

Effect of Population Outcrossing Rate on Inbreeding Depression in *Pinus contorta* var. *murrayana* Seedlings

FRANK C. SORENSEN

US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA

Scandinavian Journal
of Forest Research



Sorensen, F. C. (US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR, 97331, USA). *Effect of population outcrossing rate on inbreeding depression in Pinus contorta* var. *murrayana* seedlings. Received November 16, 2000. Accepted July 23, 2001. Scand. J. For. Res. 16: 391–403, 2001.

Self and cross families from three populations (A, low-density, ecologically marginal site for lodgepole pine, and B + C, normal sites) were cultured in a common outdoor nursery for 2 yrs. Previous results had shown higher natural selfing rates and lower inbreeding depression in embryo survival in A than in B + C. In the nursery test, selfing decreased means of size traits, retarded phenological development, increased variance among families within populations, increased the coefficient of within-family variance, and increased within-family skewness. The selfing depression of mean values was moderately less and the selfing inflation of within-plot variation was much less in A than in B + C. Results were compatible with the idea that the higher selfing rate in A had purged both severely deleterious alleles affecting embryo survival and severely deleterious alleles affecting later life-cycle traits related to plant size. Purging partially mitigated the genetic consequences associated with increased inbreeding in small populations, but even with purging, population A retained a large inbreeding depression in both embryonic and seedling traits. *Key words: deleterious alleles, family variances, lodgepole pine, purging, selfing.*

INTRODUCTION

Inbreeding is the mating together of individuals related by ancestry (Falconer & Mackay 1996). In organisms that normally outcross, inbreeding increases homozygosity and leads to a reduction in fitness, which is expressed as depression in embryo and plant survival, vigour, developmental rate and fertility, and often as an alteration in the means of other traits (Husband & Schemske 1996, Crnokrak & Roff 1999). Ritland (1996) emphasizes that “inbreeding is a biological character of no little importance, particularly in plants: inbreeding depression has implications for the evolution of mating systems, levels of genetic variation, productivity, and species (and population) conservation”.

The phenotypic consequences of inbreeding have been attributed to partial dominance (mutation), overdominance (selection), and epistatic effects (Charlesworth & Charlesworth 1987, Crow 1993, Ritland 1996). Most current experimental results seem to support the partial dominance model and suggest that alleles responsible for inbreeding effects arise and are maintained predominantly through recurrent mutation or balancing selection (Crow 1993, Fu & Ritland 1994, Husband & Schemske 1997, Charlesworth &

Charlesworth 1999), although contributions by overdominance and gene interactions are not excluded (Temin et al. 1969, Mina et al. 1991, Latta and Ritland 1994, Rumball et al. 1994).

The mutation load has been studied extensively in *Drosophila* spp. with results indicating a bimodal distribution of effects with the two modes centering on a comparatively low frequency of mutations that are lethal or highly deleterious when homozygous, and a comparatively larger to much larger number with minor effect (e.g., Crow & Simmons 1983, Lynch et al. 1999; but see also Keightley & Eyre-Walker 1999). Study of the timing of inbreeding effects has indicated that depression in early life-cycle traits (embryo and germinant seedling survival) is primarily the result of mutations of large effect, whereas inbreeding effects on subsequent vigour and morphology (metric characters) much more often are due to mildly deleterious mutations (Hadorn 1961, Mitchell-Olds & Guries 1986, Jürgens et al. 1991, Husband & Schemske 1996). Theoretical studies indicate that an increase in rate of close inbreeding will usually affect the two classes of mutants differently, tending to purge those of large, but not those of small, effect (Lande & Schemske 1985, Lande et al. 1994, Barrett & Harder 1996, Kirkpatrick & Jarne 2000).

Table 1. Mating system estimates^a for lodgepole pine in three lodgepole pine–ponderosa pine populations (additional description of stands is given in text)

Character	A	B	C
Frequency of lodgepole pine in population	0.08	0.49	0.81
Inbreeding depression in fertility ^b	0.813 (0.006) ^c	0.905 (0.004)	0.914 (0.005)
Estimated measured selfing rate ^d	0.213 (0.010)	0.017 (0.001)	0.020 (0.001)
Estimated primary selfing rate ^e	0.566 (0.010)	0.148 (0.012)	0.247 (0.010)

^a Mating system derivations and estimates are given in Sorensen & Adams (1993). Values are based on nine trees per population, and assume one embryo per ovule. The use of two embryos per ovule would have only a minor effect on the values (Sorensen & Adams 1993) and would not alter any of the comparisons.

^b $1 - (\text{Self fertility} \div \text{cross fertility})$.

^c Standard error.

^d Probability that a filled seed after wind pollination is the result of self-fertilization.

^e Probability that wind pollination results in self-fertilization.

Lodgepole pine (*Pinus contorta* Dougl. ex Loud.) is a widely distributed conifer in western North America. Four varieties are recognized (Critchfield 1957). The present study was conducted in the variety *murrayana* [*Pinus contorta* var. *murrayana* (Grev. & Balf.) Engelm.] on the east slopes of the Oregon Cascade Range.

Lodgepole pine usually regenerates after fire or other events that result in canopy openings (Stuart et al. 1989). Seed production is regular and prolific and stands are often overstocked (Dahms & Barrett 1975, Stuart et al. 1989). Although dense stocking can persist (Worrall et al. 1985), the usual pattern in initially overstocked stands is probably self-thinning approximating quasi-truncation selection for vigour traits (Crow & Kimura 1979) with only the most vigorous plants surviving and reproducing (Koch 1996).

Most seeds are disseminated within 60 m of the seed source (Mason 1915, Boe 1956, Dahms 1963), but they can disperse much farther under some conditions (Bannister 1965, Critchfield 1978) because of their slow rate of fall (Siggins 1933, Cremer 1971). The species produces unusually large amounts of pollen (Critchfield 1978) and pollen dispersal is extensive (Fall 1992). Effective population sizes are estimated to be large, ≥ 1000 (Epperson & Allard 1989) and ≥ 4000 (Yang & Yeh 1995). Spatial autocorrelation analysis in continuous stands has shown lack of population structure in distribution of most genotypes (Epperson & Allard 1989).

Lodgepole pine is mixed mating, but self fertility is low (Smith et al. 1988, Sorensen & Adams 1993). Mating system estimates indicate high measured rates of outcrossing, often not significantly different from 1

(Yeh & Layton 1979, Epperson & Allard 1984, Sorensen 1987). However, there is also evidence that outcrossing rates are directly related to density of conspecific individuals (Smith et al. 1988) or are influenced by stand density or marginality (Yeh & Layton 1979, Sorensen 1987). Investigations into several other coniferous species (Piesch & Stettler 1971, Farris & Mitton 1984, Knowles et al. 1987, Sorensen 1994, Kärkkäinen et al. 1996, Hedrick et al. 1999), but not all (Furnier & Adams 1986, Morgante et al. 1991), also indicate lower estimated rates of outcrossing in less dense stands.

