ABSTRACT.—Geneticists and silviculturists have selected over 450 black walnut clones for inclusion in the black walnut breeding program at Purdue University over the past 35 years. Most of the selections were from Indiana; a few were from other states in the Central Hardwoods Region. Selection of second and third generation clones out of this founder population was based primarily on timber traits, such as straightness and diameter growth. While maintenance of genetic diversity was considered a priority, there were no good mechanisms to quantify the presence or loss of diversity and the accumulation of inbreeding in open-pollinated populations that were not isolated from the pollen of unselected trees. Thirty-four superior clones from the Purdue walnut breeding program were evaluated for levels of inbreeding and allelic diversity using eight independently segregating dinucleotide microsatellite markers. The loci used were highly polymorphic in the 24 studied clones, with an average of 11 alleles per locus. The inbreeding coefficient (F) of the population of clones was over four times that found in a typical wild population (0.39 vs. 0.08), indicating that the superior trees may have been the product of close matings or even self-pollinations. The first-generation, mass-selected clones in the breeding program had significantly greater heterozygosity than second-generation clones, and both sub-populations from the program had significantly more homozygosity than wild trees. Surprisingly, despite their relatively high levels of homozygosity, the clones in the breeding program had greater allelic richness (one measure of genetic diversity) than a stand of native-grown trees of comparable size.