Theor Appl Genet (1985) 71:334–343

Effect of inbreeding on production of filled seed in *Pinus radiata* – experimental results and a model of gene action

A. R. Griffin[1] and D. Lindgren[2]

[1] CSIRO, Division of Forest Research, P.O. Box 4008, Canberra A.C.T. 2600, Australia
[2] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umea, Sweden

Received May 7, 1985; Accepted May 15, 1985
Communicated by P. M. A. Tigerstedt

Summary. *Pinus radiata* D. Don was inbred to different degrees, commencing with a founder population of 8 clones. Yield of filled seed was determined for each mating type. Mean yields (%), relative to outcrossing, were: half-sib 102; full-sib 97; S_1 43; S_2 42. An epistatic model was developed to predict the empty seed yield following selfing, assuming that homozygosity for several recessive co-lethals is necessary to kill an embryo. Calculations were then extended to predict the yields following different degrees of inbreeding. The proposed model gave a better fit to present results, and to other published results of similar investigations, than did an alternative based on action of independent recessive lethals. Implications for breeding and seed production strategy are discussed.

Key words: Inbreeding depression – *Pinus radiata* – Seed yield – Breeding strategy – Gene action

Introduction

Reduced seed yield following self-pollination of conifers is a well established fact (Sarvas 1962; Franklin 1970) and has been interpreted as resulting from the action of recessive lethal alleles in the homozygous state (see e.g. Koski 1971; Lindgren 1975). In conjunction with the phenomenon of polyembryony, the differential viability of selfed and outcrossed embryos maintains a high effective outcrossing rate in spite of considerable natural self-pollination (Lindgren 1975; Sorensen 1982) and the lack of demonstrable self-incompatibility systems (Hagman and Mikkola 1963).

The consequences of less severe inbreeding are less well documented and, as tree breeding progresses beyond the first generation, there is increasing need for such information. An experiment was designed to evaluate performance of *Pinus radiata* D. Don when inbred to varying degrees. In this paper we report the yields of full seed from the matings which constituted the test populations.

It was evident from preliminary inspection that published models based on action of independent recessive lethal alleles (Bramlett and Popham 1971; Koski 1971; Bishir and Pepper 1977) did not adequately account for the observed variation pattern in seed yield in this or other published experimental studies. We therefore constructed an alternative model based on interaction of alleles at two or more loci which are lethal only when each is present in the homozygous state (co-lethals).

In order to interpret the occurrence of empty seeds in pine cones it is necessary to review the processes of pollination, fertilisation, and polyembryony. The following account is specific to *Pinus radiata* but similar to other species of *Pinus* (Lill 1976).

The female strobilus (for anatomical description of which see Lill and Sweet 1977) is receptive for up to 13 days, during which time pollen may be trapped on the micropylar arms and drawn into the micropyle by the pollination drop. Up to six grains can be accommodated in the micropyle (Lill 1974). On germination, pollen tubes grow rapidly through the nucellus towards the megaspore mother cell but cease growth after about six weeks. About two months after pollination meiosis occurs in the megaspore mother cell giving rise to the functional megaspore or female gametophyte. This develops over the following year, differentiating up to four archegonia. Pollen tube growth re-commences and the egg nucleus of each archegonium may be fertilised in November/December (Lill 1976), 15 months after pollination.

Initially the embryo systems from all archegonia develop – the condition of simple polyzygotic embryony. Lill (1974) reported that 91% of immature seeds of one clone of *P. radiata* had more than one embryo yet it was rare for mature seeds to do so. One embryo evidently assumes dominance during development. Since fertilisation of eggs within one ovule appears to be simultaneous, the physiological environment for all embryos must be similar, and the genotypes of polyzygotic embryos are different, it is evident that there is an opportunity for selection to occur at this stage of the life cycle.

In order to develop models of gene action determining filled seed yield it is necessary to have realistic estimates of the frequency distribution of polyzygotes within ovules. The literature contains several estimates of numbers of developing embryos per ovule (e.g. Orr-Ewing (1957) for Douglas-fir, Koski (1971) for *Picea abies* and *Pinus sylvestris*). However, by the stage at which such assessments are made some zygote/pro-embryo mortality may already have occurred. The variable number of pollen grains received in the micropyle also sets an upper limit to the number of embryos developing. For example by counting both pollen grains and archegonia in a particular sample of *P. radiata* ovules Lill (1974) determined that, even if 100% of grains effected fertilisations, 23% of egg cells would have remained unfertilised. The number of archegonia present in an ovule is thus a more reliable estimator of potential numbers of polyzygotic embryos produced. For two species of *Pinus* the following estimates are available:

Frequency of ovules with $k = 1, 2 \ldots$ archegonia

	Clone	1	2	3	4	$\bar{k}$
Pinus radiata						
(calculated from	55	0.01	0.38	0.55	0.06	
Table 6:8,	19	0.11	0.77	0.12	0.00	
Lill (1974))	372	0.16	0.60	0.24	0.00	
	Mean	0.07	0.56	0.34	0.03	2.3
Pinus sylvestris						
(calculated from	Range for	0.02	0.61	0.35	0.02	
Table 23,	10 tree	0.30	0.67	0.03	0.00	
Sarvas (1962))	sample					
	Mean	0.19	0.71	0.10	0.00	2.1

These authors also published respective mean estimates of 1.85 and 1.7 fertilisations per ovule suggesting that pollen availability was indeed a limiting factor.

In addition to simple polyembryony *Pinus* species exhibit cleavage polyembryony (Johansen 1950; Sorensen 1982) – that is the development of more than one embryo from a single zygote. For *P. sylvestris*, Sarvas (1962) estimated that 2.5 cleavage polyembryos were formed for each zygote and, from a small sample of germinated twins of *P. radiata*, Burdon and Zabkiewicz (1973) found that about half were produced by simple and half by cleavage polyembryony. Since monozygotic embryos are genetically identical any competitive elimination cannot be ascribed to the genotypic variation addressed in this paper. Models developed thus allude only to the products of simple polyembryony.

