Theor Appl Genet (1999) 99: 346–349

D. Grivet · S. Jeandroz · J. M. Favre

Nad1 b/c intron polymorphism reveals maternal inheritance of the mitochondrial genome in *Picea abies*

Received: 5 December 1998 / Accepted: 28 December 1998

Abstract Our goal was to determine the mode of inheritance of *Picea abies* mitochondria. We studied four trees implicated in intraspecific controlled crosses. Based on a PCR-derived technique, one mitochondrial marker, the intron b/c of subunit 1 of NADH dehydrogenase (*nad1* b/c), was informative in one of the available crosses. This fragment was sequenced and exhibited several features concordant with the group-II intron, as judged from its sequence evolution and organisation. All of the 96 offspring analysed from the two polymorphic parents present a maternal profile. We conclude that in *P. abies* the mitochondrial genome is maternally inherited. This result is congruent with the commonly observed pattern of mitochondrial inheritance in the other *Picea* species studied so far. The existence of mtDNA polymorphic markers that are maternally inherited should now facilitate the study of the population genetic structure of the Norway spruce.

Key words Mitochondrial DNA · *nad1* b/c intron · Maternal inheritance · PCR-amplified RFLP · *Picea abies*

Introduction

Natural populations of Norway spruce (*Picea abies* Karst or *Picea excelsa* Link) are found from northern and western Europe to the Ural mountains where another species, *Picea obovata*, occurs. Based on paleobotanical data, and confirmed by isozymes studies, the natural range of *P. abies* is commonly divided into three areas: the Alpine area, the Hercynian Carpathic area and the Baltic area (Huntley and Birks 1983; Schmidt-Vogt 1986; Lagercrantz and Ryman 1990). A better knowledge of the genetic diversity and population structure of this species could give important information on the management of its genetic resources.

Present patterns of the genetic structure of tree populations have been influenced by historical factors (migration, colonisation from refugia, secondary contact) over the last 15 000 years (Kremer 1994). In animals, mitochondrial DNA (mtDNA) has provided a great potential for detecting population differentiation and re-tracing the populations' intraspecific phylogenetic history (Avise 1994). In fact, the mitochondrial genome appears to be well adapted for population structure analysis for two reasons: (1) due to the uniparental heredity of the cytoplasmic genome, the population size is smaller and therefore more prone to drift, compared to the biparentally inherited nuclear genome, (2) using maternally inherited genomes, a direct study of the seed dissemination pattern is possible, and seeds usually migrate over short distances compared to pollen. The smaller population sizes and the reduced gene flow leads to a higher differentiation of the cytoplasmic genes compared to the nuclear genes (Petit et al. 1993).

If the maternal inheritance of mtDNA could be demonstrated in *P. abies*, the potential usefulness of this genome to reveal population differentiation would be confirmed. In Gymnosperms, mitochondria are usually maternally inherited, while chloroplasts are biparentally or paternally transmitted (Neale and Sederoff 1988). In the genus *Picea*, several studies dealing with organelle inheritance have been carried out involving North American spruce species (*Picea rubens*, *Picea mariana*, *Picea engelmanii*, *Picea sitchensis*, *Picea*

Communicated by P. M. A. Tigerstedt

D. Grivet[1] · S. Jeandroz · J. M. Favre (✉)
Laboratoire de Biologie Forestière, Université Henri Poincaré, BP 239, 54506 Vandoeuvre lès Nancy, France
Fax: +33 3 83 90 32 77
E-mail: favre@scbiol.u-nancy.fr

Present address:
[1] INRA Recherches Forestières, Laboratoire de Génétique et Amélioration des Arbres Forestiers, BP 45, 33611 Gazinet cedex, France

glauca) and one Serbian species (*Picea omorika*). These studies confirmed that mtDNA is maternally inherited whereas cpDNA is paternally or biparentally inherited (Sutton et al. 1991a, b; Bobola et al. 1996; David and Keathley 1996). However, the inheritance pattern of the cytoplasmic genomes can exhibit important variations within a genus. In the genus *Pinus*, for instance, mtDNA is maternally inherited in *Pinus taeda* and in hybrids of *Pinus griffithii* × *Pinus strobus* (Neale and Sederoff 1988, 1989) while in *Pinus monticola*, *Pinus banksiana* and *Pinus banksiana* × *Pinus contorta* hybrids biparental inheritance patterns have been demonstrated (Bruns and Owens 1989; Wagner et al. 1991). Therefore, generalization at the whole-genus level of the inheritance patterns established from a limited number of species is inappropriate.

