# Microsatellites as indicators of genetic diversity in Black walnut (*Juglans nigra* L) across the Central Hardwood Region


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## Abstract


Black walnut (*Juglans nigra* L.) is an important species ecologically, culturally, and economically. Its lumber is valued for a variety of human uses including furniture, veneer, and gunstocks due to its coloration, machining, and wear properties. Centuries of intense harvesting and human use have presumably reduced overall levels of genetic diversity in this species. Furthermore, forest fragmentation across the central hardwood region may have increased levels of genetic differentiation among black walnut populations due to genetic isolation. The primary goal of this research was to estimate current levels of genetic diversity and population structure in wild black walnut populations across the northcentral portion of their range. In addition, we will use genetic data to make inferences about the history of this species and the recolonization processes which contributed to current patterns of genetic structure in this species. Black walnut trees from 43 populations across the central hardwood region have been genotyped at 12 microsatellite loci. Preliminary data from 9 Indiana populations suggest that there are low levels of genetic differentiation between populations and pairwise $F_{st}$ values among populations range from 0.003 - 0.086, with some evidence of regional similarities. Observed heterozygosities ranged from 0.509 - 0.918 across the 12 loci, and the total numbers of alleles per locus ranged from 5 to 40 across the 12 loci surveyed. The data presented here expands on this initial study by estimating local structure and regional variation across all 43 sampled populations within the central hardwood region.


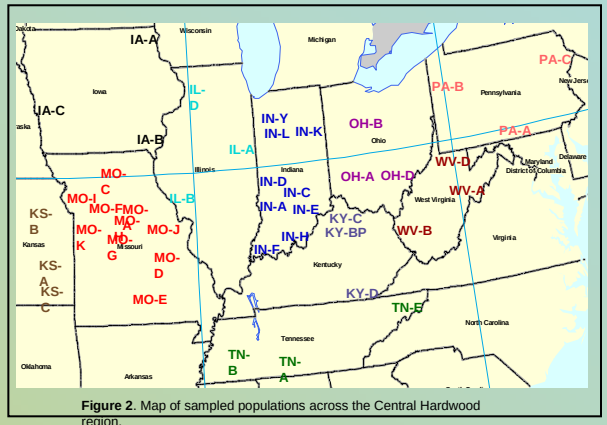


**Figure 2**. Map of sampled populations across the Central Hardwood region.

## Materials and Methods

### *Sampling*

- 1251 trees sampled across region
  - 30 trees per population
  - 4-6 leaflets per tree (Fig. 1)
- 43 natural populations across 10 states (Fig. 2)
  - Each population at least 40 acres in size
  - Each population at least 1 mi. from any known black walnut plantation
  - Sampled trees at least 100 yards apart from each other

**Figure 1**. Using a throw bag and rope to sample black walnut leaves in Kansas.

### *Laboratory*

- DNA isolated from freeze-dried leaves
  - Modified CTAB protocol (Lefort and Douglas 1999, modified with 2x PVP, 2x CTAB, and 2.0% 2-mercaptoethanol)
  - Phenol-Chloroform extraction using automated nucleic extractor (Autogen, Framingham, MA)
- Each sample amplified at 12 polymorphic nuclear microsatellite loci (Woeste et al 2002)
- PCR products run on an ABI 377 automated DNA sequencer (Perkin Elmer, Foster City, CA) (Fig. 3)

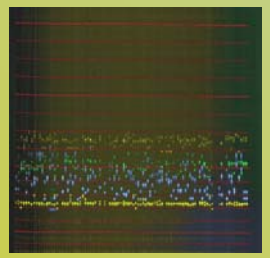

**Figure 3**. Gel image of populations IA-A,B,C on WAG 24, 72, 82, 89.

### *Analysis*

- Genotypic data extracted using Gene Scan Analysis software v. 2.1 (Perkin Elmer, Foster City, CA)
- Genotypes assigned using Genotyper v. 2.5 (Perkin Elmer, Foster City, CA) (Fig. 4)
- Statistic analyses performed with Genetic Data Analysis (GDA v. 1.1, Lewis and Zaykin 2001) , Phylip v. 3.5c (Felsenstein 1993) and Treeview v. 1.6.6 (Page 1996)

