embryonic mutations has been debated. Koski (1971) suggested a low of 75, but Namkoong and Bishir (1987) estimate closer to 10,000 loci. The latter value is biased upwards because of environmental factors and maternal effects (Namkoong and Bishir 1987). Other sources of bias in this model include a low mutation rate and a high estimate for average number of lethals. Using the higher rate of mutation reported for *Pinus sylvestris* (Kärkkäinen et al. 1996) and setting the average number of lethals to 8 (Table 2), the number of lethals is reduced from 10,000 to 1,000–2,000 even without a correction for environmental factors and maternal effects.

Seedlings to Adults

Until now, we have dealt only with the survival component of fitness. From the seedling stage onward, many quantitative characters such as growth are considered. Selfed seedlings exhibit severe early inbreeding depression (Franklin 1970, 1972, Sorensen and Miles 1982, Schemske and Lande 1985). But is inbreeding depression as severe in later life stages? How are inbreeding effects for such traits distributed over a lifetime of a tree? Early severe inbreeding with lesser

Table 2. Estimates of filled seed frequencies upon selfing and outcrossing, numbers of lethals, and number of trees studied in different coniferous species. Lethals were estimated according to Sorensen (1969).

Species	% filled seed		No. lethals	No. trees	Ref.
	Self	Outcross			
Norway spruce (*Picea abies*)	13.8	85.0	7.3	87	Koski 1973
White spruce (*Picea glauca*)	7.2	54.7	8.1	20	Fowler & Park 1983
Serbian spruce (*Picea omorika*)	32.4	52.3	1.9	13	Koski 1973
Radiata pine (*Pinus radiata*)	34.2	78.9	3.3	8	Griffin & Lindgren 1985
Red pine (*Pinus resinosa*)	28	29	0.1	9	Fowler 1965
Scots pine (*Pinus sylvestris*)	16.7	87.3	6.6	127	Koski 1973
Douglas-fir (*Pseudotsuga menziesii*)	7.9	67.2	8.6	35	Sorensen 1971
Tamarack (*Larix laricina*)	0.2	21.5	10.8	20	Park & Fowler 1982
Virginia pine (*Pinus virginiana*)	16.4	92.7	10.4	5	Bramlett & Pepper 1974

effects later on would be easier to deal with within a breeding program. However, estimates of inbreeding depression in different life stages of a plant are complicated by mortality.

It is well known that selfed seedlings have higher mortality than outcrossed seedlings. This has been documented in natural populations by studying the inbreeding coefficients at different life stages (e.g., Yadzani et al. 1985, Morgante et al. 1993, Plessas and Strauss 1986). This is also true for planted seedlings (Muona et al. 1987). Selfed progeny also exhibit strong inbreeding depression for growth at early ages (see reviews by Franklin, 1970, 1972, Sorensen and Miles 1974, Schemske and Lande 1985).

Mortality is an important factor in long-term experiments where the growth of selfed and outcrossed progeny are compared (e.g., Eriksson et al. 1973). The mortality levels vary widely by species and competitive environment (Sorensen and Miles 1982). The mortality is rarely considered when inbreeding depression for growth is compared across different life stages. In most studies, early and late measurements are not based on the same set of trees because the weakest plants die before later measurements can be taken.

For Norway spruce (*Picea abies*), selfs were 61% shorter in height at 19 yr, 54% shorter at 27 yr and 22% shorter at 61 yr (Eriksson et al. 1973). There was at least 20% mortality by age 61 yr, but it is not clear when mortality occurred. For radiata pine, inbreeding depression for nursery height was 18.2% for selfs. The estimate dropped to 8.4% when the study was remeasured at age 4 yr (Wilcox 1983), but mortality was not reported. In both cases, changes in inbreeding depression with age are biased by the death of selfed individuals.

Even in the studies where only survivors are included in early and later estimates, there is no clear trend for changes in inbreeding depression with age. In a number of studies, inbreeding depression increased with age (see review in Sorensen and Miles 1982). In other studies, inbreeding depression appeared to decline when comparisons are standardized on a common height curve than compared at a common age. This was the case for Douglas-fir, noble fir (*Abies procera*), and ponderosa pine (*Pinus ponderosa*) (Sorensen and Miles 1974, Sorensen et al. 1976). In two Scots pine (*P. sylvestris*) trials, inbreeding depression was constant from ages 6 to 14 yr (Lundkvist et al. 1987).

Adult fecundity

Inbred individuals have lower fitnesses at all life stages: embryo development, seedling to adult growth, and repro-

ductive stages. Reports on adult reproduction in inbred conifers are rare (Table 3).