The data reported in this paper also relate only to those seeds whose coats have developed to normal dimensions. Unpollinated ovules of *P. radiata* (Lill 1974) and of the majority of *Pinus* species (McWilliam 1959; Sarvas 1962) do not develop and hence would not be included in our total seed count. The literature suggests however that seed development can occur in the absence of fertilisation and that fertilisation success may be influenced by environmental factors. Righter (1945) states that in *P. ponderosa* the seed attains full size prior to fertilisation, and Hagman and Mikkola (1963) reported that a proportion of ovules in *Pinus peuce* crosses and selfs contained unfertilised eggs in spite of pollen tubes being present in the nucellus. In a paper of particular relevance to the present study, Brown (1971) showed that pollen storage and period of isolation of female strobili could both influence proportion of full seed and that yield from controlled crosses was somewhat lower than that obtained in wind pollinated cones.

It must be concluded that some of the variation in full seed present is likely to be of non-genetic origin (related to pollen quality, isolating procedures, or environmental stress during embryo development). We assume that the effect of such unspecified variation is equal for all five experimental populations in our study.

Material and methods

Genetic material

Five sets of progenies, inbred to levels of $F = 0$, 0.125, 0.25, 0.5, 0.75, were derived from a founder population of eight clones from the APM Forests breeding population in Gippsland, Victoria, Australia.

Three of these clones were selected from plantations in the Australian Capital Territory, four in New Zealand, and one in Victoria. They are assumed to be unrelated.

A 4×4 factorial mating of the founder clones constituted the outcrossed population; these 16 full-sib families had previously been established in progeny tests and provided parents for half-sib and full-sib matings. For half-sib matings, only four of the initial founder genomes were inbred. Founder clones were self pollinated to give the S_1 population and earlier outplantings of these S_1 progeny were selfed to generate the S_2 population. No selection criteria were applied in choosing parent trees other than they were flowering. Especially in the S_1 progeny test where it was necessary for a tree to produce both male and female strobili, this requirement certainly resulted in bias towards the most vigorous individuals.

A small additional population inbred to $F = 0.25$ was obtained by mating S_1 progeny of four of the founders to their outcrossed half-siblings.

Crosses were accumulated over three successive pollination seasons and with the exception of one S_2 family (derived from clone 30040 which had very weak S_1 progeny) a high level of balance was achieved in the various mating patterns.

All pollinations were made using standard techniques described in Matheson and Brown (1983). Outcrosses and S_1 matings were made on founder clones growing in a top-pruned breeding arboretum where female strobili were borne on a number of co-dominant leading shoots (Hand and Griffin 1979). Sib and S_2 matings were made by climbing plantation grown ortets which generally only flowered on the single leader. *P. radiata* is protogynous (Griffin 1984) and it is more often convenient to use pollen collected in the previous season, dried and stored at $-16\,^{\circ}\mathrm{C}$. In such cases *in vitro* tests of viability were conducted prior to use.

Assessment procedure

All seeds were extracted by hand and those from all cones of a particular mating were combined. After dewinging all normal-sized seeds were X-rayed in a Faxitron 805 unit using Polaroid type 57 positive film (3000 I.S.O.). Exposure was 180 s at 10 kVp. This high speed film was chosen as the seed was to be used for subsequent germination and growth studies and it was desirable to minimise X-ray dosage (Simak 1980). Resolution of detail within the seeds was not always optimal and classification into full and unsound was based primarily on endosperm development. In his classification of conifer seeds Simak (1980) distinguished five embryo development classes (0–IV) and two endosperm classes (A, B). In combination these classifications may be used to assign a development class to each seed. In our assessment a filled seed had to have type A endosperm, i.e. "almost filling the seed coat to capacity and easily absorbing X-rays". Embryo development was not scored, however examination of several hundred seeds of *P. radiata* suggested that it was rare to find embryo abnormalities without a corresponding change in endosperm volume or opacity. Standard germination tests of 2×25 seed samples from each of four *P. radiata* seedlots gave a correlation of $r = 0.97$ between germination % and filled seed % assessed from radiographs prior to testing.

One wind-pollinated cone was also collected from each tree used as a female parent in the experiment and seed extracted and assayed in the standard manner.

Model predicting yield of unsound seed following selfing

As noted, a model based on action of independent recessive lethal alleles did not adequately account for the observed variation pattern. A multi-locus extension, assuming epistatic action of co-lethal alleles and taking into account the phenomenon of polyembryony, was developed under the following biological assumptions:

1) A seed is formed from an ovule within which at least one embryo has developed to maturity.

2) An ovule contains 1–4 egg cells. These may be fertilised by different male gametes and hence produce genetically variable embryos. The number of such polyzygotic embryos per ovule is designated k.

3) A parental tree is heterozygous for recessive co-lethal alleles at n loci, and is self pollinated.

4) If following self-pollination the resulting embryos are homozygous for the co-lethal alleles at N or more loci they will die. If all embryos in an ovule die an unsound seed will result.

5) Embryos with < N homozygous co-lethals may die as a result of competition from other such embryos (including the products of cleavage polyembryony), but such competition will always leave a minimum of one developing embryo, and hence will not affect the proportion of unsound seed produced.

6) The percentage of unsound, full sized seed observed to occur after controlled outcrossing is ascribed to non-genetic causes which are assumed to operate uniformly across the set of all experimental matings.

