Theor Appl Genet (1992) 83:851–854

Self-fertility variation and paternal success through outcrossing in Douglas fir

R. R. Nakamura [1,*] and N. C. Wheeler [2]

[1] Department of Botany, KB-15, University of Washington, Seattle, WA 98195, USA
[2] Weyerhaeuser Company, Centralia Research Center, P.O. Box 420, Centralia, WA 98531, USA

Received August 15, 1991; Accepted September 3, 1991
Communicated by P. M. A. Tigerstedt

Summary. Douglas fir trees, *Pseudotsuga menziesii*, vary greatly in their self-fertility, but little is known about the relationship of self-fertility to outcrossing success. If low self-fertility pollen donors have lethal recessive alleles that are widespread, then in crosses with other trees they should have poor paternal success competing with high self-fertility donors that have few recessive lethals. We compared Douglas fir trees with high and low self-fertility for differences in pollen grain size, pollen number per milligram, and respiration rate. Pair-wise mixtures of pollen from individuals with high and low self-fertility were applied in controlled pollinations. Electrophoretic markers identified seed paternity. The pollen donors did differ in all three pollen traits but, as a class, the low self-fertility donors had neither inferior pollen nor low paternal success in outcrossing. Paternal success depended upon the identity of the competing pollen donors and the seed parent. It was not related to pollen grain number or respiration rate, but donors with the smaller pollen grains in a mixture had greater success.

Key words: Self-fertility – Paternal success – Pollen – Douglas fir – *Pseudotsuga menziesii*

Introduction

The mating system of many plant species combines selfing and outcrossing (Schemske and Lande 1985; Brown 1990). The reproductive success of an individual through selfing relative to outcrossing will affect the evolution of mating systems and sex allocation (Hedrick 1990). Upon self-pollination, individuals often experience high levels of inbreeding depression (Charlesworth and Charlesworth 1987). In Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, the major cause of inbreeding depression is embryo abortion, due to lethal recessive alleles rather than a loss of overall heterozygosity (Sorensen 1969, 1982; Mitchell-Olds and Guries 1986). Although inbreeding depression upon selfing is severe, the degree of self-fertility can vary widely among trees within a region or natural population (Wheeler 1989). For example, in a Douglas fir population at Santiam Pass Oregon, Sorensen (1971) measured a more than 65-fold difference among trees in relative seed production upon selfing.

Douglas fir trees have a mixed mating system that is predominantly outcrossed (Shaw and Allard 1982; Muona 1990), and pollen donors are known to have nonrandom paternal success when competing in mixed, outcross pollinations (Apsit et al. 1989). R.R. Nakamura and N.C. Wheeler (unpublished manuscript) present experimental evidence to support the view that, in controlled pollinations, differential embryo abortion is the source of nonrandom paternal success through outcrossing. Variation in self-fertility among trees raises questions about the relationship of self-fertility to paternal success through outcrossing. Are pollen donors with low self-fertility also poor competitors in outcrossing? Is their pollen of different quality from that of high self-fertility plants? These questions are of general interest because of the widespread occurrence of self-fertility variation in tree species with mixed mating systems (e.g., Franklin 1972; Koski 1973; Park and Fowler 1984; Griffin and Lindgren 1985).

Here we present the results of a study of paternal success in Douglas fir when pollen donors with high and low self-fertility compete to sire seeds on the same seed cones. If particular lethal recessive alleles are common,

* Present address: Department of Biology, California State University, Los Angeles, CA 90032-8201, USA

then trees with low self-fertility will do poorly in outcrossing, because these pollen donors will likely share the same lethal recessives with seed parents. On the other hand, if particular lethal recessives are rare, then even pollen donors with low self-fertility should not suffer in paternal success through outcrossing.