Severe inbreeding depression has been observed for age and timing of reproduction as well as number and size of male and female strobili. Most reports of adult fecundity have, however, been limited to qualitative observations. In jack pine (*Pinus banksiana*), a precociously flowering species, there was a delay in age of onset for reproduction and a reduction in strobilus number (Rudolph 1981). A 3 yr delay was observed for western white pine as well as a 15 to 26% reduction in fitness (Table 3, Bingham 1973). Age of onset was not delayed for radiata pine (Pawsey 1964) or loblolly pine (Sniezko and Zobel 1988). In Douglas-fir, no age-of-onset data were reported, but male strobili and developing conelets were smaller on selfed individuals when compared to their outcrossed half-sibs (Orr-Ewing 1965).

In a loblolly pine study, there was roughly a 30% decline in number of potential sites for seeds in female cones of selfed maternal parents (Table 4, Sniezko 1984). Outcrossed embryos that later develop on an inbred maternal parent may well exhibit reduced fitness as an indirect consequence of parental inbreeding depression. If so, this suggests that more than one generation of outcrossing may be necessary to restore vigor (Sniezko and Zobel 1988). However, the differences due to these maternal effects for loblolly pine (Sniezko and Zobel 1988) are slight. A similar study with jack pine showed no effects of inbred maternal parents on seed production upon outcrossing (Rudolph 1981). This maternal effect may be species-specific and restricted to seedlings.

The effect of inbreeding depression on adult fecundity causes more uncertainty for breeding programs than any other trait because there are so few sound estimates available. Inbreeding depression for fecundity traits will retard recurrent breeding in operational programs as well as result in the loss of valuable selections. Reduced seed potential (Sniezko and Zobel 1988) and delayed onset of reproduction (Rudolph 1981) will prolong completion of breeding cycles. Inbreeding depression in adult fecundity traits needs to be quantified in existing inbreeding studies.

Other Adult Traits

Early conifer breeding efforts emphasized growth. Today there is a growing interest in specialty traits which include wood density and bole sweep (Dean 1990, Williams and Megraw 1994, Williams and Lambeth 1990). Inbreeding

Table 3. Relative fitness estimates of selfs to outcrosses with respect to survival, height between seedling stage and given age and strobilus production.

Species	Survival (age)	Height (age)	Strobilus production	Reference
Pinus sylvestris Scots pine	0.79 (6)	0.44 (6) 0.80 (7)	—	Dengler 1939; Lundkvist et al. 1987
Pinus radiata Radiata pine	0.43 (1)	0.88	—	Pawsey 1964; Wilcox 1983
Pinus monticola Western white pin e	0.71 (20)	0.71 (10)	0.26 (female) 0.15 (male)	Bingham 1973
Picea abies Norway spruce	0.80 (20)	0.78 (61)	—	Eriksson et al. 1973
Picea omorika Serbian spruce	0.93 (24)	0.73 (15)	—	Geburek 1986

Table 4. Sound seed data for loblolly pine parents produced by selfing (S1) and outcrossing (S0) (data from Sniezko 1984). Both inbred (S1) and outcrossed (S0) parents were wind-pollinated to produce these progeny.

Category	Female fecundity (potential sites for seed per cone)	Sound seed per cone	Sound seed (%)	Seedling height (cm)
S0	99.9	79.9	78.6	28.3
S1	69.8	43.4	64.2	27.4

depression studies for these traits are rare. There is one report on radiata pine where wood density and bole straightness showed no inbreeding depression in selfed offspring (Wilcox 1983). Recent studies on Norway spruce also showed there was little inbreeding depression (6%) for wood density at age 10 yr (T. Skroppa, unpublished data). If there is indeed less severe inbreeding depression for quality traits, then selections can be made directly from inbred lines without concern for bias in breeding values. This issue merits further study.

Variability in Severity of Inbreeding Depression

Among Species

Conifers estimates of embryonic lethals range from red pine (*Pinus resinosa*) with 0.1 lethals to a maximum of 10.3 for tamarack (*Larix laricina*) (Tables 2, 5). Red pine (*Pinus resinosa*) is monomorphic and therefore homozygous for a large number of isozyme and random DNA marker loci, exhibits self-fertility, and no inbreeding depression for metric traits (Fowler 1965, Fowler and Morris 1977, Mosseler et al 1992). Deleterious alleles may have been lost during a series of early founder events in post-Pleistocene recolonization starting 10,000 yr ago. The prevalence of red pine suggests that its populations have been purged of deleterious alleles and that heterozygosity as such is clearly not necessary for any component of fitness. However, it is not clear why the high mutation rate of conifers would not have restored some of the variability at marker loci since the founder event.

