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Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine

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Summary. The inheritance of organelle DNAs in loblolly pine was studied by using restriction fragment length polymorphisms. Chloroplast DNA from loblolly pine is paternally inherited in pitch pine × loblolly pine hybrids. Mitochondrial DNA is maternally inherited in loblolly pine crosses. The uniparental inheritance of organelle genomes from opposite sexes within the same plant appears to be unique among those higher plants that have been tested and indicates that loblolly pine, and possibly other conifers, must have special mechanisms for organelle exclusion or degradation or both. This genetic system creates an exceptional opportunity for the study of maternal and paternal genetic lineages within a single species.

Key words: Chloroplast DNA – Mitochondrial DNA – Paternal inheritance – Maternal inheritance – Loblolly pine

Introduction

Cytoplasmic genomes (chloroplast and mitochondrial) are most often maternally inherited in higher plants (Gillham 1978; Kirk and Tilney-Bassett 1978; Sears 1980). Among the angiosperms, the exceptions to strict maternal inheritance of chloroplast DNA (cpDNA) show biparental inheritance, e.g., *Oenothera organensis* (Stubbe 1984) and five species of *Pelargonium* (Metzlaff et al. 1981). In contrast to the strict maternal or biparental inheritance of cpDNA in angiosperms, cpDNA seems to be paternally inherited in the family Pinaceae of gymnosperms. Paternal inheritance of cpDNA was demonstrated in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] by using restriction fragment length polymorphism (RFLP) markers in progeny from intraspecific crosses (Neale et al. 1986). By using similar techniques, others have found evidence for paternal inheritance in progeny from interspecific crosses for *Pinus* (Wagner et al. 1987), *Larix* (Szmidt et al. 1987), and *Picea* (Szmidt et al. 1988). The only exception to paternal inheritance of cpDNA is a *Larix* hybrid with a maternal cpDNA restriction pattern (Szmidt et al. 1987).

Mitochondrial DNA is maternally inherited in all higher eukaryotes that have been studied, including humans (Giles et al. 1980), other mammals (Hutchinson et al. 1974), amphibians (Dawid and Blacker 1972), fishes (Avise and Vrijerhoek 1987), and higher plants. The evidence for maternal inheritance of mtDNA in plants is based on the cytoplasmic inheritance of male-sterility (CMS) in a variety of plants (Edwardson 1971; Lonsdale 1987). Pring and Levings (1978) first attributed CMS to the mitochondrial genome of maize. Subsequently, maternal inheritance of mtDNA in *Zea* was demonstrated using RFLP markers (Conde et al. 1979). Inheritance of mtDNA in the green algae *Chlamydomonas reinhardtii*, through the mt^- (paternal) mating type, is the single known exception to maternal inheritance of mtDNA (Boynton et al. 1987).

This paper reports a study to determine the mode of chloroplast and mitochondrial DNA inheritance in the conifer, loblolly pine [*Pinus taeda* L. (Pinaceae)]. We expected that cpDNA would be paternally inherited based on evidence from other pines, but had no a priori expectation for mtDNA inheritance. The pollen cytoplasm of some conifers contains plastids as well as mitochondria (Chesnoy and Thomas 1971). Therefore, the possibility exists that paternal mitochondria are inherited as well.

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To test this hypothesis, RFLP markers were identified in chloroplast and mitochondrial genomes among loblolly pine and pitch pine [*Pinus rigida* (Mill)] parent trees used in intraspecific and hybrid crosses. Inheritance of organelle DNAs was inferred from RFLP segregations in progeny arrays.

Materials and methods

Pedigreed materials

Genetic analysis of organelle DNA inheritance was conducted by using two sets of pedigreed crosses. The first was a set of 30 select loblolly pine parent trees from a Weyerhaeuser Company seed orchard in Hot Springs, Arkansas. These trees had been mated in pair-wise combinations (15 crosses), and the full-sib progeny were planted in operational progeny test plantations. The second set was a hybrid cross between pitch pine and loblolly pine (*P. rigida* × *P. taeda*); loblolly pine was the male parent. Both parent trees and hybrid offspring were growing in an arboretum at the Institute of Forest Genetics, Placerville, California.

DNA isolation and Southern hybridizations

Total cellular DNA was isolated from 10 g fresh green needle tissue from each tree by modifying the Murray and Thompson (1980) CTAB procedure (Neale et al. 1986; Wagner et al. 1987). DNAs were digested with restriction enzymes, and approximately 1 µg of each DNA was fractionated on 0.8% agarose gels. Gels were then blotted to Biotrans (ICN, Irvine, CA) transfer membranes by standard capillary blotting methods (Maniatis et al. 1982), and the blots were hybridized with nick-translated plasmid DNAs containing cpDNA or mtDNA inserts. Two sources of probes were used: (1) a cpDNA library from *Petunia hybrida* (from J. D. Palmer) and (2) a set of mtDNA genes cloned from maize (from C. S. Levings III).

