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SHORT COMMUNICATION

Effect of fluoride pollution on genetic variability of *Larix gmelinii* (Pinaceae) in East Siberia

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Abstract The genetic variability of four natural populations of *Larix gmelinii* from East Siberia was determined and compared by RAPD analysis. Comparison of the RAPD profiles provided an estimation of variability in 193 RAPD fragments. More than 89% of these fragments were found to be polymorphic. The main genetic variability parameters of the two populations from Central Yakutia, a region free of fluoride pollution, had considerably higher values than those from East Transbaikalia, a region potentially affected by fluoride pollution (FLU, near a fluorite quarry growing on soils with a high natural content of fluorides). AMOVA revealed that 72.94% of the variation was within populations, while only 7.05% of the variation was between populations within geographical regions. The genetic diversity of the FLU fluoride-tolerant population was the lowest, but only slightly lower than that of a fluoride non-tolerant population from Chita, 50 km distant from FLU. Although this study demonstrates the absence of fundamental alterations of genetic structure within the populations of *L. gmelinii* growing on soils with a high content of fluorides, it is presumed that the reduction of genetic diversity was the genetic response of the FLU population to such an environmental stress as a constantly high concentration of fluorides within the soil.

Keywords AMOVA · Fluoride · *Larix gmelinii* · Population structure · RAPD analysis

Introduction

The Dahurian larch *Larix gmelinii* (Rupr.) Rupr. is one of the most widely distributed species of the genus, occurring in northern and eastern Siberia, north-eastern Mongolia, northern and north-eastern China, Korea and the Russian far east in a variety of site and soil conditions (Farjon 1990). Some regions of Siberia are characterized by a high level of atmospheric pollution due to industrial emissions. Atmospheric fluorides are probably the most phytotoxic compounds among other pollutants (Smith 1990; Rozhkov and Mikhailova 1993). Gaseous fluorides are absorbed through leaf stomata and directly influence the plant and may cause injury. Plants also incorporate fluorides from soil (Arnesen and Krogstad 1998; Domingos et al. 2003). In the case of fluoride pollution of both air and soil, direct absorption of airborne fluorides by plant foliage masks soil uptake (Kabata-Pendias and Pendias 1984; Vike and Habjorg 1995). Numerous investigations have been carried out to examine the influence of fluorides on plants (Miller 1993; Fornasiero 2001; Domingos et al. 2003).

Many chemical contaminants have been demonstrated to induce genetic mutations and therefore affect the genetic structure of populations (Fishbein et al. 1970). The effects of heavy metals on the genetic diversity of plant populations are well known. In particular, heavy metals were assumed to contribute to the lower genetic diversity found at the urban sites (Keane et al. 2005). It has been shown that some plant species had lower values of genetic diversity in the heavy metal-tolerant ecotypes compared with non-tolerant ones (Bush and Barrett 1993; Vekemans

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and Lefèbvre 1997; Nordal et al. 1999; Mengoni et al. 2001). To our knowledge, however, no studies to date have evaluated the effect of fluorides on the genetic structure of natural conifer populations.

In the present study, we applied RAPD analysis and the analysis of molecular variance (AMOVA) technique to investigate the genetic diversity of four natural populations of *L. gmelinii* affected to a various degree by fluoride pollution from the fluorite quarries and industrial plants.

Materials and methods

Four natural populations of *L. gmelinii* from two different regions of East Siberia were chosen for analysis (Table 1). Two populations from East Transbaikalia might in principle be affected (to a greater or lesser degree) by fluoride pollution from fluorite quarries and industrial plants. Transbaikalia is a single region in Asian Russia where numerous deposits of fluorite (at least ten) have been discovered (Eremin 2004). The individuals of the first population (code-named FLU) grew in the territory of the Solonechny fluorite quarry (Chitinskaya oblast, East Transbaikalia) on soils containing a naturally high content of the fluorite ion. The trees of the FLU population demonstrated the morphological changes compared with a normal phenotype. The second population is in the Chita region (CHIT) 50 km west of the fluorite quarry and grows on soils containing normal concentrations of the fluorite ion. The trees of the CHIT population had a normal phenotype. The sampled trees from these populations were 30 to 35 years old. Two further populations were sampled, from Pokrovsk (PKR) and Namtsy (NMT) in Central Yakutia (approximately 1,350 km distant from the CHIT and FLU populations), 30 km south of Yakutsk and approximately 40 km north of Yakutsk, respectively. The PKR and NMT populations were free of any impact of fluoride pollution as this region lacks fluoride-generating industry and fluorite deposits.

