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Paternal inheritance of chloroplast DNA in *Larix*

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Abstract

Restriction enzyme analysis was used to determine the inheritance of chloroplast DNA in conifers. The plant material studied included five individual trees of European larch (*Larix decidua* Mill.) and Japanese larch (*Larix leptolepis* Sieb. & Zucc.) and six hybrids from controlled crosses between these species. The chloroplast DNA fragment patterns generated by *Bam-HI* and *Bcl-I* were species-specific. Paternal inheritance of chloroplast DNA patterns was found in most *Larix* crosses. One hybrid showed maternal chloroplast DNA patterns. In addition, two other hybrids had mixed *Bam-HI* patterns suggesting recombination between maternal and paternal chloroplast DNA. The mechanisms favoring paternal inheritance in conifers are not known. Paternal inheritance of chloroplast DNA is suggested it to be a general phenomenon in conifers.

Introduction

Chloroplasts as a genetic system have been indicated since 1909 when Bauer [1] discovered non Mendelian heritable traits. The inheritance of cytoplasmic organelles has been studied since then, and it has been concluded that maternal inheritance of chloroplasts and mitochondria is common in the plant kingdom [12, 15].

Recently however, Medgyesy *et al.* [9] reported that the inheritance of chloroplast DNA (cpDNA) in *Nicotiana* could sometimes be both paternal and maternal. Neale *et al.* [10] recently reported paternal inheritance of chloroplast DNA in Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco.).

Our objective was to determine the inheritance of cpDNA in conifers. To facilitate the present analysis of inheritance, controlled crosses between European larch (*Larix decidua* Mill.) and Japanese larch (*Larix leptolepis* (Sieb. & Zucc.) Gord.) were studied. CpDNAs of parental species and their hybrids were investigated by restriction enzyme analysis. The results from these analyses are described, and discussed in relation to differences between conifers and other species.

Material and methods

Plant material

The plant material analysed in this study was provided by the Institute for Forest Improvement at Ekebo research station, located in southern Sweden. Controlled crosses between these species were made in 1976 and 1978.

Twigs from parental species and hybrids were collected in early September and transported by air to Umeå, in a cold box. Reciprocal crosses of *Larix* where exactly the same individuals have been used as both female and male gamete donors were not available. Hence, we had to use existing crosses of

Table 1a. Trees of *Larix* species investigated.

Larix decidua		*Larix leptolepis*	
Tree	Clone	Tree	Clone
Ld-1	N-2001	Ll-1	M-2001
Ld-2	E-2009	Ll-2	L-2005
		Ll-3	M-2002

Table 1b. Hybrid crosses between *Larix* species analysed.

Hybrid no.	Cross	
	♀ *L. leptolepis*	× ♂ *L. decidua*
1. HLlLd-1	Ll-2	× Ld-3
2. HLlLd-2	Ll-1	× Ld-4
3. HLlLd-3	Ll-1	× Ld-5
Hybrid no.	Cross	
	♀ *L. decidua*	× ♂ *L. leptolepis*
1. HLdLl-1	Ld-6	× Ll-4
2. HLdLl-2	Ld-7	× Ll-5
3. HLdLl-3	Ld-8	× Ll-6

Larix where different trees had been used as parents. A list of the five parental trees and crosses is presented in Table 1.

Isolation of cpDNA

Chloroplast DNA was isolated from needles as earlier described by Szmidt *et al.* [14]. The extraction procedure was a modification of the method presented by White [16]. A short summary of the method is given below:

Needles, 100 g fresh weight, were frozen in liquid nitrogen and ground to a fine powder. The powder was mixed with extraction medium, filtered and washed several times by centrifugation. The chloroplasts were separated by sucrose step-gradient centrifugation and lysed by adding Triton X-100. The cpDNA was recovered after CsCl gradient centrifugation, dialysed and digested with restriction enzymes.