The probability that the female gamete will carry co-lethals at exactly r loci is:

$$\frac{1}{2^n}\binom{n}{r}.$$

The probability that a male gamete will carry co-lethals at exactly m of these r loci is:

$$\frac{1}{2^r}\binom{r}{m}.$$

The probability that, following fertilisation, the embryo will carry homozygous co-lethals in N or more of these loci, and thus will die, is:

$$\sum_{m=N}^{r}\frac{1}{2^r}\binom{r}{m}.$$

The probability that all k embryos in an ovule will die (if the female gamete carries exactly r co-lethals) is:

$$\left[\sum_{m=N}^{r}\frac{1}{2^r}\binom{r}{m}\right]^k.$$

Summed over all possible r-values:

$$f(n,N,k)=\frac{1}{2^n}\sum_{r=0}^{n}\binom{n}{r}\left[\frac{1}{2^r}\sum_{m=N}^{r}\binom{r}{m}\right]^k. \tag{1}$$

For computation, formula (2) is helpful as there are fewer terms.

$$\frac{1}{2^r}\sum_{m=N}^{r}\binom{r}{m}=1-\frac{1}{2^r}\sum_{m=0}^{N-1}\binom{r}{m}. \tag{2}$$

The probability of unsound seeds is listed in Table 3 as a function of the number of co-lethals in the parent (n), the number of loci which must be homozygous for the co-lethal to cause zygote death (N), and the number of embryos per ovule (k).

Special cases

For two special cases formula (1) reduces to formulae which have been published elsewhere.

a) For $N = 1$ (the case of independent recessive lethals) formula (2) reduces to $1-\frac{1}{2^r}$ thus:

$$f(n,1,k)=\frac{1}{2^n}\sum_{r=0}^{n}\binom{n}{r}\left(1-\frac{1}{2^r}\right)^k. \tag{3}$$

This formula has been independently developed and tabulated by Koski (1971) and Bramlett and Popham (1971) although their tables give too few decimals for some applications. The present work may be regarded as a generalization of this formula.

b) For $k = 1$ (where only one embryo is formed). The probability that exactly m loci out of n will be homozygous is derived from the binomial distribution:

$$\binom{n}{m}\left(\frac{1}{4}\right)^m\left(\frac{3}{4}\right)^{n-m}. \tag{4}$$

The probability of getting more than N co-lethals ($m \geqq N$) will be:

$$f(n,N,1)=\frac{1}{4^n}\sum_{m=N}^{n}\binom{n}{m}3^{n-m}. \tag{5}$$

Values of this cumulative binomial distribution may be found in standard tables.

Assuming that all S_1 progeny with more than N co-lethals die then, provided $k = 1$ (no polyembryony) the proportion of survivors with exactly m loci ($m < N$) homozygous for any n co-lethals will be binomially distributed:

$$h(m)=\binom{n}{m}\left(\frac{1}{4}\right)^m\left(\frac{3}{4}\right)^{n-m}/(1-f(n,N,1)). \tag{6}$$

It will be assumed that this is the case also when $k > 1$, but this remains to be proven.

Prediction of unsound seed yield at other levels of inbreeding

Expected unsound seed yields following selfing given in Table 3 may be used as a basis for estimating yields from the other types of mating which we investigated. The approach outlined below, based on given values following selfing, is approximate and more accurate methods are indicated, but we suggest that, in view of the biological complexity of the system and the assumptions made, resources spent on further increasing mathematical accuracy are not justifiable.

For half-sib matings (F = 0.125) assume that the proportion of unsound seeds would be the same as that following selfing of genotypes with n/4 co-lethals, n being the number of loci carrying co-lethals carried in the common ancestor. A more accurate approach would be to use the following formula with F = 0.125, whereby the actual distribution of cases with common co-lethals is considered, instead of just using the average n/4.

$$f(n,N,k,F) = \sum_{M=0}^{n} \binom{n}{M} (2F)^{M} (1-2F)^{n-M} \cdot \frac{1}{2^{M}} \sum_{r=0}^{M} \binom{M}{r} \left[\frac{1}{2^{r}} \sum_{m=N}^{r} \binom{r}{m} \right]^{k} \qquad (7)$$

where M is the number of loci at which both parents have inherited the same co-lethal allele from the common ancestor.

For full-sib matings and S_1/half-sib matings (F = 0.25) assume that the proportion of unsound seeds would be the same as that following selfing of genotypes with n/2 co-lethals. More accurate calculations could be done using formula (7), this result probably being applicable to the full-sib case as well. Formula (7) could also be used putting F = 0.125 and summing the estimated co-lethals complement of the common parents.

The S_2 case is complex. For the special case N = 1 (the independent lethal model) calculations were performed assuming the yield of unsound seeds would be the same as after selfing of genotypes with 2n/3 lethals (n being the number of lethals in the genotype initially selfed). This follows since selfing of an *Aa* genotype will give 1*AA*:2*Aa*; 1*aa*. *aa* will die, and hence the probability is 2/3 that a surviving progeny will carry the lethal. More accurate values might be obtained by summing over different number of lethals transmitted. For the co-lethal model the calculations are approximate and tedious, and no suggestion of a more accurate procedure is made.

The probability that the surviving embryo is homozygous for m co-lethals h(m), is calculated according to formula (6). It is assumed that the distribution is independent of the number of embryos (k). The probability that an unsound seed will occur at a particular m is assumed to be:

$$f((n-m)\,2/3, N-m, k)\,.$$

Since there are already m loci homozygous for co-lethals, the additional requirement for mortality is N − m. Of the (n − m) loci not homozygous for co-lethals, two thirds are expected to be heterozygous and one third homozygous for the non-lethal allele.

The probability for an unsound seed after S_2 mating is found by summing over all m

$$f_{S_2}(n,N,k) = \sum_{m=0}^{N-1} h(m)\, f((n-m)\,2/3, N-m, k)\,. \qquad (8)$$

Results and discussion

The predictive equations were developed in terms of expected proportions of unsound seed as this simplifies the algebraic expressions. It is however the yield of filled seed which is both of more direct practical interest, and the statistic most frequently reported in the literature. Results are therefore presented in terms of filled seed.

