THE GENETIC EFFECTS OF A DIAMETER LIMITED CUT ON BLACK WALNUT

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Black walnut (*Juglans nigra* L.) trees are often selectively cut from forested stands based on their phenotype or size. This practice lowers the population density and possibly the genetic diversity of the species. Anecdotal evidence links this practice to an observed decline in the availability of high quality black walnuts.

To determine the genetic effects of a selective cut in a typical, even-aged, mixed hardwood stand, a population of 209 black walnut trees from Long Hollow, Hoosier National Forest, Crawford County, Indiana, was mapped, their diameter measured at dbh, their leaves sampled, and their DNA extracted. A random sample of 44 individuals was taken from this population and further subdivided into two size classes, those < 51 cm and those > 51 cm dbh. Twenty-six individuals represented the small class of black walnut, 18 individuals made up the large class. The different size classes were used to represent and analyze the effect of a virtual selective-cut where all the larger diameter class trees were removed.

DNA was isolated from these trees using an AutoGen™ NA-2000, automatic nucleic acid isolation system. A suite of 10 microsatellites with fluorescent labeled primers were used in genotyping these black walnuts (Woeste and others 2002). The PCR products from these 10 loci were run on Long Ranger® polyacrylamide gels using an ABI Prism™, 377 DNA Sequencer. Individuals were genotyped using Applied Biosystems GeneScan® v 3.1.2 with Genotyper® v 2.5. Once genotyped, these 44 selected trees were further analyzed using GDA v 1.0 (d16c) to calculate population and allelic statistics, levels of heterozygosity, mean number of alleles per locus, differences in F_{st}, and Nei (1978) distance. Chi-Square tests were used to test for independence and homogeneity of allelic distribution between the two size classes.

The large and small diameter classes of trees at Long Hollow were genetically similar. The number of alleles per locus ranged from 6 to 21 with a mean of 11.5 (table 1). Unique alleles were found within both the small and large classes of trees at all loci except WAG 79 (walnut-adenine-guanine dinucleotide microsatellite marker with 79 repeats). Two loci had unique alleles in the large size class only, while one locus had unique alleles in the small size class only. All the remaining loci had unique alleles in both classes.

A Chi-Square test of independence of the allelic distribution for each locus showed no significant difference between the two size classes. There was also no significant difference in the allelic distribution among loci for the two size classes based on the test of homogeneity of ratio (X^2_D=0.302, df=9, α=0.01). These results indicate that there is a high level of homogeneity for the frequency of alleles between the two size classes. Combined heterozygosity for the entire sample was 0.72 with an F_{st} of 0.001. The

Table 1.—*The number of alleles found within each microsatellite locus identified as number of adenine-guanine dinucleotide repeats*

Walnut A-G microsatellite locus	Number alleles
6	11
24	9
27	11
33	15
69	8
72	7
73	13
79	6
89	21
97	14

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observed heterozygosity by size class was H = 0.71 and H = 0.72 for the small and large size class, respectively. Nei (1973) distance measure for the two sub-samples was calculated to be 0.01. These results all indicate a high level of genetic homogeneity between the small and large size classes at Long Hollow.

The 44 individuals analyzed in this study were further subdivided into approximately equal sample sizes to evaluate the timing of pollen recruitment as the stand was maturing (table 2). We assumed the relationship between age and diameter was fairly constant within the stand. The results indicated a relative constant flow of new alleles into this population over time. Eight alleles from four loci were found to be unique to the Long Hollow stand, as compared to a previously analyzed provenance test of over 280 black walnuts, taken from 10 different states.

The black walnut population at Long Hollow was genetically diverse and homogenous in its allelic distribution. While there was no change in the level of heterozygosity when the large size class trees were (virtually) removed, a few unique alleles were lost. What effect, if any, the loss of these alleles would have on the remaining population, is unknown at this time. The rate of recruitment of new alleles into the population over time was found to be relatively constant. The impact of recruitment on stand diversity and regeneration are still under investigation.

LITERATURE CITED

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings, National Academy of Science USA. 70: 3321-3323.

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Table 2.—*Number of unique alleles by size class*

Subpopulations	Sample size	Dbh (cm)	Unique alleles[1]
LH1	9	7.1 – 27.9	6
LH2	13	28.0 – 46.0	4
LH3	11	46.1 – 55.0	11
LH4	11	55.1 – 71.0	7

[1]Unique alleles are unique to that sample in comparison to the others.