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Note

Oak chloroplast-DNA polymorphisms detected by restriction fragment length polymorphism (RFLP)

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Summary — *Petunia hybrida* chloroplast (cp) DNA probes were used to find restriction fragment length polymorphisms (RFLPs) in the cp DNA of the oak species *Quercus robur* and *Quercus petraea*. Five individuals have been analysed (2 *Q robur* and 3 *Q petraea)* with 18 different restriction enzymes, and with 7 *P hybrida* cp DNA probes. Only 2 probes (P6 and P8) detected polymorphisms, probe P6 detected 4 polymorphisms with the restriction enzymes *Ava*I, *Bgl*II, *Cla*I and *Xba*I, while probe P8 detected a *Bgl*II polymorphism.

oak / chloroplast / restriction fragment length polymorphism (RFLP)

Résumé — Polymorphisme de longueur des fragments de restriction de l'ADN chloroplastique chez les chênes. *Des sondes du génome chloroplastique de* Petunia hybrida *ont été utilisées pour la recherche de polymorphisme de longueur de fragment de restriction dans l'ADN chloroplastique (cp) de* Quercus robur *et* Quercus petraea. *L'ADNcp de 5 arbres (2* Q robur *et 3* Q petraea*) a été digéré par 18 enzymes de restriction et hybridé avec 7 sondes de* P hybrida. *Deux sondes seulement ont révélé du polymorphisme (P6 et P8) : P6 avec les enzymes de restriction* Ava*I,* Bgl*II,* Cla*I et* Xba*I; P8 avec l'enzyme* Bgl*II.*

Quercus / *chloroplaste / polymorphisme de longueur de fragment de restriction (RFLP)*

INTRODUCTION

Climatic variations as well as human activities (*eg* environmental pollution) greatly influence natural ecosystems, frequently restricting the habitat, reproductive potential and number of individuals of many species.

These restrictions may reduce genetic variability within populations, which may in turn diminish the capacity of populations to respond to new selective pressures. Therefore, we need better insight into the genetics and ecology of populations in order to minimize the negative effects of human activity.

Genetic markers are needed to understand the population's genetic events taking place in forest tree species, *eg,* to study inheritance patterns, however, the numbers of available genetic markers are

very limited in forest tree species. In particular, little information is available on the oak pecies *Quercus robur* and *Quercus petraea,* which are the focus of our interest, as the 2 most important oak species in Austria.

Direct DNA analysis by restriction fragment length polymorphism (RFLP), produces a theoretically infinite number of DNA markers. These markers can be chosen from coding or non-coding regions of nuclear and cytoplasmic genomes (chloroplast and mitochondrial).

As far as oak species are concerned, few RFLP data are available (*ie,* Bellarosa, 1990; Petit *et al,* 1990; Whittemore and Schaal, 1991).

However, development of probes detecting polymorphisms in the chloroplast (cp) DNA of oak species, would enable us to follow the inheritance of the most conservative DNA sequences of plants. cpDNA polymorphisms could provide information on evolutionary distances among oak species, the origins of populations and the occurrence of interspecies crosses.

In this paper, we report on DNA probes which detect RFLP variation in *Q robur* and *Q petraea.*

MATERIALS AND METHODS

Leaf material of both *Quercus robur* and *Quercus petraea* was collected in the southeastern part of province Burgenland in the forest domain Prince of Bavaria in Austria.

DNA was extracted as described previously by Kreike *et al* (1991). DNA samples of 1 μg were digested by Boehringer–Mannheim restriction endonucleases according to the manufacturer's recommendations. The digested samples were loaded onto 0.8% agarose gels (Sigma, A-9539) and run overnight at approximately 1 V/cm. Alkaline capillary blotting to Hybond N filters (Amersham) was done according to the manufacturer's recommendations. DNA was fixed on the filter by heating at 80°C for 2 h.

In our experiments, we used a *Petunia hybrida* cpDNA library (originally described by Palmer *et al,* 1983), kindly provided by Dr D Neale, to identify oak cpDNA polymorphisms. DNA probes were labeled with [α-^{32}P]dATP by random priming (Boehringer–Mannheim). Filter hybridization was performed as described by Church and Gilbert (1984), at 50°C overnight. Three washes were made in 2 x SSC, 0.1% (w/v) sodium dodecyl sulfate (SDS) at room temperature (5 min each), followed by 1 x SSC, 0.1% SDS washes at 50°C for 30 min. The wet filters were exposed to Kodak X-Omat autoradiographic films with 2 intensifying screens (DuPont) at –80°C.

RESULTS

Since cpDNA sequences are rather conservative, it was possible to use heterologous petunia cpDNA probes.