Perennial plant species have more lethal equivalents for early viability than most other reported plant and animal species (Table 5). There are some exceptions, such as Wied's red-nosed rat (*Wiedomys pyrrhorhinos*), which has the highest reported number of lethal equivalents (Table 5).

The high numbers of lethals observed for conifers are due in part to high mutation rates. Genome-wide mutation rates

(U) for albinism, a chlorophyll-deficient lethal allele, are 10 to 20 times higher for some conifers than the mutation rates measured in angiosperm annuals (Eiche 1955, Table 6, Koski and Malmivaara 1974, Kärkkäinen et al. 1996).

It is worthwhile to note that all estimates based on lethals such as chlorophyll-deficiency may be lower than the mutation rates for mildly deleterious alleles. Also, lethal alleles in *Drosophila* are less frequent than mildly deleterious alleles (Simmons and Crow 1977, Caballero and Hill 1992).

High mutation rates in woody perennials have been partly explained by the transfer of somatic mutations into the germline, a phenomenon which does not happen in animals. Mutations accumulate through mitotic divisions in meristematic initials as the plants age (Klekowski 1988). In long-lived plants, there are more mitotic divisions and presumably a higher rate of mutations per generation than in annuals. Since meristematic tissue gives rise to gametes, these somatic mutations are transmitted to the next generation (Klekowski and Godfrey 1989). If so, gametes from a 20-yr-old tree will be expected to have fewer mutations than the same tree at a later age.

Variability within a Species

Variability in the number of lethals is as important as the mean number of lethals in a conifer breeding programs. A parent that exhibits little decline in fertility or progeny performance upon inbreeding may have few lethals or other deleterious recessives. If this parent also exhibits high performance for traits of direct economic value, then it may be highly prized for advanced-generation breeding.

Within a population, individuals will vary in the number of lethals. Each individual receives a sample of lethals from its parents' gametes. If the total number of loci which carry deleterious alleles is n, and the average allele frequency is p, then sampling approximates a Poisson distri-

Table 5. Number of lethal equivalents per zygote in different species. All estimates are solely based on the offspring viability component. Lethal equivalents for animals and dioecious plants are based on Morton et al. (1956) using single-generation data; other estimates of lethal equivalents are based on Sorensen (1969) using single-generation data.

Species	Lethal equivalents	References
Wied's red-nosed rat (*Wiedomys pyrrhorhinos*)	30.4	Ralls et al. 1988
Eld's deer (*Cervus eldi thamin*)	15.1	Ralls et al. 1988
Tamarack (*Larix laricina*)	10.8	Park & Fowler 1982
High-bush blueberry (*Vaccinium corymbosum*)	10.0	Krebs & Hancock 1991
Douglas-fir (*Pseudotsuga menziesii*)	8.6	Sorensen 1971
Loblolly pine (*Pinus taeda*)	8.5	Franklin 1972
Scots pine (*Pinus sylvestris*)	6.6	Koski 1973
Speke's gazelle (*Gazella spekei*)	6.2	Ralls et al. 1988
Willow (*Salix viminalis*)	1.8	Kang et al. 1994
Phlox (*Phlox drummondii*)	1.2	Anderson et al. 1992
Japanese quail (*Coturnix coturnix japonica*)	3.6	Sittmann et al. 1966
Pygmy hippopotamus (*Choeropsis liberiensis*)	3.2	Ralls et al. 1988
Fruit flies (*Drosophila melanogaster*)	0.3–2.3	Lewontin 1974
Humans (*Homo sapiens*)	2.2	Cavalli-Sforza & Bodmer 1971

Table 6 Estimates of diploid genome-wide mutation rates per generations, *U,* for annuals and perennials based on albinism, a class of chlorophyll-deficient lethals. In Scots pine and Norway spruce, the selfing rate is assumed to be 20% .

Species	U	References
Monkeyflower (*Mimulus guttatus*)	1.5×10^{-3}	Willis 1992
Barley (*Hordeum vulgare*)	6.3×10^{-4}	Jorgenson & Jensen
Maize (*Zea mays*)	4.0×10^{-3}	Crumpacker 1967
Mangrove (*Rhizophora mangle*)	1.5×10^{-2}	Klekowski & Godfrey 1989
	1.2×10^{-2}	
Scots pine (*Pinus sylvestris*)	3.0×10^{-2}	Kärkkäinen et al. 1996; Eiche 1955
Norway spruce (*Picea abies*)	2.5×10^{-2}	Kärkkäinen et al. 1996; Koski and Malmivaara 1974

bution with mean and variance *pn.* For example, if the average number of lethals equals is 10, 1 in roughly 20,000 trees is expected to have no lethal alleles. Similarly, if the average number of lethals is 3, then 1 in 20 trees is expected to have no lethal alleles.

