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Joel S. Shore; Karina L. McQueen; Stephen H. Little

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INHERITANCE OF PLASTID DNA IN THE *TURNERA ULMIFOLIA* COMPLEX (TURNERACEAE)[1]

JOEL S. SHORE,[2] KARINA L. MCQUEEN, AND STEPHEN H. LITTLE

Department of Biology, York University, 4700 Keele Street, North York, Ontario, Canada M3J 1P3

We used PCR to amplify most of the *rbc*L gene and identified restriction fragment length polymorphisms to study the inheritance of chloroplast DNA (cpDNA) in the cross between two taxonomic varieties of the *Turnera ulmifolia* L. complex, vars. *angustifolia* and *velutina*. We identified an *Alu* I restriction site polymorphism that distinguished the parental plants. All 23 progeny from the cross var. *angustifolia* × *var. velutina*, where var. *angustifolia* was the maternal parent, possessed the paternal cpDNA. Results for the reciprocal cross were more varied, and the 16 progeny showed maternal, paternal, or biparental inheritance. We believe this represents the first study of plastid inheritance for any species in the Turneraceae. The results are unusual and warrant further investigation using other species in this family.

Chloroplast DNA (cpDNA) has proven to be a useful molecule for phylogenetic studies of plants at a number of levels of the taxonomic hierarchy (Clegg and Zurawski, 1992; Downie and Palmer, 1992), and there has been a rapid proliferation of such studies. A knowledge of the patterns of inheritance of cpDNA is necessary to correctly interpret phylogenetic information, particularly for taxa undergoing polyploidization and hybridization (Harris and Ingram, 1991). Patterns of transmission of cpDNA may also be of significance for the population genetics of organelle genes (Birky, Maruyama, and Fuerst, 1983; Birky, Fuerst, and Maruyama, 1989), particularly where there are asymmetries in gene flow via pollen vs. seeds.

Different approaches have been used to study the inheritance of plastids, including: 1) the use of plastid mutants that alter the plant's phenotype: 2) ultrastructural methods and fluorescence microscopy that examine the potential for different kinds of plastid transmission; and 3) the study of cpDNA directly, using restriction fragment length polymorphisms (RFLPs) (Corriveau and Coleman, 1988; Harris and Ingram, 1991). The latter method has allowed the investigation of plastid inheritance in an increasing number of species, and it also removes the potential transmission bias that might occur for mutant plastids (Schumann and Hancock, 1989).

Recent studies have shown that while maternal inheritance is most common in angiosperms (Hagemann and Schröder, 1989), strict paternal inheritance is common in the Coniferales (Neale, Wheeler, and Allard, 1986; Szmidt, Alden, and Hällgren, 1987; Neale, Marshall, and Sederoff, 1989; Wagner et al., 1989; but see Govindaraju et al., 1988), and largely unknown in the angiosperms (although see Medygesy, Páy, and Márton, 1986; Schumann and Hancock, 1989; Boblenz, Nothnagel, and Metzlaff, 1990). Biparental and trace paternal transmission have, however, been documented in a number of angiosperms (e.g., Metzlaff, Börner, and Hagemann, 1981; Smith, Bingham, and Fulton, 1986; Moon, Kao, and Wu, 1987; Lee, Blake, and Smith, 1988; Johnson and Palmer, 1989; Sewell et al., 1993). Some studies have revealed that variation in inheritance patterns is determined by plastid genotype, nuclear genes, or both (Tilney-Bassett and Birky, 1981; Cornu and Dulieu, 1988; Smith, 1989; Tilney-Bassett and Almouslem, 1989; Chiu and Sears, 1993).

Here we investigate the inheritance of cpDNA in the Turneraceae using two taxonomic varieties of the *Turnera ulmifolia* L. complex. *T. ulmifolia* var. *angustifolia* and var. *velutina* are both self-compatible hexaploids ($2n = 30$) (Barrett and Shore, 1987). Previous crossing studies revealed that hybrids were viable (Barrett and Shore, 1987), and therefore, reciprocal crosses could be made to study the inheritance of cpDNA. Autopolyploidy, allopolyploidy, and hybridization play important roles in speciation in this complex. Diploid, tetraploid, hexaploid, and octoploid species and varieties are known to occur (Arbo and Fernandez, 1983; Shore and Barrett, 1985; Barrett and Shore, 1987; Fernandez, 1987; Shore, 1991). A knowledge of plastid inheritance will be of importance in interpreting phylogenetic studies based upon chloroplast DNA in this group.