In this paper, we present the first demonstration of maternal inheritance of mtDNA in *P. abies*. Contrary to the previous reports cited above, we used a PCR-derived technique consisting of a restriction study of the mtDNA fragments amplified by PCR with one pair of universal mitochondrial primers.

Materials and methods

Plant material

Three controlled crosses, performed in Denmark, involving four parent trees (A, B, C, and D) were supplied by CEMAGREF (Nogent sur Vernisson, France). Tree A was used as the female parent; trees B, C, and D were used as male parents. Ninety six seeds (supplied by INRA Orléans) from the cross A × D were used to verify maternal inheritance of the mtDNA.

Needles were frozen at −80°C until DNA extraction. Seeds were germinated in Petri dishes of filter paper. Seedlings of about 2-cm high were kept frozen until they were analysed.

DNA extraction

DNA was obtained from parents and offspring. Total genomic DNA was extracted from frozen needles (about 1 g) of young plants using a method adapted from Doyle and Doyle (1988) for small amounts of material, with PVP 40 000 1% (p/v) and proteinase K (0, 5 mg/ml) added to the extraction buffer.

PCR amplification, restriction and electrophoresis

DNA samples were characterized by restriction studies of a mtDNA fragment [subunit 1 of NADH dehydrogenase: *nad1* (b/c) intron] amplified by PCR with one pair of universal mitochondrial primers located in the two flanking exons b and c (Demesure et al. 1995). Conditions for mtDNA amplification were: 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2 mM $MgCl_2$, 100 µM dNTP, 0.2 µM of each primer, 0.0025 U *Taq* polymerase, 20 ng of DNA in a final reaction volume of 25 µl. Amplifications were conducted in a DNA thermocycler (PTC 100, MJ Research) for 30 cycles, each consisting of a denaturing step of 45 s at 92°C, an annealing step of 45 s at 60°C and an extension step of 3 min at 72°C. The first cycle was preceded by 4 min at 94°C. The last one was followed by 10 min at 72°C to ensure that the primer extension reactions proceeded to completion.

Thirteen microliters of the PCR products were digested with 5 U of a single restriction enzyme, *Hae*III or *Msp*I, over 3 h. DNA fragments were then separated by electrophoresis on 1.5% agarose gels. Gels were photographed on a MP4 + box with Ilford FP4 plus 125 film after ethidium bromide staining.

Sequencing

PCR products were concentrated, then purified with a Prep-A-Gene Purification System (Biorad). The *Nad1* b/c intron of trees A and D were sequenced by Genome Express SA (Grenoble, France). Sequences were aligned and analyzed with the GCG (Genetic Computer Group, University of Wisconsin; Devereux et al. 1984) computer program. The sequences were deposited in the Genbank database (Accession numbers AF 142641-AF 142642).

Results

Nad1 b/c intron organisation (Fig. 1 A)

Sequencing showed a length of 2217 pb for the female *nad1* b/c PCR fragment (tree A), and 2146 pb for the male *nad1* b/c PCR fragment (tree D). Excluding the two regions 5′ and 3′ corresponding to the exons, the real sizes of the introns in trees A and D were respectively 2036 bp and 1968 bp.

Alignment between the sequences of parents A and D reveals eight substitutions. In addition, a large insertion/deletion event of 68 bp is apparent (Fig. 1 A). A 25-bp motif is present three times in the indel of tree A but only once in tree D (Fig. 1 B). The *nad1* b/c intron presents at least two characteristic features of group-II introns: one sequence (GCGCG) near the 5′ cleaving site and one sequence of domain V (GAAA loop) near the 3′ cleaving site (Chapdelaine and Bonen 1991).