Provenances also differ in their estimated lethal numbers. For example, there are fewer lethals in the northern Finnish populations of Scots pine (*P. sylvestris*) than in southern populations (Kärkkäinen et al. 1996). This supports an earlier study where Bishir and Namkoong (1987) cataloged means and variances for embryonic lethal estimates. In many instances they found variances which exceeded the Poisson expectation. This was attributed to those datasets which included heterogeneous populations, composed of individuals drawn from widely distributed stands (Bishir and Namkoong 1987).

Inbreeding Depression in Future Generations

Multiple generation studies of inbreeding are needed to guide decisions for operational conifer breeding programs. They will also contribute to understanding the genetic basis of inbreeding depression. Ideally, these long-term experiments would extend over the entire lifespan to include adult fecundity, but this is difficult with long-lived conifers. Multiple-generation studies can also be used to test whether it is possible to eliminate or purge deleterious alleles through inbreeding.

There are few data on inbreeding depression after the first generation of inbreeding. One Douglas-fir study has been reported through the third-generation (S3) (Orr-Ewing 1976) and there are some second-generation selfing studies (Pawsey 1964, Orr-Ewing 1965, Bingham 1973, Andersson et al. 1974, Rudolph 1981, Griffin and Lindgren 1985, Sniezko and Zobel 1988, Woods and Heaman 1989). Studies using milder forms of inbreeding have not been carried though more than one generation. The question of selfing for purging deleterious alleles is addressed using these conifer studies as well as reports based on model organisms.

Purging Deleterious Alleles

Can conifer populations be purged of their deleterious alleles? In the simplest case, after selfing of a parent with *n* lethals, the expected number of lethals in sound seed is 2/3 *n* (e.g., Namkoong and Bishir 1987). If the lethal allele is not fully recessive, selection can be made even more efficient because heterozygotes will also be affected (e.g., Lande and Schmeske 1985). However, this situation is of course an

oversimplification. There will be many loci with alleles with different effects, lethal, semilethal, and mildly detrimental These loci may be linked and even epistatic. It is also possible that there is some proportion of overdominant loci, which will have different dynamics.

Several analytical and simulations studies have explored the interactions of mode of selection, linkage, population size, and mutation on inbreeding depression (Charlesworth et al. 1990, Barrett and Charlesworth 1991, Charlesworth et al 1992, Hedrick 1994). These studies have demonstrated the many complex interactions among these factors. These results are relevant for tree breeding, even if mutation need not be considered in short-term studies.

These studies allow some general predictions about the possibility of purging deleterious alleles from breeding populations. First, it should be fairly easy to purge a population of its lethals and highly deleterious alleles, even with moderate progeny sizes (Hedrick 1994). There will be a concomitant and rapid increase in fitness. Second, when inbreeding depression is due to mildly detrimental alleles, purging will be more difficult. Instead, there will be an initial decline then population fitness will increase very slowly. These alleles may be fixed and cause a permanent decline in fitness. Third, any lethal or deleterious alleles which become fixed will increase the risk of extinction. This will lead to a loss of adaptive potential. Thus, large progeny sizes per mating are needed to maintain viable lines (Hedrick 1994).

There are many other concerns when planning actual inbreeding schemes for conifers. First, even if early acting lethals are common, most inbreeding depression in later life stages may be due to slightly deleterious alleles, as suggested by Lande (1994). It will difficult to purge these alleles. Second, purging forest tree populations will require large numbers of progeny per founder to also meet other objectives: to buffer against extinction losses, to reduce the probability of random fixation of slightly deleterious alleles, to conserve alleles of future adaptive potential and to maximize chances of fixing favorable alleles for one or more economic traits.

Strong selection must be applied not only to fitness traits such as offspring survival and adult fecundity, but also for economic traits. Negative genetic correlations between survival, adult reproductive fitness, and economic traits could increase the probability of extinction, requiring even larger family sizes. Until now, most conifer selfing programs have been started with such low numbers of founders such that strong selection would not be possible (e.g., Orr-Ewing 1965, 1976).

Further, inbreeding depression is often not fully expressed on optimum growing sites or in greenhouses (e.g., Dudash 1990). If inbreeding depression is not fully expressed in these environments then selection against deleterious recessive alleles will be weakened. Intense plant-to-plant competition can also alter estimates of inbreeding depression.