Table 1. Yield of filled seeds (%) following crosses between trees related to varying degrees. Each entry corresponds to a different sample of trees from the respective parental families

a) Outcross (OC, F = 0.00)

		Male parent			
		5	6	7	8
Female parent	1	93[a]	83	53	84
	2	77[a]	80[a]	91[a]	85
	3	95[a]	71	78[a]	74
	4	62	78[a]	74	83

b) Half-sib mating (HS_1, F = 0.125)

Common grandparent	
1	98, 93, 83, 70, 90, 84, 76
2	81, 82, 83, 51, 72, 62, 77
3	84, 88, 83, 71, 85, – –
4	69, 92, 94, 70, 91, 84, –

c) Half-sib with one parent and S_1 individual (HS, F = 0.25)

Selfed grandparent	
5	93, 82
6	66
7	93
8	73, 92, 90, 90

d) Full-sib mating (FS, F = 0.25)

Grandparents	5	6	7	8
1	76, 82	79, 92	64, 74	87, 86
2	89, 52	95, 91	81, 82	94, 64, 71
3	77, 73	66, 74	56, 86	94, 85
4	56, 64	80, 60	95, 00	83, 46

e) Selfing (S_1, F = 0.5) and second generation selfing (S_2, F = 0.75)

Parent/grandparent	S_1[b]	S_2
1	1	0[c]
2	53	52, 11, 38, 47, 25, 33
3	68, 33	55, 66
4	63	73, 69, 70, 58, 13
5	39, 31	11, 6, 34
6	28, 28	17, 27, 80, 48
7	6, 28, 6	2, 32, 15
8	47, 15	27, 57, 30

[a] Reciprocal cross

[b] Data refer to repeat of selfing on same trees in different seasons

[c] Two cones with no filled seed – case not distinguishable from cone mortality for non-genetic reasons

Experimental results

The filled seed yield from each individual cross is presented in Table 1, and averages for different types of crosses over different inbred founder clones are presented in Table 2. Number of cones and total seed number are also given to indicate the size of the study.

Table 2. Mean yield of filled seed %, and yield % relative to outcross, following crosses between trees related to varying degrees

Founder clone			Mating type					
No.	Plus tree[a]		OC F = 0	HS 0.125	HS_1 0.25	FS 0.25	S_1 0.5	S_2 0.75
1	30040	Yield	78	85	–	80	1	0
		Rel. yield	100	109		103	1	0
2	80080		83	73	–	80	53	34
			100	88		96	64	41
3	80086		80	82	–	76	50	60
			100	102		95	63	75
4	10948		74	83	–	69	63	57
			100	112		93	85	77
5	80055		82	–	88	71	35	17
			100		107	87	48	21
6	12038		78	–	66	80	28	43
			100		85	102	36	55
7	12374		74	–	93	77	13	16
			100		126	104	8	22
8	80121		82	–	86	79	31	38
			100		105	96	38	46
Mean yield			78.9	80.7	83.2	76.5	34.2	33.1
Rel. yield			100	102.3	105.5	97.0	43.3	42.0
No. cones assayed			146	80	12	89	123	60
No. seeds assayed			11,320	8,726	1,115	9,442	10,421	6,081
Total seed per cone			77.5	109.1	92.9	106.1	84.7	101.4

[a] Australian plus tree register no.

Yields from half- and full-sib matings were not significantly different from outcrosses. The average difference for the eight founders between outcrosses and full-sib matings was 3.0% with the standard deviation of the mean of 1.8. Thus, a difference of around 4% would have been significant.

S_1 and S_2 gave comparable yields of sound seed, 43.3 and 42.0%, respectively relative to outcross. There was a correlation between S_1 and S_2 yield for individual founders (rank correlation coefficient $r = 0.67^*$) confirming a common genetic effect on the yield of sound seeds.

The mean filled seed yield from wind-pollinated cones of the founder clones (mother of matings $F = 0$ and $F = 0.5$) was 89.8 ± 1.8%; from the outcrossed progeny which were the maternal parents of crosses with $F = 0.125$ and $F = 0.25$, 90.5 ± 1.2%; and from the S_1 parents of the S_2 ($F = 0.75$) progeny, 89.7 ± 1.2%. All samples of parent trees used in the experiment evidently have comparable potential for seed set and observed variation at different levels of inbreeding is therefore ascribable to mating type.

Predicted seed yield at different levels of inbreeding

In order to demonstrate the effects of varying parameters on the seed yield function, numerical results are presented in Table 4 for conditions $k = 1, 2, 3$, $N = 1, 2, 3, 4$, and relative seed yield following selfing of 0.10, 0.25, 0.50, and 0.75.

As noted, k is likely to lie between 2 and 3 for *P. radiata*. In Fig. 1a and b are plotted the respective estimated seed yield curves given the observed mean filled seed yield of 43% after selfing, together with the experimentally determined values.

In order to test the general validity of the model, we have reviewed published reports of seed yield following inbreeding other than selfing. These are summarised in Table 5, together with expected yields based on the experimental S_1 seed yields, assuming $k = 2$, for the $N = 1$ and $N = 3$ cases. For $F = 0.125$ the independent lethal model predicted lower filled seed yield than was obtained in each study evaluated, the co-lethal model providing a better fit. At the $F = 0.25$ level results are ambiguous with each model most closely fitting the data in two cases.