Some of these studies suggest severely deleterious alleles affecting embryo survival are purged when selfing rate is increased (Hedrick et al. 1999). To the author's knowledge, no previous study has looked at the relation between stand density characteristics and inbreeding effects on plant growth traits. Embryo survival is an early life-cycle trait and decreased embryo survival in conifers following self-pollination is attributed primarily to recessive lethal and highly deleterious alleles (Orr-Ewing 1957, Hagman & Mikkola 1963, Mitchell-Olds & Guries 1986, Kuang et al. 1999, Remington & O'Malley 2000). Seedling vigour and other nursery characters are representative of later life-cycle traits with inbreeding effects probably due predominantly to weakly deleterious alleles (Falconer & Mackay 1996, Husband & Schemske 1996). If these assumptions are correct, the lodgepole populations, even though they differ in inbreeding depression at the seed stage (Table 1), may be expected to have comparable inbreeding depression in vigour traits (Lande et al. 1994, Ritland 1996).

In the present study, self and outcross progenies from several seed trees in three populations were

grown for 2 yrs in favorable nursery conditions, and populations compared for the effect of selfing on mean performance and on variances. Of the three populations, one (population A) is a low-density, ecologically marginal stand with a low frequency of lodgepole pine; while the two others (B and C) are non-marginal and representative of the main distribution (Table 1, top row). Self fertility and estimated rates of primary and measured selfing are higher in A than in B and C (Table 1, from Sorensen & Adams 1993).

MATERIALS AND METHODS

Pollinations were done in three stands: A (43°46'N, 121°28'W, 1370 m) with self and outcross families from eight seed parents; B (43°46'N, 121°29'W, 1430 m) from seven, and C (43°47'N, 121°31'W, 1540 m) from four. All three stands were mixed lodgepole-ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) on the east slopes of the Cascade Range in central Oregon, USA. Mature tree (trees capable of seed production) components were 8, 49, and 81% lodgepole pine for stands A, B, and C, respectively. Population A was on a marginal site for lodgepole, and stand density of all trees of reproductive age was low. This situation appeared to be historical. Populations B and C were separated from A by 2–4 km, at a higher elevation, on more mesic sites and more densely stocked. In population A, lodgepole pine appeared to be present as a minor, persistently seral species; in B and C, it was a dominant, persistently seral species (Pfister & Daubenmire 1973). The amount of pollen inflight into population A is unknown. According to pollination records, floral phenology was slightly delayed in populations B and C compared to A (average dates of artificial pollination 3–5 days earlier in population A). Phenology and proximity (first pollen to arrive most effective, Sorensen & Webber, 1997) probably contributed to isolation of the marginal population.

Progenies from eight of the original seed parents were not included in the nursery test for the following reasons: seed packet mislabeling (one parent), self families segregated for recessive markers and seed previously germinated to obtain segregation ratios (Sorensen 1987) (three parents), and insufficient self seed for the nursery test because of low self fertility (four parents). Thus, estimates of mating system parameters in Table 1 are based on 27 parent trees, whereas nursery estimates are based on only 19. For

the most part, different gene sets appear to influence self fertility and later inbreeding depression (Snyder 1968, Sorensen & Miles 1974). Therefore, it is assumed that the loss of low-fertility parents will only negligibly bias the contrast between marginal and non-marginal populations in the nursery. Original mating-system values from Sorensen & Adams (1993) are shown in Table 1, because it seemed biologically more straightforward to give observed population estimates at each stage even though some families subsequently were lost.

Pollinations were done at the field locations in 1967, 1970, and 1973. All were years of abundant flowering. Thirty to 70 female flower buds were isolated on each tree. About half were pollinated by self-pollen and half by a polymix of eight to 12 trees from the same site but separated from the seed trees by a minimum of 0.5 km. Isolation bags were installed before the opening of female buds. Stands were visited twice weekly during the receptive period, and flowers in individual bags were pollinated on two or three successive visits. Isolation bags were removed at the end of the first summer and replaced by cloth bags in the following spring to provide protection from insects.

Cones were collected just before separation of cone scales and stored in a dehumidified room at 30°C until scales flared. Seeds were extracted by hand, with round seeds separated into filled and empty classes through X-ray. Seeds were stored in sealed plastic envelopes at –10°C until sowing in spring 1994.

In February 1994, seeds were removed from storage and each family was placed in a small mesh bag. Bags were soaked for 22 h in aerated water, excess water was drained off, and mesh bags were placed in large plastic containers for 60 days' chilling at 2–3°C. Long chilling increases the uniformity of emergence and is similar to what would occur naturally.

In mid-April, seeds were surface dried and sown in small depressions on the soil surface of raised nursery beds. Seeds were covered with a thin layer of granite grit and the soil surface was moistened as necessary to maintain suitable conditions for germination. Spacing was 10 cm between rows by 8 cm within rows. Competition effects were minimal at this spacing. Two rows of border spots were sown around the test by using extra seeds from the outcrosses. Where sufficient seeds were available, two seeds were sown per spot and systematically thinned back to one in late summer of the first year by removing the north seedling in each spot. Seedlings were grown for 2 yrs

Table 2. Description of seedling traits analyzed for inbreeding depression

Trait	Description
Size	
Height-1	Final height, year 1 (cm)
Height-2	Final height, year 2 (cm)
Diameter	Final diameter at cotyledon scar, year 2 (mm)
Top weight	Fresh weight of top at harvest, ^a year 2 (g)
Root weight	Fresh weight of root at harvest, year 2 (g)
Elongation rate	Relative elongation rate, year 2 (mm·mm ⁻¹ ·yr ⁻¹)
Phenology	
Emergence	Days from sowing to emergence above ground surface
Budset	Days from sowing to visible budset, year 1
Other	
Cotyledon number	Number of cotyledons
Variance _w ^b	Family within-plot variance
Standard deviation _w ^b	Family within-plot standard deviation
Coef. of variation _w ^b	Family within-plot coefficient of variation
Skewness _w ^b	Family within-plot skewness

^a Plants were harvested in January after the second growing season.

^b Variance, standard deviation, coefficient of variation, and skewness were calculated for each trait; only the last two are discussed.

in the nursery. The traits measured are listed in Table 2.

The experimental design was a split plot with 10-plant family plots and four replications. The main plots were seed parents, which were randomized within replications. Subplots were self and outcross families within seed parents. Family plots were non-contiguous; i.e. seedlings were completely randomized within seed-parent main plots.

