Inheritance of chloroplast DNA in Magnolia

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Abstract

Restriction fragment analysis was used to determine the mode of chloroplast DNA inheritance in *Magnolia*. Crosses made between *M. virginiana* var. *virginiana* x *M. tripetala*, *M. sieboldii* x *M. virginiana* var. *virginiana*, and *M. virginiana* var. *virginiana* x *M. grandiflora* demonstrate uniparental-maternal inheritance. Maternal inheritance of the chloroplast genome in *Magnolia* agrees with similar observations for most other angiosperms.

Introduction

During the past few years there has been a remarkable increase in the application of DNA analysis to problems in plant systematics. One approach involves comparing the number and size of fragments produced by the digestion of chloroplast DNA (cpDNA) with restriction endonucleases. Restriction endonucleases are enzymes that cut DNA at a constant position within a specific recognition sequence. The specificity of cleavage by restriction enzymes means that a complete digestion of the cpDNa will yield a reproducible array of fragments. These fragments are than sorted according to their size of gel electrophoresis. Changes in the number or size of the fragments are the consequence of heritable changes in the DNA; point mutations create or abolish restriction endonuclease sites, while DNA rearrangements, insertions, or deletions alter their relative positions. These variations in number or size of the fragment are called restriction fragment length polymorphisms (RFLPs).

Early studies of cpDNA RFLPs were accomplished by direct inspection of the fragments on a gel. Presently, most investigators use a radioactive, cloned cpDNA fragment (probe) that is hybridized to filter blots containing digests of genomic DNA.

Magnolia x thompsoniana (Loudon) C. de Vos

This approach has two advantages. (1) The use of probes decreases the complexity of fragment differences and thereby allows a more critical analysis of fragment differences, and (2) the use of total DNA as compared to cpDNA requires less plant material.

The principal goal of our research is to use cpDNA to produce a phylogenetic treatment for *Magnolia* in eastern North America. The chloroplast genome is ideal for systematic and phylogenetic investigations because it is: (1) conservative in molecular arrangement and sequence and is relatively easy to isolate (Palmer, 1987), and (2) relatively small (ca. 150-200 kb) and thus, it can be completely cloned using restriction endonucleases in relatively few plasmid clones.

Evolutionary relationships among taxa of a number of angiosperm families have been resolved using cpDNA analyses (Palmer, 1986, 1987). It is important to note that these reconstructed phylogenies reflect the phylogeny of the chloroplast genome and may not be a good marker for the species phylogeny in the absence of other data (Nei, 1987). Introgres-

sive hybridization (Riesenberg *et al*, 1990) and polyploidy (Doyle *et al*, 1990) can influence the position a taxon assumes in cpDNA based phylogeny (Harris and Ingram, 1991). However, cpDNA analysis has been useful in identifying introgressive hybridization (Riesenberg *et al*, 1990) and the origin of polyploids (Doyle *et al*, 1990).

Phylogenies constructed from cpDNA data will trace maternal, paternal, or bipaternal ancestries, depending on whether the cpDNA of the group being studied is maternally, paternally, or bipaternally inherited. The mode of inheritance of cpDNA is cytoplasmic and varies in the plant groups studied thus far. Understanding the inheritance pattern of the cpDNA is of fundamental biological importance for making phylogenetic inferences. Chloroplast DNA is inherited maternally or biparentally in angiosperms (Harris and Ingram, 1991) and paternally in gymnosperms (Neale and Sederoff, 1988). Corriveau and Coleman (1988), using a rapid screening procedure involving DNA fluorochrome to detect potential biparental inheritance of chloroplast DNA in pollen, reported maternal inheritance in *Magnolia* sp. The objective of the study was to unequivocally determine the mode of cpDNA inheritance in *Magnolia* using restriction enzyme markers.

Materials and Methods

Plant Material

This study is based on three different crosses. A list of the taxa examined, their sectional affiliations, and distribution is as follows:

Taxon	Section (Dandy, 1976)	Distribution
M. virginiana var. *virginiana*	Magnolia	E. North America
M. grandiflora	Theorhodon	E. North America
M. tripetala	Rhytidospermum	E. North America
M. sieboldii	Oyama	Eastern Asia

The parents and hybrids analyzed for cpDNA inheritance are as follows:

Hybrid	Female parent	Male parent	Source
1	*M. sieboldii*	*M. virginiana*	Hendersonville, NC
2	*M. virginiana*	*M. tripetela*	Hendersonville, NC
3	*M. virginiana*	*M.grandiflora*	National Arboretum

Voucher herbarium specimens are deposited in the Clemson University Herbarium (CLEMS).

Extraction of cpDNA and Southern hybridization

Leaves were collected from the F_1 hybrids and the parents. Approximately 100 grams of fresh leaf material was homogenized in ice-cold buffer to extract the chloroplasts using a procedure from Palmer (1986). Chloroplast DNA was extracted by the method of Bernatzky and Tanksley (1986). The chloroplasts were isolated, lysed and the cpDNA purified as described in Palmer (1986). A 15 to 20 µg sample was digested with restriction enzymes (*Hin*dIII, *Eco*RI, *Bam*HI, *Hae*III, *Alu*I, *Rsa*I, *Sac*I) and the digested cpDNA were then electrophoresed on 0.8% agarose gels at 50 volts for ≈20 hours. The gels were treated and blotted onto Amersham High Bond-N membranes according to the manufacturer's instructions.

