A CHLOROPLAST DNA PROBE IDENTIFIES UNEXPECTEDLY HIGH LEVELS OF POLYMORPHISM IN PINUS BANKSIANA AND PILAUS CONTORTA

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Abstract.--A cloned, 500 base pair (bp) restriction fragment from the chloroplast genome of Pinus contorta hybridized to restriction fragments of a previously known polymorphism when used in molecular mapping experiments with total cellular DNAs of P. banksiana and P. contorta. Unexpectedly however, a large number (as many as 20) of additional, extremely polymorphic, DNA fragments were also identified by this probe. Study of a large number of individuals of P. banksiana and P. contorta, using the cloned 500 bp P. contorta fragment, leads to the following conclusions: 1) in random samples from across the allopatric ranges of the two species, as well as in samples from two populations of a sympatric region shared by P. banksiana and P. contorta, many individuals have identifiably unique autoradiogram patterns; 2) in a restricted set of germplasm, representing seven individuals from a single P. banksiana provenance, nearly every individual has an identifiably unique genotype; and 3) at least some of the highly-variable bands appear to reside in the chloroplasts. The 500 bp probe may be useful for clonal identification in tree improvement programs. Because of the inheritance of chloroplasts through pollen in conifers, this cloned fragment may also be suitable for paternity resolution.

Keywords: jack pine, lodgepole pine, DNA fingerprinting, hypervariability, RFLP.

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INTRODUCTION

Genetic markers that could unambiguously distinguish all genotypes in populations of forest trees would be of significant value in forest genetics. If such markers became widely available in forest trees, as they are in animals (Jeffreys 1987), then clonal identification and paternity analyses (e.g., Adams et al. 1988) would no longer need to rely on presently available, often ambiguous, genetic markers such as allozymes. Unambiguous markers would also provide unprecedented precision for studies of evolutionary processes in natural populations.

A recent report in the literature suggests that it may be possible to extend the most powerful methods of genotypic identification (DNA fingerprinting) to plant species (Rogstad et al. 1988). These authors presented preliminary results for several taxa, including the forest trees Pinus torreyana, Populus deltoides and Populus tremuloides.

The data reported below support the notion that DNA fingerprinting may indeed be possible in the genus Pinus, using sequences from the chloroplast genome as probes. However, the identity of such probes may differ among taxa within the genus.

METHODS

Genetic Material

The material studied was obtained from both natural and experimental populations of Pinus banksiana and P. contorta in Canada, the United States and Sweden. Details regarding the origin of this material have been published previously, and the specific relevant citations are provided below in the figure legends.

Analyses of DNA Polymorphisms

Total cellular DNA was purified from each individual as described by Wagner et al. (1987), and chloroplast (cp) DNA was purified from individual trees by the method of Szmidt et al. (1986). Restriction fragments produced by double digestion (to completion) with the restriction endonucleases Hamill and SstI were separated by electrophoresis through agarose, transferred to Biotrans membranes, and visualized by Southern (1975) hybridization and autoradiography. The ^{3}2P-labeled (Feinberg and Vogelstein 1983) probe for the molecular hybridizations was a 500 bp BamHI - Smal fragment, cloned from the region between psbAl and psbA2 in the chloroplast genome of P. contorta (Lidholm et al. 1988).

RESULTS AND DISCUSSION

High Levels of Detectable Genetic Variation

The chosen combination of restriction endonucleases and probe permitted the visualization of many bands in each DNA sample. These bands produced a large number of autoradiogram patterns in samples drawn from the allopatric and sympatric ranges of P. *banksiana* and P. *contorta*. Moreover, in a sample of seven individuals of P. *banksiana* from a single provenance, at least six different genotypes were evident (e.g., Figure 1).

Figure 1. Autoradiogram of total cellular DNA samples, doubly digested with BamHI and *SstI*, probed with the 500 hp cpDNA fragment cloned from P. *contorta*. The lanes contain (left-to-right): samples from seven different individuals of P. *banksiana* from a single provenance in Ontario, Canada (lanes 1 - 7); samples from three different individuals of P. *contorta* from a single provenance in British Columbia, Canada (lanes 8 - 10); and samples from five different individuals of P. *banksiana* - P. *contorta* from a single population in a sympatric region in Alberta, Canada (lanes 11 - 15). The fragment sizes range approximately from 500 bp to 4500 bp in size. The DNA samples represented on the autoradiogram originated from studies reported elsewhere (Wagner et al. 1987; Govindaraju et al. 1989; Wagner et al. 1989).

Subcellular Location of the Polymorphism

Two lines of evidence suggest that at least some of the highly-variable bands must reside in the chloroplasts of P. banksiana and P. contorta. First, these variable banding patterns of P. banksiana are inherited paternally in a controlled cross (data not shown), as is cpDNA in P. banksiana (Wagner et al. 1989) and other conifers (Neale and Sederoff 1988). Second, the 500 bp probe is homologous to many fragments in DNA purified from chloroplasts of P. contorta (Figure 2). This second observation indicates that a sequence within the probe fragment may occur several times in the chloroplasts of P. banksiana and P. contorta.

Figure 2. Autoradiogram of DNA purified from chloroplasts, doubly digested with BamHI and S^{s}tI, probed with the 500 bp cpDNA fragment cloned from P. contorta. The two samples were prepared from individuals of P. contorta, growing in Sweden and originating from two provenances in Yukon Territory, Canada. These individuals are two of the plus-tree selections described by Fries and Lindgren (1986).

Potential Pitfalls and Limitations

The diversity of autoradiogram patterns identified could be explained by several factors. For example, small recombination products of the chloroplast enome, resulting from homology among short repeats, might persist in the chloroplasts and be detected by autoradiography.

In principle, the polymorphism may also be due to differential, incomplete enzyme digests. However, repeated DNA purifications from three single trees produced consistent results among samples within trees, and all progeny from a controlled cross of P. banksiana consistently exhibited their paternal genotype. These two facts are evidence that the high level of polymorphism documented by Figure 1 is not the result of random partial digestions.

We have screened individuals of four other species in the genus Pinus (P. ponderosa, P. resinosa, P. rigida, P. taeda), using the same probe that reveals polymorphism in P. banksiana and F. contorta. Although this screen revealed inter-specific differences, polymorphism did not occur within any of the other four species studied. This indicates that probes which identify hypervariability are likely to differ among taxa in the genus Pinus.

CONCLUSIONS

Regardless of the precise molecular mechanism which generates the polymorphism we have discovered, its potential utility is obvious. A genetic marker, which is consistent within individuals and known pedigrees, yet which unambiguously distinguishes many individuals in populations of forest tree:;, offers great power for the solution of problems associated with clonal identification, paternity resolution, and population/evolutionary genetics. The hypervariable markers we have identified may be particularly significant 1 cause of their paternal inheritance through chloroplasts.

Clearly, more work is needed to determine the frequencies of the hypervariable genotypes in natural and breeding populations before these markers could be recommended for application. Additionally, we can do no more than speculate that similar markers exist in other plant species. Nonetheless, the present example, involving small repeated sequences in the chloroplasts of P. banksiana and P. contorta, may be instructive for systematic searches for hypervariable markers in other coniferous taxa.

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