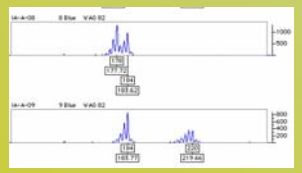

**Figure 4**. Genotyper file for population IA-A on WAG 82.

## Results and Discussion

### *Results - Genetic Diversity*

- Observed heterozygosities range from 0.590 – 0.914 (Table 1)
- Allele numbers per locus range from 9 - 49
- Black walnut appears to be a very genetically diverse species

**Table 1**. Sample size, allele ranges, number of alleles and expected and observed heterozygosities by locus.

| Locus | n | Allele Size | # Alleles | $H_e$ | $H_o$ |
|---|---|---|---|---|---|
| AAG 01 | 1235 | 148 - 172 | 9 | 0.700 | 0.662 |
| WAG 06 | 1233 | 130 - 176 | 21 | 0.613 | 0.590 |
| WAG 24 | 1215 | 221 - 253 | 17 | 0.876 | 0.836 |
| WAG 27 | 1228 | 200 - 248 | 25 | 0.892 | 0.868 |
| WAG 32 | 1238 | 163 - 235 | 34 | 0.935 | 0.909 |
| WAG 69 | 1235 | 160 - 186 | 21 | 0.660 | 0.598 |
| WAG 72 | 1235 | 134 - 162 | 14 | 0.606 | 0.600 |
| WAG 76 | 1236 | 224 - 254 | 15 | 0.745 | 0.721 |
| WAG 82 | 1232 | 144 - 256 | 49 | 0.966 | 0.914 |
| WAG 89 | 1210 | 181 - 239 | 29 | 0.925 | 0.909 |
| WAG 90 | 1240 | 132 - 190 | 28 | 0.907 | 0.898 |
| WAG 97 | 1226 | 148 - 190 | 23 | 0.901 | 0.861 |
| Overall | 1230 | --- | 23.75 | 0.811 | 0.780 |

### *Results - Genetic Structure*

- The data indicate very little evidence of genetic differentiation among populations across the Central Hardwood region (Fig. 5)
- The lack of obvious genetic structure could indicate homoplasy, or that black walnut is essentially panmictic across broad areas

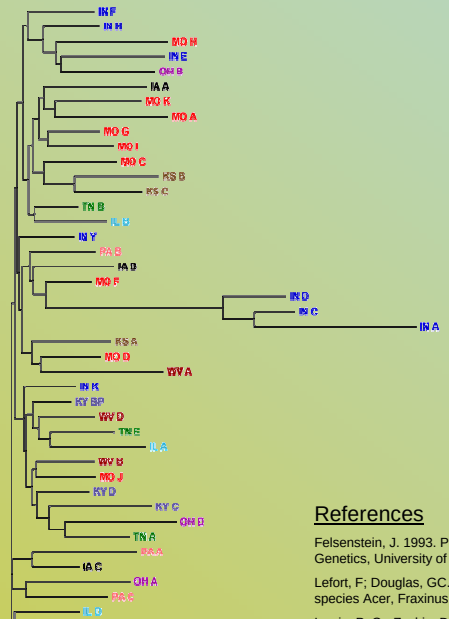


**Figure 5**. Neighbor-joining tree depicting Nei's genetic distance estimates for all 43 populations.

### *Results - Genetic Differentiation*

- All the F values are significantly different from zero (Table 2)
- Theta-P is lower than one might expect across such a broad range, indicating either high levels of gene flow or homoplasy
- f indicates small heterozygote deficiencies within populations

**Table 2**. Overall estimates of fixation, and associated 95% confidence intervals (based on 1000 bootstraps).

| | f* | F** | Theta-P*** |
|---|---|---|---|
| Overall | 0.017 | 0.038 | 0.021 |
| Upper Bound | 0.026 | 0.051 | 0.029 |
| Lower Bound | 0.009 | 0.026 | 0.016 |

* f is equivalent to $F_{IS}$; ** F is equivalent to $F_{IT}$; *** Theta-P is equivalent to $F_{ST}$

## References


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