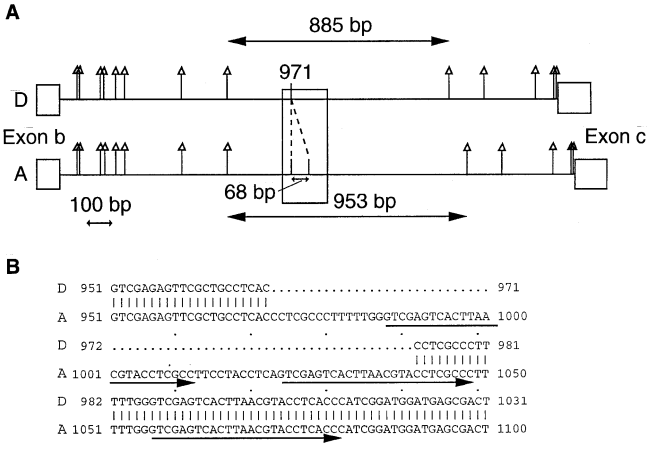

Fig. 1A Comparison of the *nad1* b/c intron organisation in two individuals of *P. abies*. The *nad1* b/c intron of the tree A (female parent) and *nad1* b/c intron of the tree D (male parent) differ by a 68-bp indel. *: polymorphic fragment revealed by electrophoresis after a *Msp*I digest. ↑: *Msp*I restriction sites. **B** The expanded region depicts in detail the indel in A and D *nad1* b/c introns and the two flanking regions (box in **A**). *Arrows* represent a 25-bp repeated sequence

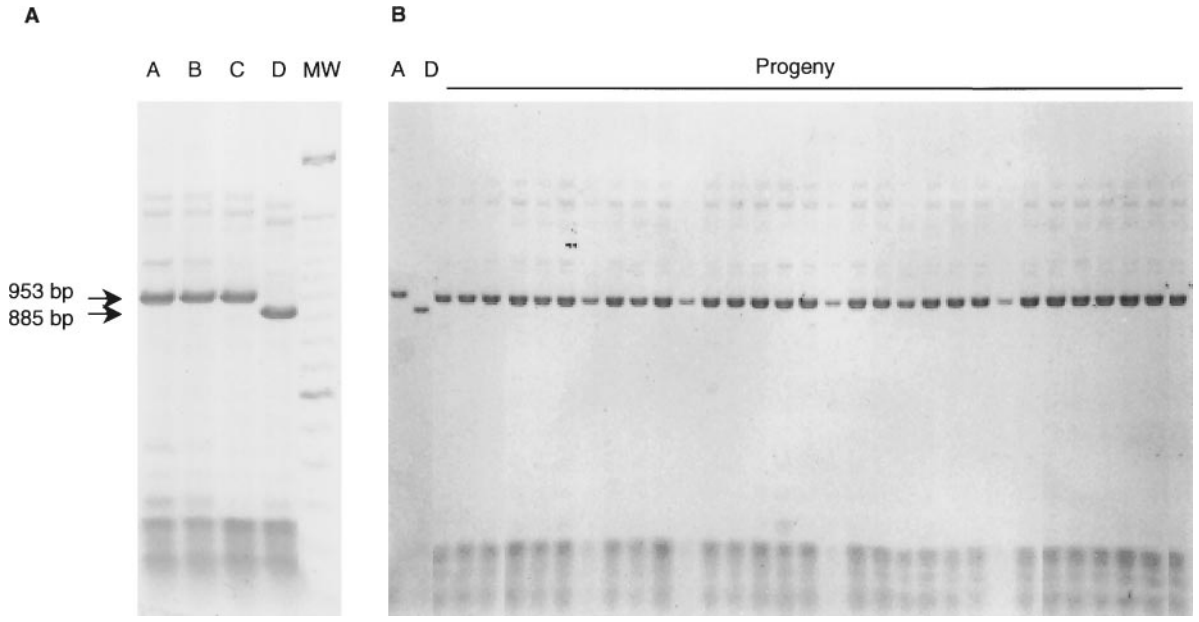

Fig. 2A, B Analysis of the mtDNA *nad1* b/c intron restricted with *Msp*I. **A** Polymorphism is only detected between trees A and D. *Msp*I polymorphic fragments are indicated by *arrows*. A is used as female parent and D as male parent in the intraspecific cross. **B** Analysis of inheritance of the *nad1* b/c intron in the intraspecific A × D cross. The progeny show the same maternal fragment as the female parent D

Mitochondrial inheritance

PCR-RFLP polymorphic patterns were revealed only in the cross involving parents A and D for the *nad1* b/c *Hae*III and *nad1* b/c *Msp*I combinations. For example, *Msp*I generated 14 fragments for each of the four parents. The polymorphism consists in length variation: the more discriminant fragment has a length of 953 bp in trees A, B and C, and of 885 bp in tree D (Fig. 2 A). All the 96 analysed offspring from the A × D cross contained the maternal fragment (Fig. 2 B).