Restriction enzyme analysis of cpDNA

The following restriction enzymes were used: *Bam-HI, Bcl-I, Hind-III, Sac-I* and *Xho-I* (Boehringer, Mannheim). One-μg samples of cpDNA were digested with 10 μ of each enzyme according to the supplier's instruction. The cpDNA fragments were separated by agarose gel electrophoresis as described by Maniatis *et al.* [7]. Gels were photographed in UV using 665 or 667 films (Polaroid). The length of cpDNA fragments was determined according to the method described by Shaffer and Sederoff [12] using a BASIC computer program designed by A.E. Szmidt.

Results and discussion

No variation in the restriction pattern of cpDNAs was found among individual trees of *L. leptolepis* or of *L. decidua*, with the five restrictases tested. Similar result has earlier been observed for *Pinus sylvestris* and *Pinus contorta* [14]. Conservative nature of cpDNA has also been reported for many angiosperms [2].

Comparison of cpDNA restriction patterns between different *Larix* species also showed much homology. In this study, *Hind-III, Sac-I* and *Xho-I* gave identical cpDNA patterns in the two species of *Larix* as well as in their hybrids and were not investigated further. However, there were consistent differences in cpDNA patterns generated by *Bam-HI* and *Bcl-I* between the two species. Evidence for interspecific variation among other gymnosperms has earlier been provided [5, 14].

Restriction patterns generated by *Bcl-I* and *Bam-HI* are presented in Figs. 1 and 2. The size (in kb) of cpDNA restriction fragments in the two species is demonstrated in Table 2. *L. leptolepis* and *L. decidua* were different in *Bcl-I* restriction patterns with respect to one fragment of approximately 2.68 kb which was absent in the latter species but always present in *L. leptolepis* (Table 2). As regards *Bam-HI* pattern the two parental species differed with respect to size of two fragments, 4.89/4.74 kb and 2.18/2.23 kb, respectively (Table 2).

Figure 1 shows the *Bcl-I* restriction enzyme patterns of the two *Larix* species and the hybrids. Except for one individual, lane 6, Fig. 1, all hybrids tested showed paternal inheritance of cpDNA. The

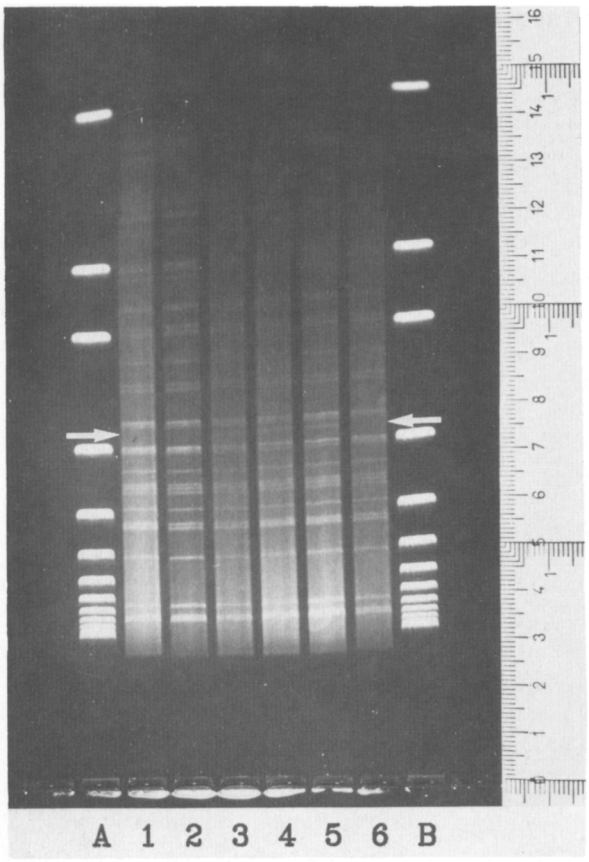

Fig. 1. Bcl-I digests of cpDNA from *Larix leptolepis* (Ll) and *Larix decidua* (Ld) and their hybrids separated by 0.8% agarose gel electrophoresis. Lane 1: Ld-1, Lane 2: HLlLd-3, Lane 3: HLlLd-1, Lane 4: Ll-1, Lane 5: HLdLl-3, Lane 6: HLlLl-1. Lanes A and B: 1 kb DNA ladder (BRL) used as molecular weight standard. Regions showing differences between the samples are indicated by arrows.