MATERIALS AND METHODS

Plant material—To investigate the inheritance of chloroplast DNA, a reciprocal cross was made between two parental plants (following Barrett and Shore, 1987). One plant was from a population of *T. ulmifolia* var. *angustifolia* Willd. (referred to as var. *angustifolia*) collected in Stoney Hill, Jamaica (collection of Barrett #1260), while the second was from a population of *T. ulmifolia* var. *velutina* Urban (referred to as var. *velutina*), from Tuxtla Gutierrez, Mexico (collection of Koch and Fryxell #78341). These populations correspond to populations A12 and V1 in Barrett and Shore (1987), respectively. Twenty-three progeny from the cross var. *angustifolia* × *velutina*, where var. *angustifolia* was the maternal parent, and 16 progeny from the reciprocal cross were grown in standard potting soil in 12-cm pots in a greenhouse.

PCR protocol—When the progeny had reached roughly 20 to 30 cm in height, total cellular DNA was extracted

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[2] Author for correspondence.

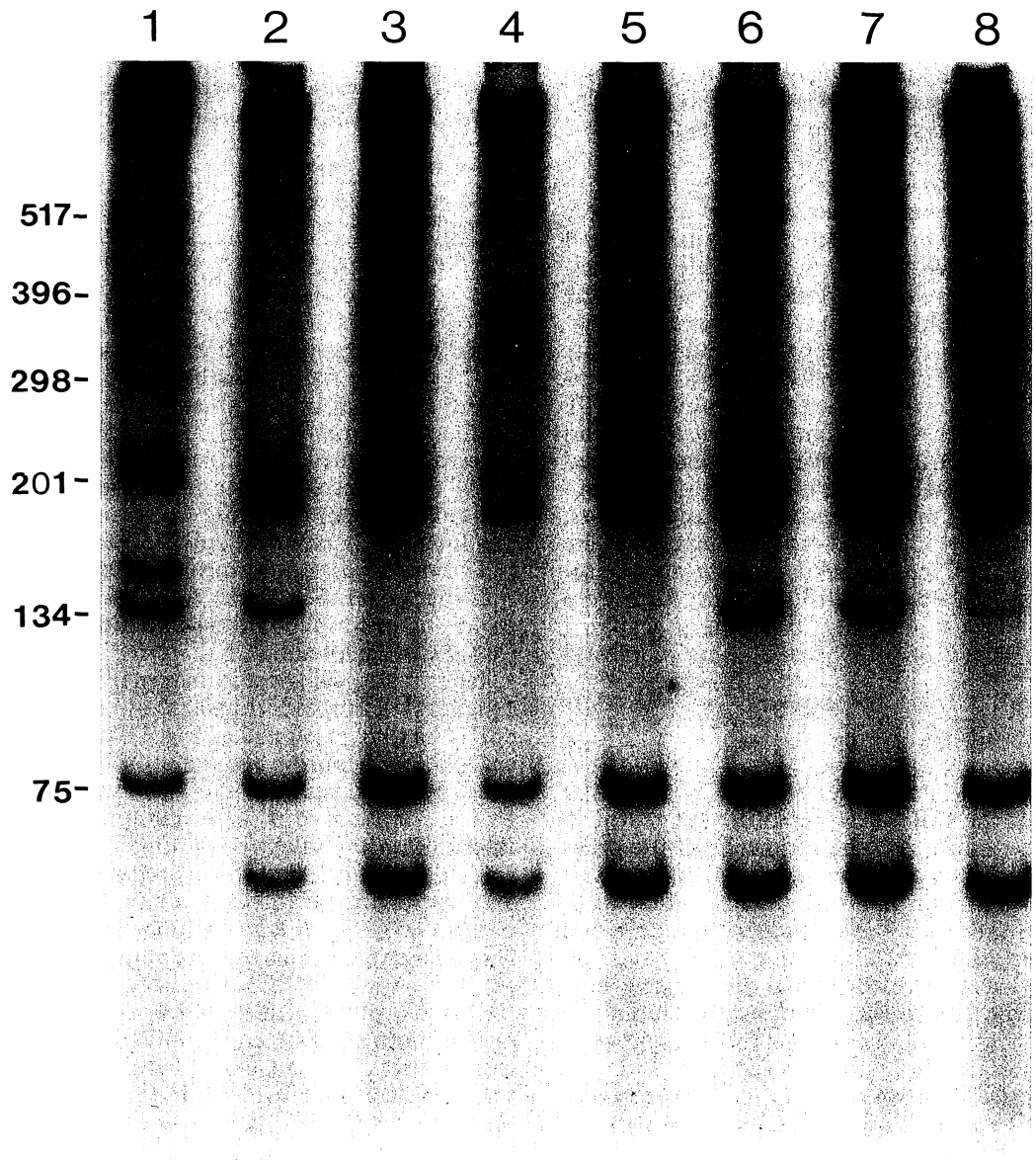

Fig. 1. Autoradiograph of end-labeled *Alu* I digests of the PCR amplified gene, *rbcL*, from progeny and parental plants. Lane 1 is a molecular size marker (corresponding numbers of base pairs are indicated at the left side of the figure). Lanes 2 (var. *angustifolia*) and 3 (var. *velutina*) show digests from the parental plants. Lane 4 shows fragment patterns of an individual from the cross *angustifolia* × *velutina* and indicates paternal inheritance. Lanes 5–8 are progeny from the cross var. *velutina* × *angustifolia*. Lane 5 shows maternal and lane 6 shows paternal inheritance. Progeny in lanes 7 and 8 are heteroplasmic.

from each individual using approximately 1 g of young leaf tissue (approximately ten to 20 young leaves) and the hexadecyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987). To identify a restriction site polymorphism that uniquely marked the parental chloroplast genomes, two oligonucleotide primers, homologous to the *rbcL* (the large subunit of ribulose bisphosphate carboxylase) gene in maize, were synthesized: 5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′ (*rbcL*-Z1F) and 5′-AATTTGATCTCCTTCCATATTTCGCA-3′ (*rbcL*-Z1375R). The primer *rbcL*-ZlF corresponds to positions 1–30, and *rbcL*-Z1375R corresponds to positions 1375–1401 of the maize sequence.

