Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 9347–9349, December 1989
Evolution

Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don Endl.

(organelle inheritance/restriction fragment length polymorphisms)

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Communicated by R. W. Allard, August 14, 1989

ABSTRACT **Restriction fragment length polymorphisms in controlled crosses were used to infer the mode of inheritance of chloroplast DNA and mitochondrial DNA in coast redwood (*Sequoia sempervirens* D. Don Endl.). Chloroplast DNA was paternally inherited, as is true for all other conifers studied thus far. Surprisingly, a restriction fragment length polymorphism detected by a mitochondrial probe was paternally inherited as well. This polymorphism could not be detected in hybridizations with chloroplast probes covering the entire chloroplast genome, thus providing evidence that the mitochondrial probe had not hybridized to chloroplast DNA on the blot. We conclude that mitochondrial DNA is paternally inherited in coast redwood. To our knowledge, paternal inheritance of mitochondrial DNA in sexual crosses of a multicellular eukaryotic organism has not been previously reported.**

The mode of inheritance of chloroplast and mitochondrial genomes in gymnosperms has, until recently, been unknown. In angiosperms, mitochondrial DNA (mtDNA) is strictly maternally inherited, whereas chloroplast DNA (cpDNA) is strictly maternally or biparentally inherited (reviewed in refs. 1–3). There have been a number of recent investigations of cpDNA inheritance in the Pinaceae of gymnosperms. cpDNA is strictly paternally inherited in intraspecific crosses of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] (4) as well as in interspecific crosses of *Pinus* (5, 6), *Larix* (7), and *Picea* (8). We are not aware of any reports of strict maternal inheritance of cpDNA in Pinaceae. mtDNA inheritance has been tested in only one member of the Pinaceae, loblolly pine (*Pinus taeda* L.), in which it was shown to be maternally inherited (6).

We have extended our investigation of organelle DNA inheritance to a second family of conifers, the Taxodiaceae. We identified cpDNA and mtDNA restriction fragment length polymorphisms (RFLPs) among coast redwood (*Sequoia sempervirens* D. Don Endl.) parent trees and inferred inheritance from intraspecific crosses. cpDNA was paternally inherited in coast redwood, as in Pinaceae, but, surprisingly, mtDNA was paternally inherited as well. We know of no prior reports of paternal inheritance of mtDNA in sexual crosses of a eukaryotic organism.

MATERIALS AND METHODS

Foliage samples were obtained from parents and offspring of three coast redwood crosses (ARC154 × ARC28, R42 × R49, and R41 × R46) from the Simpson Timber Company, Korbel, CA. Total cellular DNA was isolated from 10 g of fresh weight needle tissue by small modifications of the Murray and Thompson (9) cetyltrimethylammonium bromide procedure. DNAs were digested with restriction enzymes, fractionated on agarose gels, and blotted to nylon transfer membranes. Plasmids containing cpDNA inserts from *Petunia hybrida* (10) were labeled by nick-translation. A purified fragment containing the cytochrome oxidase II gene from maize mtDNA (11) was labeled by random-primer labeling (12). Hybridizations were conducted in 5× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), 50 mM phosphate, 0.4% SDS, 5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 2.5 mM EDTA, and 100 μg of herring sperm DNA per ml at 65°C. Hybridization washes were conducted in 2× SSC/0.1% SDS at 65°C. Hybridized blots were exposed to x-ray film with intensifier screens for 24–72 hr at −70°C.

RESULTS

Paternal Inheritance of cpDNA. Parent trees of each of the two crosses ARC154 × ARC28 and R42 × R49 differed by small insertions/deletions in hybridizations with the P6 cpDNA clone from petunia. P6 is a 15.3-kilobase (kb) *Pst* I fragment from the large single-copy region of the petunia chloroplast genome. For example, the female parent ARC154 had a 3890-base-pair (bp) fragment in *Eco*RI digests, whereas the male parent ARC28 had a deletion of ≈140 bp, giving a fragment of 3750 bp (Fig. 1). This deletion was also seen in *Bcl* I digests (Fig. 1). All 10 offspring of the cross ARC154 × ARC28 and 12 offspring of the cross R42 × R49 had the same restriction fragments as the male parent, thus demonstrating the paternal inheritance of cpDNA in these coast redwood crosses (Table 1).