Table 3. Probability[a] of producing an empty seed following selfing for varying number of parental loci carrying co-lethal alleles (n); number of embryos per ovule (k); and threshold number of homozygous co-lethals (N) (from Equation 1)

k	n	No. of homozygous co-lethals necessary to kill embryo (N)						
		1	2	3	4	6	8	10
1	1	0.2500	0	0	0	0	0	0
	2	0.4375	0.0622	0	0	0	0	0
	3	0.5781	0.1562	0.0156	0	0	0	0
	4	0.6836	0.2617	0.0508	0.0039	0	0	0
	6	0.8220	0.4661	0.1694	0.0376	0.0002	0	0
	8	0.8999	0.6329	0.3215	0.1138	0.0042	0.0000	0
	10	0.9437	0.7560	0.4744	0.2241	0.0197	0.0004	0
	12	0.9683	0.8416	0.6093	0.3512	0.0544	0.0028	0.0000
	16	0.9900	0.9365	0.8029	0.5950	0.1897	0.0271	0.0016
	20	0.9968	0.9757	0.9087	0.7748	0.3828	0.1018	0.0139
	30	0.9998	0.9980	0.9894	0.9626	0.7974	0.4857	0.1966
2	1	0.1250	0	0	0	0	0	0
	2	0.2656	0.0156	0	0	0	0	0
	3	0.4004	0.0547	0.0020	0	0	0	0
	4	0.5198	0.1155	0.0100	0.0002	0	0	0
	5	0.6208	0.1921	0.0280	0.0017	0	0	0
	6	0.7036	0.2778	0.0579	0.0061	0.0000	0	0
	7	0.7703	0.3664	0.1002	0.0153	0.0000	0	0
	8	0.8231	0.4530	0.1535	0.0311	0.0002	0.0000	0
	9	0.8644	0.5344	0.2153	0.0548	0.0008	0.0000	0
	10	0.8965	0.6084	0.2829	0.0869	0.0022	0.0000	0
	12	0.9402	0.7311	0.4241	0.1747	0.0099	0.0001	0.0000
	14	0.9658	0.8212	0.5581	0.2859	0.0294	0.0008	0.0000
	16	0.9805	0.8840	0.6736	0.4071	0.0661	0.0034	0.0001
	18	0.9889	0.9262	0.7664	0.5260	0.1231	0.0104	0.0003
	20	0.9937	0.9537	0.8371	0.6335	0.1990	0.0252	0.0012
	22	0.9965	0.9713	0.8889	0.7247	0.2892	0.0512	0.0038
	24	0.9980	0.9824	0.9256	0.7984	0.3873	0.0910	0.0096
	26	0.9989	0.9893	0.9509	0.8556	0.4863	0.1453	0.0209
	28	0.9994	0.9935	0.9681	0.8985	0.5803	0.2131	0.0401
	30	0.9996	0.9961	0.9794	0.9298	0.6653	0.2912	0.0691
	33	0.9998	0.9982	0.9896	0.9607	0.7712	0.4188	0.1337
3	1	0.0625	0	0	0	0	0	0
	2	0.1680	0.0039	0	0	0	0	0
	3	0.2888	0.0215	0.0002	0	0	0	0
	4	0.4084	0.0574	0.0024	0.0000	0	0	0
	6	0.6132	0.1802	0.0239	0.0013	0.0000	0	0
	8	0.7595	0.3418	0.0839	0.0109	0.0000	0.0000	0
	12	0.9146	0.6465	0.3138	0.0995	0.0026	0.0000	0.0000
	16	0.9715	0.8388	0.5791	0.2977	0.0289	0.0007	0.0000
	20	0.9907	0.9335	0.7780	0.5336	0.1180	0.0085	0.0002
	30	0.9995	0.9942	0.9700	0.9006	0.5701	0.1931	0.0307
4	1	0.0312	0	0	0	0	0	0
	2	0.1104	0.0010	0	0	0	0	0
	3	0.2154	0.0093	0	0	0	0	0
	4	0.3291	0.0311	0.0007	0	0	0	0
	6	0.5415	0.1235	0.0111	0.0003	0	0	0
	8	0.7055	0.2669	0.0499	0.0044	0.0000	0.0000	0
	12	0.8911	0.5789	0.2415	0.0616	0.0008	0.0000	0.0000
	16	0.9628	0.7991	0.5063	0.2272	0.0144	0.0002	0.0000
	20	0.9878	0.9148	0.7277	0.4587	0.0758	0.0035	0.0000
	30	0.9993	0.9924	0.9610	0.8741	0.4975	0.1363	0.0157

---

[a] In the table distinction is made between zero probability (0) and $0 < P < 0.00005$, (0.0000)

Table 4. Expected percentage filled seeds for specified yield following S_1 mating

Yield filled seed at S_1	N^a	No. embryos per ovule (k)/mating type k = 1			2			3		
		HS	FS	S_2	HS	FS	S_2	HS	FS	S_2
75%	1	94	86	83	94	88	84	95	90	84
	2	100	94	71	100	95	69	100	97	70
	3	100	96	62	100	97	58	100	99	57
	4	100	98	53	100	99	49	100	99	47
50%	1	85	71	63	88	75	66	90	80	68
	2	96	82	47	98	86	48	99	89	48
	3	99	90	37	100	94	35	100	94	35
	4	100	92	30	100	95	27	100	96	26
25%	1	71	50	40	80	55	42	83	60	46
	2	89	64	25	92	65	26	97	74	27
	3	98	70	17	98	79	17	99	81	18
	4	99	79	13	100	85	12	100	90	12
10%	1	56	32	22	66	37	24	71	39	26
	2	79	44	12	86	52	13	90	57	13
	3	89	53	7	94	63	8	98	69	8
	4	95	62	2	98	71	5	99	76	5

[a] Number of homozygous co-lethals required to kill embryo

Table 5. Published observations of filled seed in conifers at different levels of inbreeding (% of outcross (C) or open-pollinated (OP) checks), together with expected values under alternate models, for k = 2 case