During this study we analyzed 5 oak individuals (2 *Q robur* and 3 *Q petraea*) with 18 different restriction enzymes (table I) and with 7 different *P hybrida* cpDNA clones (table I). RFLPs were found only with the probes P6 and P8. Hybridizations with the probe P6 revealed several polymorphisms. In the case of the enzyme *Ava*I this probe detected 8 constant fragments in both species (12.5, 7.6, 4.7, 3.8, 2.3, 2.0, 1.8 and 1.65 kilobases (kb) in size). Additionally, in *Q robur* samples there were 2 fragments (3.0 and 1.0 kb) which were not present in *Q petraea* samples. Instead there was a 7.0-kb fragment and in *Q petraea* samples (fig 1A). The same probe revealed 3 common *Bgl*II fragments (11.0, 3.3 and 1.1 kb in size) and a variable one which of either 3.6 or 5.0 kb in the *Q robur* and *Q petraea* samples, respectively (fig 1B). In the case of *Xba*I digestion, 6 constant fragments were found (17, 6.5, 3.1, 2.8, 1.4 and 1.25 kb) and a 9.0-kb band was also present in the *Q petraea* samples (fig 1D). Four constant *Cla*I fragments were found (3.5, 3.2, 3.0 and 1.8 kb), while a 2.5-kb restriction fragment

Table I. Restriction endonucleases applied and *Petunia hybrida* cpDNA library clones used.

Restriction endonucleases
4-cutters: *Cfo*I, *Hae*III, *Hpa*II, *Taq*I
6-cutters: *Acc*I, *Ava*I, *Bam*HI, *Bgl*II, *Cla*I, *Eco*RI, *Hind*III, *Kpn*I, *Sac*I, *Sau*3A, *Sma*I, *Xba*I, *Xho*I
8-cutter: *Not*I
cpDNA clones
Single-copy region: P6, P8, P10, P16, P20
Repetitive region : P1, P4

was detected only in the *Q petraea* samples (fig 1E). In the latter two cases, however, the hybridization signal of the polymorphic fragment was weaker than that of the constant ones.

Probe P8, one of the fragments adjacent to P6 in *Petunia*, detected a *Bgl*II fragment similar to that of P6 in *Q petraea* samples (5-kb fragment). However, the smaller polymorphic fragment found in *Q robur* samples (3.6-kb fragment) was not detected (fig 1C).

CONCLUSIONS

In the present report, we described 5 polymorphic sites within the oak cpDNA. Four of these 5 polymorphisms were detected by the *Petunia* cpDNA probe P6, while the 5th one was identified by the neighboring *Petunia* probe P8. The other *Petunia* cpDNA probes did not show polymorphisms with the restriction enzymes used. The polymorphism detected by the P6 *Petunia* probe with *Bgl*II had been described earlier (Kreike *et al,* 1991). The other polymorphisms are new ones not reported to date.

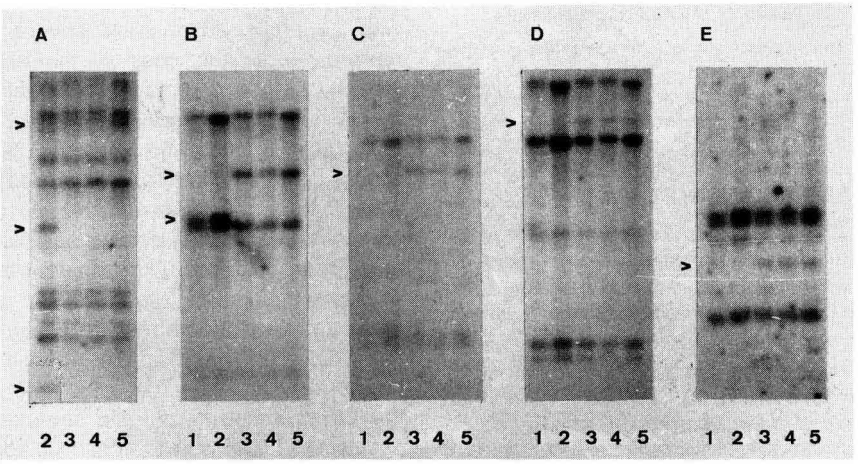

Fig 1. Restriction fragments detected by *Petunia hybrida* cpDNA probes. Autoradiograms represent the following restriction enzyme digests of the oak DNA samples : **A**: *Ava*I; **B**: *Bgl*II; **C**: *Bgl*II (the same filter); **D**: *Xba*I and **E**: *Cla*I. Lanes 1 and 2 are DNA samples of non-related individuals of *Q robur,* while lanes 3–5 represent those of *Q petraea.* Autoradiograms A, B, D and E were obtained after hybridization with the *P hybrida* cpDNA probe P6, while autoradiogram C shows the hybridization pattern of probe P8. Polymorphic bands are indicated by the arrowheads.

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