The predictions on the efficacy of purging should be tested in experimental populations, independent of operational breeding programs. The sheer size and economy of scale needed for operational breeding populations mitigates against their experimental use. Operational breeding programs will not be useful for testing breeding strategy assumptions about inbreeding depression. Experimental inbred populations will yield useful results which can be applied to operational programs.

Multiple-Generation Studies for Conifers

Changes of inbreeding depression in embryo viability over two generations have been reported. Most studies report that S2 sound seed proportions are lower than in S1 parents (Pawsey 1964, Orr-Ewing 1965, Andersson et al. 1974, Rudolph 1981, Woods and Hearhan 1989). One exception to this is found in Griffin and Lindgren (1985) who reported similar sound seed yields for S2 relative to the S1 for radiata pine (*Pinus radiata*).

Comparisons among S0, S1, and S2 should be based on the same set of parents or lines. In some cases, difficulty in obtaining viable selfs has lead to unbalanced (and biased) comparisons. Fitness estimates across generations are then confounded by differences in the number of lethals among founders. This was the case for a jack pine (*Pinus banksiana*) study of the S1 and S2 generations (Rudolph 1981). Reports for a radiata pine (*P. radiata*) S2 study (Pawsey 1964 p. 12–13), and a western white pine (*P. monticola*) S2 study (Bingham 1973) documented similar problems.

Maternal effects from low-vigor S1 parents could also increase estimates of S2 embryo lethals. However, there is sparse evidence to support this source of bias. Loblolly pine S1 and S0 parents were outcrossed, and their outbred seedlings showed negligible inbreeding depression and full vigor compared to outcrossed S0 parents (Sniezko 1984). Similar results were reported for jack pine (*P. banksiana*) (Rudolph 1981).

Multiple-Generation Studies for Other Species

Empirical data on long-term changes in inbreeding depression are scarce in other organisms as well. After 5 generations of selfing, deleterious alleles were eliminated in a water hyacinth (*Eichhornia paniculata*) population. There was an initial decline in flower number then fitness increased by the fifth generation. The mean fitness of the progeny derived from intercrossing among the inbred lines was much higher than that of the original outcrossed population (Barrett and Charlesworth 1991). These data are in accordance with the partial dominance model (Barrett and Charlesworth 1991).

Similar results have been reported for maize. Early in a maize selfing program, lines were severely depressed, but they began to regain vigor after 20 generations (Jones 1939). Inbreeding and selection were also effective for reducing the

frequency of deleterious, visible mutants in maize. In local races which were maintained as outcrossing populations, the frequency of chlorophyll-deficient heterozygotes was 24.4%, but in a synthetic of inbred lines, the frequency dropped to 5.3% (Crumpacker 1967).

In strawberry (*Fragaria* spp.), similar results were reported for a comparison among lines with similar inbreeding coefficients but different generations of inbreeding (Shaw 1995). Inbreeding depression was significant (14–15% for selfs) for lines developed from one generation of close inbreeding. Lines with inbreeding accrued over several generations had no inbreeding effects. This suggests the deleterious alleles had been eliminated over multiple generations of inbreeding.

Selfing as a Breeding Tool

The first selfing study for a conifer was reported in 1910 by Swedish silviculturalist Nils Sylven (Sylven in Eriksson et al. 1973) near the same time when deleterious recessives were first proposed as the cause of inbreeding depression (Davenport 1908, Bruce 1910). Since then, a number of selfing programs have been outlined for conifers (Righter, 1960, Orr-Ewing 1965, Bingham 1973, Barker and Libby 1974, Lindgren 1975b). Selfing and sib-mating as a breeding tool has been revived because of the growing interest in small elite breeding populations.

Selfing has traditionally been justified as a breeding method for several reasons: (1) mating is completely assortative, (2) efficacy of selection among selfed lines is maximized, and (3) the expected approach to uniformity within a family is faster than for any other type of inbreeding (Barker and Libby 1974, Lindgren 1975b). These advantages of selfing are based on a strictly additive genetic model. Many traits have some degree of nonadditive inheritance, and thus practice does not always bear out theoretical advantages. This departure from theory has resulted in some notable failures to attain pure breeding lines in other species (Robertson 1949, Sittmann et al. 1966).

Perfect Assortative Mating

Selfing represents perfect positive assortative mating for all traits and ensures that the best parent will be mated to itself. In other mating schemes, particularly those that avoid related matings, there is some disadvantage to having to mate the top-ranked selection with the next selection. The next selection is often a relative, so skipping to the next best selection means a trade-off between genetic value and relatedness, and it may indeed entail using a mediocre parent. Under selfing, the superlative parent's genes are transmitted to the next generation without a mediocre contribution from the second-best individual.

