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杉木叶绿体和线粒体遗传的研究*

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摘要 利用PCR技术分别以亲本杉木(*Cunninghamia lanceolata* (Lamb.) Hook.)、柳杉(*Cryptomeria fortunei* Hooibrenk)和杉木×柳杉杂种的总DNA为模板,扩增了叶绿体 *trnL-trnF* 和线粒体 *Cox* Ⅲ基因片段,比较了这些扩增片段的限制性内切酶 *Alu* Ⅰ, *Dde* Ⅰ, *Hinf* Ⅰ, *Mse* Ⅰ和 *Rsa* Ⅰ的酶切片段多态性,结果表明:F_1 代的叶绿体DNA为母系遗传,而线粒体DNA为父系遗传。杉木线粒体DNA父系遗传方式与杉科其他植物一致,而叶绿体DNA母系遗传则为在松柏类植物中首次发现。

关键词 杉木,柳杉,叶绿体DNA遗传,线粒体DNA遗传,限制性片段长度多态性

Inheritance of Chloroplast and Mitochondrial DNA in Chinese Fir (*Cunninghamia lanceolata*)*

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Abstract The inheritance of mitochondrial (mt) DNA and chloroplast (cp) DNA was investigated in intergeneric hybrids from crossing between *Cunninghamia lanceolata* (Lamb.) Hook. and *Cryptomeria fortunei* Hooibrenk. The chloroplast *trnL-trnF* region and one intra-genic segment of the mitochondrial gene, *Cox* Ⅲ, were amplified from those of the parents and hybrids by PCR using gene-specific primers. Cp- and mtDNA polymorphisms of the amplified regions were detected between the parents after restriction digestions. Restriction fragment length polymorphism (RFLP) analysis revealed that all the F_1 individuals possessed *Cox* Ⅲ restriction fragment patterns (characteristic of the paternal parent *Cryptomeria fortunei*) and the *trnL-trnF* region (identical to the maternal parent *Cunninghamia lanceolata*) showing that a different mode of inheritance for organelle DNA has occurred in the hybrids. Furthermore, the maternal inheritance of chloroplast DNA is reported here for the first time in coniferophyta.

Key words *Cunninghamia lanceolata*, *Cryptomeria fortunei*, Inheritance of chloroplast DNA, Inheritance of mitochondrial DNA, Restriction fragment length polymorphism

Organelle DNAs are extensively used for studies of plant population and evolutionary biology. In angiosperms, chloroplast DNA (cpDNA) is inherited maternally in over 70% of the plant genera, biparentally in about 25% of the genera[1] and paternally in only a few genera studied, such as *Medicago*, *Daucus* and *Pharbitis*[2]. Except the *Ephedra*, *Ginkgo*, and the cycads most gymnosperms exhibit uniparental, i.e. maternal inheritance of both plastids and mitochondria[3], however cpDNA shows an exclusive or predominant paternal inheritance in the conifers, including *Abies*[4], *Pseudotsuga*[5], *Picea*[6~9], *Larix*[10], *Pinus*[11~16], *Cryptomeria*[17,18], *Sequoia*[19] and *Calocedrus*[20]. Cytological studies showed that the paternal inheritance of cpDNA is due to the elimination of maternal chloroplasts during fertilization in gymnosperms. This result is consistent with the genetic findings[21]. The pattern of mitochondrial DNA (mtDNA) inheritance is

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predominantly maternal in angiosperms and also in the Pinaceae and probably the Taxaceae of gymnosperms, but in four coniferous families, viz. Araucariaceae, Cephalotaxaceae, Taxodiaceae and Cupressaceae, the mitochondrial inheritance appears to be predominantly paternal[3,8,14,19,20,22~25]. In this paper, we analysed the inheritance of organelle DNA in a controlled cross between *Cunninghamia lanceolata* and *Cryptomeria fortunei*. RFLP analysis revealed a paternal inheritance of mtDNA and maternal inheritance of cpDNA in the intergeneric hybrids, suggesting the existence of a different mode of inheritance for organelle DNA in the crosses. Furthermore, the maternal inheritance of chloroplast DNA is reported here for the first time in Coniferophyta.

