

MATING SYSTEM AND GENETIC DIVERSITY IN NATURAL POPULATIONS OF KNOBCONE PINE (*PINUS ATTENUATA*)

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ABSTRACT

Mating system and genetic diversity were investigated in three natural populations of knobcone pine (*Pinus attenuata* Lemmon.) located at the northern limits of the species range near the California-Oregon border. Multilocus estimates of outcrossed progeny (t_m) were high in all populations (mean $t_m = 0.927$, range 0.890–0.968). Although t_m values were not statistically heterogeneous ($P > 0.05$) over populations, they varied in magnitude consistent with stand density; that is, t_m decreased with increased spacing between trees. Mean single-locus estimates of t differed only slightly from t_m suggesting that there is little inbreeding other than selfing, despite expected clustering of family members in these stands. The genetic structure of adult trees was consistent with high outcrossing and with the high levels of genetic diversity typically observed within populations of conifer species.

Key words: *Pinus attenuata*, allozymes, outcrossing, mixed-mating model, genetic diversity

INTRODUCTION

Coniferous forest tree species are wind-pollinated and typically have high proportions of outcrossed progeny ($t > 0.80$) (MUONA 1990; ADAMS & BIRKES 1991; MITTON 1992) and high levels of genetic diversity within populations (HAMRICK *et al.* 1992). Population outcrossing rates lower than 0.80, however, are occasionally reported (PERRY & KNOWLES 1990; XIE *et al.* 1991; EL-KASSABY *et al.* 1994), and levels of genetic diversity within populations vary widely both among and within species (HAMRICK *et al.* 1992).

Knobcone pine (*Pinus attenuata* Lemmon.) is a conifer widely dispersed throughout the mountainous region in southern Oregon and northern California (MILLAR *et al.* 1988), where it grows on dry, submarginal forest sites. Cones of knobcone pine are born on the main stem and open usually only after the occurrence of forest fires. The result is that trees often exist in dense clusters, probably of close relatives, since seed dispersal may be limited. If mating occurs primarily between near neighbors, clustering of relatives is expected to result in high levels of inbreeding and small effective population size. A study of effective pollen dispersal and the mating system in one knobcone pine stand near the California-Oregon border (Sanger Peak);

however, indicated that less than half of the matings were with near neighbors (*i.e.*, within 11 m), and that outcrossing was on par with observations in other conifers ($t = 0.92$) (BURCZYK *et al.* 1996).

In this note, we expand estimation of the mating system in knobcone pine to two additional stands in the California-Oregon border region, because no other estimates of outcrossing have been reported in this species. We were particularly interested in the extent to which tree density influences t , since density varies 10-fold among the three stands. In addition, we provide estimates of parameters describing genetic structure and levels of genetic diversity in each population. Previous estimates of genetic diversity in this species were based on samples from only four populations (MILLAR *et al.* 1988). The California-Oregon border is near the northern limit of the species distribution.

METHODS

The three natural populations included in this study are Cedar Flat (CF) (N42°13'; W123°23'), Rough & Ready (RR) (N42°05'; W123°41') and Sanger Peak (SP) (N41°58'; W123°39'). RR is a very dense (2050 trees ha^{-1}), nearly pure, stand of knobcone pine found on a valley floor at 450 m elevation. Both CF and SP consist

of patches of knobcone pine at approximately 1200 m elevation, but tree density varies widely between the two stands: 1110 trees/ha at SP and only 200 trees · ha⁻¹ at CF. Cones were collected in the fall of 1992 from the upper crowns of individual mother trees (23, 22 and 26 for RR, CF and SP, respectively). An equal number of 11 seeds per tree were sampled for genetic analyses.

Fifteen loci (*Aco*, *Adh-2*, *Aat-1*, *Aat-2*, *Aat-3*, *Fdp-2*, *Fest*, *Idh*, *Lap-1*, *Lap-2*, *Mnr-3*, *6-Pgd*, *Pgm-1*, *Pgi-1* and *Pgi-2*) were used to assess genetic diversity, with all but four loci (*Aat-2*, *Aat-3*, *Fdp-2*, *Pgi-1*) also used for mating system estimation. Electrophoretic techniques were similar to those described in CONKLE *et al.* (1982). Banding patterns, verification of their Mendelian inheritance, and linkage relationships were reported previously (STRAUSS & CONKLE 1986).