Selection Efficacy Among Lines

In theory, selection is also more effective among and within selfed lines. The total additive genetic variance is doubled upon complete inbreeding ($F = 1$). In practice, few

traits are completely additive, and it is the non-additive component that causes problems. Using a strictly additive model, selfs and outcrosses from common parents can be used to estimate a self-sib genetic covariance (Matzinger and Cockerham 1963):

$$\text{Cov}(HS, S) = 1/2\,{}^2_A + 1/4\,{}^2_{AA} \qquad (3)$$

where Cov *(HS,S)* is the covariance between selfed and outcrossed sibs, σ^2_A is the additive genetic variance, σ^2_{AA} is the additive × additive genetic variance. There are no dominance or other epistatic terms. Half-sib performance alone is an estimate of breeding value or general combining ability (GCA), so in the absence of nonadditive genetic variance, we can expect a perfect correlation between selfed (S1) and outcrossed breeding value (GCA).

In practice, an additive genetic model cannot be assumed for most conifer traits of economic interest. Selfed performance tends to be a poor indicator of general combining ability, and this reduces the advantage of selfing as a practical tool in the early generations (Gallais 1988, 1990, Barker and Libby 1974). Selection on selfs for traits of economic value will not be effective if there is a strong nonadditive genetic component. Additional genetic testing of outcrossed sibs must be conducted to obtain unbiased GCA estimates, and this offsets the advantage of enhanced selection efficacy.

The departure from a perfect genetic correlation between a parent's selfed and outcrossed progeny performance measures how much selection efficacy will be reduced for traits of direct economic interest. If the correlation between selfs and outcrossed sibs approaches unity, then a selfed line becomes the preferred option. For example, Corn Belt maize lines exhibit a S1-GCA correlation near 0.50 (Hallauer et al. 1988). If the S1-GCA correlation is lower, it is prudent to initially use lower levels of inbreeding before developing selfed lines (Gallais 1990).

Conifer S1-GCA correlation estimates for growth are nearly zero (Wilcox 1983, Sniezko and Zobel 1988). We have found no explicit reports of S1-GCA correlations for straightness or wood quality traits although it seems likely that the S1-GCA correlations will be higher. For improvement of growth traits, use of full- or half-sibbing in early generations would seem more prudent than selfing even though an additional set of outcrosses may also be required to obtain unbiased GCA estimates.

It has been argued that selfing is best even in the early generations if culling weak selfed offspring is used to improve the S1-GCA correlation (Orr-Ewing 1965, Wilcox 1983). It is difficult to test this assertion because no estimates of S1-GCA correlations after early selection have been reported to date. Culling selfed seedlings for reforestation was favorably evaluated for two species, Douglas-fir and ponderosa pine (Sorensen and Miles 1974) but the effect on selfed-outcross correlations was not reported. Early selection in the nursery stage must be evaluated as a two-stage selection method before it can be used to justify selfing.

Uniformity Within an Inbred Line

In theory, a notable advantage to selfing is the increase in uniformity within a line as well as the improved selection efficacy among lines (Barker and Libby 1974, Wright 1980).

If the population is divided into lines, and there is only additive genetic variance, the total genetic variance (defined as V_g) in the base population will increase in proportion to the inbreeding coefficient: $(1 + F)V_g$. The within-line variance (defined as V_w) will decline as a function of $(1 - F)V_g$, and the among-line variance (defined as V_b) will gradually become $2FV_g$ (Falconer 1981).

Empirical results do not support theoretical predictions. Within-line variance increases with inbreeding for many species including conifers after one single generation of selfing; twofold increases are common (Rudolph 1981, Sniezko and Zobel 1988, Geburek 1986). Similar increases for within-line variance have been observed for several generations with other organisms such as house flies (Bryant et al. 1986, 1992).

There are several explanations for observed within-line variance increases. First, within-line variance is allele frequency-dependent. Expression of rare, recessive alleles during the transition from an outbred to an inbred mating system causes an increase in within-line variance if recessive allele frequencies are less than 0.3 (Robertson 1952). This has been demonstrated experimentally; population bottlenecks lead to increased additive genetic variance (Bryant et al. 1986).

A second explanation is that during a bottleneck event, additive genetic variance increases in a transient manner with the conversion of additive × additive epistasis to additive genetic variance (Bryant et al. 1986, Goodnight 1988). This depends on the rate of recombination across successive generations.

A third explanation is that environmental variance increases as inbred plants become less buffered against environmental stress (Robertson 1952, Falconer 1981, p. 268), offsetting the decrease of genetic variance within a line.