Analyses were conducted in two stages. First, to determine whether the selfing effect was significant, the data were analyzed as a split-plot experiment by using the model (Snedecor & Cochran, 1967):

$$Y_{ijk} = \mu + R_i + S_j + \epsilon_{ij} + P_k + (PS)_{jk} + \delta_{jk} \quad (1)$$

where μ is the overall mean; $i = 1, \dots, 4$ random replications, R ; $j = 1, \dots, 19$ random seed parents, S ; $k = 1, 2$ fixed pollen treatments, P ; main plot error, $\epsilon_{ij} = N(0, \sigma_s)$, and subplot error $\delta_{ijk} = N(0, \delta_p)$. Pollen mean square (MS_p) was tested against MS_{PS} . The effect of inbreeding on within-plot variation was evaluated using (1) the coefficient of family within-plot variation (σ_w /plot mean) (Lewontin, 1966, Bendel et al., 1989) corrected for small numbers (Haldane, 1955), and (2) within-plot skewness. Within-plot coefficient of variation and skewnesses were calculated for each plot and values analysed

according to the above model. Pooled variance among families within populations (F/P) was also calculated separately for outcross and self families. These values could not be compared statistically, but the ratio, $MS_{F/P(\text{self})}/MS_{F/P(\text{outcross})}$, would indicate the direction in which selfing affected variation among families.

The second step in analysis was to test the population contrasts: A vs. (B + C) and B vs. C (B and C were pooled in the first contrast, because they had common mating system estimates, Table 1). This was done by entering the value for inbreeding effect (IE) of each progeny-pair (self and cross) in each replication into an hierarchical model with three populations of eight, seven, and four self-cross pairs per population and four replications. The value for each pair in each replication was calculated as $IE = 1 - (W_s/W_x)$, where W is the performance of the self ($_s$) and polymix outcross ($_x$) progenies (Lynch 1988, Johnston & Schoen 1994). If $W_s > W_x$, $IE = 1 - (W_x/W_s)$ (Ågren & Schemske, 1993, Holtsford, 1996). Ågren & Schemske (1993) used the term "relative performance" instead of inbreeding depression. Here, the terms "inbreeding effect" or "response to selfing" are used in cases where $W_s > W_x$. A nested model was used for the population contrast:

$$Y_{ijk} = \mu + R_i + L_j + S_{k(j)} + \epsilon_{ijk}, \quad (2)$$

Table 3. Mean outcross value, significance of inbreeding effect, mean inbreeding effect, significance of location contrasts [A vs. (B+C)]^a and (B vs. C), and significance of variation among families within locations for inbreeding effect

Trait ^b	Mean outcross value	Inbreeding effect	Mean inbreeding effect (%)	Among seed trees within populations	Among populations	
					A vs. (B+C)	B vs. C
Size traits						
Height-1	6.50	<0.0001	17.7	0.0063	0.1051	0.0974
Height-2	20.8	<0.0001	24.4	<0.0001	0.0155	0.4935
Elongation rate	1.17	<0.0001	9.6	<0.0001	0.0382	0.9601
Diameter	5.69	<0.0001	21.9	0.0036	0.0235	0.4811
Top weight	19.9	<0.0001	44.9	0.0099	0.0632	0.5706
Root weight	6.39	<0.0001	43.1	0.0319	0.0484	0.9112
Phenological traits						
Emergence	15.3	0.0033	-5.4 ^c	<0.0001	0.0064	0.2626
Budset	140.1	<0.0001	-4.4	<0.0001	0.2556	0.8319
Other trait						
Cotyledon no	4.68	0.0358	-2.9	<0.0001	0.7190	0.7056
Within-plot coefficients of variation						
Height-1	0.183	0.6565	5.5	0.1848	0.0481	0.5948
Height-2	0.160	0.0086	-17.6	0.0379	0.0128	0.3366
Elongation rate	0.122	0.0404	-15.5	0.0022	0.0160	0.0124
Diameter	0.146	0.0077	-17.7	0.0188	0.0432	0.2460
Top weight	0.310	0.0024	-18.8	0.1676	0.0189	0.3168
Root weight	0.356	0.0152	-12.8	0.0294	0.1204	0.1604
Emergence	0.0139	0.0484	-9.5	0.7908	0.0010	0.9386
Budset	0.0313	0.0004	-22.1	0.2067	0.0367	0.2269
Cotyledon no.	0.0979	0.0006	-17.9	0.0761	0.5662	0.6761

^a Population A is low-density, ecologically marginal lodgepole stand (8% lodgepole pine in a mixed-conifer stand), populations B and C are higher density, nonmarginal stands, 49% and 81% lodgepole, respectively.

^b Traits and units of measurement are described in Table 2.

^c Negative sign indicates that value for self families is larger than value for outcross families, e.g. emergence is later for self than for cross families and, for within-plot coefficients of variation, negative sign means coefficients are larger for self than for cross families.

where μ is the overall mean, $i = 1, \dots, 4$ replications; $j = 1, 2$ fixed locations (or populations), L ; $k = 1, \dots, n$ random seed trees within populations, S/L ; and $\epsilon_{ijk} = N(0, \sigma)$. MS_L was tested against $MS_{S/L}$, and the latter against MS_e .

RESULTS

General selfing effects

Selfing significantly reduced plant size and the relative rate of elongation in the second year, and depressed rate of development (i.e., delayed seedling emergence and date of budset) (Table 3). Inbreeding depression in height and diameter were about equal, as were depressions in top and root weights. Plant weights had about twice the depression of height and

diameter. Depression in percentage was larger for size than for phenology traits, but this may have been a scaling effect. Phenology was measured on time scales with arbitrary starting points that may not have been appropriate for determining the magnitudes of inbreeding. Mean emergence time was 0.9 day later and mean bud set date 6.9 days later for selfs than for outcrosses. Inbreeding effects were similar to those reported for other conifers in comparable nursery test environments (Sorensen & Miles, 1974, Sorensen et al., 1976, Sorensen, 1997).

As has been observed in most evaluations of inbreeding, progenies within populations differed significantly in response to selfing (Table 3, Among seed trees within populations). As two examples, mean family inbreeding depression in second-year height

ranged from 18.2% to 47.6% in population B. In the same population, inbreeding delayed bud set from -0.5 days (selfs set buds before crosses) to 23.4 days.

Inbreeding also strongly influenced variances. First, within-plot (i.e. within-family) coefficients of variation were significantly larger for self than for cross families for all traits except first-year height (Table 3, values of mean inbreeding effect on within-plot coefficients of variation are negative). Second, the pooled mean square term for families-in-populations was larger for self than for cross families for all traits except for weights and days to emergence (Table 4, $MS_{F/L}$) and was much larger for second-year height. When this variance was expressed as coefficient of variation [Table 4, $(\sqrt{MS_{F/L}/\text{mean}})100$], which adjusts for size differences between self and cross families, variation among self families was larger than variation among cross families for all traits except for days to emergence. Within-plot skewness was also affected by selfing, significantly so for second-year relative elongation rate and diameter. Size traits were more negatively skewed (larger tail of small values) and phenological traits more positively skewed in self than in cross families.

Population contrast

In spite of highly significant variation among seed trees within populations, the population contrast, A vs. (B + C) was often significant; the contrast B vs. C was not significant except in one case (Table 3). In the following presentation, B and C are considered to be samples of a common population for inbreeding purposes. For all traits, the response of the mean to selfing was less in population A than in population B + C (Table 5).

