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PATERNALLY BIASED cpDNA INHERITANCE IN *TURNERA ULMIFOLIA* (TURNERACEAE)[1]

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We end-labeled *Hin* fI restriction digests of a PCR-amplified plastid encoded gene, the large subunit of ribulose bisphosphate carboxylase, to investigate patterns of cpDNA inheritance in *Turnera ulmifolia.* A total of 70 progeny from crosses among plants taken from ten populations revealed varying patterns of inheritance. A majority of progeny inherited the paternal cpDNA (64%), while 19% exhibited maternal and 17% biparental inheritance. Eight variegated progeny showed biparental inheritance and were analyzed in greater detail. We extracted and analyzed the cpDNA content of light- vs. dark-green leaf sectors from these plants. The results showed that vegetative segregation of cpDNA had occurred for seven of the eight plants.

Key words: biparental inheritance; chloroplast DNA; *rbcL*; *Turnera ulmifolia*; Turneraceae.

A majority of flowering plants exhibit maternal inheritance of cpDNA, although there are numerous exceptions (e.g., Medgyesy, Páy, and Márton, 1986; Smith, Bingham, and Fulton, 1986; Hagemann and Schröder, 1989; Schumann and Hancock, 1989; Tilney-Bassett and Almouslem, 1989; Boblenz, Nothnagel, and Metzlaff, 1990; Chiu and Sears, 1993; Birky, 1994; Mogensen, 1996). A knowledge of patterns of cpDNA inheritance is very important in phylogenetics (Harris and Ingram, 1991), hybridization studies (Cruzan et al., 1993), and the measurement of gene flow among populations of a single species (McCauley, 1994, 1995). For example, if cpDNA is strictly maternally inherited, then variation in plastid DNA can be used to infer patterns of gene flow via seeds. If paternally inherited, then cpDNA markers can provide useful information on gene flow via pollen. Different patterns of organelle inheritance may also bias estimates of quantitative genetic parameters, and specific breeding designs may be required to alleviate this problem (Mazer and Gorchov, 1996). Given the variation among angiosperms, specific patterns of inheritance must be determined for taxa under investigation if cpDNA data are to be interpreted correctly.

Patterns of inheritance of cpDNA are also important as they influence, differentially, the population genetics of organellar as opposed to nuclear genes (Birky, Fuerst, and Maruyama, 1989). Birky (1994) has provided hypotheses to account for nonMendelian inheritance patterns in these "relaxed" organellar genomes. The lack of stringent control and inclusion of stochastic processes in the replication and partitioning of cpDNA (and other cytoplasmic genes) are important determinants of the various patterns of inheritance. Nuclear genes are also known to influence the transmission of cpDNA (Cornu and Dulieu, 1988; Chiu and Sears, 1993; Tilney-Bassett, 1994). The more proximate cytological bases of various mechanisms of cpDNA inheritance have been reviewed recently. A wide diversity of cytological processes occur to exclude paternal transmission in species exhibiting maternal inheritance (Hagemann and Schröder, 1989; Mogensen, 1996).

The inheritance of cpDNA in the angiosperm family Turneraceae and in allied families in the order Passiflorales (sensu Takhtajan, 1969), is poorly known. In the Passifloraceae, Corriveau and Coleman (1988) used cytological methods to show that there is a potential for biparental inheritance. Shore, McQueen, and Little (1994) studied the inheritance of plastid DNA in a cross between two species in the genus *Turnera* (Turneraceae). They found contrasting patterns of inheritance, where progeny of the cross in one direction (using *T. ulmifolia* L. as the maternal parent) all possessed the paternal chloroplast genome of *T. velutina* Presl. (formerly *T. ulmifolia* var. *velutina*). For the reciprocal cross, approximately equal numbers of progeny possessed the maternal, paternal, or both cpDNA types (i.e., showed biparental inheritance). Because their study involved a cross between two species, it is not clear whether the results are an outcome of the fact that the two species might differ in their patterns of inheritance of chloroplasts. Alternatively, the two species might differ in the distribution of plastids within egg cells, which might have important consequences for plastid inheritance (Zhu, Mogensen, and Smith, 1993; Mogensen, 1996).

In this paper we investigate the inheritance of cpDNA within the perennial neotropical weed *T. ulmifolia* L. We do so using the radioactive end-labeling protocols detailed in Shore, McQueen, and Little (1994), which allow increased sensitivity in detecting trace amounts of maternal and paternal cpDNA when biparental inheritance is a possibility.