Managing Experimental Inbred Populations

Today, the appropriate inbreeding methods can easily be explored via pilot-scale experimental populations, developed in *advance* of operational conifer programs. Experimental inbred lines or populations use breeding strategies that are quite different from present-day operational breeding programs. Here is ample opportunity to test genetic models and collect data on high-risk breeding strategy options for the larger and more costly operational breeding programs. We propose an experimental inbred population using accelerated breeding technology developed for loblolly pine (*Pinus taeda*) as an example of this concept.

Systematic and Random Mating

Selfing has been proposed for some conifer breeding programs which emphasize growth and volume traits. This is advisable only for species, provenances, or pedigrees known to express modest inbreeding depression for growth and for fitness traits. For example, a large ($N = 500$ to 1000) number

of parents might be selected from existing selfed-offspring studies as well as outcrossed progeny or family performance. Parents which were successfully bred after 2 seasons would be retained, and large numbers of their selfed progeny would be screened within the first year. Vigorous selfed progeny which had superior outcrossed sib performance would be the basis for the next generation. It should be noted that the breeding workload is heavy for an experimental inbred population because it includes outcrossings as well as inbred matings. General combining ability estimates for family and or parental selection must be based on outcrossed offspring performance in order to make genetic gain.

Multiple-Stage Selection

Under this selection scheme, a different set of traits is used at each stage of selection. Multiple-stage selection is appropriate when traits are expressed at different ages and when traits differ widely in cost and difficulty of measurement (Xu and Muir 1992). This selection method will increase genetic gain if there is strong selection at all stages, if there is at least a modest genetic correlation between stages and if the trait with the highest heritability is used first (Cotterill and James 1981). Multiple-stage selection is useful for an experimental inbred population for *P. taeda* because many abnormalities are expressed after germination (Franklin 1970) but other traits, like long-term adaptability, are expressed later. Also, adult fecundity traits are more expensive and difficult to measure although it may be possible to assess them early.

Greenhouse Testing

In an experimental inbred population, one must use large numbers of offspring per self-fertile parent to select strongly against loss of vigor, mutants, and malformation. Juvenile traits and markers linked to older-tree traits could be selected in the first-stage culling (Williams 1987, Williams and Neale 1992, Williams and Megraw 1994). Adult fecundity, long-term testing, and older-tree measurements would constitute the basis for the second stage of selection.

Adult Fecundity

Adult fecundity can be assessed rapidly on conifer species that have precocious reproduction such as jack pine (*Pinus banksiana*) and some of the Cupressaceae. It can also be assessed within a year after grafting for some late-flowering conifer species using the surrogate strobilus induction technique (Bramlett et al. 1995). For example, inbred selections (plus outcrossed controls) from the first stage would be grafted onto mature, heavy-flowering orchard ramets. Within a year after grafting, strobili will appear on these selections so breeding as well as an assessment of fecundity traits can be completed. Those selections with late reproductive onset or a complete absence of reproductive structures would be culled.

Long-Term Testing

The second stage of selection would be based on adult fecundity and older-tree measurements of traits that are of economic value but not unduly subject to inbreeding depres-

sion. Long-term testing is critical to ensure that purging has not randomly fixed mildly deleterious alleles, thereby restricting adaptability in future generations. Quantifying reduction in adaptability with inbreeding is critical to breeding and testing plans.

Genetic testing of inbreds will require more plants per entry than for outbred testing. More plants will be needed at each location to offset mortality, and more locations will be needed to select for broadly adapted genotypes. For selfing, Sniezko and Zobel (1988) estimated that at least 2.5 times more seed would be needed for genetic testing than for outcrossed offspring. If adult fecundity is limited for inbred parents, vegetative propagation for genetic testing will warrant consideration for some species. Vegetative propagules provide better estimates of adaptability as well as reduce sound-seed requirements. For conifer species where vegetative propagation is not an option, long-term testing for adaptability is likely to increase the inbred seed requirements and possibly the generation length if seed potential declines with inbreeding. This would sharply reduce justification of a rapidly cycled experimental inbred population. Experimental inbred populations are one example of an emerging research area in forest genetics and breeding. The genetic basis of inbreeding depression is the foundation for population improvement programs in forestry.

Implications for Research

Studying the Genetic Basis of Inbreeding Depression

Previous studies on the genetic basis of inbreeding depression have been based on biometrical approaches (e.g., Frankel 1983) or studies using small numbers of isozyme loci (e.g., Bush and Smouse 1992). The numerous molecular markers that are now being developed for conifers will allow new approaches. The mode of gene action can be studied while mapping quantitative trait loci (Bradshaw and Stettler 1994). The question of overdominance versus dominance can be addressed by comparing correlations of growth with genotypic distributions in different parts of the genome, e.g., enzyme loci versus random coding regions. Studying mollusks, Pogson and Zouros (1994) used this approach to test whether the candidate enzyme loci were responsible for observed correlations between heterozygosity and growth, or whether the correlations were due to associative overdominance.