Materials and methods

Crossing design and pollination procedures

To study the effects of variation in self-fertility on paternal success through outcrossing, we cross-pollinated Douglas fir trees in April 1988 at the Weyerhaeuser Co. seed orchard in Rochester/WA. The trees originated from natural populations in western Washington and represented parents selected on the basis of the growth traits of their progeny in genetic tests. The pollen donors were divided into two self-fertility classes according to the number of lethal equivalents. Three donors had high self-fertility (lethal equivalents: 5.3 to 6.5), and three donors had low self-fertility (lethal equivalents: 14.2 to 25). The two seed parents were of intermediate self-fertility. The method of Morton et al. (1956), as illustrated by Sorensen (1969), was used to estimate the number of lethal gene equivalents per zygote ($2B$) for each tree, where $2B = -4 \ln R$ and R is relative self-fertility (percentage of filled seeds from selfing/percentage of filled seeds from outcrossing).

We made pollen mixtures for each pair of donors with high and low self-fertilities, for a total of nine pollen mixtures. Each mixture contained a 50:50 combination by mass of pollen from a pollen donor with high self-fertility and a donor with low self-fertility.

Pollination procedures followed standard protocols. Two to three weeks before seed parents were receptive, we isolated seed cone buds with paper breeding bags after removing pollen buds. When pollen donors started to shed pollen, pollen cones were picked and processed to extract pollen grains (for details of the method used see Apsit et al. 1989). A mean of 8.4 seed cones in each breeding bag was brush pollinated at the peak of receptivity. We pollinated a total of 54 bags (nine pollen mixtures × three replicates × two seed parents). Seeds were extracted from the mature cones in October 1988.

Identification of paternity

Starch gel electrophoresis of embryos determined seed paternity. In each pollen mixture the two pollen donors were distinguished by different allozymes at one of four loci: glucose-6-P-dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase-3, and phosphoglucomutase. The two seed parents were homozygous. Electrophoretic and staining procedures followed Conkle et al. (1982) and Jech and Wheeler (1984), except that the morpholine-citrate buffer was pH 8.0. Allozymes of the loci in our study exhibited Mendelian inheritance (El-Kassaby et al. 1982). For each breeding bag the paternal success of a pollen donor was the proportion of seeds it sired. On average, we identified the paternal parent of 45.4 seeds from each bag with mature cones, 2,314 seeds in all.

Pollen traits of different donors

To investigate the relationship between self-fertility and pollen quality, we measured three pollen traits and correlated them with a pollen donor's paternal success. An Elzone 180XY particle analysis system (Particle Data, Inc.; Elmhurst/IL) counted the number of pollen grains per unit mass and measured modal pollen grain size (μm^3) for four subsamples from each pollen donor (Young and Stanton 1990a). A YSI model 53 oxygen monitor (Yellow Springs Instrument Co., Yellow Springs/OH) measured pollen respiration rates ($\mu l\ O_2$/min/g), an indicator of pollen viability, for two subsamples from each pollen donor (Binder and Ballantyne 1975).

Results

Pollen traits

As a class, the low self-fertility pollen donors did not have a distinct type of pollen. The pollen traits we measured differed significantly among the pollen donors, but the degree of self-fertility had no effect (Table 1). For the pollen donors mean pollen grain size was 98.1 μm^3 (range: 95.2 to 100.2), mean pollen grain number per milligram was 4,960 (range: 4483 to 5840), and mean respiration rate was 24.1 $\mu l\ O_2$/min/g (range: 8.5 to 34.2).

Paternal success

Although paternal success significantly varied among the pollen donors (Table 2), the self-fertility class of the pollen donor did not consistently predict paternal success in the pollen mixtures. Pollen donors with low self-fertility were not poor competitors against high self-fertility donors. Rather, some of these donors did well in particular pollen mixtures but not in others (Table 3). For instance, low self-fertility donor 1 sired 81% of the seeds when competing against high self-fertility donor 4, but only 30% against donor 6. Also, we detected pollen donor by seed parent interaction effects (Table 2). The relative performance of a pollen donor depended upon the identity of the seed parent.