The most striking population contrast, however, was in the response of within-plot variation to selfing. For all traits except cotyledon number and first-year height, the selfing effect on within-plot coefficient of variation was much less in population A than in B + C (Table 5, larger negative values under B + C than under A mean that selfing increases coefficients of variation more in population B + C families than in A families). Similarly, the selfing effect on within-plot skewness was much less in A than in B + C. For example, within-plot skewness for second-year height was much greater (longer tail) for B + C self families (-0.495) than for A self families (-0.112). This is in contrast to within-plot skewness for cross families, which was similar for both populations (0.161 and 0.096 for A and B + C, respectively).

DISCUSSION

Inbreeding depression in vigour and population contrast

Selfing significantly depressed size, slowed the rate of development, increased phenotypic variance and skewness within families, and increased variance among families within populations. The following discussion will focus on second-year height and relative elongation rate as fitness traits and bring in other traits as appropriate. First-year height and to a lesser extent second-year height are influenced by seed size and emergence time (Sorensen & Campbell 1993), which means that elongation rate may best reflect the fitness associated with vigour.

Phenotypic responses to inbreeding depend on the gene action underlying the trait (Falconer & Mackay, 1996). If genetic variance is additive, selfing will not change the mean, but will change variances, increasing variance among and decreasing variance within families. A change in mean associated with selfing is evidence for nonadditive gene action. If the change in mean is directional and the loci affecting a trait combine additively, primarily dominance variance is indicated; if the mean changes curvilinearly with inbreeding coefficient, interlocus interactions, also involving dominance, are implied (Crow & Kimura, 1970). As concerns the variance response to selfing, dominance effects after selfing initially increase phenotypic variance both within and among families (Robertson, 1952).

The large inbreeding depression in vigour (e.g., 25% in second-year height) is in line with that reported for many other conifers (e.g., Bingham & Squillace, 1955, Squillace & Kraus, 1962, Sorensen & Miles, 1974). Conifer tests also have indicated a predominantly linear relation between inbreeding coefficient and inbreeding depression in size traits (Squillace & Kraus, 1962, Matheson et al., 1995, McKeand & Jett, 1995, Durel et al., 1996, Sorensen, 1997). These responses to inbreeding indicate largely dominance variance. This, however, is in contrast with designed mating tests, in which dominance variance (specific combining ability) is much lower than additive variance (general combining ability) (Yanchuk, 1996, King et al., 1998, McKeand & Bridgwater, 1998). The contrast, large inbreeding dominance effects and low outcross dominance variance, is evidence that the deleterious recessives contributing to inbreeding depression are at low, probably very low, frequencies in the population (Falconer & Mackay, 1996, Skrøppa, 1996).

The large effect of selfing on within-plot variation, increasing both coefficient of within-plot variance and within-plot skewness, indicates phenotypic outliers and suggests that some of the recessive alleles have severely deleterious effects. Severe vigour defects (e.g., dwarfism, chlorophyll abnormalities, needle and root abnormalities) (Eiche, 1955, Orr-Ewing, 1969, Franklin, 1969, Sorensen, 1987, and unpubl. results) have been observed in several coniferous species.

These population-genetics characteristics of lodgepole pine and at least some other widespread conifers suggest that a portion of the inbreeding depression in vigour is due to rare, severely deleterious, partially dominant alleles. The contrast between populations A and B + C in inbreeding response (Tables 3 and 5) indicated that the higher natural selfing rate in A has purged severely deleterious alleles associated with the phenotypic outliers, but not deleterious alleles of small effect. The primary evidence that purging has involved very rare alleles associated with outliers is that self families in A, compared with B + C, have a greatly reduced inbreeding effect on within-plot variance and skewness, but only a moderately reduced inbreeding effect on mean values. A few outliers inflate variances much more than they change means (Snedecor & Cochran, 1967, Hawkins, 1980). These results are in good agreement with other observations and with current theory (Dubinin, 1946, Lande and Schemske, 1985, Charlesworth & Charlesworth, 1987, Crow, 1993, Lande et al., 1994, Husband & Schemske, 1996, Kirkpatrick & Jarne, 2000).

Other traits

Fitness and non-fitness traits often differ in genetic background and in inheritance pattern (Falconer & Mackay 1996). Non-size traits may or may not fit into the "fitness" category for various reasons. In this test, with the exception of first-year height, the population contrast in response to selfing was always much larger for within-plot variation than for means. First-year height, which is partially determined by seed size, a maternal influence that decreases with age (Sorensen & Campbell, 1993), had somewhat less response to selfing than did other size traits. Because seed weight differs both among and within families (Silen & Osterhaus, 1979), it adds a confounding effect to first-year height growth and may contribute to lower expression of selfing depression.

Plant size in conifers is determined by both rate and duration of growth (Cannell et al., 1981, Rehfeldt & Wykoff, 1981). Date of budset, therefore,

would be a component of vegetative vigour (Skrøppa & Magnussen, 1993), because it contributes to the duration of growth. In agreement with this, self progeny had larger within-plot coefficient of variation for date of budset than did cross progeny (Table 3) and greater within-plot skewness (0.636 and 0.381 for self and cross families, respectively), and self families had greater variation among families within locations than did cross families (Table 4). The population contrast was somewhat less for date of budset than for the pure vigour traits (Table 5), but its response was that of a partial fitness trait.

Days to emergence showed a similar pattern to date of budset. Days to emergence depends on rate of embryo development after sowing. It is a function of seed weight, a maternal trait, but also of embryo size and vigour, and of response to pretreatments that overcome dormancy, also a maternal trait in conifers (Downie & Bewley, 1996). Germination rate has significant additive genetic variance (Morgenstern, 1974, Bramlett et al., 1983) and is conditioned by major rare recessive alleles functioning in the embryo (Sorensen, 1971). Populations differed significantly for this trait (Tables 3 and 5) indicating that decreased population size and increased selfing in population A probably purged severely deleterious alleles affecting the rate of embryo development.

Cotyledon number appears to have the characteristic of a threshold trait, either an additional cotyledon develops or it does not. Heritability is moderately

Table 4. Variation among families-within-populations given in two forms, pooled mean square_{family/location} ($M/S_{F/L}$) and its coefficient of variation, ($\sqrt{MS_{F/L}}/\text{mean}$) 100

Trait ^a	$MS_{F/L}$		$(\sqrt{MS_{F/L}}/\text{mean}) 100$	
	Self	Cross	Self	Cross
Height-1	0.320	0.301	10.8	8.44
Height-2	3.67	1.48	12.2	5.85
Elongation rate	0.0104	0.00431	9.68	5.62
Diameter	0.174	0.138	9.43	6.52
Top weight	5.06	8.28	20.5	14.5
Root weight	0.674	0.742	23.5	13.5
Emergence	1.02	1.51	6.31	8.41
Budset	88.3	18.6	6.28	3.06
Cotyledon number	0.213	0.0770	9.53	5.93

^a Traits and units of measurement are described in Table 2.