1 Materials and Methods

1.1 Plant materials

Seeds from *Cunninghamia lanceolata* (Lamb.) Hook., *Cryptomeria fortunei* Hooibrenk and their hybrids were collected from Nanjing Botanical Garden. The cross between *Cunninghamia lanceolata* and *Cryptomeria fortunei* was performed as described in Li *et al*[26]. Linear leaves were obtained after seed germination in soil for 3 months.

1.2 Molecular techniques

The leaf samples were dehydrated with SiO_2 for one week before genomic DNA extraction. The DNA was prepared as described previously but using a different extraction buffer (100 mmol/L Tris-HCl (pH 8.0), 50 mmol/L EDTA, 500 mmol/L NaCl, 2% SDS (W/V), 1% PVP-40, 1% β-mercaptoethanol)[27].

Polymerase chain reaction (PCR) was carried out in a reaction volume of 50 μL containing 10 ng template DNA, 0.4 mmol/L each of two gene-specific primers, 200 μmol/L each of four dNTP, 5 μL 10×PCR buffer (100 mmol/L Tris-HCl (pH 8.3), 500 mmol/L KCl, 20 mmol/L $MgCl_2$, 0.1% (W/V) gelatin), and 2.5 units of Taq polymerase on a Perkin-Elmer 9600 DNA Thermal Cycler according to the following procedure[28]: 94 ℃, 3 min for initial denaturation, followed by 35 cycles of denaturation at 94 ℃ for 30 sec, annealing (59 ℃ for *Cox* Ⅲ, 55 ℃ for *trnL-trnF*) for 1 min, extension at 72 ℃ for 1 min and 30 sec, and terminated at 72 ℃ for 10 min. One pair of the primers specific for mitochondrial gene *Cox* Ⅲ or one pair of the primers for the chloroplast *trnL-trnF* region capable of amplifying the corresponding mt- or cpDNA regions were derived from the sequences published by Wang *et al*[28]. The *Cox* Ⅲ primers are: 5′GGTAGATCCAAGTCCATGGC3′, 5′CAGTACCATGCAGCTGCTTC3′ and the *trnL-trnF* region 5′CGAAATCGGTAGACGCTACG3′ and 5′ATTTGAACTGGTGACACGAG3′. 5 μL out of the total 50 μL PCR products were separated on 0.8% agarose gel and stained with ethydium bromide. The rest of the PCR products were purified using WizardTM PCR preps DNA purification system kit (Promega).

Purified PCR products (8 μL) after digestion by *Alu* Ⅰ, *Dde* Ⅰ, *Hinf* Ⅰ, *Mse* Ⅰ or *Rsa* Ⅰ were separated on 6% non-denaturing polyacrylamide gels[29]. The restriction fragment patterns were visualized by the silver staining method[30]. The gel was fixed in 10% acetic acid for 20 min, silver stained for 30 min in 0.1% $AgNO_3$ and 0.15% formaldehyde (37%), and the image developed in a solution of 3% Na_2CO_3, 0.15% formaldehyde (37%) and 0.02% $Na_2S_2O_3$ (10 mg/mL) for 3~5 min.

2 Results and Discussion

2.1 Detection of mt- and cpDNA restriction fragment polymorphisms

Genomic DNAs were isolated from ten dried young needles of *Cunninghamia lanceolata*, *Cryptomeria fortunei* and their three hybrids respectively. When the fresh needles were directly ground in liquid nitrogen, the quantity of genomic DNA obtained was not sufficient for further analysis(data not shown). This was overcome by using the drying method here described. 10~20 μg of genomic DNA was obtained from ten dry needles. This could be due to the fact that the fresh leaves contain more fibers and are hard to grind in liquid nitrogen. For small quantities of materials, i.e. conifer leaves, it is best to dry them before grinding.