Total genomic DNA was extracted from 50–70 mg of fresh young needles according to Doyle and Doyle (1987). RAPD analysis was carried out using primers OPA-11, OPA-19, OPA-04, OPA-09, OPB-10, OPB-11, OPC-19 and OPC-02, chosen from a set of primers which were effective in the PCR reactions with the larch DNA templates and generated the highest number of the fragments (Sazonova et al. 2001). Each reaction was repeated twice or four times and only those bands that could be scored without ambiguity were scored.

For each primer, bands were scored as either present (1) or absent (0). Both POPGENE (Yeh and Boyle 1997) and TFPGA (Miller 1997) software was used to estimate the frequency of each band, the proportion of polymorphic loci at $P < 0.05$ (P_{95}), expected heterozygosities (He) and Shannon's index of gene diversity (SI). A dendrogram was constructed based on the Nei's genetic distance (Nei 1978) between populations by applying the unweighted pair group method (UPGMA). Binomial RAPD matrix was also applied to an analysis of molecular variance (AMOVA, implemented in ARLEQUIN 3.01; Excoffier et al. 2005). The hierarchical analysis was conducted at three levels: (1) among geographic groups; (2) among populations within groups; and (3) within populations. For each analysis, 50,000 permutations were performed to test the significance of the variance components.

Results and discussion

RAPD is a relatively fast and low-cost technique that has found the widest application in analyses of genetic variation below the species level, despite their drawbacks (Renau-Morata et al. 2005). Recent comparisons of different nuclear DNA markers for estimating intraspecific genetic diversity in plants demonstrated that estimates derived by the dominantly inherited markers (RAPD, AFLP and ISSR) are very similar and may be directly comparable (Nybom 2004). RAPDs proved to be a successful method for the detection

Table 1 Geographic information on four populations of *L. gmelinii* from East Siberia included in the study

Region	Population (code)	Latitude, longitude	No. of samples	Concentration of fluorite ion in soil (%)[a]
Transbaikalia	Fluorite (FLU)	51°30′N, 118°50′E	21	0.195–1.540
	Chita (CHIT)	51°35′N, 118°20′E	14	0.044–0.046
Yakutia	Pokrovsk (PKR)	61°48′N, 129°31′E	14	0.060–0.063[b]
	Namtsy (NMT)	62°20′N, 129°48′E	14	0.060–0.063[b]

[a] Fluorite ion mass/dried soil mass ratio expressed as a percentage

[b] Average concentration in the Earth's crust (Dobrovolsky 1983), precise data are absent

of genetic variability in natural populations of *Larix* species (Sazonova et al. 2001).

Comparison of the RAPD profiles provided an estimation of the variability of 193 RAPD fragments. As a result, 185 of these fragments (89.4%) were found to be polymorphic. The number of fragments in the RAPD profiles varied from 16 to 33 depending on the primer used, constituting on average 24.1 loci per primer. The population's diagnostic fragments of *L. gmelinii* were totally absent. All diversity parameters (P_{95}, He and SI) for individual populations showed the same patterns FLU < CHIT < NMT < PKR, and these same parameters for the Yakutia group were more than 1.3–1.4 times higher than those for the Transbaikalia group (Table 2). Nearly as wide a range of genetic variability has already been reported in this species. Semerikov et al. (1999) examined allozyme diversity in six populations of *L. gmelinii* from East Siberia, and found that *P* varied from 66.0 to 73.3% and He from 0.140 to 0.186. The lowest values of these parameters were derived from the Nerungri population (56°44′N, 124°42′E), whose environmental condition is unfavorable (i.e., coal-mining and a dressing plant for the production of metallurgical coal concentrate).

The extent of variation Shannon's indices recorded in *L. gmelinii* populations (0.24–0.35) was quite concordant with that in *Cedrus atlantica* (0.210–0.316; Renau-Morata et al. 2005) and lower than in *Fitzroya cupressoides* (0.349–0.648; Allnut et al. 1999). The genetic distances calculated over 193 RAPD loci for pairs of populations varied to a considerable degree, reaching a sixfold difference. The population PKR was the most genetically distant from the population FLU (0.0909), while FLU and CHIT were closest to each other (0.0132). The genetic distance between the two groups was 0.0582. In an UPGMA cluster analysis, the populations were grouped together according to their geographic location (Fig. 1).