MATERIALS AND METHODS

Plants from each of ten populations of *T. ulmifolia* (Table 1) were used in a reciprocal crossing scheme to generate progeny for the study of cpDNA inheritance. Eight populations were sampled previously from Jamaica and plants were grown in the greenhouse at York University.

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TABLE 1. Populations used in crosses, their *rbcL* haplotype, collection number, and locality. All plants were collected in Jamaica unless indicated otherwise.

Popn.	Haplotype	Collection locality	Collection number
33	A	Sandy Bay, Hanover,	Shore and Schappert #103
39	A	Cave, Westmoreland,	Shore and Schappert #104
40	A	Portland Point, Clarendon,	Shore and Schappert #109
41	A	Irish Town, St. Andrew,	Shore and Schappert #113
3	B	Falmouth, Trelawny,	Shore and Schappert #107
6	B	Ochos Rios, St. Ann,	Shore and Schappert #112
17	B	Pleasant Hill, St. Thomas,	Shore and Schappert #114
Qua	B	Quaco Rock, Trelawny,	Barrett #1337
Mad	B	Madruga, Lanabana, Cuba,	Shore #99
Bah	B	Pelican Lake, Bahamas,	Correll #40638

Our recent studies have revealed that one of these, the population from Quaco Rock, Jamaica (Table 1), warrants recognition as a new species endemic to Jamaica (Baker and Shore, 1995; J. S. Shore, unpublished data). Additional populations were sampled from Madruga, Cuba, and Grand Bahama Island. The Bahamian collection represents a distinct taxonomic variety, *T. ulmifolia* var. *acuta* (Spreng.) Urban, and does produce fertile F_1 progeny in crosses with most Jamaican populations (J. S. Shore, unpublished data). We used 1–4 plants per population for the crosses. Plants used in crosses from three of the populations (Bah, Mad, and Qua) were inbred descendants derived from two or more generations of selfing of a single plant originally sampled from the field. Seeds from reciprocal crosses were sown and varying numbers of progeny were grown in 5-cm pots for ~5 mo.

We extracted total DNA from parental plants and from varying numbers of progeny from each cross following the methods of Doyle and Doyle (1987). We used ~1 g of young leaves (10–20 leaves) per plant for DNA extraction. Our previous work had revealed variation at a *Hin* fI restriction site within *rbcL* (the chloroplast gene encoding the large subunit of ribulose bisphosphate carboxylase), and we used this genetic marker to study the inheritance of cpDNA. We used the polymerase chain reaction (PCR) to amplify *rbcL* (~1400 bp) using the primers and protocols detailed in Shore, McQueen, and Little (1994). Products of the PCR reactions were purified, restriction digested with *Hin* fI (a restriction enzyme with a four-bp recognition sequence), end-labeled with [^{32}P]dATP (Dowling, Moritz, and Palmer, 1990; Shore, McQueen, and Little, 1994), and run on 12% polyacrylamide gels following protocols detailed in Shore, McQueen, and Little (1994). End-labeled fragments were detected using x-ray films and/or electronic autoradiography, using an Instant Imager (Canberra Packard Co. Ltd., Meriden, Connecticut, USA). We intentionally overexposed our autoradiographs to increase the probability of detecting trace amounts of maternal or paternal cpDNA in progeny exhibiting biparental inheritance.

Progeny from some crosses were variegated, showing dark- and light-green sectors on their leaves. We tested the hypothesis that this visible sectoring was the result of vegetative segregation of plastids in progeny that had biparentally inherited both maternal and paternal cpDNA. For eight variegated plants, we cut out dark-green sectors and light sectors and extracted DNA (as above) from each of the sectors. These samples were subjected individually to PCR, restriction digestion, and electrophoresis, as above.

