

Exploring Gene Flow in Two Black Walnut Populations in a Fragmented Landscape

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Abstract

Over the last 200 years, deforestation of the central hardwood forest for agriculture has left a heavily fragmented landscape across much of Indiana. The creation of isolated forest patches is thought to lead to loss of genetic variability because of increased genetic drift or inbreeding. The potential negative effects of fragmentation can be mitigated by between-fragment pollen flow. We are investigating the effects of forest fragmentation on pollen-mediated gene flow within and between populations of black walnuts (*Juglans nigra* L.). Using a suite of highly polymorphic microsatellites, we have genotyped every individual black walnut tree within two small (3.6 and 8.5 hectare), adjacent, mixed hardwood stands located in central Indiana (176 trees in total). To estimate the level of pollen mediated gene flow into these isolated populations; parentage analysis was performed on each population. First, each population was subdivided into an offspring (individuals with dbh < 20 cm) and an adult cohort. Once the most-likely parent was determined for each offspring, the most-likely parent and offspring genotypes were used in a paternity analysis to find the second parent. At least one parent was found for every offspring, but only 20% of the offspring cohort had both parents within their respective fragments. This indicates pollen flow into the fragments from outside and therefore limited or no genetic isolation.

Introduction

Forest fragmentation occurs when large continuous stands of forest become subdivided into smaller sections either through natural or anthropologic processes. Depending on the severity of the fragmentation, populations of trees may become genetically isolated due to increased genetic drift or inbreeding. Between-fragment pollen flow can moderate the potential negative effects of fragmentation and help to prevent the loss of genetic variability.

We present here our initial findings into the effects of forest fragmentation on pollen-mediated gene flow within and between populations of black walnuts (*Juglans nigra* L.). We selected two small (3.6 and 8.5 hectare), adjacent, mixed hardwood stands located in central Indiana, and genotyped every individual black walnut tree (176 trees in total) using a suite of highly polymorphic microsatellites. An earlier analysis into population differentiation indicated that both fragments had randomly mating populations, but limited gene flow between them (Table 1). To further investigate the level of pollen gene flow into these isolated populations, parentage analysis was performed on each population by first subdividing each population into an offspring and an adult cohort. Once the most-likely parent was determined, its genotype was used with the associated offspring genotype in a paternity analysis to try and find a genetically corresponding second parent (Table 2). We also checked to see if a pollen parent could be found in the adjacent fragment. Presently, we are in the process of genotyping approximately 100 open-pollinated progeny from each of six maternal seed sources in each fragment (Table 3). These future results will hopefully lead to better estimates of the true levels of pollen flow within, between, and from outside these two isolated stands of black walnut.

Fragment B, Near Flora, IN



Forest fragmentation over time

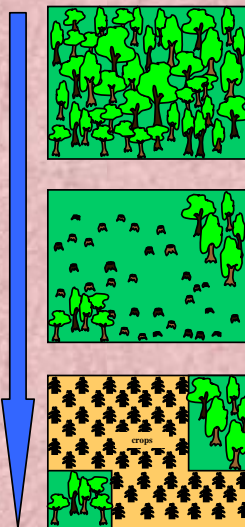


Table 1. Diversity Levels and Population Differentiation

Population Index	Allelic Richness		Heterozygosity		Fixation
	Expected	Observed	Expected	Observed	
Fragment A	14.3	0.802	0.812	-0.0123	
Fragment B	14.2	0.793	0.811	-0.0224	
$F_{ST} = 0.0206$					
Exact test for population differentiation Chi-square: Infinity p-value: 0.0000000					

Table 2. Parentage Analysis

Offspring Parent ²	DBH (cm)	Most-likely Parent ¹	Second
FA02 ³	18.0	FA17	Not found
FA32	11.2	FA20	Not found
FA72	6.9	FA05	Not found
FA74	1.3	FA17	Not found
FA75	1.3	FA17	Not found
FA76	1.3	FA18	FA19
FA77	1.3	FA18	Not found
FA78	1.3	FA20	FA53
FA79	1.3	FA17	Not found
FA80	1.3	FA17	Not found
FA82	5.6	FA70	Not found
FA83	1.3	FA45	Not found
FA84	6.4	FA05	Not found
FA85	1.3	FA05	Not found
FA86	1.3	FA01	Not found
FB03	5.6	FB47	Not found
FB06	16.5	FB05	Not found
FB58	11.4	FB77	Not found
FB79	13.5	FB73	Not found
FB83	11.7	FB66	FB67
FB84	10.2	FB66	Not found
FB85	16.8	FB63	Not found
FB88	16.5	FB63	FB69
FB89	1.3	FB53	Not found
FB90	1.3	FB64	FB69