Case no.	Species/references	Check type	Mating type						
			HS F = 0.125	FS 0.25	HS_1 0.25	S_1 0.5	$S_1 \times S_1$ 0.5	$S_1 \times$ parent 0.5	S_2 0.75
1.	*Pinus elliotii*	C (1, 2)	92	52	–	22	–	–	–
	Squillace and	Expec- N = 1	77	54		22			
	Kraus (1963)	tation N = 3	98	78		22			
2.	*P. thunbergii*	OP (1)	–	–	–	77	36	50	30
	Katsuta (1966)	N = 1				77			80
		N = 3				77			60
3.	*P. thunbergii*	C (3)	87	74	–	–	66	–	59
	Saito et al. (1973)	(5)							
4.	*P. monticola*	OP	102	81	78	60	32	40	19
	Bingham (1973)	N = 1	91	80		60			73
		N = 3	100	94		60			47
5.	*Picea abies*	C	137	80	–	25	26	–	7
	Andersson et al.	N = 1	78	55		25			41
	(1974)	N = 3	98	81		25			17
6.	*Pinus radiata*	C + OP (4)	–	–	–	74	–	–	78
	Pawsey (1964)	N = 1				74			83
		N = 3				74			57
7.	*P. radiata*	C	102	97	105	43	–	–	42
	(present study)	N = 1	88	73		43			60
		N = 3	99	90		43			30
Unweighted mean			104	77	91	50	40	45	39

(1) = Cited by Franklin (1970)
(2) = Germination test data
(3) = Part of material omitted as no check
(4) = Estimated from report that mean plant % of S_2 progeny was 105% of S_1
(5) = Expected values not computed for case 3 as no S_1 yield reported

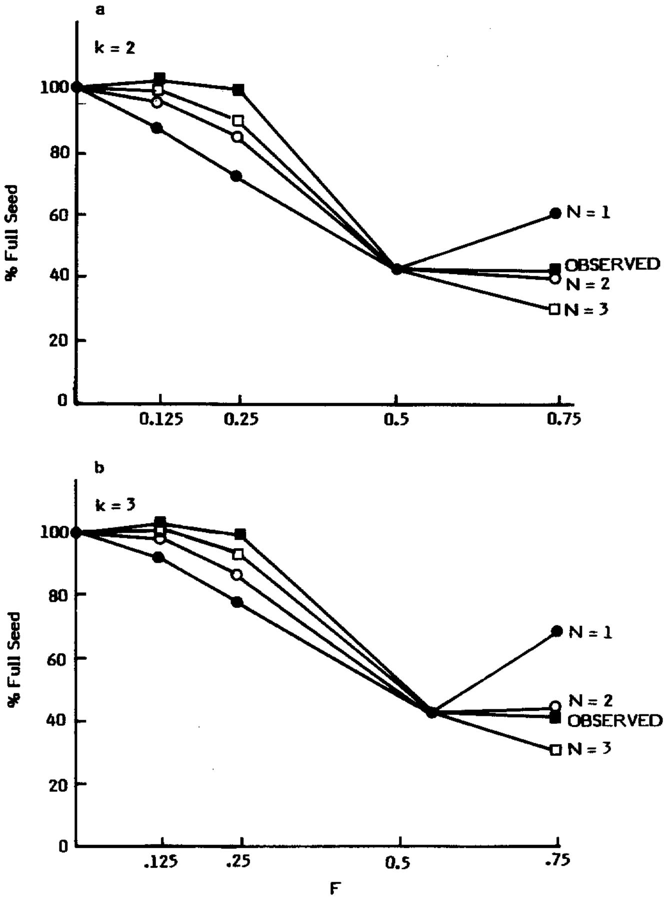

Fig. 1. Comparison of observed variation in full seed yield of *P. radiata* with expected values for $k = 2$, 3 and $N = 1$, 2, 3 models where S_1 yield = 43%

Under the independent lethal model filled seed yield after second generation selfing should be considerably greater than that following S_1 mating (see Fig. 1). No cases fulfilled this expectation. The co-lethal model with $N = 2$ predicts approximately the same filled seed yield in S_1 and S_2 as was the case for the two *P. radiata* studies (cases 6, 7 in Table 5). Investigations with other species (cases 2, 4, 5) showed a considerable decrease in yield in the S_2, compatible with a co-lethal model with a requirement of homozygosity at considerably more loci in order for mortality to occur.

In practice it is likely that S_2 filled seed yield relative to S_1 will be overestimated, thus underestimating N. It is difficult, if not impossible, to avoid selection for reproductive traits in the S_1 population. Those founder clones (such as 30040) which show greatly reduced seed set in the S_1 will have correspondingly fewer plants to act as parents for S_2 mating and thus contribute less to the S_2 population mean. Also with S_1 progeny those individuals which show greatest inbreeding depression (possibly associated with co-lethal allele complement) are less likely to flower and so be chosen as parents for S_2's. The effects of co-lethals at different loci may of course not be equal, in which case the more lethal will be eliminated in the S_1 to a greater extent.

According to the independent lethal model the filled seed yield of $S_1 \times S_1$ (selfed full-sib mating) would approximate S_1 with $(4n/9)$ as many heterozygous loci. This is similar to that expected from full-sib mating. For $S_1 \times$ parent the same result is expected as for S_1 with $2n/3$ heterozygous loci, the same change in filled seed yield as expected in the S_2. All published values (cases 2, 3, 4, 5) are below these expectations. We have not calculated predicted values under the co-lethal model but as selection against the lethals is less strong, values between S_1 and those predicted by the independent lethal model are expected. This is still not adequate to explain the published values, but the fit is improved. Further experimental and theoretical work with such complex mating types would be worthwhile.

Taking into account the scant knowledge of archegonium frequency distribution in *P. radiata* (Lill 1974); the relative fit of the $N = 2$ and $N = 3$ curves in Fig. 1a and b; and the above comment on effects of selection in the population of S_2 parents, the experimental data are best explained by a $k = 2$, $N = 3$ model with co-lethal alleles carried at 14 loci (see Table 3) and the assumption that selection in the S_1 raises S_2 filled seed yield to the equivalent of that under an $N = 2$ model.