RESULTS

We detected two different *Hin* fI restriction fragment profiles (haplotypes) among plants from different populations (Table 1, Fig. 1). Plants from four Jamaican populations exhibited six restriction fragments (referred to as haplotype A; fragment sizes are 640, 274, 210, 143, 97, and 36 bp (base pairs) in length; Fig. 1, lane 2), while those from the remaining populations, including plants from Jamaica, Cuba, and the Bahamas possessed seven restriction fragments (haplotype B, Table 1; Fig. 1, lane 1). There is an additional restriction site in plants possessing haplotype B, which results in cleavage of the 210-bp fragment that occurs in haplotype A, into two additional fragments of sizes of 164 and 46 bp. We had a single marker that distinguished two different cpDNA haplotypes so we carried out crosses among plants where the maternal and paternal plants differed in their *Hin* fI restriction fragment patterns (i.e., plants from populations 33, 39, 40, and 41 were crossed reciprocally with plants from the remaining six populations).

We assayed a total of 70 progeny from 43 crosses, including reciprocals, for their cpDNA inheritance pattern. From one to nine progeny were analyzed for each cross and the mean number of progeny analyzed was small (mean 1.6), since our main purpose was to investigate general patterns of cpDNA inheritance in this species. The results demonstrate that cpDNA inheritance is quite variable (Table 2). A majority of progeny, 45 (64%), uniparentally inherited the paternal cpDNA, 13 (19%) uniparentally inherited the maternal cpDNA, and 12 (17%) showed biparental inheritance. Two of these biparental progeny possessed largely the paternal cpDNA and probably a very small amount of the maternal cpDNA, based on the relative intensity of bands on autoradiographs (Table 2). For two particular crosses, a larger number of progeny were assayed. For the cross 40 × 3, three progeny showed paternal, two maternal, and three biparental inheritance. For the cross 40 × 6, four progeny showed paternal and five biparental inheritance. There were 11 other crosses where two or more progeny were assayed. In two of these, mixed patterns of inheritance were observed.

To examine the inheritance of cpDNA for each population, we summed the numbers of progeny exhibiting maternal, paternal, or biparental inheritance for each parental population (considered both as a maternal and paternal parent), over all crosses it was involved in. These results are presented as row and column totals in Table 2. Frequently, all plants showed varying proportions of different inheritance patterns. In three instances, however, all progeny exhibited paternal inheritance. This occurred when population 17 and Qua were involved as parents (both maternal and paternal) and when population 40 was used as a paternal parent.