¹ The most-likely parent was chosen based on exclusion, aided through a parentage analysis using CERVIS software, and having at least one matching allele across all loci with its associated offspring (Marshall et al., 1998).
² The second parent was determined based on the most-likely parent's genotype with its associated offspring's and accepting only those candidates that had the matching allele across all loci to generate the offspring's genotype (i.e. "FA" and "FB" are short for Fragment A and Fragment B respectively).

Table 3. Inheritance of four microsatellites in one open-pollinated family

Individual	WAG24	WAG72	WAG82
Mother Tree WAG89	241/247	148/148	174/198
205/205 progeny1	241/247	148/148	174/198
205/205 progeny2	241/241	148/148	166/174
191/205 progeny3	241/241	146/148	170/174
191/205 progeny4	237/241	148/148	186/198
205/207 progeny5	241/247	148/148	174/198
205/207 progeny6	237/247	148/148	188/198
205/207 progeny7	241/245	148/148	156/174
205/205	241/245	148/148	191/205

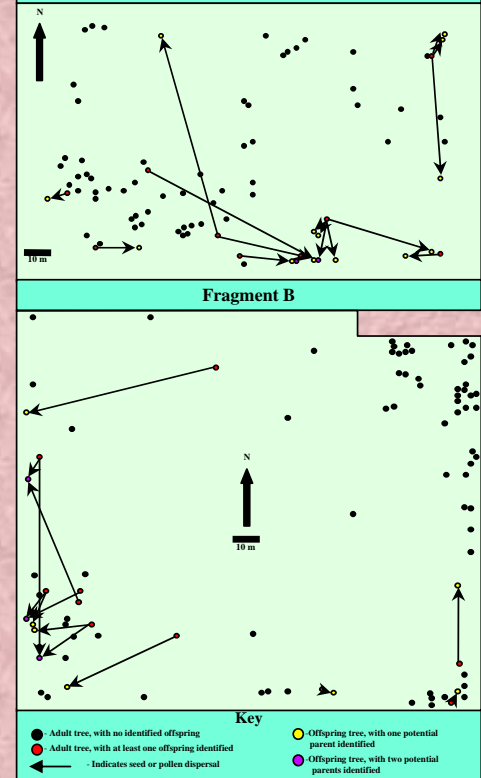
Male black walnut flowers close to pollen shed



Walnuts ready for harvest



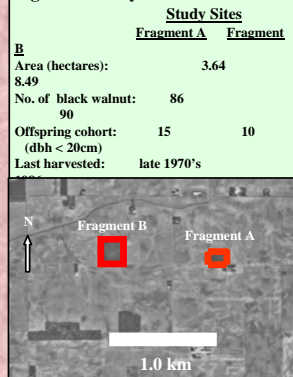
Figure 2. Black Walnut Map for Fragment A and B



Materials and Methods

- Located a severely fragmented forest landscape (Figure 1).
- Mapped and sampled all black walnut trees within each stand (Figure 2).
- Genotyped each individual across 12 microsatellites.
 - Extracted DNA from sampled tissue.
 - PCR each individual DNA across the 12 microsatellites.
 - Electrophoresed samples onto a polyacrylamide gel using an ABI Prism™, 377 DNA Sequencer.
 - Constructed a unique genetic fingerprint using themicrosatellite data and Genotyper® v 2.5.
- Genetic analysis.
 - Calculated population genetic parameters using GDA v 1.0 (Table 1).
 - Performed parentage and paternity analysis using Cervus v 2.0 (Tables 2 and 3).

Figure 1. Study site location



Results and Conclusions

- At least one parent was found for every offspring from both populations.
- A second potential parent was found for only five offspring, two in Fragment A and three in Fragment B (only 20% of the combined offspring cohort).
- This indicates pollen flow into the fragments and therefore limited or no genetic isolation.
- Past logging, mortality, and genotyping error may have affected our ability to discern a second parent.
- Future paternity analysis using open-pollinated seed will lead to better estimates of pollen flow.

Literature Cited

- Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7: 639-655.