Table 5. *Selfing effect on family means and on within-family coefficients of variation for the population contrast, population A vs. populations B + C pooled*

Trait ^a	Selfing effect on			
	Means		Coefficients of variation (%)	
	A ^b	B + C	A	B + C
Height-1	15.0 ^c	19.6	16.2	-2.3
Height-2	18.6	28.6	-3.1	-28.2
Elongation rate	4.9	12.9	-2.0	-25.7
Diameter	18.0	24.7	-4.0	-27.6
Top weight	40.5	48.1	-5.0	-28.8
Root weight	37.5	47.2	-3.9	-19.2
Emergence	-1.3 ^d	-8.4	4.9	-19.9
Budset	-3.4	-5.2	-11.5	-29.8
Cotyledon no.	-2.3	-3.4	-20.8	-15.8

^a Traits and units of measurement are described in Table 2.

^b Population A is a low-density, ecologically marginal lodgepole pine stand (8% lodgepole mixed with ponderosa pine). Locations B and C are higher density (49% and 81%, respectively), less marginal stands mixed with ponderosa pine and white fir.

^c Probabilities that the population contrast is statistically significant are given in Table 3.

^d Negative sign indicates that values for self families are larger than values for cross families.

high ($h_{\text{family}}^2 = 0.58$, Morgenstern, 1974). The population contrast, A vs. B + C, was not significant for either mean inbreeding depression or selfing effect on within-plot variation (Table 3). This suggests no purging in population A, and that cotyledon number is behaving more as a morphological than as a fitness trait.

Comparison with other conifers

Because inbreeding depresses plant height, the measured degree of depression to some extent will depend on how plants respond to changing light conditions and to developing height inequalities. Different coniferous species respond differently to the former (Chen et al., 1996) and probably differently to the latter. Lodgepole pine showed inbreeding depression in the trait relative elongation rate and an increase in inbreeding depression between first- and second-year heights (Table 3). Second-year relative elongation rate in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], by contrast, was non-significantly greater for inbred than for outcross seedlings (Sorensen 1997). This was attributed to a photomorphogenic response

in Douglas-fir that sensed increasing neighbor proximity as plant size increased. The light-mediated plastic response partially dampened the development of size inequality (Ritchie 1997). It is possible that physiological plasticity will influence the relation between ontogeny and expression of inbreeding effect.

Comparison with other plant species

Several studies have compared mating system and inbreeding effects in closely related taxa (reviewed in Parker et al. 1995, Byers & Waller 1999). Inbreeding effects are often lower by a few percent in populations with higher rates of selfing. Results perhaps most comparable to lodgepole pine are reported by Willis (1999). He compared self and outcross populations of the annual monkeyflower (*Mimulus guttatus*) for several measures of survival and fertility, both before (ancestral) and after (purged) five generations of selfing. In all traits, mean inbreeding depression was a few percentage points less in the purged than in the ancestral population. The difference was not significant but was very similar to the difference in mean inbreeding depression in vigour between populations A and B + C in lodgepole pine. Willis (1999) concluded that deleterious alleles of large effect (lethals and steriles) are probably purged during the five cycles of selfing. This seems compatible with the large interpopulation difference in self-family variances in the lodgepole pine test.

In monkeyflower, much of the inbreeding load was retained after selfing (Willis 1999), as it was in the marginal population of lodgepole pine. This is in agreement with the theory that most of the inbreeding load expressed later in ontogeny is due to weakly deleterious alleles, and that little purging of these alleles should be expected when rate of inbreeding is increased.

Genetics of marginal populations

It is not known for how many generations population A has existed as such, only that the current situation is marginal both ecologically and in population numbers. As noted in the introduction, conifers are wind pollinated, and many widespread species, within their main distribution, occur in stands where one species dominates or at least occurs at moderately high frequencies. Under these conditions, high outcrossing rates are usually estimated and inbreeding loads are so large that many conifer populations at the adult stage have no evidence of selfing (Savolainen 1994). Marginal populations have decreased density or spe-

cies frequency and lower estimated outcrossing rates (Farris & Mitton 1984, Sorensen & Adams 1993, Hedrick et al. 1999). The population contrast for lodgepole pine (Tables 1 and 5) indicates that the marginal population has experienced a bottleneck sufficient to purge both embryonic lethals (Table 1) and severely deleterious alleles affecting vigour (Table 5) (Barrett & Charlesworth 1991, Hedrick 1994, Latta & Ritland 1994, Latter et al. 1995, Kirkpatrick & Jarne 2000). This indicates that purging has a role in mitigating the negative effects of increased inbreeding in marginal populations. The large reduction in within-plot coefficient of variation in the marginal compared with the non-marginal populations suggests that purging has primarily involved extreme individuals within families (i.e. alleles present at very low frequency in the population), but the very low self fertility of some seed trees and the highly significant difference among families (Table 3) suggests that purging may also occur among maternal lineages (Holtsford 1996, Carr & Dudash 1997, Willis 1999). In summary, it appears that the selection against rare deleterious alleles should enhance the ability of marginal populations to persist in the face of increased inbreeding.

How far can this process proceed? Within-plot coefficients of variation (Table 5, under A) and within-plot skewnesses (not given) show that for size-related traits except first-year height (see above), self-family values in population A do not differ greatly from cross-family values (2% larger to 5% smaller). Apparently, most of the extreme alleles have been removed. This suggests that the remaining inbreeding depression in vigour traits in population A is due to detrimental alleles of relatively small effect. Given the mating system of the species (outcrossing and selfing) this load probably is resistant to further selection (Lande et al. 1994, Hedrick 1994, Willis 1999).

There is some evidence that purging of detrimental alleles may be enhanced if the rate of inbreeding is reduced (Ehiobu et al. 1989, Latter et al. 1995, Sewalem et al. 1999). Because of increased spacing among lodgepole trees in a marginal population like A and because pollen dispersal is extensive, it seems probable that most of the increased inbreeding in population A would come from increased rate of selfing rather than biparental matings or lower level inbreeding. In other words, in this situation it is probable that most of the inbreeding will continue to be selfing even though the population size is greatly reduced.

In addition, increased environmental stress may influence the mitigating effect of purging. Tests with *Drosophila* (Bijlsma et al. 1999, 2000) indicate that "inbreeding and environmental stress are not independent but can act synergistically." In other words, although purging may increase the persistence of marginal populations given stable environmental conditions, the effects of the remaining inbreeding load may be enhanced if environmental conditions become more stressful (Bijlsma et al. 2000).

One factor that may bias the marginal–non-marginal contrast is the effect of previous inbreeding in the marginal population on the genetic structure of the outcross families (Waller 1993, Fazekas & Yeh 2001). If outcross families are less heterozygous in the marginal than in the nonmarginal population, this could reduce the inbreeding depression of the marginal self families. Because outcross males in all populations were separated from sced parents by 0.5 km or more, it seems unlikely that differences in outcross inbreeding contribute greatly to the population contrast. This is supported by the similar within-family skewnesses for outcross families from all populations, and by the fact that the skewnesses for size traits were marginally positive, with no indication of small outliers in either population.