We observed distinct patterns of variegation for prog-

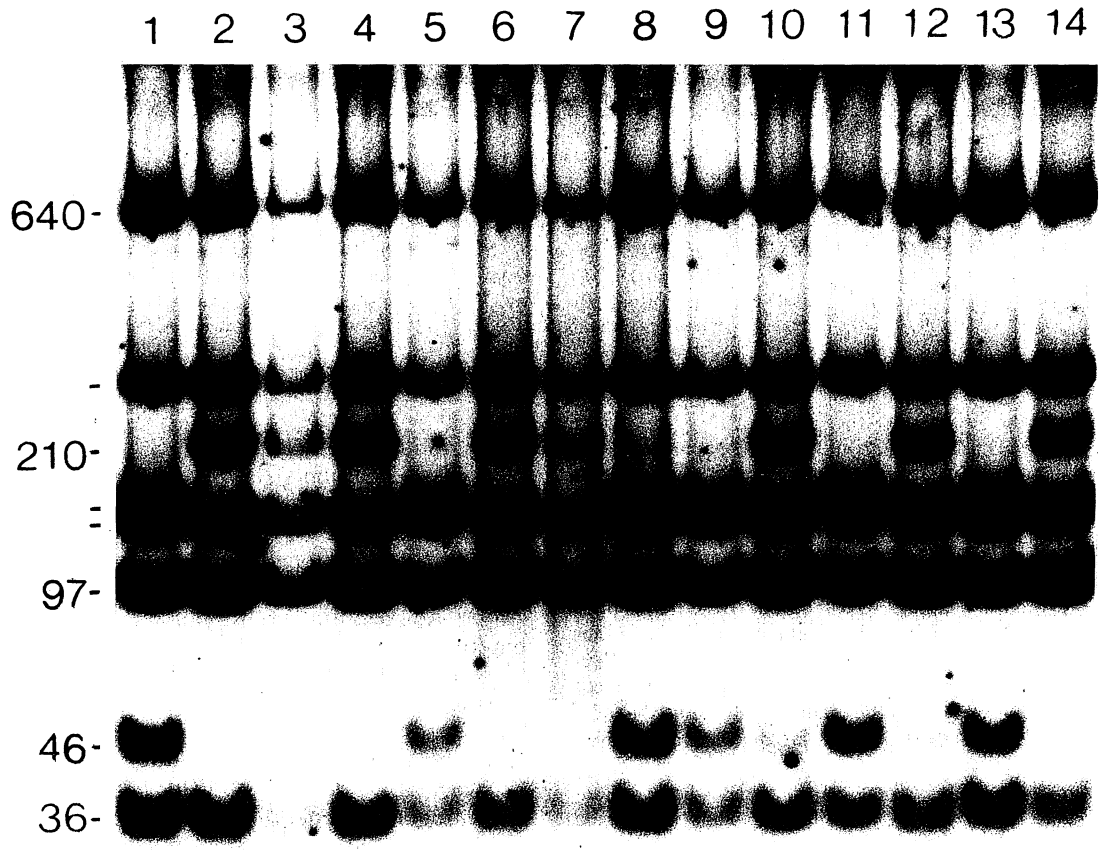

Fig. 1. Autoradiograph of a gel showing *Hin* fI restriction digests of PCR-amplified *rbcL* for seven progeny, three from cross 40 × 3 (lanes 1–6), and four from cross 40 × 6 (lanes 7–14). The size of various restriction fragments (number of base pairs) is provided. Each pair of adjacent lanes represents the results of restriction digests from a dark- and then light-green sector from the same plant (with the exception of lanes 7 and 8 where the results of light sector are in lane 7 followed by the dark sector, lane 8). Lane 1 (from a dark-green sector resulting from paternal cpDNA) illustrates haplotype B, while lane 2 shows haplotype A (from a light-green sector inherited from the maternal cpDNA). This plant clearly shows biparental inheritance. With the exception of lanes 3 and 4, a similar pattern occurs for all progeny. Traces of the alternative haplotype appear as faint bands in lanes 8, 10, 12, and 14.

eny derived from the crosses 40 × 3 and 40 × 6, but not their reciprocals. We exploited these patterns to determine whether vegetative segregation of plastids was occurring. Digests from light- vs. dark-green sectors obtained from the same plant were run in adjacent lanes on gels (Fig. 1). We assayed three variegated progeny from the cross 40 × 3 and five progeny from the cross of 40 × 6. For seven of the eight variegated plants, there was a clear difference in the cpDNA haplotype found in light- vs. dark-green sectors. Dark-green sectors possessed the paternal cpDNA haplotype B of populations 3 or 6 and in one instance a trace amount of maternal cpDNA (Fig. 1,

TABLE 2. Numbers of progeny inheriting the maternal (m), paternal (p), or biparental (b) cpDNA are indicated for each cross and reciprocals. Each parental population is listed both as a maternal (♀) and paternal (♂) parent. For rows, the first line presents the results when the plant was a maternal parent and the second when used as a paternal parent. For columns, the first values in each cell are the results when the plant was used as a paternal parent and the second when used as a maternal parent.

Parental popn.	3			6			17			Mad			Qua			Bah			Total		
	m	p	b	m	p	b	m	p	b	m	p	b	m	p	b	m	p	b	m	p	b
40♀	2	3	3	0	4	5	0	1	0	0	1	0	0	1	0	0	1	1[a]	2	11	9
40♂	0	1	0	0	3	0	0	1	0	0	1	0	—	—	—	0	2	0	0	8	0
41♀	0	1	0	1	1	0	—	—	—	1	0	0	—	—	—	—	—	—	2	2	0
41♂	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	5	0
33♀	0	0	1	2	0	0	0	2	0	0	1	0	0	1	0	0	1	1[a]	2	5	2
33♂	0	1	0	0	2	0	0	2	0	0	1	0	0	2	0	1	1	0	1	9	0
39♀	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	1	3	2	1
39♂	1	0	0	1	0	0	0	1	0	0	1	0	—	—	—	0	1	0	2	3	0
Tot♂	3	4	4	4	5	5	0	4	0	2	2	0	0	3	0	0	2	3	13	45	12
Tot♀	1	3	0	1	6	0	0	5	0	0	4	0	0	3	0	2	4	0			

[a] These progeny possessed a trace amount of maternal cpDNA.

lane 8). Light-green sectors showed the opposite pattern and possessed the cpDNA (haplotype A) of the maternal parent (population 40). In some instances trace amounts of the paternal haplotype B, from populations 3 or 6 (Fig. 1, lanes 10, 12 and 14) was present and most easily recognized by the occurrence of a faint 46-bp restriction fragment. The one marked exception to the pattern was from a single plant from the cross of 40 × 3 (Fig. 1, lanes 3 and 4). For this plant, both sectors showed the maternal haplotype. For this particular plant, we noted that the degree of sectoring was not as distinct, and there was an intermingling of small light and dark sectors throughout its leaves.