Table 1. Nested analyses of variance for pollen traits. The random effect pollen donor was nested within the fixed effect self-fertility class

Source	*df*	SS	F	*P*
Pollen number (grains/mg):				
Self-fertility class	1	0.00507	0.01	NS
Pollen donor within class	4	0.20749	5.72	0.0037
Error	18	0.16317		
Model $R^2 = 56.6\%$				
Pollen size (μm^3):				
Self-fertility class	1	0.00004	0.02	NS
Pollen donor	4	0.00977	6.41	0.0022
Error	18	0.01667		
Model $R^2 = 58.9\%$				
Pollen respiration ($\mu l\ O_2$/min/g):				
Self-fertility class	1	0.06260	0.08	NS
Pollen donor	4	3.08222	24.51	0.0007
Error	6	0.18860		
Model $R^2 = 94.3\%$				

Table 2. Analysis of variance of paternal success of low self-fertility pollen donors in pollen mixtures with high self-fertility donors

Source	*df*	SS	F	*P*
Low self-fertility donor	2	0.3289	14.4	0.0001
High self-fertility donor	2	2.5157	110.2	0.0001
Low donor × high donor	4	0.0406	0.9	NS
Seed parent	1	0.3656	32.0	0.0001
Seed parent × low donor	2	0.1392	6.1	0.0056
Seed parent × high donor	2	0.3313	14.5	0.0001
Three-way interaction	4	0.1344	3.0	0.0347
Error	33	0.3766		
Model $R^2 = 91.4\%$				

Table 3. Mean paternal success per seed parent of low self-fertility pollen donors competing against high self-fertily donors. Paternal success is the proportion of seeds sired by the low self-fertility donor. SF = self-fertility

Low SF donor	High SF donor	Mean paternal success
1	4	0.808
1	5	0.478
1	6	0.301
2	4	0.911
2	5	0.734
2	6	0.512
3	4	0.867
3	5	0.632
3	6	0.447

Paternal success of the low self-fertility donors was related to pollen size but not to the other pollen traits. For each of the nine pollen mixtures, we calculated the difference between the two donors in their pollen traits (value of the low donor minus the value of the high donor). Thus, a positive correlation with paternal success would mean that the larger the value pollen of the low self-fertility donor with respect to its competitor, the greater the low donor's paternal success would be. Instead, the mean paternal success of the low self-fertility donor was negatively correlated with the difference in pollen size (Spearman $r = -0.90$, $P = 0.0009$, $N = 9$). A low self-fertility donor sired a greater proportion of the seeds the relatively smaller its pollen grains were compared to those of the high self-fertility donor. Paternal success of the low self-fertility donor was not correlated with differences between donors in pollen number ($r = 0.00$, $P = 1.00$, $N = 9$) or respiration rate ($r = 0.12$, $P = 0.76$, $N = 9$).

Discussion

Self-fertility variation in Douglas fir trees did not accurately predict paternal success through outcrossing. Pollen donors with low self-fertility were not generally poor competitors with high self-fertility donors. Rather, paternal success depended upon the particular pollen donors in a pollen mixture and the identity of the seed parent (see also Apsit et al. 1989). These results indicate that individual lethal recessive alleles may exist at only low frequencies among Douglas fir trees. Otherwise, the low self-fertility trees with larger numbers of recessive lethals would have had poorer paternal success in outcrossed pollinations than donors with fewer recessive lethals. Future crossing designs with a much larger number of pollen donors and seed parents are needed to further test our conclusions.

The degree of self-fertility was unrelated to variation in pollen traits. Evidently, the number of recessive lethal equivalents per donor did not affect pollen number, pollen grain size, or respiration rate. Orr-Ewing (1957) found that under selfing, the expression of lethality in Douglas fir came at the embryo stage rather than during pollen growth. No other published study has compared the pollen characteristics of conifers from different self-fertility classes.