In order to detect the restriction fragment polymorphisms of mtDNA or cpDNA between the two parents, the DNAs were used as templates for PCR amplification using mtDNA (*Cox* Ⅲ) or cpDNA (*trnL-trnF* region) specific primers (see Materials and Methods). One fragment of approximately 660 bp in length, which has the size similar to that observed in *Pinus nigra*, *P. sylvestris* and their hybrids[28], was amplified by the *Cox* Ⅲ primers in *Cunninghamia lanceolata* and *Cryptomeria fortunei* (Fig. 1A). The fragment amplified by the *trnL-trnF* primers in the maternal parent was about 870 bp and the two fragments in the paternal parent were 870 bp and 660 bp respectively (Fig. 1B). Previous amplification of the *trnL-trnF* region in gymnosperms resulted in one fragment[28, 31]. The two fragments amplified in *Cryptomeria fortunei* indicates that the organization of the

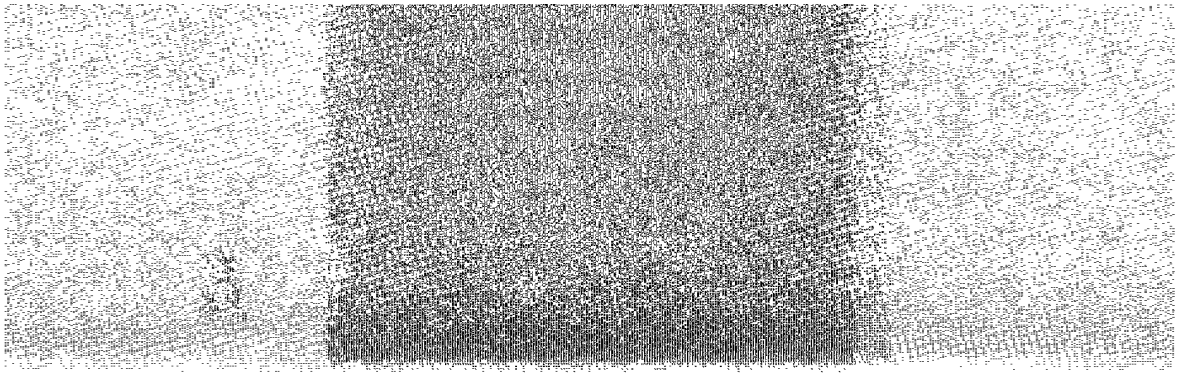

Fig.1 mtDNA (A) and cpDNA (B) segments amplified by PCR in *Cunninghamia lanceolata*, *Cryptomeria fortunei* and three F_1 progenies M. DNA marker; ♀. Maternal parent *Cunninghamia lanceolata*; 1～3. *Cunninghamia lanceolata* × *Cryptomeria fortunei*; ♂. Paternal parent *Cryptomeria fortunei*.

chloroplast genome of this species might be different from that of other conifer species.

The restriction digestion of the *Cox* Ⅲ and *trnL-trnF* region restriction fragments produced differences between the two parents. These differences generated by *Rsa* Ⅰ, *Dde* Ⅰ *or Alu* Ⅰ were shown in Fig. 2. To confirm that the observed mtDNA and cpDNA polymorphisms between the two species are truly species specific, DNA samples from 5 additional individual seedlings of *Cunninghamia lanceolata* and *Cryptomeria fortunei*, respectively, were further analyzed. No intra-specific variation in the restriction fragment patterns was observed (data not shown).

2.2 Mitochondrial DNA in the hybrids displays a paternal inheritance

Restriction fragment patterns of the *Cox* Ⅲ fragment by five restriction enzymes employed in this study evidenced that the mode of mtDNA inheritance in *Cunninghamia lanceolata* is paternal. The fragment sizes of mtDNA (*Cox* Ⅲ) amplified by PCR in all three F_1 progenies were the same as the two parents (660 bp) (Fig. 1A). The fragment (398 bp) specific for the paternal parent *Cryptomeria fortunei* produced by *Rsa* Ⅰ was observed in all progenies except for one progeny, which could be due to the loss of one *Rsa* Ⅰ site in the fragment caused by PCR artifact. However, the fragment (355 bp) specific for the maternal parent *Cunninghamia lanceolata* produced by *Dde* Ⅰ was not observed in all of the progeny (Fig. 2A).

Except for one example found in a cross between *Pinus banksiana* and *P. contorta*, where 7% of the F_1 progeny contained mtDNA identical to the paternal parent[24], the mode of mtDNA inheritance in Pinaceae is predominately maternal, similar to that found in most plants and animals[32]. However, in the Taxodiaceae and Cupressaceae, mtDNA displays a paternal inheritance in the species examined (*Sequoia sempervirens*[19] and *Calocedrus decurrens*[20]). Our results provided additional information on this subject. All F_1 progenies analyzed possessed only paternal mtDNA pattern. Furthermore, this finding further confirms that the progenies were of hybrid nature.