Paternal Inheritance of mtDNA. Parent trees of the crosses R41 × R46 and R42 × R49 also differed for a mtDNA RFLP. This polymorphism was revealed in *Bam*HI digests hybridized with the cytochrome oxidase II clone. The female parent R41 had an 8.2-kb fragment, whereas the male parent R46 and the offspring had fragments of 4.7, 4.3, and 3.5 kb (Fig. 2). The RFLP markers were reversed in the cross R42 × R49. All progeny of both crosses had the same fragments as the male parents (Fig. 2 and Table 1), thus demonstrating paternal inheritance of mtDNA in these coast redwood crosses. We have not been able to determine what type of rearrangement caused this polymorphism. *Eco*RI and *Hin*dIII digests of parents and offspring also revealed paternal inheritance of RFLPs (data not shown).

An alternative explanation for the result shown in Fig. 2 is that the cytochrome oxidase II probe had actually hybridized to nuclear DNA or cpDNA on the blots, which were made from total DNA preparations. It seems unlikely that the probe revealed nuclear DNA sequences based on the strict uniparental inheritance patterns. There is no evidence for

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Abbreviations: cpDNA, chloroplast DNA; mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism.

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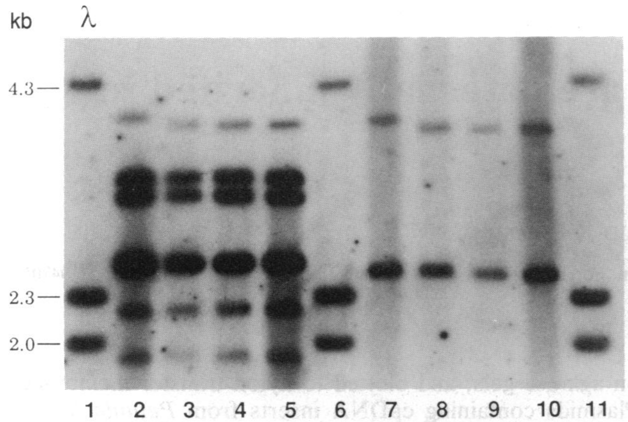

FIG. 1. Paternal inheritance of cpDNA in *Sequoia sempervirens*. Lanes 1, 6, and 11, λ/*Hin*dIII size markers; lane 2, maternal parent ARC154/*Eco*RI digest; lanes 3 and 4, progeny of ARC154 × ARC28/*Eco*RI digest; lane 5, paternal parent ARC28/*Eco*RI digest; lane 7, maternal parent ARC154/*Bcl* I digest; lanes 8 and 9, progeny of ARC154 × ARC28/*Bcl* I digest; lane 10, paternal parent ARC28/*Bcl* I digest. The blot was hybridized with the P6 clone from *Petunia hybrida* cpDNA.

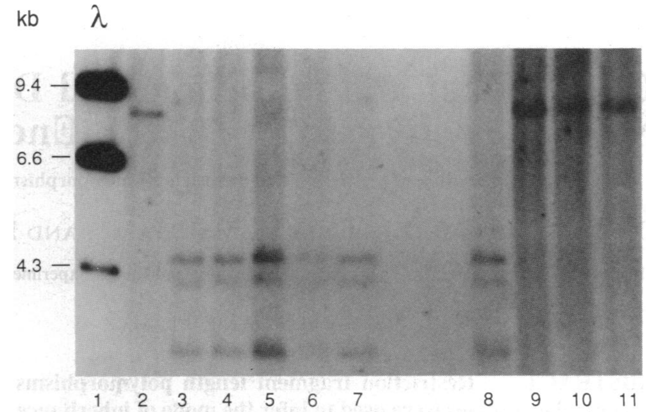

FIG. 2. Paternal inheritance of mtDNA in *Sequoia sempervirens*. Lane 1, λ/*Hin*dIII size markers; lane 2, maternal parent R41/*Bam*HI digest; lanes 3–6, progeny of R41 × R46/*Bam*HI digest; lane 7, paternal parent R46/*Bam*HI digest; lane 8, maternal parent R42/*Bam*HI digest; lanes 9 and 10, progeny of R42 × R49/*Bam*HI digest; lane 11, paternal parent R49/*Bam*HI digest. The blot was hybridized with a 1.9-kb mtDNA fragment from maize containing the cytochrome oxidase II gene.

promiscuous mtDNA sequences in the chloroplast genomes of higher plants (13), although the possibility of sequence homology between the maize mtDNA fragment and coast redwood cpDNA cannot be absolutely excluded. To test this hypothesis, 14 blots were prepared that contained total DNA of parent tree R41 and parent tree R46, each digested with *Bam*HI. The 14 small blots were then hybridized with one each of the 14 cpDNA clones from petunia. Both parent trees showed the same bands in all hybridizations; the polymorphism revealed by the cytochrome oxidase II clone was not found in hybridizations with cpDNA clones comprising the entire chloroplast genome from petunia. We conclude that the cytochrome oxidase II clone from maize did in fact hybridize to mtDNA sequences from redwood and that mtDNA is paternally inherited in the redwood crosses.