Only a few recent studies have addressed the relationship between pollen size and success in siring seeds. The results so far have been equivocal. In our study, pollen grain size was the only pollen trait correlated with paternal success in the mixed outcrossed pollinations. Although this correlation was negative, R.R. Nakamura and N.C. Wheeler (unpublished manuscript) reported a positive size correlation in other outcrossed pollinations with Douglas fir. Cruzan (1990) did see a positive relationship between pollen size and paternal success in glacier lily, but Young and Stanton (1990b) found no relationship in wild radish.

Acknowledgements. We thank the personnel of the Rochester Seed Orchard. We are also indebted to D. Lello and K. Jech for laboratory and field assistance; to H. Young and M. Stanton for measurement of pollen size and number; to T. Mitchell-Olds, D. Schemske, and the Department of Botany of the University of Washington for sharing their facilities; and to G. Namkoong for advice. Financial support came from Weyerhaeuser Forestry Research and USDA Forest Biology Competitive Grant 87-FSTY-9-0266 to N.C. Wheeler.

References

Apsit VJ, Nakamura RR, Wheeler NC (1989) Differential male reproductive success in Douglas fir. Theor Appl Genet 77:681–684

Binder WD, Ballantyne DJ (1975) The respiration and fertility of *Pseudotsuga menziesii* (Douglas fir) pollen. Can J Bot 53:819–823

Brown ADH (1990) Genetic characterization of plant mating systems. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland/MA, pp 145–162

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Conkle MT, Hodgskiss PD, Nunnally LB, Hunter SC (1982) Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For Serv Genet Techn Rep PSW-64

Cruzan MB (1990) Variation in pollen size, fertilization ability, and postfertilization siring ability in *Erythronium grandiflorum*. Evolution 44:843–856

El-Kassaby YA, Yeh FC, Sziklai O (1982) Inheritance of allozyme variants in coastal Douglas-fir, (*Pseudotsuga menziesii* var. *menziesii*). Can J Genet Cytol 24:325–335

Franklin EC (1972) Genetic load in loblolly pine. Am Nat 106:2621–265

Griffin AR, Lindgren D (1985) Effect of inbreeding on production of filled seed in *Pinus radiata* – experimental results and a model of gene action. Theor Appl Genet 71:334–343

Hedrick PW (1990) Mating systems and evolutionary genetics. In: Wohrmann K, Jain J (eds) Population biology. Springer, New York, pp 83–114

Jech KS, Wheeler NC (1984) Laboratory manual for horizontal starch gel electrophoresis. Weyerhaeuser Tech Rep 050-3210/6, Centralia/WA

Koski V (1973) On self-pollination, genetic load, and subsequent inbreeding in some conifers. Comm Inst For Fenn 78:1–42

Mitchell-Olds T, Guries RP (1986) Genetic load and heterozygosity in the Pinaceae. Can J Genet Cytol 28:942–946

Morton NE, Crow JF, Mueller HJ (1956) An estimate of the mtuational damage in man from data on consanguineous marriages. Proc Natl Acad Sci USA 42:855–863

Muona O (1990) Population genetics in forest tree improvement. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland/MA, pp 282–298

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

Park YS, Fowler DP (1984) Inbreeding in black spruce (*Picea maiana* (Mill.) B.S.P.): self-fertility, genetic load, and performance. Can J For Res 14:17–21

Schemske, DW, Lande R (1985) The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. Evolution 39:41–52

Shaw DV, Allard RW (1982) Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theor Appl Genet 62:113–120

Sorensen F (1969) Embryonic genetic load in coastal Douglas-fir, *Pseudotsuga menziesii* var. *menziesii*. Evolution 103:389–398

Sorensen F (1971) Estimate of self-fertility in coastal Douglas-fir from inbreeding studies. Silvae Genet 20:115–120

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

Wheeler NC (1989) Characterization of select pedigrees for genetic load. Weyerhaeuser Tech Rep 050-3210/26, Centralia/WA

Young HJ, Stanton ML (1990a) Temporal patterns of gamete production within individuals of *Raphanus sativus* (Brassicaceae). Can J Bot 68:480–486

Young HJ, Stanton ML (1990b) Influence of environmental quality on pollen competitive ability in wild radish. Science 248:1631–1633