2.3 Chloroplast DNA in the intergeneric hybrids shows a novel maternal inheritance

The amplified products of the *trnL-trnF* region in all progenies were similar to the maternal parent *Cunninghamia lanceolata*, with a size of 870 bp (Fig. 1B). The *trnL-trnF* restriction fragment patterns in the hybrids were similar to that in the maternal parent, suggesting a maternal inheritance of cpDNA in *Cunninghamia lanceolata* and *Cryptomeria fortunei* hybrids (Fig. 2B).

The current knowledge of organelle DNA inheritance in gymnosperms is based on a limited number of species and individuals, mostly belonging to Pinaceae family. In general, cpDNA is predominantly paternally inherited, including species from Pinaceae, Taxaceae, Araucariaceae, Cephalotaxaceae, Taxodiaceae and Cupressaceae[3,20]. Genetic and cytological studies showed the paternal plastid inheritance in *Cryptomeria japonica*[17,18], a closely related species to *Cunninghamia lanceolata*. In contrast to these results, the present study provides direct evidence for maternal inheritance of cpDNA in *Cunninghamia lanceolata*, concordant with the mode of inheritance of cpDNA in angiosperms and lower plants[33,34].

The possible mechanisms of cytoplasmic inheritance in plants include exclusion, loss and degradation of organelle DNA. In many angiosperms, the maternal inheritance of cpDNA is accounted to the absence of cpDNA in pollens[35,36]. However, cytological observations and genetic evidence indicated that the

Coniferale pollen contains both chloroplasts and mitochondria[28, 37～39] which can be transmitted along with the male nuclei at fertilization, rendering the possible paternal inheritance of cpDNA and mtDNA in Coniferale. However, the chloroplast and mitochondrial transmission is by no mean the same as the inheritance of organelle DNA. The mode of cp⁻ and mtDNA inheritance is determined ultimately by the precise mechanism(s) aiding elimination of the alternative organelle DNA during embryogenesis or possibly controlled by nuclear genes. Cytological studies on this project which may further discover the mechanism of organelle DNA inheritance in *Cunninghamia lanceolata* are in progress.

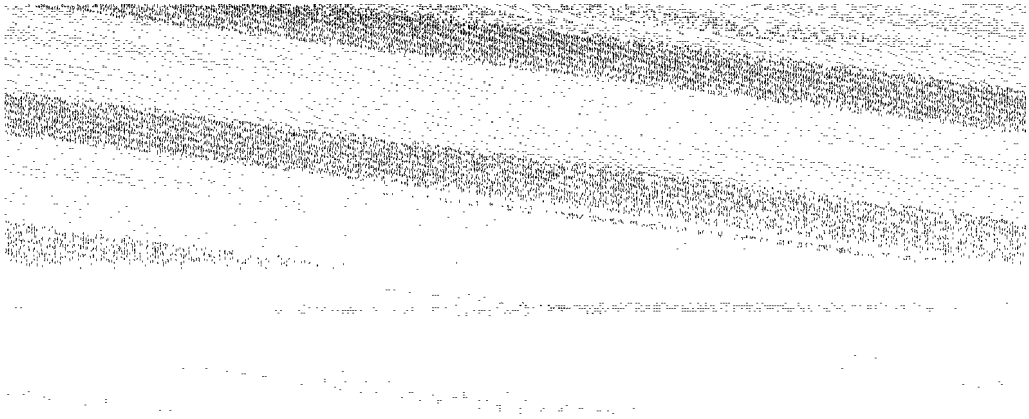

Fig. 2 *Cox* Ⅲ (A) and *trnL-trnF* (B) restriction fragment patterns generated by *Rsa* Ⅰ, *Dde* Ⅰ and *Alu* M. DNA marker; ♀. *Cunninghamia lanceolata*; 1～3. *Cunninghamia lanceolata* × *Cryptomeria fortunei*; ♂. *Cryptomeria fortunei*.

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