Finally, the estimated inbreeding depression did not differ between populations B and C, even though the lodgepole pine frequency was intermediate in B (Table 1). Similarly, filled seed production following wind pollination did not differ between these stands and pure lodgepole pine in the same general area (Sorensen & Adams 1993). Lodgepole pine, even as a 50% component in a mixed forest with ponderosa pine, responded as a large, random mating population. According to theory, the reduction in number of intermating individuals must be large before there is an effect on measures of inbreeding, and even at extreme reduction in numbers the effect is small (Kirkpatrick & Jarne 2000). Lodgepole pine appears to behave in agreement with this model both for alleles associated with embryo survival and for severely deleterious alleles associated with seedling vigour and development.

ACKNOWLEDGEMENTS

I thank Richard S. Miles for his help with pollinations and maintenance and management of the nursery, and Drs. O. Savolainen and D. Byers and two anonymous referees for their comments.

REFERENCES

- Ågren, J. & Schemske, D. W. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. *Evolution* 47: 125–135.
- Bannister, M. H. 1965. Variation in the breeding system of *Pinus radiata*. In Baker, H. G. & Stebbins, G. L. (eds.). *The Genetics of Colonizing Species*, pp. 353–372. Academic Press, New York.
- Barrett, S. C. H. & Harder, L. D. 1996. Ecology and evolution of plant mating. *Trends Ecol. Evol.* 11: 73–79.
- Bendel, R. B., Higgins, S. S., Tedberg, J. E. & Pike, D. A. 1989. Comparison of skewness coefficient, coefficient of variation and Gini coefficient as inequality measures within populations. *Oecologia* 78: 394–400.
- Bijlsma, R., Bundgaard, J. & van Putten, W. F. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* 12: 1125–1137.
- Bijlsma, R., Bundgaard, J. & Boerema, A. C. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *J. Evol. Biol.* 13: 502–514.
- Bingham, R. T. & Squillace, A. E. 1955. Self-compatibility and effects of self fertility in western white pine. *For. Sci.* 1: 121–128.
- Boe, K. N. 1956. Regeneration and slash disposal in lodgepole pine clear cuttings. *Northwest Sci.* 30: 1–11.
- Bramlett, D. L., Dell, T. R. & Pepper, W. D. 1983. Genetic and maternal influences on Virginia pine seed germination. *Silv. Genet.* 32: 1–4.
- Byers, D. L. & Waller, D. M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Evol. Syst.* 30: 479–513.
- Cannell, M. G. R., Thompson, S. & Lines, R. 1981. Heights of provenances and progenies of *Pinus contorta* in Britain correlated with seedling phenology and the duration of bud development. *Silv. Genet.* 30: 166–173.
- Carr, D. E. & Dudash, M. R. 1997. The effects of five generations of enforced selfing on potential male and female function in *Mimulus guttatus*. *Evolution* 51: 1797–1807.
- Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genet. Res. (Camb.)* 74: 329–340.
- Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18: 237–268.
- Chen, H. Y. H., Klinka, K. & Kayahara, G. J. 1996. Effects of light on growth, crown architecture, and specific leaf area for naturally established *Pinus contorta* var. *latifolia* and *Pseudotsuga menziesii* var. *glauca* saplings. *Can. J. For. Res.* 26: 1149–1157.
- Cremer, K. W. 1971. Speeds of falling and dispersal of seed of *Pinus radiata* and *P. contorta*. *Australian For. Res.* 5: 29–32.
- Critchfield, W. B. 1957. Geographic variation in *Pinus contorta*. Maria Moors Cabot Foundation Publication No. 3, 118 pp. Harvard University, Cambridge, MA.
- Critchfield, W. B. 1978. The distribution, genetics and silvics of lodgepole pine. In *Proceedings of the IUFRO joint meeting of Working Parties. Vol. 1. Background Papers and Douglas Fir Provenances*, pp. 65–94. British Columbia Ministry of Forests, Victoria, BC, Canada. ISBN 0-7719-8311-5.
- Crnokrak, P. & Roff, D. A. 1999. Inbreeding depression in the wild. *Heredity* 83: 260–270.
- Crow, J. F. 1993. Mutation, mean fitness, and genetic load. In Futuyama, D. & Antonovics, J. (eds). *Oxford Surveys in Evolutionary Biology. Vol. 9*, pp. 3–42. Oxford University Press, Oxford. ISBN 0-19-508492-6.
- Crow, J.F. & Kimura, M. 1970. *An Introduction to Population Genetics Theory*, 571 pp. Harper & Row, New York.
- Crow, J. F. & Kimura, M. 1979. Efficiency of truncation selection. *Proc. Nat. Acad. Sci. USA* 76: 396–399.
- Crow, J. F. & Simmons, M. J. 1983. The mutation load in *Drosophila*. In Ashburner, M., Carson, H. L. & Thompson, J. N. (eds). *The Genetics and Biology of Drosophila. Vol. 3c*, pp. 1–35. Academic Press, London. ISBN 0-12-064847-0.
- Dahms, W. G. 1963. Dispersal of lodgepole pine seed into clear-cut patches. Pacific Northwest Forest and Range Experiment Station Res. Note PNW-3. USDA Forest Service, Portland, OR.
- Dahms, W. G. & Barrett, J. W. 1975. Seed production of central Oregon ponderosa and lodgepole pines. Pacific Northwest Forest and Range Experiment Station Res. Pap. PNW-191. USDA Forest Service, Portland, OR.
- Downie, B. & Bewley, J. C. 1996. Dormancy in white spruce (*Picea glauca* [Moench.] Voss.) seeds is imposed by tissues surrounding the embryo. *Seed Sci. Res.* 6: 9–15.
- Dubin, N. P. 1946. On lethal mutation in natural populations. *Genetics* 31: 21–38.
- Durel, C. E., Bertin, P. & Kremer, A. 1996. Relationship between inbreeding depression and inbreeding coefficient in maritime pine (*Pinus pinaster*). *Theoret. Appl. Genet.* 92: 347–356.
- Ehiobu, N. C., Goddard, M. E. & Taylor, J. F. 1989. Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*. *Theoret. Appl. Genet.* 77: 123–127.
- Eiche, V. 1955. Spontaneous chlorophyll mutations in Scots pine (*Pinus sylvestris* L.). *Medd. Stat. Skogsforsk. Inst.* 45 (13): 1–69.
- Epperson, B. K. & Allard, R. W. 1984. Allozyme analysis of the mating system in lodgepole pine populations. *J. Hered.* 75: 212–214.
- Epperson, B. K. & Allard, R. W. 1989. Spatial autocorrelation analysis of the distribution of genotypes with populations of lodgepole pine. *Genetics* 121: 369–377.
- Falconer, D. S. & Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics*, 4th edn, 464 pp. Longman, Essex, ISBN 0582-24302-5.
- Fall, P. L. 1992. Pollen accumulation in a montane region of Colorado, USA: a comparison of moss polsters, atmospheric traps, and natural basins. *Rev. Palaeobot. Palynol.* 72: 169–197.

