WHOLE-GENOME CHARACTERIZATION OF INBREEDING DEPRESSION IN A SELFED LOBLOLLY PINE FAMILY

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Inbreeding depression has long been recognized as an important factor in the evolution of plant populations and mating systems (Charlesworth and Charlesworth 1987). The degree of inbreeding depression in a population may affect the evolution of selfing *vs.* outcrossing modes of reproduction. Conversely, a history of inbreeding may result in purging of deleterious alleles and reduce the degree of inbreeding depression if selection against individual alleles is sufficiently strong (Husband and Schemske 1996; Lande and Schemske 1985). The equilibrium level of inbreeding depression is in part a function of the genomic deleterious mutation rate U, but is also affected by the extent to which overdominance and epistasis contribute to inbreeding depression. Consequently, the roles of dominance vs. overdominance, the distribution of selection effects against individual alleles, and the extent of epistasis are important and longstanding issues surrounding inbreeding depression.

Most gymnosperms exhibit high levels of inbreeding depression, especially at the embryonic stage. Embryonic lethality generally results in empty seed, and embryonic inbreeding depression is typically estimated from the ratio of filled seed frequencies under inbreeding compared with outcrossing. Recent advances in molecular markers now make genetic mapping a powerful additional tool to study the genetic architecture of inbreeding depression. In this study, we conducted a genome-wide evaluation of loci affecting embryonic viability in selfed offspring of a loblolly pine *(Pinus taeda* L.) parent. We scored both germinating and non-germinating selfed individuals for AFLP markers from a previously constructed linkage map with essentially complete genome coverage (Remington *et al.* 1999).

Selfed seed from loblolly pine clone 7-56 (NCSU-Industry Cooperative Tree Improvement Program) were provided by Westvaco Corporation and the USDA Forest Service. A total of 373 filled seeds were germinated under sterile conditions. Germinated seedlings were transferred to growing media and placed in a greenhouse. Embryos were harvested from non-germinating seed. The surviving seedlings were transferred to 10 L containers the summer of the first growing season, and transplanted to a field site at the South Carolina Forestry Commission Niederhoff Seed Orchard at Tillman, SC during the second growing season. DNA preparations suitable for AFLP templates were made from the needles of 270 surviving seedlings and from 57 embryos of non-germinating seeds. These 327 individuals were scored for 226 AFLP markers, including framework markers from the original linkage map plus some alternate markers that were added to improve coverage of both linkage phases. All markers were scored as dominant markers.

Genomic regions with distortion from the expected 3:1 segregation ratios were identified as prospective embryonic viability loci. Coupling phase markers (i.e. those in which the recessive band-absent marker allele is linked to a recessive allele affecting viability) are expected to show a deficit of band-absent individuals. Repulsion phase markers show a corresponding deficit of band-present individuals if the viability allele is recessive. We used an iterative maximum likelihood procedure to estimate the linkage group position and fitness coefficient w at each candidate viability locus. A likelihood ratio test statistic threshold of 11.60, corresponding to an experimentwise 0.05 significance level, was chosen using methods of Lander and Botstein (1989). The fit to a recessive model of gene action was tested by comparing the frequency of band-absent individuals at nearby repulsion phase markers to expected

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values using a chi-square test. Finally, we tested for epistasis using the method of Fu and Ritland (1996), in which the log-transformed fitness of individuals homozygous for i markers tightly linked to viability alleles is regressed on i. A significant quadratic term suggests epistasis.

We identified a total of 19 embryonic viability loci with LR values that exceeded the test threshold. Each of the twelve linkage groups contains at least one viability locus. Five linkage groups (LGs 1,4,5,8, and 12) have two viability loci and one linkage group (LG 10) has three. One additional interval on LG9 had an LR value of 9.37, which is suggestive of an additional viability locus. Three loci harbor complete lethals (Cs = 1- 0.99). Alleles at the remaining loci are semi-lethal with estimated selection coefficients ranging from 0.42 to 0.93. Six of the 20 identified or suggestive viability loci show deviations from the recessive model, all in the direction of overdominance. However, the deviations appear to be caused by linkage in repulsion to other identified viability loci in three of these cases. Deviations at the remaining three loci are significant at a 0.05 comparisonwise level but not at a 0.05 experimentwise level. The regression of fitness on number of homozygous viability alleles strongly suggests the absence of appreciable epistasis. A regression model with only a linear term resulted in an R^2 of 0.9973 (p = 1.31 x 10^{-v}). A quadratic term added to the model was insignificant.

The 19 identified viability loci represent 13.13 lethal equivalents, which increases to 13.52 lethal equivalents when the additional suggestive locus is added. The average number of lethal equivalents for loblolly pine reported by Franklin (1972) is only 8.5, and only 7.8% of individual parents had estimates of more than 14 lethal equivalents. This suggests that slightly deleterious alleles do not contribute much to embryonic inbreeding depression, at least for this individual. On the other hand, complete lethals account for less than one quarter of the mapped inbreeding depression. Models in which inbreeding depression is primarily explained either by completely lethal alleles or by slightly deleterious alleles do not appear to hold for loblolly pine. Intense inbreeding in small sublines may carry a high risk of fixation of semi-lethal alleles, which may drastically reduce seed yields after several generations. It is noteworthy that all but 21 of the 292 progeny included in the regression analysis for epistasis were homozygous for at least one of the markers linked to viability alleles. Marker assisted breeding may be an important component of subline breeding strategies, not so much to select for desirable quantitative trait loci as to minimize the numbers of deleterious viability alleles in parents for the next generation of breeding.

Our data suggest that high levels of inbreeding depression in pines are probably related to high rates of deleterious mutation per generation, as there is no evidence for epistasis and weak support at most for overdominance at viability loci. However, the existence of high levels of U in tree species does not seem unreasonable, as trees have long generation times with many more germline cell divisions between meioses than most animals or plants with short lifespans.

We have shown that genetic mapping can be a powerful tool for gaining new insights on the genetic architecture of inbreeding depression. When a map with essentially complete coverage is available, genome-wide inferences can be made that are not feasible with markers that merely sample the genome. Whole-genome studies such as this are now feasible in a wide variety of organisms with marker techniques such as AFLP that allow rapid *de novo* construction of genetic maps.

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