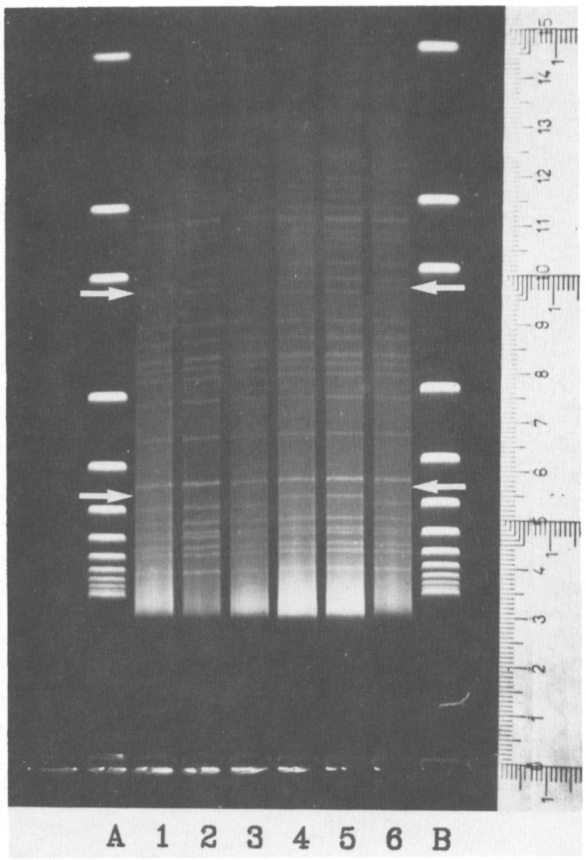

Fig. 2. Bam-HI digests of cpDNA from *Larix leptolepis* (Ll) and *Larix decidua* (Ld) and their hybrids separated by 0.8% agarose gel electrophoresis. Lane 1: Ld-1, Lane 2: HLlLd-3, Lane 3: HLlLd-1, Lane 4: Ll-1, Lane 5: HLdLl-3, Lane 6: HLlLl-1. Lanes A and B: 1 kb DNA ladder (BRL) used as molecular weight standard. Regions showing differences between the samples are indicated by arrows.

restriction pattern of cpDNA in the deviating tree showed a maternal inheritance. Similar result was observed for the *Bam-HI* cpDNA pattern in the same hybrid, lane 6, Fig. 2.

Analysis of inheritance of *Bam-HI* fragments was more complicated. Three hybrids showed strictly paternal inheritance of *Bam-HI* fragments. Two of these hybrids are shown in Fig. 2, lanes 2 and 5. The cpDNA patterns in the two remaining hybrids were of a mixed character, one of them is shown in Fig. 2, lane 3. In these hybrids a *L. decidua*-specific (4.74 kb) fragment was observed. However, they also possessed a 2.18 kb fragment which was specific for *L. leptolepis*.

The results presented show that paternal inheritance of cpDNA dominates among *Larix* hybrids. However, cpDNA patterns found in one hybrid indicate that maternal inheritance cannot be excluded. It is possible however, that isolation of flowers was imperfect. Contamination with foreign pollen can thus not be excluded in this case.

Paternal inheritance of cpDNA was also recently observed in *Pseudotsuga menziesii* [10]. These results are in contrast to the maternal inheritance of cpDNA observed in angiosperms [15]. However, recently 0.07 to 2.5% paternal inheritance of

Table 2. Size in kb of cpDNA fragments from *L. decidua* and *L. leptolepis* generated by digestions with the restriction endonucleases *Bcl-I* and *Bam-HI*. Numbers in brackets refer to multiple bands.

