Ten microsatellite markers identify cultivars of black walnut 
(Juglans nigra L.) in a nut breeding orchard

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Abstract
Black walnut (Juglans nigra L.) is a large tree native throughout the eastern United States from New England to Texas. It is a valuable hardwood species that also produces a high quality edible nut. The black walnut nut breeding orchard at the University of Missouri contains about 70 accessions used for breeding. Nearly all of these accessions were obtained as named cultivars (clones) propagated by small scale, private, nut growers. To maintain the integrity of the breeding program, it was important to verify the identity of the accessions. We combined morphological, phenological, and genotypic data from ten microsatellites to group individual accessions by cultivar (clones). We have found many clones that shared similar characteristics and did not have identical phenotypes or genotypes and not conversely. For example, some accessions of ‘Clarendon’, were found to have an identical genotype with the cultivars Victoria and trame, which were found to be identical. On the other hand, other accesses of ‘Clarendon’ had a genotype identical to the cultivars ‘Thomas’ and ‘Thatcher’, which were also identical to each other. Our results indicate that multiple errors were committed during the propagation of these important cultivars, and that it may be difficult or even impossible to properly assign a name to a genotype. This set of microsatellite loci clearly provides a powerful tool for the management of black walnut nut breeding populations

Introduction
Black walnut (Juglans nigra L.), is a large tree native throughout the eastern United States from New England to Texas (Powells 1965). (Fig. 1). Black walnut is prized as a multipurpose species: it provides valuable timber, produces a high quality edible nut, and is attractive to wildlife. The black walnut nut breeding orchard (Fig. 2) at the University of Missouri contains about 70 accessions used for breeding. Phenological and morphological data indicated that some of the cultivars in the breeding orchard were incorrectly labeled, leading to uncertainty concerning their breeding value. We used 10 microsatellite markers to evaluate the genotypes of the accessions in the orchard and compared them to standards.

Materials and Methods

Plant material
Genotypes used in this study were obtained from the Black Walnut breeding orchard in Missouri University (Table 2)

DNA Extraction
DNA was extracted from leaf samples using a modified version of the methods of Doyle and Doyle (1987), and Robichaud et al. (1997) and stored at -20°C.

Microsatellite analysis
Primer pairs designed for this research were derived by further sequencing of 70 a black walnut microsatellite library described by Woeste et al. (2002); Dangl et al. (2005) and Victory et al. (2006) (Table 1, Fig. 4). Polymerase chain reaction (PCR) was conducted in a total volume 10 μl and genotyping was performed as described in Victory et al. (2006) except products were separated using an ABI3730 sequencer. Two positive and one negative control were run with each PCR to ensure accurate scoring. Failed reactions were repeated for accuracy. Used software Genemapper to score genotyping data (Fig. 5).

Results and Discussion

To maintain the integrity of the black walnut breeding program, it was important to verify the identity of accessions in the nut breeding orchard. Ten microsatellite markers were used to characterize 22 reference samples provided by Coggeshall. We identified 17 unique genotypes. One hundred additional samples of cultivars from the University of Missouri nut breeding orchard were also genotyped. All of these accessions were obtained as named cultivars (clones) propagated by small-scale, private, nut growers. The results showed that many clones shared similar characteristics and did not have identical genotypes, and conversely, some genotypes had multiple cultivar names. For example, some accessions of ‘Victoria’, ‘Hare’, and ‘Clarendon’, had identical genotypes. Other accessions of ‘Clarendon’ had the same genotypes as the cultivars ‘Thomas’, ‘Dambough’, ’Flutter’, ’VY’ and ’Thatcher’, which were identical to each other (Table 1).

The most likely reasons for these results are mistakes in labeling and mistakes in sample collection. Most black walnut cultivars were first identified as wild trees or chance seedlings by amateur breeders. Over 750 black walnut cultivars have been selected and named since ‘Thomas’, the first black walnut selected for nut production, was propagated in 1881 (Higges 1896). Black walnut has great genetic potential for improved nut yield and quality because the extensive native range of the species ensures a large gene pool from which advanced selections can be made. Genetic traits that would lead to advances in a tree improvement program include: lateral bud fruitfulness, late leafing, resistance to anthracnose Gnomonia kypistophora (F.-E.) C. & R. Not, precocity and productivity, and improved nut quality. We will combine genotype data with morphological and phenological data to characterize the most important black walnut nut cultivars for breeding.

![Fig 1 Range of black walnut (Juglans nigra L.) in U.S.](image)

![Fig 2 Breeding Orchard](image)

![Fig 3 Black walnut buds and nuts](image)

![Fig 4 DNA Extraction and Polymerase PCR](image)

![Fig 5 GeneMapper analysis of PCR products.](image)