Table 2 Genetic variability in natural populations of *L. gmelinii*

Population	P_{95} (%)	He	SI
FLU	46.4	0.153 (0.16)	0.241 (0.23)
CHIT	48.8	0.170 (0.17)	0.261 (0.25)
Transbaikalia samples	52.7	0.165 (0.16)	0.262 (0.23)
PKR	63.8	0.228 (0.19)	0.335 (0.26)
NMT	59.9	0.205 (0.18)	0.309 (0.26)
Yakutia samples	69.1	0.231 (0.17)	0.351 (0.24)
Total	68.1	0.211 (0.16)	0.346 (0.22)

Standard deviations are in parentheses

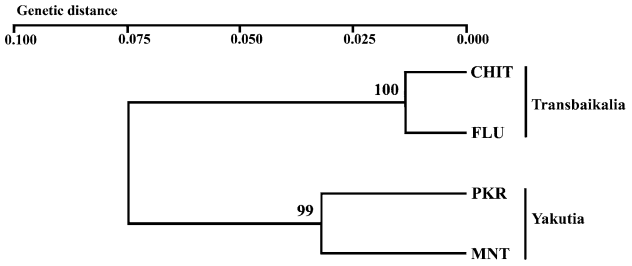

Fig. 1 UPGMA dendrogram of genetic relationships between four populations of *L. gmelinii* from East Siberia. The numbers at the nodes indicate the clustering reliability values (bootstrap index)

AMOVA revealed that 72.94% of the total variation was within populations (Table 3), a result congruous with those from most other woody perennial, outbreeding plant species, especially conifers (Hamrick et al. 1992). Genetic variation among Yakutia and Transbaikalia regions was higher (20%) than the genetic variation among populations within these regions (7%). Such a pattern of genetic differentiation may be explained by both geographic and environmental effects.

RAPD analysis detected no significant difference in genetic diversity parameters between the two populations from Transbaikalia. CHIT and FLU populations are characterized by the lowest values of between-population differentiation and genetic distance. This clearly indicates that these populations are genetically very similar. Considerable morphological differences, however, were found between them: the trees of the FLU population are characterized by some variation compared with a normal phenotype (Rozhkov and Mikhailova 1993). The genetic diversity of the FLU population was lower than that of the CHIT population. This demonstrated that the FLU population is situated in a less favorable environmental condition than that of the CHIT population and is under stronger selection pressure (Altukhov 2004). Some morphological peculiarities of the FLU tree population (e.g., the morphology of needles) are probably adaptations to a high soil concentration of fluorides. As a result, the fluoride-tolerant population arose under selective pressure during thousands of years. An analysis of responses to fumigation with hydrogen fluorides and other experiments showed that the FLU population is indeed fluoride-tolerant (Rozhkov and Mikhailova 1993). The relationship of the FLU and CHIT populations towards fluorides agree well with different relationships of tolerant and non-tolerant populations towards chemical agents in other plant species (Bush and Barrett 1993; Vekemans and Lefèbvre 1997; Nordal et al. 1999; Mengoni et al. 2001).

In general, the within-population genetic diversity of both the FLU and CHIT populations compared with the Yakutia populations is low. These populations may theoretically be affected by fluoride emissions, but to differing degrees. The CHIT population is situated about 150 km to the east of the Zabaikal mining and concentrating combine

Table 3 Hierarchical analysis of molecular variance for 63 individuals of *L. gmelinii* representing four populations in two geographic regions (Yakutia and Transbaikalia)

Source of variation	*df*	Sum of squares	Variance components	Percentage total	Fixation index
Among regions	1	267.96	6.64	20.02	$F_{CT} = 0.200$*
Among populations within geographic regions	2	120.42	2.34	7.05	$F_{SC} = 0.088$*
Within populations	59	1,427.90	24.20	72.94	$F_{ST} = 0.271$*

df Degrees of freedom

* $P < 0.00001$

(JSC ZabGOK) that produces fluorite–tantalum and niobium concentrates, about 100 km north-east of the Kalanguy fluorite deposit, and about 50 km west of the Solonechny fluorite quarry. These, as well as other possible sources of fluorides, might theoretically affect this population, as particulate fluorides may be transferred by wind for a distance of more than 100 km (Rozhkov and Mikhailova 1993). If there is any such effect, however, it should be minimal, because of the great distance from potential sources of fluorides, and also as particulate fluorides are less toxic than gaseous.

It was assumed in a previous, non-genetic study of this population that because it adapted to a high soil concentration of fluorides during thousands of years, this should be reflected in its genotype (Rozhkov and Mikhailova 1993). Our results indicate that the high concentration of fluoride in the soil did not fundamentally alter the genetic structure of *L. gmelinii*, although some reduction of genetic diversity in the FLU population compared with the other populations was apparently the genetic response to this environmental stress. Future investigations should clarify if lower parameters of genetic variability in the Transbaikalia populations are connected with higher gene flow between them or with general fluoride "infection" in this region because of numerous fluorite deposits.

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