Fragment no.	*L. decidua* *Bam-HI*	*L. leptolepis* *Bam-HI*	*L. decidua* *Bcl-I*	*L. leptolepis* *Bcl-I*
1.	8.40	8.40	9.72	9.72
2.	7.01	7.01	9.42	9.42
3.	6.57	6.57	7.71	7.71
4.	6.01	6.01	4.93	4.93
5.	5.86	5.86	4.19	4.19
6.	5.45	5.45	4.11	4.11
7.	>4.74	>4.89	3.67	3.67
8.	4.35 (×2)	4.35 (×2)	3.48	3.48
9.	3.59	3.59	3.42	3.42
10.	3.16	3.16	3.35	3.35
11.	2.94 (×2)	2.94 (×2)	3.09	3.09
12.	2.85	2.85	2.85 (×2)	2.85 (×2)
13.	2.78 (×2)	2.78 (×2)	-	2.68
14.	2.66	2.66	2.60 (×2)	2.60 (×2)
15.	2.49	2.49	2.36	2.36
16.	>2.23	>2.18	2.33	2.33
17.	2.09	2.09	2.15	2.15
18.	1.97	1.97	1.98	1.98
19.	1.68	1.68	1.91	1.91
20.	1.61	1.61	1.86 (×2)	1.86 (×2)
21.	1.51 (×2)	1.51 (×2)	1.76	1.76
22.	1.47	1.47	1.62	1.62
23.	1.34	1.34	1.56 (×2)	1.56 (×2)
24.			1.50	1.50
25.			1.31	1.31
26.			1.25	1.25
27.			1.21	1.21
28.			1.17	1.17
29.			1.02	1.02
30.			0.96	0.96
31.			0.88	0.88
32.			0.78	0.78
33.			0.76	0.76

cpDNA was reported in *Nicotiana* [9]. It is possible that paternal cpDNA inheritance is characteristic for conifers. As shown in Fig. 3, pollen cytoplasm containing plastids is transferred to the egg cell in conifers [6]. Thus, the male organelles are likely to be passed on to the zygote. This observation supports our suggestion on the paternal cpDNA inheritance. However, it is unclear why such inheritance should be favored in these species. Similarly, nothing is known about the processes responsible for elimation of female cpDNA from the hybrid trees. They may be of similar nature to some of the processes elimating male cpDNA in angiosperms [3, 13, 15].

An unexpected result observed in this study was the presence of a mixed *Bam-HI* pattern in two hybrids. At least three different suggestions can be advanced to explain this observation.

The first is the occurrence of spontaneous point mutation. We find it unlikely however, that similar mutations occurred independently in two different individuals.

The second is the existence of intraspecific variation in *Larix* with respect to *Bam-HI* recognition

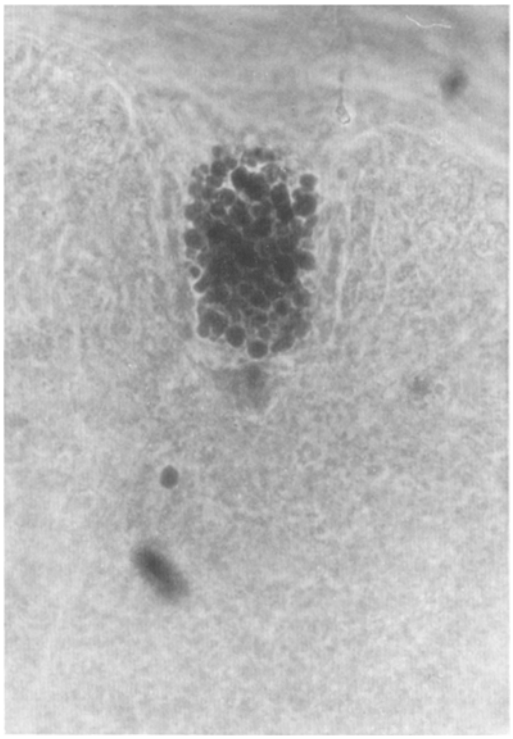

Fig. 3. Pine pollen tube carrying massive amounts of proplastids, stained for starch with iodine, during fertilization of the egg cell.

sites. If this were the case, cpDNA patterns observed in the two particular hybrids could be delivered by the male *L. decidua* parent. However, two individuals of *L. decidua* available for analysis showed *Bam-HI* patterns which were different to those found in the deviating hybrids, but identical to those in hybrids showing paternal cpDNA inheritance.