DISCUSSION

Patterns of cpDNA inheritance in *T. ulmifolia* are variable and strongly biased towards paternal transmission. If it can be categorized, cpDNA inheritance is perhaps best described as biparental, despite our findings that only 12% of progeny assayed contained both maternal and paternal cpDNA. It is likely that the true proportion of progeny inheriting both maternal and paternal cpDNA is larger than our estimate. On technical grounds, it may be difficult to detect the presence of both the maternal and paternal cpDNAs if they are highly asymmetrically represented, even though we are using sensitive radioactive labeling methods. In addition, if vegetative segregation occurs sufficiently early in development, it is possible that only one plastid type will be represented in older plants (or among most leaves of older plants). The occurrence of progeny that uniparentally inherited just the maternal or paternal cpDNA supports the possibility that vegetative segregation does indeed occur early in development. This hypothesis could be tested by analyzing seedlings at the dicotyledonous stage of development, or perhaps even embryos within developing seeds.

Inheritance patterns in *T. ulmifolia* appear most similar to those in *Medicago sativa,* in showing a considerable frequency of paternal transmission and varying amounts of maternal and biparental transmission, as well (Schumann and Hancock, 1989; Smith, 1989). In *M. sativa* there is a correlation between egg cytology and plastid transmission frequencies between two genotypes (Zhu, Mogensen, and Smith, 1993; Mogensen, 1996). The differential distribution of plastids in eggs of different genotypes might account for variable patterns of inheritance in that species. Variable plastid inheritance patterns in *Oenothera* have been largely attributed to differential competitive abilities of the plastids (Kirk and Tilney-Bassett, 1978), but Chiu and Sears (1993) have demonstrated that the interaction between plastid genome and nuclear genome also plays a significant role in inheritance. Furthermore, they have suggested that lower rates of pollen tube growth (mediated by differences in style length) might increase the degeneration of pollen plastids and contribute to variable inheritance patterns in crosses among strains of *Oenothera.*

Given the variation we have observed in inheritance patterns among populations (Table 2) and species of *Turnera* (Shore, McQueen, and Little, 1994), this genus might provide another useful system for investigating the proximate mechanisms of plastid inheritance including the effects of differences in egg cytology and features of the pollination process. Considerable genetic variation for style length occurs among Jamaican populations (Belaoussoff and Shore, 1995), and there are marked differences in pollen competitive abilities among populations of *T. ulmifolia* (Baker and Shore, 1995).

Cytological and genetic studies indicate that *T. ulmifolia* is an allohexaploid (Fernandez, 1987; Barrett and Shore, 1989; Belaoussoff and Shore, 1995), but the ancestry of this hybrid species is unknown. Given that recent analyses of other polyploid groups (both auto- and allopolyploids) have revealed multiple origins of polyploidy using cpDNA variation (e.g., Soltis and Soltis, 1989; Soltis, Soltis, and Ness, 1989; Doyle et al., 1990; Wolf, Soltis, and Soltis, 1990; Soltis, Doyle, and Soltis, 1992; Soltis, Soltis, and Milligan, 1992), we speculate that the variation observed in cpDNA (and plastid phenotypes) might be the result of multiple origins of polyploidy. This hypothesis could be tested by carrying out an extensive survey of cpDNA variation, preferably using more variable noncoding regions, such as introns and intergenic spacers (Soltis, Soltis, and Milligan, 1992; McCauley, 1994, 1995) as well as a survey of putative diploid and/or tetraploid progenitors.

There are additional possibilities for the observed restriction fragment length polymorphism, including that polyploidy originated once, yet as a result of biparental inheritance two cpDNAs were "injected" into the new polyploid species, or alternatively, the polymorphism arose after the origin of the polyploid. While we have discovered only a single restriction fragment length polymorphism, the appearance of variegation indicates that there are some functional differences between chloroplasts in this species (at least in some nuclear backgrounds) and this could indicate there are other genetic differences among plastids.

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