- Farris, M. A. & Mitton, J. B. 1984. Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. *Evolution* 38: 1151–1154.
- Fazekas, A. J. & Yeh, F. C. 2001. Random amplified polymorphic DNA diversity of marginal and central populations in *Pinus contorta* subsp. *latifolia*. *Genome* 44: 13–22.
- Franklin, E. C. 1969. Mutant forms found by self-pollination in loblolly pine. *J. Hered.* 60: 315–320.
- Fu, Y.-B. & Ritland, K. 1994. Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus*. *Genetics* 136: 323–331.
- Furnier, G. R. & Adams, W. T. 1986. Mating system in natural populations of Jeffrey pine. *Am. J. Bot.* 73: 1002–1008.
- Hadorn, E. 1961. *Developmental Genetics and Lethal Factors*, 355 pp. Wiley, New York.
- Hagman, M. & Mikkola, L. 1963. Observations of cross-, self- and interspecific pollinations in *Pinus peuce* Griseb. *Silv. Genet.* 12: 73–79.
- Haldane, J. B. S. 1955. The measurement of variation. *Evolution* 9: 484.
- Hawkins, D. M. 1980. *Identification of Outliers*. 188 pp. Chapman & Hall, London. ISBN 0-412-21900-X.
- Hedrick, P. W. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* 73: 363–372.
- Hedrick, P. W., Savolainen, O. & Kärkkäinen, K. 1999. Factors influencing the extent of inbreeding depression: an example from Scots pine. *Heredity* 82: 441–450.
- Holtsford, T. P. 1996. Variation in inbreeding depression among families and populations of *Clarkia tembloriensis* (Onagraceae). *Heredity* 76: 83–91.
- Husband, B. C. & Schemske, D. W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54–70.
- Husband, B. C. & Schemske, D. W. 1997. The effect of inbreeding in diploid and tetraploid populations of *Epi-lobium angustifolium* (Onagraceae): implications for genetic basis of inbreeding depression. *Evolution* 51: 737–746.
- Johnston, M. O. & Schoen, D. J. 1994. On the measurement of inbreeding depression. *Evolution* 48: 1735–1741.
- Jürgens, G., Mayer, U., Ruiz, R. A. T., Berleth, T. & Miséra, S. 1991. Genetic analysis of pattern formation in the *Arabidopsis* embryo. *Development* 1991 (Suppl 1): 27–38.
- Kärkkäinen, K., Koski, V. & Savolainen, O. 1996. Geographic variation in the inbreeding depression in Scots pine. *Evolution* 59: 111–119.
- Keightley, P. D. & Eyre-Walker, A. 1999. Terumi Mukai and the riddle of deleterious mutation rates. *Genetics* 153: 515–523.
- King, J. N., Carson, M. J. & Johnson, G. R. 1998. Analysis of disconnected diallel mating designs. II – Results from a third generation progeny test of the New Zealand radiata pine improvement programme. *Silv. Genet.* 47: 80–87.
- Kirkpatrick, M. & Jarne, P. 2000. The effects of a bottleneck on inbreeding depression and the genetic load. *Am. Nat.* 155: 154–167.
- Knowles, P., Furnier, G. R., Aleksiuk, M. A. & Perry, D. J. 1987. Significant levels of self-fertilization in natural populations of tamarack. *Can. J. Bot.* 65: 1087–1091.
- Koch, P. 1996. *Lodgepole pine in North America*. Vol. 1, 344 pp. Forest Products Society, Madison, WI, ISBN 0-935018-78-6.
- Kuang, H., Richardson, T. E., Carson, S. D. & Richardson, B. C. 1999. Genetic analysis of inbreeding depression in plus tree 850.55 of *Pinus radiata* D. Don. II. Genetics of viability genes. *Theoret. Appl. Genet.* 99: 140–146.
- Lande, R. & Schemske, D. W. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24–40.
- Lande, R., Schemske, D. W. & Schultz, S. T. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution* 48: 965–978.
- Latta, R. & Ritland, K. 1994. The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. *Evolution* 48: 806–817.
- Latter, B. D. H., Mulley, J. C., Reid, D. & Pascoe, L. 1995. Reduced genetic load revealed by slow inbreeding in *Drosophila melanogaster*. *Genetics* 139: 287–297.
- Lewontin, R. C. 1966. On the measurement of relative variability. *Syst. Zool.* 15: 141–142.
- Lynch, M. 1988. Design and analysis of experiments on random drift and inbreeding depression. *Genetics* 120: 791–807.
- Lynch, M., Blanchard, J., Houle, D., Kibota, T., Schultz, S., Vassilieva, L. & Willis, J. 1999. Perspective: spontaneous mutation rate. *Evolution* 53: 645–663.
- Mason, D. T. 1915. *The life history of lodgepole pine in the Rocky Mountains*. USDA Bulletin 154. Washington, DC.
- Matheson, A. C., White, T. L. & Powell, G. R. 1995. Effects of inbreeding on growth, stem form and rust resistance in *Pinus elliotii*. *Silv. Genet.* 44: 37–46.
- McKeand, S. & Jett, J. B. 1995. Effect of inbreeding on superior loblolly pine. In Lavereau, J. (ed.). *Proceedings of the Twenty-Fifth Meeting of the Canadian Tree Improvement Association*, Part 2, p. 52 (Abstract). August 28–September 1, 1995, Victoria, BC, Canada.
- McKeand, S. E. & Bridgwater, F. E. 1998. A strategy for the third breeding cycle of loblolly pine in the southeastern U.S. *Silv. Genet.* 47: 223–234.
- Mina, N. S., Sheldon, B. L., Yoo, B. H. & Frankham, R. 1991. Heterozygosity at protein loci in inbred and outbred lines of chicken. *Poultry Sci.* 70: 1864–1872.
- Mitchell-Olds, T. & Guries, R. 1986. Genetic load and heterozygosity in the Pinaceae. *Can. J. Genet. Cytol.* 28: 942–946.
- Morgante, M., Vendramin, G. G. & Rossi, P. 1991. Effects of stand density on outcrossing rate in two Norway spruce (*Picea abies*) populations. *Can. J. Bot.* 69: 2704–2708.