The third suggestion includes the possibility of intermolecular recombination between the parental cpDNAs. The occurrence of both intra- and intermolecular recombination between cpDNAs was suggested by several authors [4, 11]. Recently, experimental evidence of cpDNA recombination has been provided [8]. We found no difference in the cpDNA patterns between the deviating hybrids.

If the observed mixed cpDNA patterns were a result of recombination it must have occurred within the same regions of cpDNA in both hybrids. As discussed by Palmer [11] the existence of site-specific recombination of cpDNA can not be excluded. In such a case however, the recombinant molecules should have an advantage over the parental cpDNA types. Otherwise, both parental cpDNA fragments should be found, which was not the case in this study.

The data presented here do not allow us to make a clear choice between the last two suggestions put forward to explain mixed *Bam-HI* patterns. Further studies of mechanisms of cpDNA inheritance in conifers and comparisons with angiosperms are of interest.

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References

1. Bauer E: Das Wesen und sie Erblichkeitverhältnisse der "Varietates albomarinatae hort" von *Pelargonium zonale*. Z Vererb Lehre, 1: 333–351 (1909).
2. Dyer TA: The chloroplast genome and its products. Oxford Surv Plant Mol & Cell Biol 2: 147–177 (1985).
3. Hagemann R: Unequal plastid distribution during the development of male gametophyte of angiosperms. Acta Soc Bot Pol 50: 321–327 (1981).
4. Kolodner R, Tewari KK: Inverted repeats in chloroplast DNA from higher plants. Proc Natl Acad Sci USA 76: 41–45 (1979).
5. Kondo T, Ishibashi T, Shibata M, Hirai A: Isolation of Chloroplast DNA from Pinus. Plant Cell Physiol 27: 741–744 (1986).
6. Hamnede M: Fröforskning V. En metod att studera fröutveckling. K Skogs- o Lantbr akad tidskr 125: 193–202 (1986).
7. Maniatis T, Fritsch EF, Sambrook J: Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory (1982).
8. Medgyesy P, Fejes E, Maliga P: Interspecific chloroplast recombination in a *Nicotiana* somatic hybrid. Proc Natl Acad Sci USA 82: 6960–6964 (1985).

9. Medgyesy P, Pay A, Marton L: Transmission of paternal chloroplasts in *Nicotiana*. Mol Gen Genet 204: 195–198 (1986).
10. Neale DB, Wheeler NC, Allard RW: Paternal inheritance of chloroplast DNA in Douglas-fir. Can J For Res 16: 1152–1154 (1986).
11. Palmer JD: Chloroplast DNA exists in two orientations. Nature 301: 92–93 (1983).
12. Schaffer HE, Sederoff R: Improved estimation of DNA fragment lengths from agarose gels. Anal Biochem 115: 113–122 (1981).
13. Sears BB: Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4: 233–255 (1980).
14. Szmidt AE, Lidholm J, Hällgren J-E: DNA extraction and preliminary characterization of chloroplast DNA from *Pinus sylvestris* and *Pinus contorta*. In: Lindgren D (ed) Provenances and Forest Tree Breeding for High Latitudes, pp 269–280. Proc of the Frans Kempe symposium, Umeå, June 10–11 (1986).
15. Tilney-Bassett RAE: The inheritance and genetic behaviour of plastids. In: Kirk JTO, Tilney-Basset RAE (eds) The Plastids. Their Chemistry, Structure, Growth and Inheritance, pp 251–254. Amsterdam New York Oxford: Elsevier/North Holland (1978).
16. White EE: A method for extraction of chloroplast DNA from conifers. Plant Mol Biol Rep, in press (1986).