Variation in TaqI-digested DNA of Sugar and Black Maples Is Independent of Taxon and Plant Origin

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Abstract. Morphological distinctions between sugar maples and black maples are not consistently evident, and molecular assessment of genetic diversity is lacking for these taxa. We examined restriction-site polymorphisms in the ndhA intron of the chloroplast DNA (cpDNA) in populations of sugar maples and black maples representing their zones of allopatry and sympatry in eastern North America. Restriction-site analysis of the ndhA intron after digestion with Hinfl and Sau3A1 yielded no polymorphisms. Restriction digestion of the ndhA intron with TaqI revealed two cpDNA haplotypes that were neither geographically localized nor taxon specific. Although testing additional accessions of sugar maples and black maples for cpDNA variation will further elucidate patterns of genetic variation, our initial results suggest that the taxa are either exchanging genes or share an ancestral cpDNA polymorphism.

Sugar maples (Acer saccharum Marsh.) are highly valued trees indigenous to eastern North America. They are frequently planted in designed landscapes because of their colorful foliage and the uniformity of their growth habit. Black maples (Acer nigrum Michx. f. (Rehder, 1940)) share many attributes of sugar maples and may be particularly resilient when planted in harsh environments (St. Hilaire and Graves, 1999). Similarities in the traits and distribution of these maples lead to confusion about their taxonomy and genetic relatedness. The two taxa are sympatric from eastern Iowa to Vermont. Sugar maples are allopatric to black maples east of Vermont, while populations of black maple are allopatric to sugar maples west of eastern Iowa (St. Hilaire and Graves, 1999). Improving our understanding of the relationship of these taxa would establish their phylogeny and would facilitate the selection and description of cultivars.

Molecular assessment of genetic diversity is lacking among sugar maples and black maples across a wide geographical region. Chloroplast DNA (cpDNA) variation is useful for studying genetic diversity because cpDNA is haploid, nonrecombinant, and often uniparentally and maternally inherited (McCauley, 1995; Olstead and Palmer, 1994). Data on cpDNA variation have been used to address many biological and evolutionary issues, such as the phylogeny of angiosperms (Olstead and Palmer, 1994), phyleogeography of common beech (Fagus sylvatica L.) (Demereur et al., 1996), and the postglacial colonization routes of European species of oaks (Quercus L.) (Petit et al., 1997). The ndhA intron, which resides in the small single-copy region of cpDNA, can be useful for research on phylogeny (Small et al., 1998). We screened several cpDNA noncoding regions (data not presented) as described in Small et al. (1998), but only obtained reliable amplification or detected polymorphism in the ndhA region. Our objective was to conduct a preliminary assessment of the potential for using restriction-site variation in the ndhA intron of cpDNA to assess genetic diversity of sugar maples and black maples in their zones of sympathy and allopatry.

Materials and Methods

We germinated seeds of the two taxa, arranged the individually potted seedlings randomly in a greenhouse, and grew them for 1 year. The seedlings were indigenous to zones of sympathy and allopatry for the taxa (Table 1). Young leaf blades were taken from each plant, frozen in liquid nitrogen, and stored at −80 °C. Total genomic DNA was extracted from 1.5 g of frozen tissue by using the method of Doyle and Doyle (1987), except that the extraction buffer was modified to contain 10% (w/v) PVP-40 (Eastman Kodak, Rochester, N.Y.). DNA was cleaned by using a protocol for tissues rich in polysaccharides, polyphenolics, and other PCR-inhibiting compounds (Lamboy and Alpha, 1998). DNA concentration was determined with a UV-VIS spectrophotometer (Lambda Bio; Perkin-Elmer, Norwalk, Conn.).

The degenerate primer-pair sequence for ndhA is ndhF (5′-GGW CTT CTY ATG –F (5′-GGW CTT CTY ATG

Table 1. Regions of the United States and geographical origins of the 24 accessions that yielded amplification products from among the 107 sugar maples and black maples from which DNA was extracted. All accessions were native to the sites where they were sampled. The two maple taxa are sympatric in eastern Iowa. Black maples are allopatric to sugar maples in central Iowa. Sugar maples are allopatric to black maples in New Hampshire. Sugar maples are much more prevalent than black maples in Vermont and New York.

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Restriction digestion with TaqI revealed one or two restriction sites depending on the population that was sampled. We designated the cpDNA haplotype as A when one restriction site was present (Table 1). All accessions within a geographical location had the same number of restriction sites. Also, digests did not detect taxon-specific markers. For example, two TaqI restriction sites were present in all the sugar maples from the north-eastern United States. These restriction sites were shared with some sugar maples in eastern Iowa, and with some black maples indigenous to central Iowa (Table 1).

Discussion

Although many studies of variation in cpDNA involve very small sample sizes (Whittemore and Schaal, 1991), sampling additional accessions, and use of other locus–enzyme combinations, are needed to reveal taxonomic or geographical patterns of genetic variation among sugar maples and black maples. Our initial assessment of the potential for using restriction-site variation with these taxa indicates that polymorphisms cannot be obtained by using HinfI or Sau3AI. We also have demonstrated that variation at the ndhA locus after digesting with TaqI is independent of taxon and geographical region. Similarities in cpDNA from sugar maples in eastern Iowa and black maples in central Iowa (Table 1) indicate that either the taxa are exchanging genes via introgressive hybridization, or that they share an ancestral cpDNA polymorphism. Observations of trees with morphological characters intermediate between the two taxa led Desmarais (1952) to suggest that the species may introgressively hybridize. The lack of variation in cpDNA among multiple accessions within a location (Table 1) indicates that future research should be designed to examine plants from as many locations as possible, even if the number of samples within locations must be minimized to allow this.

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