- Morgenstern, E. K. 1974. A diallel cross in black spruce, *Picea mariana* (Mill.) B.S.P. *Silv. Genet.* 23: 67–70.
- Orr-Ewing, A. L. 1957. A cytological study of the effects of self-pollination of *Pseudotsuga menziesii* (Mirb.). *Franco. Silv. Genet.* 6: 179–185.
- Orr-Ewing, A. L. 1969. Inbreeding to the S₂ generation in Douglas-fir. In *Second World Consultation of Forest Tree Breeding, Section III. FO-FTB-69-8/6*, pp 1–13. Washington, DC.
- Parker, I. M., Nakamura, R. R. & Schemske, D. W. 1995. Reproductive allocation and the fitness consequences of selfing in two sympatric species of *Epilobium* (Anagraceae) with contrasting mating systems. *Am. J. Bot.* 82: 1007–1016.
- Pfister, R. D. & Daubenmire, R. 1973. Ecology of lodgepole pine. In *Baumgartner, D. M. (ed.). Management of Lodgepole Pine Ecosystems, vol. 1*, pp 27–46. Washington State University Cooperative Extension Service, Pullman, WA.
- Piesch, R. F. & Stettler, R. F. 1971. The detection of good selfers for haploid induction in Douglas-fir. *Silv. Genet.* 20: 144–148.
- Rehfeldt, G. E. & Wykoff, W. R. 1981. Periodicity in shoot elongation among populations of *Pinus contorta* from the northern Rocky Mountains. *Ann. Bot.* 48: 371–377.
- Remington, D. L. & O'Malley, D. M. 2000. Whole-genome characterization of embryonic stage inbreeding depression in a selfed loblolly pine family. *Genetics* 155: 337–348.
- Ritchie, G. A. 1997. Evidence for red:far red signaling and photomorphogenic growth response in Douglas-fir (*Pseudotsuga menziesii*) seedlings. *Tree Physiol.* 17: 161–168.
- Ritland, K. 1996. Inferring the genetic basis of inbreeding depression in plants. *Genome* 39: 1–8.
- Robertson, A. 1952. The effect of inbreeding on the variation due to recessive genes. *Genetics* 37: 189–207.
- Rumball, W., Franklin, I. R., Frankham, R. & Sheldon, D. L. 1994. Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics* 136: 1039–1049.
- Savolainen, O. 1994. Genetic variation and fitness: conservation lessons from pines. In *Loeschcke, V., Tomiuk, J. & Jain, S. K. (eds.). Conservation Genetics*, pp 27–36. Birkäuser, Basel. ISBN 0817629394
- Sewalem, A., Johansson, K., Wilhelmson, M. & Lillpers, K. 1999. Inbreeding and inbreeding depression on reproduction and production traits of White Leghorn lines selected for egg production traits. *Br. Poultry Sci.* 40: 203–208.
- Siggins, H. W. 1933. Distribution and rate of fall of conifer seeds. *J. Agric. Res.* 47: 119–128.
- Silen, R. & Osterhaus, C. 1979. Reduction of genetic base by sizing of bulked Douglas-fir seed lots. *Tree Planters Notes* 30 (1): 24–30.
- Skrøppa, T. 1996. Diallel crosses in *Picea abies*. II. Performance and inbreeding depression of selfing families. *For. Genet.* 3: 69–79.
- Skrøppa, T. & Magnussen, S. 1993. Provenance variation in shoot growth components of Norway spruce. *Silv. Genet.* 42: 111–120.
- Smith, C. C., Hamrick, J. L. & Kramer, C. L. 1988. The effects of stand density on frequency of filled seeds and fecundity in lodgepole pine (*Pinus contorta* Dougl.). *Can. J. For. Res.* 18: 453–460.
- Snedecor, G. W. & Cochran, W. G. 1967. *Statistical Methods*, 6th edn. The Iowa State University Press, Ames, IA.
- Snyder, E. B. 1968. Seed yield and nursery performance of self-pollinated slash pines. *For. Sci.* 14: 68–74.
- Sorensen, F. C. 1971. "White seedling": a pigment mutation that affects seed dormancy in Douglas-fir. *J. Hered.* 62: 127–130.
- Sorensen, F. C. 1987. Estimated frequency of natural selfing in lodgepole pine (*Pinus contorta* var. *murrayana*) from central Oregon. *Silv. Genet.* 36: 215–216.
- Sorensen, F. C. 1994. Frequency of seedlings from natural self-fertilization in Pacific Northwest ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.). *Silv. Genet.* 43: 100–108.
- Sorensen, F. C. 1997. Effects of sib mating and wind pollination on nursery seedling size, growth components, and phenology of Douglas-fir seed-orchard progenies. *Can. J. For. Res.* 27: 557–566.
- Sorensen, F. C. & Adams, W. T. 1993. Self fertility and natural selfing in three Oregon Cascade populations of lodgepole pine. In *Lindgren, D. (ed.). Pinus contorta-From Untamed Forest to Domesticated Crop*. Dept of Forest Genetics and Plant Physiology, Swed. Univ. of Agri. Sci., Report 11: 358–374. Umeå, Sweden.
- Sorensen, F. C. & Campbell, R. K. 1993. Seed weight–seedling size correlation in coastal Douglas-fir: genetic and environmental components. *Can. J. For. Res.* 23: 275–285.
- Sorensen, F. C. & Miles, R. S. 1974. Self-pollination effects on Douglas-fir and ponderosa pine seeds and seedlings. *Silv. Genet.* 23: 135–138.
- Sorensen, F. C. & Webber, J. E. 1997. On the relationship between pollen capture and seed set in conifers. *Can. J. For. Res.* 27: 63–68.
- Sorensen, F. C., Franklin, J. F. & Woollard, R. 1976. Self-pollination effects on seed and seedling traits in noble fir. *For. Sci.* 22: 155–159.
- Squillace, A. E. & Kraus, J. F. 1962. Effects of inbreeding on seed yield, germination, rate of germination, and seedling growth in slash pine. In *Proceedings of a Forest Genetics Workshop*, pp 1–5. October 25–27, 1962, Macon, GA, USA.
- Stuart, J. D., Agee, J. K. & Gara, R. I. 1989. Lodgepole pine regeneration in an old, self-perpetuating forest in south central Oregon. *Can. J. For. Res.* 19: 1096–1104.
- Temin, R., Meyer, H.-U., Dawson, P. & Crow, J. F. 1969. The influence of epistasis on homozygous viability depression in *Drosophila melanogaster*. *Genetics* 61: 389–393.
- Waller, D. M. 1993. The statics and dynamics of mating system evolution. In *Thornhill, N. W. (ed.). The Natural*

- History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives, pp. 97–117. University of Chicago Press, Chicago, IL. ISBN 0-226-79854-2.
- Willis, J. H. 1999. The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* 53: 1678–1691.
- Worrall, J., Draper, D. A. & Anderson, S. A. 1985. Shoot characteristics of stagnant and vigorous lodgepole pines, and their growth after reciprocal grafting. *Can. J. For. Res.* 15: 365–370.
- Yanchuk, A. D. 1996. General and specific combining ability from disconnected partial diallels of coastal Douglas-fir. *Silv. Genet.* 45: 37–45.
- Yang, R.-C. & Yeh, F. C. 1995. Patterns of gene flow and geographic structure in *Pinus contorta* Dougl. *For. Genet.* 2: 65–75.
- Yeh, F. C. & Layton, C. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* var. *latifolia*. *Can. J. Genet. Cytol.* 21: 487–503.