EFFECTS OF REGENERATION METHODS ON GENETIC DIVERSITY IN SHORTLEAF PINE (*Pinus echinata* Mill.)

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Continuing demand for forest products, the increasing demand for use of forested areas for non-traditional purposes, and the general increase in awareness of the need for maintaining landscape diversity and biodiversity, wildlife conservation, protection of old growth forests, control of ecodegradation and global climate change has put a complex array of often conflicting demands, priorities and conditions on forest managers. The choice of suitable management strategies applicable to the climatic, political and public situation of the forests under their care has become exceedingly difficult. Therefore, an evaluation of within-species genetic diversity of natural stands compared to that of stands regenerated by various management schemes would help in understanding man's effect on these stands, and may suggest suitable management strategies. This study examined the effect of regeneration methods on genetic diversity in shortleaf pine (*Pinus echinata* Mill.) by quantifying the changes in genetic composition of shortleaf pine stands following harvest by monitoring changes in allele number and frequency at heterozygous loci over time. The study involved two steps. The first step examined isoenzyme variation and genetic structure in natural populations of shortleaf pine. The second step examined genetic variation in two stands and it's changes due to different regeneration management systems.

The first part of the study, reported in detail by Raja *et al.* (1997) examined seed from populations representing 15 geographic locations covering much of the natural range of shortleaf pine using 23 enzyme systems and 39 loci. Populations were polymorphic (*p) at* 87.2% of the loci, had 2.18 alleles *(A)* per locus and 2.35 alleles per polymorphic locus (A_n). Mean expected heterozygosity (H_e) was 0.194 and mean observed heterozygosity (H_0) was 0.174. Western populations had a higher p, higher A, similar A_p and a higher H_0 and H_e than eastern populations. Interpopulation genetic variation was 9% percent, meaning 91% of the genetic variation in shortleaf pine resides within populations. Interpopulation gene flow was relatively high and explains the small interpopulation genetic variation in the species. Shortleaf pine populations exist in naturally outcrossing random-mating populations and have a relatively large amount of natural variability.

For the second part of the study, seed from 48 trees was collected from each of two shortleaf pine stands in the Ouachita Mountains near Mt. Ida, Arkansas. These 40-acre 60-80 year old stands contained shortleaf pine and a mixture of other deciduous species, predominantly *Quercus* and *Carya* species. Each stand was subdivided into quarters of approximately equal area arranged perpendicular to the elevation gradient and each quarter further subdivided into thirds along the elevation gradient, as part of a large ecosystem management research study on the Ouachita and Ozark National Forests in west-central Arkansas and eastern Oklahoma. A plot center was marked in each of the 12 plots and 4 healthy trees with abundant cones were selected from each plot for seed collection in this study. Seed-tree and single tree selection harvest / regeneration systems were applied to the two stands, respectively, following the first seed collection. The subsequent crop of seed representing genetic variation after management was then collected. The seed samples were assayed to detect changes in genetic variation due to management. Twenty-five seeds from each of the 48 trees from each stand for pre- and post-treatment were assayed for the 34 isoenzyme loci that were found polymorphic in stage one of this study (Raja et al. 1997). Fifty seeds each from the bulked seeds of Ouachita and Ozark seed orchards were also analyzed to represent artificial regeneration. Seed extraction and storage procedures, sample preparation, starch gel electrophoresis, enzymes staining and isoenzyme detection procedures followed protocols described by Raja et al. (1997).

Megagametophytes and embryos from each seed were scored for each locus. Identification of pollen genotype was accomplished by comparing megagametophyte and embryo data. Haploid pollen allele frequencies and diploid embryo genotypic frequencies were calculated. Allele frequencies for pre-treatment, post-treatment and artificial regeneration were compared with χ^2 tests (Snedecor and Cochran 1967, p. 250). When expected values were too small for χ^2 tests, Fisher's exact test was used (Sokal and Rohlf 1981, p. 740). Genetic diversity was estimated by percent polymorphic loci `p', mean number of alleles per locus *'A'* and mean number of alleles per polymorphic locus *`A_P'.* Diploid embryo data from each stand were pooled for pre- and post-treatment to calculate the observed (H0) and expected (H_e) heterozygosities, and the fixation index using the formula $F = 1 - H_0 / H_e$. H_e and H_e were calculated using the BIOSYS-1 computer program (Swofford and Selander 1981).

Both natural regeneration treatments resulted in higher genetic variation post-treatment, indicating a richer pollen cloud after management. Artificial regeneration showed much lower variation compared to both natural regeneration treatments. Frequency of alternate alleles increased at several loci in the seed-tree stand after treatment, which is an indication of less inbreeding or consanguineous mating. Single tree selection resulted in an increase in alternate allele frequencies at a relatively fewer loci and at some loci alternate allele frequencies decreased,

indicating that the treatment may result in more inbreeding than seed tree. Artificial regeneration showed a considerable increase in alternate allele frequencies at several loci and hence can be considered outbred. The above mentioned observations were confirmed by comparing H_o, H_e and F values for the two stands before and after treatment. The seed tree method resulted in a decrease in inbreeding, whereas single tree selection did not alter it. Artificial regeneration showed a value indicative of high levels of heterozygosity and outbreeding. Our results generally agree with the results of Neale and Adams (1985).

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