Results

Paternal inheritance of cpDNA

Loblolly pine parent tree DNAs were digested with six restriction enzymes (*Hind*III, *Eco*RI, *Bam*HI, *Eco*RV, *Sma*I, *Bcl*I) and hybridized with each of six petunia cpDNA probes [P3, P4, P6, P8, P10, S8, S6 (notation of Sytsma and Gottlieb 1986)]. No polymorphisms were detected in any of these hybridizations. This result was unexpected because of the number of polymorphism that have been detected in other species of Pinaceae (Neale and Sederoff 1988; Wagner et al. 1987). These probes comprise 68% of the petunia chloroplast genome (or 86% if P1 is also included in the calculation, because it is nearly identical to P4). We, therefore, discontinued the search for cpDNA polymorphisms among the 30 parent trees.

Because of the lack of intraspecific polymorphism, we inferred inheritance from *P. rigida* × *P. taeda* hybrids. A 200 base pair (bp) deletion was found in a pitch pine parent tree relative to loblolly pine in *Bam*HI and *Hind*III digests hybridized with petunia cpDNA clone S8. All three existing hybrid offspring of the *P. rigida* × *P. taeda* cross had the same restriction fragments as loblolly pine (Fig. 1), demonstrating paternal inheritance of cpDNA from loblolly pine.

Maternal inheritance of mtDNA

The maternal inheritance of mtDNA in loblolly pine was demonstrated in intraspecific crosses. Loblolly pine parent tree blots prepared for cpDNA inheritance were

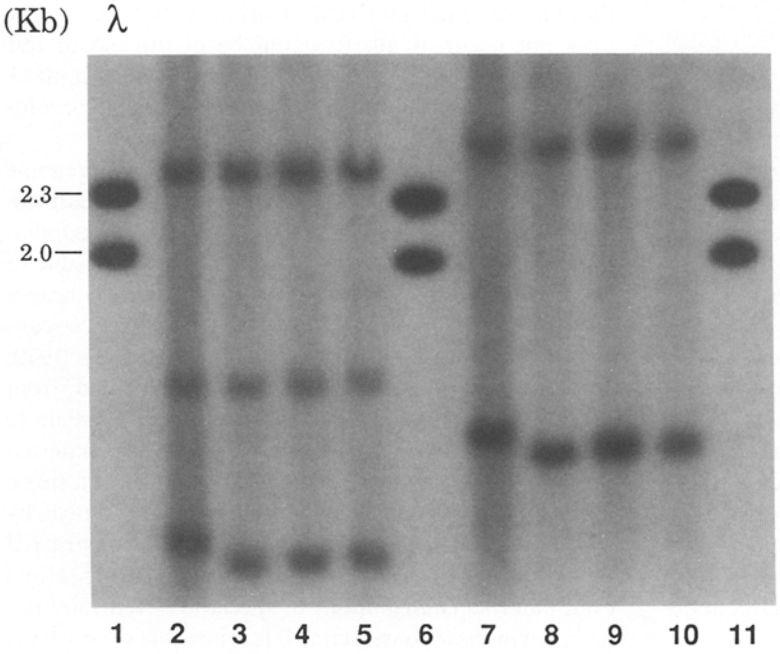

Fig. 1. Paternal inheritance of chloroplast DNA restriction fragments in progeny from a *Pinus rigida* × *P. taeda* cross. *Lanes 1, 6, 11, Hind*III/Lambda DNA size marker; *lane 2, Hind*III/pitch pine female parent; *lanes 3,4, Hind*III/full-sib offspring; *lane 5, Hind*III/loblolly pine male parent; *lane 7, Bam*HI/pitch pine female parent; *lanes 8, 9, Bam*HI/full-sib offspring; *lane 10, Bam*HI/loblolly pine male parent

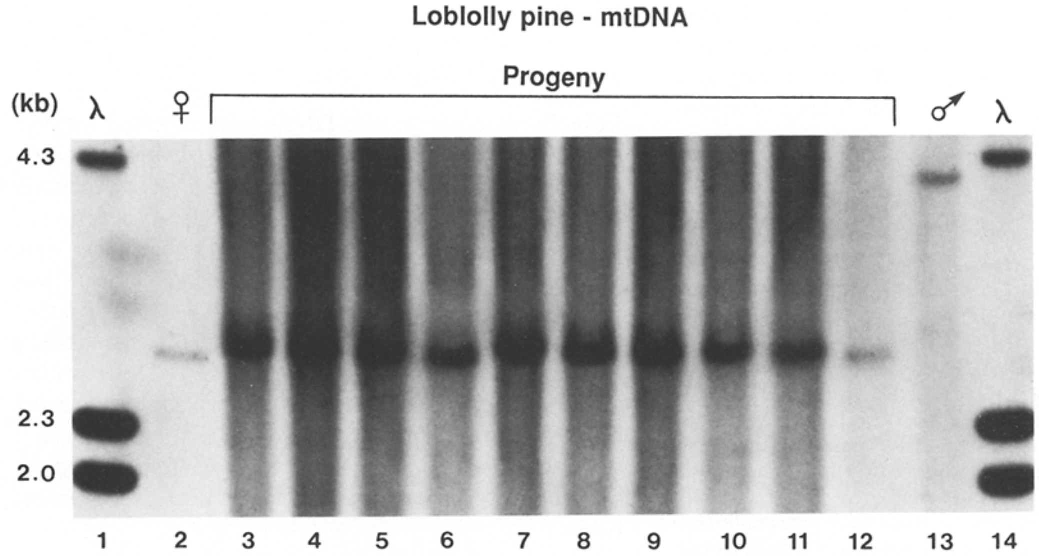

Fig. 2. Maternal inheritance of mitochondrial DNA restriction fragments in progeny from a loblolly pine cross. *Lanes 1 and 14, Hin*dIII/Lambda DNA size marker; *lane 2, Eco*RI/loblolly pine female parent; *lanes 3–12, Eco*RI full-sib offspring; *lane 13, Eco*RI/loblolly pine male parent