Another option is to examine segregation ratios in progeny after selfing. A lethal gene close to a marker will result in a deviation from Mendelian expectation at the marker locus. This can allow for mapping of completely recessive lethals (Sorensen 1969, Hedrick and Muona 1990). This approach has been further developed using a graphical approach to allow a test of the mode of gene action (Fu and Ritland 1994). Until the advent of molecular markers, there has been no way to track individual genes causing inbreeding depression. These markers are linked to alleles causing inbreeding depression and can be used to characterize distribution of these genes throughout the conifer genome.

Inbreeding Depression in Adult Fecundity Traits

The evaluation of selfing as a breeding tool is hampered by the limited knowledge about adult fecundity traits. If inbreeding depression is more severe for adult fecundity than for offspring viability or growth, then losses due to inbreeding will be higher than expected. Inbreeding depression for juvenile and adult fecundity traits need to be comparatively studied in existing inbreeding studies.

Inbreeding Depression for Wood Quality and Adaptability Traits

Quality traits such as bole straightness and wood properties receive more emphasis in conifer breeding programs than they once did. The advent of specialty populations bred specifically for quality and for disease resistance has created a great need for studies on inbreeding depression in these traits. Only initial reports for wood density are available, which suggest low inbreeding depression. There is no information on adaptability traits such as cold-hardiness or drought-tolerance.

Inbreeding depression for adaptation is a critical research area, particularly for programs based on exotic species or nonlocal seed sources. Even if inbreeding is limited to the breeding population, genetic testing must be increased to compensate for poor survival in inbred plants. If exotic species or nonlocal seed sources are planted on harsh sites, mortality of inbred plants could increase substantially.

Computer Simulation Models for Conifer Inbred Lines

The simulation study reported by Hedrick (1994) provides some guidelines for management of captive animals. Such effort needs to be adapted to the particulars of conifer biology, e.g., high genetic load and large progeny numbers. Computer simulation studies are under development for management of small conifer populations (Mahalovich 1990), but there is still a need to extend these multilocus models to experimental inbred populations.

Summary and Conclusions

Interest in inbreeding depression in conifers has been renewed by a shift to smaller breeding populations and by seed production in controlled-pollinated seed orchards. This has renewed interest in inbreeding as a method rather than as a problem. Inbreeding depression changes across generations, and these directional changes depend on the genetic basis of inbreeding depression.

Under the partial dominance model, inbreeding depression is caused by deleterious recessives which can be eliminated through strong selection. Inbreeding depression is expected to decrease in future generations. Purging is not predictable because the incidence of lethals in conifers is typically high compared to reports for other organisms and mildly detrimental alleles will be difficult to purge. Conversely, if overdominant loci contribute, fitness will decrease continuously over generations.

Based on this model, selfing in early generations of breeding does not seem to be the best option for most conifers. Possible exceptions may be conifers with a low lethal load. The theoretical advantages of selfing are perfect

assortative mating, increased selection efficacy among lines, and increased uniformity within lines, but these advantages are weakened in the presence of nonadditive genetic variance. Also, selfing programs require extremely large population sizes for several reasons: (1) strong selection pressure is required for effective purging, (2) selfing may well entail extinction losses exceeding the 97% rate initially observed for maize, and (3) valuable selfed lines in the initial generations, as with maize, will be very rare. Rapidly cycled experimental inbred populations are quite useful for testing these assertions. Otherwise, circular half-sib and random mating in small closed populations seem preferable, particularly for conifers with high lethal loads.

For conifers, testing this model requires an experimental system independent of operational breeding programs. Operational conifer programs are not suited to experimental use because random losses to attrition are too costly. Experimentation in *advance* of operational conifer programs is therefore critical. Experimental inbred populations represent a major departure from the breeding strategies currently used in operational programs. Within these small populations, there is ample opportunity to test genetic models, to test high-risk breeding strategy options, and to optimize breeding methods for larger, more costly operational breeding programs. We have described how an experimental inbred population might be managed using a multiple-stage selection scheme which combines greenhouse testing, rapid screening for adult fecundity and long-term adaptability.

There are pressing needs for both applied and basic research in inbreeding depression. Experimental inbred populations will provide timely, first-hand knowledge and lend confidence to decision-making in large operational programs.

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