Variation between progeny of different founder clones

The model should, of course, apply to relative seed yields of the progeny lines derived from each founder clone in addition to the total population. There are considerable differences in S_1 seed yield (Table 2), which should be reflected at other levels of inbreeding. For S_2 such a correspondence exists, compatible with the $N = 2$ model, if we accept rather broad sampling limits. For example, clone 4 has relatively high seed yields in S_1 and S_2 and 1 and 7 low yields in each generation. The low yields of S_1 progeny of clones 1 and 7 are not reflected in decreased yields after half- and full-sibing, as would certainly be expected with the independent lethal model (see Table 4, S_1 yield = 0.1, $N = 1$, $k = 2$ case). The higher order co-lethal models provide a better fit but are still not adequate to fully explain the data, particularly the full-sib case.

The individual founder line data also shed some light on the question of whether the yield of empty seed after outcrossing can be ascribed to action of common (co-)lethal alleles as suggested by Koski (1971). It has been assumed in this paper that this is not the case and that the effect is predominantly environmental. We conclude that our data confirm this view, for the following reasons:

In our sample of founder clones the apparent number of lethals varied considerably. The lowest filled seed yield in the S_1 corresponds to $n = 26$ and the highest to $n = 4$. Assuming the $k = 2$, $N = 2$ model, then an average of $n = 5$ common lethals in the outcrossing founders would result in 81% filled seed (Table 3), consistent with the observed average of 78.9% (Table 2). Variation in this number should be proportional to the number of lethals per founder, say from $n = 3 < 8$. This corresponds to a range in outcrossed filled seed of 47–95% (Table 3). The actual observed range of 74–83% (Table 2) does not approach this magnitude, nor is there the expected correlation between outcross and self seed yield. Sorensen (1969); Franklin (1972) and Johnsson (1976) noted a similar lack of correlation for Douglas-fir, *Pinus taeda* and *Pinus sylvestris*, respectively.

A similar argument can be developed to show that common lethals would result in a greater decrease in filled seed yield following low levels of inbreeding than was actually observed. The apparent lower seed yield following outcrossing c.f. half-sibing is obviously anomalous if a purely genetic interpretation is sought, but is more understandable if it is accepted that maternal environmental conditions may influence seed yield. The outcrosses and first generation selfs differed from the other matings in being carried out in a hedged breeding arboretum (Hand and Griffin 1979). Ramets treated in this way typically carry cones on a number of co-dominant leaders, while the unpruned trees used for the other types of mating were mainly flowering on a single leader. Competition for nutrient resources may therefore have been more severe within and between cones in the breeding aboretum, leading to a greater loss of developing embryos. This hypothesis obviously warrants further work, but it is interesting to note that our study is not alone in the finding that outcrossing did not always result in maximum seed yield (see cases 4 and 5, Table 5). The total seed number per cone for the OC and S_1 matings was lower than that from the other matings carried out on plantation grown progenies (Table 2), suggesting that cones were smaller in the arboretum.

Implications for tree breeding

The experimental results of this study have implications for both seed production and breeding strategy.

It will be reported elsewhere that the progeny derived in this experiment exhibit a linear decline in vigour with increasing F (Griffin, unpublished) and it is clearly desirable to minimise inbreeding in production seed orchards. Whether it should be eliminated entirely by restricting entries to unrelated individuals, or permitted to some degree in order to increase selection intensity at the family level, is a matter for assessment by individual breeders. In the latter case genetic gain estimates must be adjusted for the consequences of inbreeding on growth, and our data suggest that the appropriate adjustment will vary according to the intensity of inbreeding practiced. At higher intensity ($F \geqq 0.5$) the breeding system of *P. radiata* (in particular the combined effects of polyembryony, mortality of embryos through interaction of co-lethal alleles, and dichogamy) effectively reduces the proportion of inbred seed produced in orchards. Moran et al. (1980) estimated an effective outcrossing rate of about 0.90 in an orchard of unrelated clones and Matheson (1980) has shown that, in experiments with self and outcross pollen mixtures, the proportion of selfed seed harvested is below that predicted from frequency in the pollen.

At lower levels of inbreeding the practical effects may actually be more serious. The co-lethal allele system does not result in reduced seed set and, although we cannot discount the possibility that embryos from sib-matings will be selectively eliminated in competition with outcrosses in the same ovule, this is less likely because of the relatively smaller degree of inbreeding depression of these mating levels. Further, inter-family variation in flowering time will increase the probability that male and female flowering of relatives will be synchronised and the moderately high heritability of fecundity and phenology trials may mean that at any particular period in the flowering season the actively flowering individuals will tend to be relatives. At our present state of knowledge we suggest that gain estimates should be adjusted downward at least in proportion to the expectation for relative number of sib-matings, if the breeder were to include multiple selections from full- or half-sib families in the production orchards.

Franklin (1974) has argued against the use of a classical line breeding approach to forest tree improvement, largely on the grounds of increased cost and constraints on ability to select other traits which are imposed by strong inbreeding depression for embryo and seedling viability. Our experimental results suggest that such conclusions apply to *P. radiata* if selfing were adopted as the inbreeding mating system (see also Wilcox 1983). However since seed yield is maintained following matings of half- or full-sibs and even S_1 progeny produce normal seed yields following wind ($\triangleq$ outcross) pollination, the *P. radiata* breeder has no compelling biological reason for avoiding lower levels of inbreeding in breeding populations. Breeding strategies such as sub-lining (Burdon and Namkoong 1983; Matheson and Brown 1983) would certainly be more practicable if restrictions on the increase in average coancestry over generations were relaxed. Mild inbreeding may indeed be a useful procedure in forest tree breeding.