stripped completely of cpDNA probe and rehybridized with mtDNA probes. A complex polymorphism among parent trees was detected in EcoRI digests hybridized with the 18S–5S rRNA probe from maize (Chao et al. 1984). The female parent and all offspring had a 2.7 kilobase (kb) fragment, whereas the male parent had a 2.8 kb and a 4.0 kb fragment (Fig. 2). A total of 40 offspring (20 from each of two crosses) were sampled, all of which had the restriction fragment of the maternal parent.

Discussion

This is the first report on the inheritance of mtDNA in any gymnosperm. The maternal inheritance of mtDNA in loblolly pine does not depart from that in virtually all sexually reproducing organisms where inheritance has been studied. However, it is surprising that there was no inheritance of paternal mtDNA in our samples given that cpDNA was paternally inherited, and there is clear evidence from ultrastructural observations that the pollen of several pines contains mitochondria (Chesnoy and Thomas 1971). Further studies will be needed to determine if mtDNA is strictly maternally inherited in all conifers. If mtDNA is strictly maternally inherited in loblolly pine and other conifers that have strict paternal inheritance of cpDNA, then many questions arise as to the mechanisms of exclusion which control the inheritance of organelles. Specifically, there must be separate mechanisms that exclude or degrade mitochondria in the pollen and plastids in the egg cytoplasm.

Ohba et al. (1971) first proposed paternal inheritance of plastids in a gymnosperm [*Cryptomeria japonica* D. Don (Taxodiaceae)] based on crosses with a yellow shoot mutant. Inheritance of the variant was strongly biased towards the paternal parent. Recent studies using molecular markers have shown paternal inheritance of cpDNA in four genera of the Pinaceae: *Pseudotsuga* (Neale et al. 1986), *Pinus* (Wagner et al. 1987), *Larix* (Szmidt et al. 1987) and *Picea* (Szmidt et al. 1988). We can now add *Pinus taeda* to this list. Accordingly, paternal inheritance of cpDNA may be a common feature of all members of the Pinaceae, and maternal inheritance may be rare or does not occur at all. It would be of interest to test cpDNA inheritance in more distantly related conifers and other gymnosperms to further understand the evolution of organelle inheritance in higher plants.

The maternal inheritance of one organelle genome and the paternal inheritance of the other genome presents an unique opportunity for evolutionary and population geneticists. This genetic system makes it possible to determine and compare maternal and paternal lineages in the same organism. Molecular phylogenies constructed from mtDNA in animals (Avise et al. 1979; Lansman et al. 1981; Wilson et al. 1985) and from cpDNA in angiosperms (Palmer and Zamir 1982; Palmer et al. 1985; Sytsma and Gottlieb 1986) are maternal lineages. These phylogenies are based primarily on single base-pair substitutions and are not complicated by meiotic recombination, such as phylogenies constructed from nuclear-encoded characters (Palmer 1985). However, paternal contributions to speciation events are not included in these constructions. It is possible to construct independent maternal and paternal phylogenies in conifers which could be more informative than either one alone. However, more data is needed on the inheritance and variability of mtDNA in other conifers before mtDNA polymorphisms can be used for phylogenetic analyses.

The inheritance patterns of cpDNA and mtDNA in loblolly pine (and possibly other conifers) will allow population genetic studies that are not possible in angiosperms. The contributions of maternal and paternal parents can be distinguished in hybrid and introgressed populations (Wagner et al. 1987). Organelle DNA RFLPs can also be used to identify putative hybrids in seed lots collected in hybrid or introgressed populations (Szmidt et al. 1988).

The population genetic structure of organelle DNA variation can be contrasted with that of nuclear DNA (nDNA). In angiosperms with strict maternal inheritance of mtDNA and cpDNA, the organelle genomes are transmitted along with the nDNA in the maternal gamete. Therefore, patterns of organelle DNA variation are not affected by dispersal of pollen. In contrast, the chloroplast genome of loblolly pine, and possibly other Pinaceae, is effectively dispersed twice, once by pollen and then again by seed. Therefore, cpDNA gene flow parallels that of nuclear DNA in the paternal gamete and mtDNA follows that of nDNA in the maternal gamete. Population geneticists can compare the effects of evolutionary forces such as natural selection, genetic drift, gene flow, and mutation on each of three genomes that operate in a coordinated fashion within the cell, but have different modes of inheritance.

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