DISCUSSION

We expected that *Sequoia sempervirens* would show paternal inheritance of cpDNA; however, we were surprised to observe that mtDNA is paternally inherited as well. These data are based on just two crosses of 10–12 progeny each for each genome; therefore, we cannot unequivocally conclude that these organelles are strictly paternally inherited. They do provide, however, evidence for strong paternal bias at the very least. mtDNA is strictly maternally inherited in a wide range of animals, including *Homo* (14), *Equus* (15), *Rattus* (16–18), *Mus* (19), *Poeciliopsis* (20), *Xenopus* (21), *Drosophila* (22), and *Heliothis* (23). In sexual crosses of plants, mtDNA appears to be strictly maternally inherited as well: *Zea* (24), *Hordeum* (25), *Epilobium* (26), *Pinus* (6), and *Triticum* (27). One possible exception to strict maternal inheritance of mtDNA is in alfalfa (*Medicago sativa*) in which mitochondrial RNAs of paternal origin were observed in progenies of sexual crosses (28). Only in protoplast fusions of *Nicotiana* (29–31), *Petunia* (32–34), *Cruciferae* (35), and *Solanum* (36) and in the hybrid sexual cross *Hordeum* × *Secale* (25) has the transmission of paternal mtDNA been shown. How and why could the mechanisms for paternal inheritance of cpDNA and mtDNA have evolved in *Sequoia sempervirens*?

Table 1. Inheritance of cpDNA and mtDNA in intraspecific crosses of coast redwood (*Sequoia sempervirens* D. Don Endl.)

	Cross	No. of progeny: Total	Paternal	Maternal
cpDNA	ARC154 × ARC28	10	10	0
	R42 × R49	12	12	0
mtDNA	R41 × R46	10	10	0
	R42 × R49	12	12	0

Ultrastructural investigations of gymnosperm fertilization (reviewed in refs. 2, 37, and 38) provide some insights into how chloroplasts and mitochondria might be inherited in *Sequoia sempervirens*. The presence of plastids and mitochondria of paternal origin in the fertilized egg cytoplasms of two taxa of Taxodiaceae [*Sciadopitys verticillata* (39) and *Cryptomeria japonica* (40)] has been reported as well as for two species of the closely related Cupressaceae: *Thuja orientalis* (41, 42) and *Chamaecyparis lawsonia* (43). These observations make it apparent that paternal inheritance of cpDNA and mtDNA in Taxodiaceae and Cupressaceae should not be unexpected. In fact, a shoot color mutant was shown to be paternally inherited in *Cryptomeria japonica* (Taxodiaceae) many years ago (44).

The fate of the maternal plastids and mitochondria in coast redwood is unknown. In Pinaceae, maternal plastids appear to be excluded by being sequestered in inclusions within the egg cytoplasm (reviewed in ref. 37), but similar observations have not yet been reported in Taxodiaceae. Mechanisms must also be present to exclude maternal mitochondria as well in Taxodiaceae. These mechanisms may be unique to Taxodiaceae in that mtDNA is maternally inherited in at least one species of Pinaceae, *Pinus taeda* (6).

Sears (2) and Whatley (38) have traced the evolution of plastid inheritance in the plant kingdom. There is a general trend toward exclusion of plastids during earlier stages of reproductive development in green algae and at later stages in angiosperms. There is also somewhat of a trend toward strict uniparental inheritance in angiosperms versus biparental inheritance in lower plants. However, these trends are marked by exceptions. The most obvious exception is the Coniferales. This order appears to have paternal inheritance of cpDNA and both maternal (*Pinus taeda*) and paternal (*Sequoia sempervirens*) inheritance of mtDNA. Two other orders of gymnosperms (Ginkgoales and Cycadales), however, appear to have maternal inheritance of organelles (38). The paternal inheritance of organelle DNA in Coniferales provokes a need for an evolutionary explanation as to why this mode of inheritance evolved in this group of plants versus the strict maternal or biparental inheritance associated with all other plants that have been studied.

We thank Bill Libby, Kim Rodrigues, and Simpson Timber Co. for providing plant materials and Jeff Palmer and Glenn Furnier for comments on the manuscript. This work was supported by U.S. Department of Agriculture Forest Biology Grant 87-FSTY-9-0239 to D.B.N. and R.R.S.

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