For polymerase chain reaction (PCR) amplification of this cpDNA region, we used 100 μl of the following reaction mixture: 2.75 mM $MgCl_2$, 50 pmol of each primer, 0.2 mM dNTPs, 1–2 μg of total DNA, and 2.5 units Taq DNA polymerase (Perkin Elmer Corp., Branchburg, NJ). We carried out the amplification using a thermal cycler (Ericomp, Inc., San Diego, CA) that was programmed for 30 cycles of 1.5 min at 94 C, 2 min at 48 C, 3 min at 72 C, followed by one cycle of 15 min at 72 C. PCR amplified samples were then purified using Geneclean (Bio 101, Inc., La Jolla, CA), and samples were resuspended in 20 μl of sterile distilled water prior to restriction digestion.

The 1,400 base pair (bp) PCR products of the parental

plants were initially digested using the following restriction enzymes: *Alu* I, *Eco* RI, *Hae* III, *Hin* fI, *Mbo* I, *Rsa* I, *Sau* 96, and *Taq* I. The digests were run on 2% agarose gels to visualize the fragments. Only *Alu* I showed a difference in restriction sites between the parental plants. DNA obtained from PCR amplification of all progeny was then digested for 2 to 16 hours at 37 C, using five to ten units of *Alu* I. These restriction digests were initially run either on 2% agarose or 12% polyacrylamide gels, and fragments were stained with ethidium bromide and photographed. Later, to increase the sensitivity of detecting plants that possess both chloroplast DNAs (heteroplasmic plants), the entire procedure was repeated and fragments were end-labeled with [^{32}P]dATP following a modification of the methods in Dowling, Moritz, and Palmer (1990). All progeny from the cross *velutina* × *angustifolia* and 11 of 23 progeny from the reciprocal cross were reanalyzed in this way. Agarose and polyacrylamide gels were prepared and run following procedures outlined in Maniatis, Fritsch, and Sambrook (1982). End-labeled fragments were detected using autoradiography. After initial autoradiographic exposure (2 to 4 days), additional x-ray films were exposed for 3 to 4 weeks (at −80 C using an intensifying screen), to allow detection of trace amounts of plastid DNA.

RESULTS

Progeny phenotype—A total of 39 progeny from the reciprocal cross of the two parental plants was grown to flowering in a greenhouse. The hybrid status of the progeny was easily confirmed as F_1 progeny have pale yellow flowers in contrast to the parental plants, which have either deep yellow flowers (var. *angustifolia*) or white flowers (var. *velutina*). In addition, previous investigation of progeny from this cross revealed that the progeny were highly pollen sterile (Barrett and Shore, 1987) and the shriveled anthers were readily apparent for these progeny as well.

Interestingly, progeny from the cross var. *angustifolia* × *velutina* were normal in appearance, all having dark green leaves, while a number of progeny from the reciprocal cross (*velutina* × *angustifolia*) showed varying degrees of variegation. Leaves of some of these progeny were pale green while others showed marked sectoring of their leaves.