Mother tree genotypes were inferred from allelic arrays in megagametophytes (MORRIS & SPIETH 1978). Single-locus (t_s) and multilocus (t_m) estimates of proportions of outcrossed progeny were calculated for each population based on the mixed-mating model (FYFE & BAILEY 1951) and maximum-likelihood procedures developed for conifers (RITLAND & EL-KASSABY 1985), using MLTF computer program. Heterogeneity of outcrossing estimates among populations was evaluated using Fisher's test for heterogeneity (RAO 1973).

Genetic structure and diversity were assessed for the adult trees in each population by estimating the proportion of polymorphic loci (95 % criterion), number of alleles per locus (A), and expected (H_e) and observed (H_o) heterozygosities. Estimates of H_e were

corrected for small sample sizes (NEI 1978). Observed genotype frequencies were compared to those expected under Hardy-Weinberg equilibrium using a goodness-of-fit test (G -statistics, SOKAL & ROHLF 1981). All tests of significance were conducted at the 0.05 probability level.

RESULTS

Multilocus estimates of outcrossed progeny ranged 0.890 to 0.968 among the three populations, but were not significantly heterogeneous ($P = 0.072$) (Table 1). In two of the three populations (CF and SP), however, \hat{t}_m was significantly less than 1. Although \hat{t}_s ranged widely among loci in each population (significantly so in CF and SP), mean values \hat{t}_s were similar to \hat{t}_m . Genetic diversity was high among adults ($A = 0.196$, $H_e = 0.235$), and varied only slightly among populations (Table 1). Mean estimates of observed heterozygosity were very similar to H_e ; in only 3 cases, out of the 37 that could be tested, were genotypic frequencies significantly different from Hardy-Weinberg expectations.

DISCUSSION

Mating system estimates in this study support our earlier findings based on only one of the three populations (BURCZYK *et al.* 1996); that is, the proportion of outcrossing in northern populations of knobcone pine ($\bar{t}_m = 0.927$) is similar to high levels observed in most

Table 1. Estimated proportion of polymorphic loci (95 % criterion), mean number of alleles per locus (A) and expected (H_e) and observed (H_o) heterozygosity among adult trees, and single (t_s) and multilocus (t_m) estimates of proportions of outcrossed progeny, in each of three knobcone pine populations.

	Population		
	Cedar Flat (CF)	Rough & Ready (RR)	Sanger Peak (SP)
P (%) ^a	66.7	73.3	66.7
A^a	1.87	2.00	2.00
H_e^a	0.231	0.244	0.230
H_o^a	0.214	0.220	0.236
t_s mean ^b	0.860	0.970	0.914
(range over loci)	(0.676–1.102)	(0.851–1.129)	(0.713–1.107)
t_m (SE) ^c	0.890 (0.027) ^d	0.968 (0.022)	0.923 (0.022) ^d
Tree density (trees·ha ⁻¹)	200	2,050	1,110

^a Based on 15 polymorphic loci.

^b Unweighted mean of 11 loci in RR and SP; 10 loci in CF.

^c Based on 11 polymorphic loci.

^d Significantly different from $t = 1$ ($P < 0.001$)

other conifers (MUONA 1990; ADAMS & BIRKES 1991; MITTON 1992). Although heterogeneity of t_m among populations was not significant, the estimates varied in magnitude consistent with the density of trees in the populations. Outcrossing might be expected to decrease with increasing distance between trees (*i.e.*, with lower density), as found in this and other studies (RUDIN *et al.* 1977; FARRIS & MITTON 1984; KNOWLES *et al.* 1987); but, this relationship is not always observed (NEALE & ADAMS 1985; FURNIER & ADAMS 1986).

Single-locus estimates of t are sensitive to violations of the mixed-mating model (SHAW & ALLARD 1982). In particular, the mixed-mating model assumes that pollen pool allele frequencies are homogeneous in space and that there is no inbreeding other than selfing. If either of these assumptions are violated, t_s is expected to be less than t_m . Pollen pool heterogeneity is not a likely explanation for $t_s < t_m$ in SP since all mother trees were sampled within a 15 m radius, but might explain $t_s < t_m$ in CF, where mother trees were sampled over a distance of 200 m. In both cases, however, the difference between t_s and t_m is very small. Thus, despite the potential for family clusters in these stands, inbreeding other than selfing is not evident. This is consistent with our earlier observations indicating broad effective pollen dispersal in this species (data from SP; BURCZYK *et al.* 1996), which suggest that even if there is strong family clustering, inbreeding is minimized because most mating is not with near neighbors.

The genetic structure of the adults in these populations is consistent with the high observed outcrossing and with high levels of genic diversity found previously in this species (MILLAR *et al.* 1988). There is no evidence that the location of these populations on a margin of the species distribution has resulted in significant inbreeding or reduced genetic diversity.

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