Discussion

The sequencing results shows that the *nad1* b/c introns of *P. abies* exhibit characteristic features of the group-II introns. They also show a high degree of similarity between the two trees analysed (A and D). Both extremities (5′ and 3′ ends) are well conserved, in contrast with the middle part of the introns which is divergent (an insertion/deletion event of 68 bp explaining an important part of the sequence divergence). This result is concordant with previous studies on the organisation of this type of intron group in plants (Clegg et al. 1994; Gielly and Taberlet 1994). Indeed, group-II introns are subdivided into six different domains exhibiting variable evolutionary rates in relation to, and the functional importance of, intron structural features (Wissinger et al. 1991; Clegg et al. 1994). For example, domains I and V are important for intron processing and evolve at the slowest rate.

Comparison of the *P. abies nad1* b/c intron with the homologous *Pinus sylvestris* sequence (from Genbank) showed that more than 95% similarity could be observed in the first 750 bp (5′ end) and in the last 650 bp (3′ end) of the alignment. As previously described in the intraspecific comparison (trees A and D), the sequence divergence between the *P. abies* and *P. sylvestris* introns is mostly located in the central part of the intron. In contrast, comparison with four angiosperms (sequences from Genbank and Embl) showed strong differences in length. In *Arabidopsis thaliana*, *Triticum aestivum*, *Oenothera berteriana*, and *Petunia hybrida* the intron length is respectively 893 bp, 1422 bp, 1463 bp and 986 bp. Compared to the sequences of tree A (2035 bp), tree D (1967 bp) and *P. sylvestris* (2410 bp) the angiosperm sequences are much shorter. Hence, similarity scores could not be computed between angiosperms and gymnosperms for the complete sequence. However, high degrees of similarity (respectively 92% and 93%) restricted to the first 323 bp (5′ end) and to the last 67 bp of the intron (3′ end) can be observed.

Comparison of *nad1* b/c introns between *P. abies* and *P. sylvestris* could help to design additional primers located in conserved regions. These primers could be used to selectively amplify the more polymorphic central region, and to identify polymorphism without the need of an additional restriction step. Such a marker could then be employed to examine inter- or intrageneric phylogenetic relationships in conifers. Actually, the mtDNA region that we have studied showed additional polymorphisms between trees from different provenances of *P. abies* (Sperisen et al. 1998; Grivet et al., unpublished results).

Analysis of the *nad1* b/c intron pattern in the A × D controlled cross confirmed the non-Mendelian inheritance of the mitochondrial genome. A maternal inheritance for mtDNA, corroborating earlier studies in the *Picea* genus, was demonstrated. However, it is noteworthy that some examples of the partial parental transmission of mitochondria have been reported in a number of gymnosperms. This is the case in the interspecific controlled crosses between *P. banksiana* × *P. contorta*, or of the intraspecific controlled crosses in *P. contorta* where about 7.1% of the offspring have inherited their mitochondria from the male parent (Wagner et al. 1991). Similarly, 10% of the mitochondria of *Pseudotsuga menziesii* were shown to be paternally inherited (Marshall and Neale 1992). Low levels of paternal leakage in an otherwise predominantly maternal lineage could have dramatic effects on the observed genetic structure (Petit et al. 1993; Dumolin-Lapègue et al. 1998). Therefore, it seems important to take into account the possibility of paternal or biparental inheritance in which at least some offspring contain the paternal organelle type. According to the binomial model of organelle inheritance (Milligan 1992), and taking into account the fact that no offspring containing paternally derived organelles were found among the 96 samples tested in the A × D *P. abies* cross, it can be concluded that, at the 95% confidence limit, the degree of paternal transmission is no more than 3% in the studied cross. Hence, rare events of paternal contribution cannot be discarded with the sample size. Other mtDNA markers should be identified and additional crosses analysed to confirm these results on the inheritance of mtDNA and to reveal additional mtDNA diversity in *P. abies*.

Acknowledgments The authors thank Bernard Héois (CEMAGREF, Nogent sur Vernisson) for providing the plant material used in this work and Rémy Petit (INRA, Pierroton) for comments on a previous draft of this manuscript.