Construction of cpDNA library

A cpDNA clone library was prepared by digesting *Magnolia grandiflora* cpDNA with *Bam*HI and inserting the resulting fragments into the *E. coli* plasmid vector pUC8. Probes were prepared by labeling the isolated insert fragments with [α–^{32}P] dCTP (Dupont) by random primer labeling (Feinberg and Vogelstein, 1983). Hybridizations were carried out at 65°C for ≈20 hours. Hybridization membranes were washed according to the manufacturer's instructions and X-ray film (Kodak X-OMAT XAR-5) were exposed to the hybridized filter at -80°C.

Results and Discussion

The cpDNA library was screened to select probes that easily distinguish the parents, and these probes were used to determine the mode of cpDNA inheritance in progenies of interspecific *Magnolia* hybrids. In all cases, all of the F_1 hybrid progenies examined had cpDNA restriction fragments that only their maternal parents had. As an example of our results,

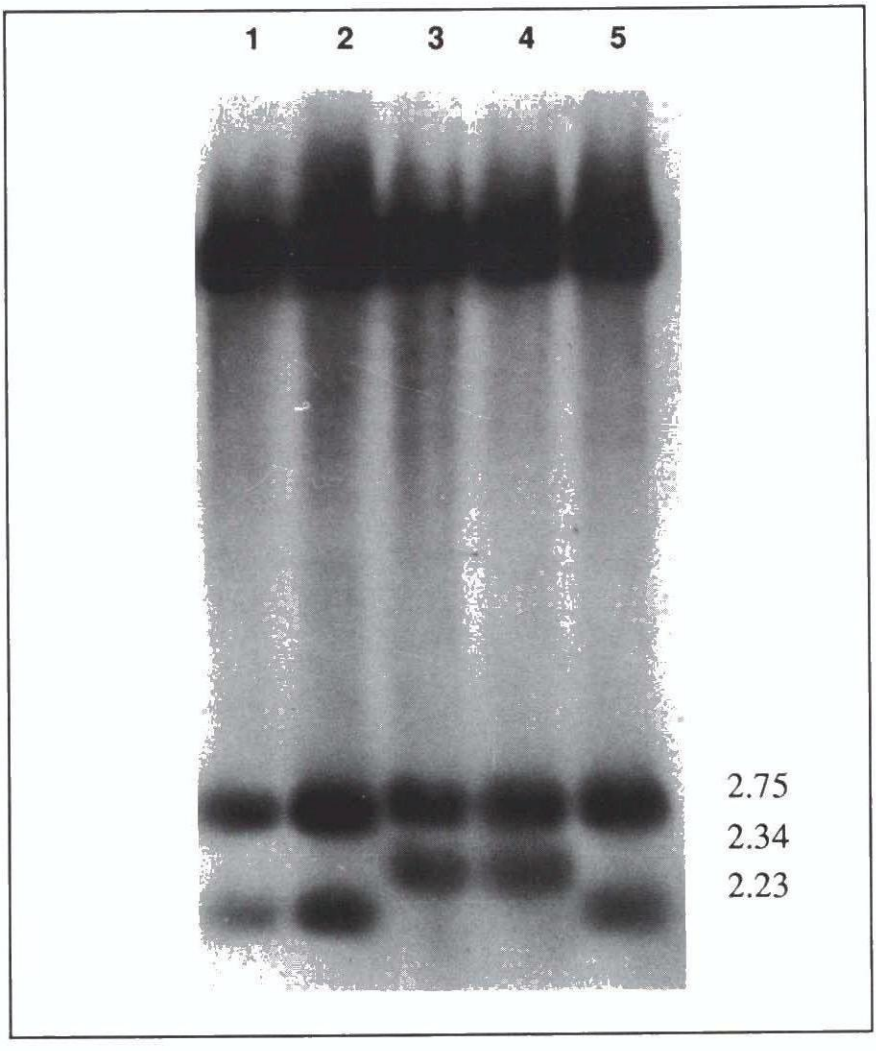

Figure 1. *Autoradiograph of chloroplast DNA probed with 32P-labeled cpDNA clone 2C5 from* M. grandiflora; *restriction fragment sizes labeled in kb. Southern blot hybridization of* Hae*III digested cpDNA from* Magnolia.

Lanes: 1. M. sieboldii 2. M. sieboldii x M. virginiana 3. M. virginiana 4. M. virginiana x M. tripetala 5. M. tripetala.

Figure 1 shows an autoradiograph of cpDNA restriction fragments of the parents and progeny of *M. sieboldii* x *M. virginiana* and *M. virginiana* x *M. tripetala* (= *M.* x *thompsoniana*). This probe (2C5)—enzyme (*Hae*III) combination demonstrates maternal inheritance. Note that all taxa yield fragments of 2.75 kb, but the 2.23 kb fragment is common to *M. sieboldii*, *M. sieboldii* x *M. virginiana* and *M. tripetala* while the 2.34 kb fragment is common to *M. virginiana* and *M. virginiana* x *M. tripetala* (= *M.* x thompsoniana). The polymorphic bands in both F_1 hybrids are present only in the maternal parents providing evidence of maternal inheritance. Other hybrids between *M. tripetala* x *M. sieboldii* and *M. virginiana* x *M. grandiflora* showed similar results.

Once the inheritance of the cpDNA is known, at least one of the parents of allopolyploid taxa can be distinguished (Gastony and Yatskievych, 1992). This is especially important in the investigation of phylogeny in sections containing polyploid taxa, such as Theorhodon.

Preliminary investigation, using cpDNA probes from *M. grandiflora* has shown a surprisingly low variability in the genetic divergence of cpDNA among populations of eastern North American *Magnolia* taxa, making the cpDNA molecule ideal for tracing the evolutionary history of *Magnolia*. ❧

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