Acknowledgements. The work was carried out whilst Dag Lindgren was a visiting scientist at Division of Forest Research, CSIRO and visiting fellow at Department of Forestry, Australian National University funded by Fonden for Skogsvetenskaplig forskning and 1959 ars fond. We are grateful to Carolyn Raymond for computing the values condensed in Table 3, to Shane Dineen for X-radiography, and to Rick Hand and colleagues at Division of Forest Research, Traralgon for successfully completing a laborious series of controlled pollinations.

References

Andersson E, Jansson R, Lindgren D (1974) Some results from second generation crossings involving inbreeding in Norway spruce (*Picea abies*). Silvae Genet 23:34–43

Bingham RT (1973) Possibilities for improvement of western white pine by inbreeding. USDA For Serv Res Pap INT-144

Bishir J, Pepper WD (1977) Estimation of numbers of embryonic lethal alleles in conifers. 1. Self-pollinated seed. Silvae Genet 26:50–54

Bramlett DL, Popham TW (1971) Model relating unsound seed and embryonic lethal alleles in self-pollinated pines. Silvae Genet 20:192–193

Brown IR (1971) Flowering and seed production in grafted clones of Scots pine. Silvae Genet 20:121–132

Burdon RD, Namkoong G (1983) Multiple populations and sub-lines. Silvae Genet 32:221–222

Burdon RD, Zabkiewicz JA (1973) Identical and non-identical seedling twins in *P. radiata*. Can J Bot 51:2001–2004

Franklin EC (1970) Survey of mutant forms and inbreeding depression in species of the family *Pinaceae*. USDA For Serv Res Pap SE-61

Franklin EC (1972) Genetic load in loblolly pine. Am Nat 106:262–265

Franklin EC (1974) Classical inbreeding in forest tree improvement. Proc Joint IUFRO Meeting S.02.04.1-3, Royal College of Forestry, Stockholm

Griffin AR (1982) Clonal variation in radiata pine seed orchards. 1. Some flowering cone and seed production traits. Aust For Res 12:295–302

Griffin AR (1984) Clonal variation in radiata pine seed orchards. 2. Flowering phenology. Aust For Res 14:5–19

Hagman M, Mikkola L (1963) Observations on cross-, self- and inter-specific pollinations in *Pinus peuce*. Silvae Genet 12:73–79

Hand FC, Griffin AR (1979) Top pruning of *Pinus radiata* in breeding arboreta. Aust For Res 9:1–7

Johansen DA (1950) Plant embryology. Chronica Botanica Co, Waltham USA

Johnsson H (1976) Contribution to the genetics of empty grains in the seeds of pine (*Pinus sylvestris*). Silvae Genet 25:10–15

Katsuta M (1966) Further observations on the seed yield in self-pollination of *Pinus thunbergii* and *P. densiflora*. Misc Inf Tokyo Univ For 16:35–41

Koski V (1971) Embryonic lethals of *Picea abies* and *Pinus sylvestris*. Commun Inst For Fenn 75:1–30

Lill BS (1974) Development of the female cone of *Pinus radiata* D. Don. PhD Thesis, University Canterbury, NZ

Lill BS (1976) Ovule and seed development in *P. radiata* D. Don: post meiotic development, fertilisation and embryogeny. Can J Bot 54:2141–2154

Lill BS, Sweet GB (1977) Pollination in *P. radiata*. NZ J For Sci 7:21–34

Lindgren D (1975) The relationship between self-fertilisation, empty seeds and seeds originating from selfing as a consequence of polyembryony. Stud For Suec 126:1–24

Matheson AC (1980) Unexpectedly high frequencies of outcrossed seedling among offspring from mixtures of self and cross pollen in *P. radiata*. Aust For Res 10:21–27

Matheson AC, Brown AG (1983) Radiata pine breeding manual. CSIRO, Div For Res, Canberra

McWilliam JR (1959) Interspecific incompatibility in *Pinus*. Am J Bot 46:425–433

Moran GF, Bell JC, Matheson AC (1980) The genetic structure and levels of inbreeding in a *Pinus radiata* seed orchard. Silvae Genet 29:190–193

Orr-Ewing AL (1957) A cytological study of the effects of self-pollination in *Pseudotsuga menziesii*. Silvae Genet 6: 179–185

Pawsey CK (1964) Inbreeding in Radiata pine. Commonwealth of Australia, For Res Inst Leafl 87, 31 pp

Righter FI (1945) Pinus: the relationship of seed size and seedling size to inherent vigour. J For 43:131–137

Saito M, Iwakawa M, Watanabe M (1973) Effects of inbreeding on cone, seed and seedling yields in *Pinus thunbergii* (English summary). Bull Gov For Exp Stn No 255:31–46

Sarvas R (1962) Investigations of the flowering and seed crop of *Pinus sylvestris*. Commun Inst For Fenn 53:1–198

Simak M (1980) X-radiography in research and testing of forest tree seeds. Department of Silviculture, Swedish University of Agricultural Sciences

Sorensen FC (1969) Embryonic genetic load in coastal Douglas-fir. Am Nat 103:389–398

Sorensen FC (1982) The role of polyembryony and embryo viability in the genetic system of conifers. Evolution 36: 725–733

Squillace AE (1973) Comparison of some alternative second-generation breeding plans for slash pine. In: Proc 12th Southern For Tree Imp Conf. Baton Rouge, pp 2–13

Squillace AE, Kraus JF (1963) Effects of inbreeding on seed yield, germination, rate of germination, and seedling growth in Slash pine. In: Proc For Gen Workshop. Southern For Tree Imp Committee, Macon Ga, pp 59–63

Wilcox MD (1983) Inbreeding depression and genetic variances estimated from self- and cross-pollinated families of *P. radiata*. Silvae Genet 32:89–96