References

Avise JC (1994) Molecular markers, natural history and evolution. Chapman and Hall, New York

Bobola MS, Eckert RT, Klein AS, Stapelfeldt K, Smith DE, Guenette D (1996) Using nuclear and organelle DNA markers to discriminate among *Picea rubens*, *Picea mariana* and their hybrids. Can J For Res 26 : 433–443

Bruns D, Owens JN (1989) Mechanisms of cytoplasmic inheritance in western white pine. In: Bailey GW (ed) Proc 47th Meeting Electron Microscopy Society of America. San Francisco Press, San Francisco, pp 766–767

Chapdelaine Y, Bonen L (1991) The wheat mitochondrial gene for subunit I of the NADH dehydrogenase complex: a *trans*-splicing model for this gene-in pieces. Cell 65 : 465–472

Clegg MT, Gaut BS, Learn G, Morton BR (1994) Rates and patterns of chloroplast DNA evolution. Proc Natl Acad Sci USA 91 : 6795–6801

David AJ, Keathley DE (1996) Inheritance of mitochondrial DNA in interspecific crosses of *Picea glauca* and *Picea omorika*. Can J For Res 26 : 428–432

Devereux J, Haeberli P, Smithies O (1994) A comprehensive set of sequence analysis program for the VAX. Nucleic Acids Res 12 : 387–395

Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol Ecol 4 : 129–131

Doyle JJ, Doyle JL (1988) Isolation of plant DNA from fresh tissue. Focus 12 : 13–15

Dumolin-Lapègue S, Pemonge M-H, Petit RJ (1998) Association between chloroplast and mitochondrial lineages in oaks. Mol Biol Evol 15 : 1321–1331

Gielly L, Taberlet P (1994) The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbc*L sequences. Mol Biol Evol 11 : 769–777

Huntley B, Birks HJB (1983) An atlas of past and present pollen maps for Europe: 0–13 000 years ago. Cambridge University Press, Cambridge, UK

Kremer A (1994) Diversité génétique et variabilité des caractères phénotypiques chez les arbres forestiers. Genet Selec Evol 26 : 105s–123s

Lagercrantz U, Ryman N (1990) Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. Evolution 44 : 38–53

Marshall KA, Neale DB (1992) The inheritance of mitochondrial DNA in Douglas-fir (*Pseudotsuga menziesii*). Can J For Res 22 : 73–75

Milligan BG (1992) Is organelle DNA strictly maternally inherited? Power analysis of a binomial distribution. Am J Bot 79 : 1325–1328

Neale B, Sederoff RR (1988) Inheritance and evolution of conifers organelle genomes. In: Hanover JW, Keathley DE (eds) Genetic manipulation of woody plants. Plenum Press, New York, pp 251–264

Neale B, Sederoff RR (1989) Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor Appl Genet 77 : 212–216

Petit RJ, Kremer A, Wagner DB (1993) Finite island model for organelle and nuclear genes in plants. Heredity 71 : 630–641

Schmidt-Vogt (1986) Die fichte. Band II/I. Ed. Parey, Hamburg Berlin

Sperisen C, Buchler U, Gabor M (1998) Genetic variation of mitochondrial DNA reveals subdivision of norway spruce (*Picea abies* (L.) KARST). In: Molecular tools for screening biodiversity. Chapman and Hall, UK, pp 413–417

Sutton BCS, Flanagal DJ, El-Kassaby YA (1991 a) A simple and rapid method for estimating the representation of species in spruce seedlots using chloroplast DNA restriction fragment length polymorphism. Silvae Genet 4 : 119–123

Sutton BCS, Flanagan DJ, Gawley JR, Newton CH, Lester DT, El-Kassaby YA (1991b) Inheritance of chloroplast and mitochondrial DNA in *Picea* and composition of hybrids from introgression zones. Theor Appl Genet 82 : 242–248

Wagner DB, Dong J, Carlson MR, Yanchuk AD (1991) paternal leakage of mitochondrial DNA in *Pinus*. Theor Appl Genet 82 : 510–514

Wissinger B, Schuster W, Brennicke A (1991) *Trans*-splicing in *Oenothera* mitochondria: *nad1* mRNAs are edited in exon and *trans*-splicing group-II intron sequences. Cell 65 : 473–482