Inheritance studies—PCR amplification of much of the *rbcL* gene (positions 1–1,400 of the maize sequence) allowed us to identify restriction site differences between cpDNA of the parental plants. Following digestions with eight different restriction enzymes, only *Alu* I revealed a single restriction site difference between the parental plants (Fig. 1, lanes 2, 3). The parental plant of *T. ulmifolia* var. *velutina* yielded six restriction fragments (approximately 55, 75, 190, 215, 345, and 520 bp in length, see Fig. 1, lane 3), indicating the presence of five *Alu* I sites, while var. *angustifolia* showed seven fragments (Fig. 1, lane 2). Var. *angustifolia* possesses an additional *Alu* I site that results in further digestion of the 345-bp fragment found in var. *velutina* into two fragments, 140- and 205-bp in length (Fig. 1, lane 2).

The results of our inheritance studies are provided in Table 1, and representative progeny are shown in Fig. 1. All 23 progeny from the cross involving var. *angustifolia* as the maternal parent showed the restriction fragment profile of var. *velutina* (e.g., Fig. 1, lane 4) indicating that paternal transmission of plastid DNA had occurred. For 11 of the 23 progeny, we used end-labeling to increase the sensitivity of detecting trace maternal contributions but found only paternal cpDNA fragment patterns.

TABLE 1. Results of plastid inheritance from the cross of var. *angustifolia* × var. *velutina* and reciprocal. The restriction fragment patterns obtained from *Alu* I digests of the amplified *rbcL* gene were used to determine plastid type(s) in each progeny individual.[a]

Maternal parent	Paternal parent	Number of progeny showing cpDNAs of		
		velutina	*angustifolia*	Both
angustifolia ×	*velutina*	23^{p}	0	0
velutina ×	*angustifolia*	6^{m}	6^{p}	4^{mp}

[a] p = paternal transmission; m = maternal transmission.

The results for the reciprocal cross (where var. *velutina* was the maternal parent) were more varied. Based on ethidium bromide staining and end-labeling, we found that six progeny showed maternal inheritance (e.g., Fig 1, lane 5) and six showed paternal inheritance (e.g., Fig. 1, lane 6). Four individuals showed evidence that both cpDNAs were present (e.g., Fig. 1, lanes 7, 8). The paternal cpDNA contribution for the individual in Fig. 1, lane 8 must be quite small as the 140- and 205-bp fragments, that are indicative of paternal cpDNA, are quite faint. Based upon the relative staining intensity of the 345-bp fragment (diagnostic for var. *velutina*) and the 140- and 205-bp fragments (diagnostic for var. *angustifolia*), the individual in Fig. 1, lane 7 is heteroplasmic and has roughly equal contributions of both maternal and paternal plastid types. We have PCR amplified and digested the DNA from these progeny at least four times to verify the presence of both cpDNA types, and on each occasion similar results were obtained. These heteroplasmic fragment patterns are reproducible. While the lanes showing heteroplasmic progeny in Fig. 1 show some evidence of incomplete digestion, replicate gels substantiated the heteroplasmic nature of these plants.

DISCUSSION

As a prelude to phylogenetic studies of the Turneraceae, we investigated the inheritance of cpDNA in a cross of two taxonomic varieties. We discovered somewhat unusual patterns of inheritance of cpDNA. One cross yielded 23 progeny, all of which exhibited uniparental inheritance of the paternal cpDNA. For the reciprocal cross, maternal, paternal, and biparental transmission were all observed. While strong paternal inheritance has been observed in *Medicago sativa,* variable modes of inheritance also occur among reciprocal crosses of two subspecies (Schumann and Hancock, 1989). We did not attempt to extract DNA from particular sectors on variegated progeny, but this would be informative to see if vegetative segregation of plastid types had occurred. Our DNA samples were, however, extracted from ten or more leaves, increasing the chances of our including and detecting both plastid types. It is also possible that a greater number of the progeny

show biparental inheritance, but the maternal and paternal contributions could be so highly asymmetrical that the minority cpDNA can't be detected.

Turnera ulmifolia vars. *angustifolia* and *velutina* are reproductively isolated as F_1 hybrids obtained from crosses between the varieties are completely sterile (Barrett and Shore, 1987). Thus it is possible that the patterns of inheritance seen here are characteristic of interspecific crosses, and intraspecific crosses might show a different pattern. We have now detected a restriction site polymorphism within var. *angustifolia* and are currently analyzing inheritance patterns.

As far as we are aware, this is the first investigation of plastid inheritance in the Turneraceae. Corriveau and Coleman (1988) have, however, shown that in the allied family, the Passifloraceae, there is at least the potential for biparental inheritance of plastids, a suggestion consistent with our findings for the Turneraceae.

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