patterns. Genome **34**: 693–703 (1991). — GILLET, E.: Genmarker als Entscheidungshilfen für die Genkonservierung. Allg. Forst- u. J.-Ztg. **164** (2/3): 30–35 (1993). — JEFFREYS, A. J., WILSON, V. and THEIN, S. L.: Hypervariable minisatellite regions in human DNA. Nature **314**: 67:73 (1985b). — JEFFREYS, A. J.: Hypervariable DNA and Fingerprints. In: Current Communications in Molecular Biology: DNA probes: Application in Genetic and Infectious Disease and Cancer. LERMAN, L. S. (ed.). Conference in Cold Spring Harbour, N. Y., USA, April 20 to 23, 1986: 57–61 (1986). — JEFFREYS, A. J.: Highly variable minisatellites and DNA fingerprints. Biochem. Soc. Trans. **15**: 309–317 (1987). — JEFFREYS, A. J., WILSON, V. and THEIN, S. L.: Individual-specific 'fingerprints' of human DNA. Nature **314**: 76–79 (1985a). — JEFFREYS, A. J., WILSON, V. and THEIN, S. L.: Hypervariable minisatellite regions in human DNA. Nature **314**: 67–73 (1985b). — JERMSTAD, K. D., REEM, A. M., HENIFIN, J. R., WHEELER, N. C. and NEALE, D. B.: Inheritance of restriction length polymorphisms and random amplified polymorphic DNAs in coastal Douglas-fir. Theor. Appl. Genet. **89**: 758–766 (1994). — KAWCHUK, L. M., LYNCH, D. R., HACHEY, J., BAINS, P. S. and KULCSAR, F.: Identification of a codominant amplified polymorphic DNA marker linked to the Verticillium wilt resistance gene in tomato. Theor. Appl. Genet. **89** (6): 661–664 (1994). — KAZAN, K., MANNERS, J. M. and CAMERON, D. F.: Inheritance of random amplified polymorphic DNA markers in an interspecific cross in the genus *Stylosanthes*. Genome **36**: 50–56 (1993). — KENNARD, W. C., POETTER, K., DIJKHUIZEN, A., MEGLIC, V., STAUB, J. E. and HAVEY, M. J.: Linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. Theor. Appl. Genet. **89**: 42–48 (1994). — KVARNHEDEN, A. and ENGSTRÖM, P.: Genetically stable, individual-specific differences in hypervariable DNA in Norway spruce, detected by hybridization to a phage M13 probe. Can. J. For. Res. **22**: 117–123 (1992). — LIN, D., HUBBES, M. and ZSUFFA, L.: Differentiation of poplar and willow clones using RAPD fingerprints. Tree Physiology **14**: 1097–1105 (1994). — LYNCH, M.: Estimation of relatedness by DNA-fingerprinting. Mol. Biol. Evol. **5**: 584–599 (1988). — MILGROOM, M. G., LIPARI, S. E. and POWELL, W. A.: DNA fingerprinting and analysis of population structure in chestnut blight fungus, *Cryphonectria parasitica*. Genetics **131**: 297–306 (1992). — NYBOM, H. and HALL, H. K.: Minisatellite DNA 'fingerprints' can distinguish *Rubus* cultivars and estimate their degree of relatedness. Euphytica **53**: 107–114 (1991). — NYBOM, H. and ROGSTAD, S. H.: DNA "fingerprints" detect genetic variation in *Acer negundo* (Aceraceae). Plant Systematics and Evolution **173** (1–2): 49–56 (1990). — NYBOM, H., ROGSTAD, S. H. and SCHAAL, B. A.: Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus*, *Prunus*, and *Rubus* (Rosaceae). Theor. Appl. Genet. **79**: 153–156 (1990). — PELEMAN: Molecular genetic screening technologies. In: Molecular Screening News. Commission of the European Communities (ed.) No. 7, August. Supplement, 31–32 (1995). — PRABHU, R. R. and GRESSHOFF, P. M.: Inheritance of polymorphic markers generated by DNA amplification fingerprinting and their use as genetic marker in soybean. Plant Mol. Biol. **26** (1): 105–116 (1994). — ROGSTAD, S. H., NYBOM, H. and SCHAAL, B. A.: The tetrapod "DNA fingerprinting" M13 repeat probe reveals genetic diversity and clonal growth in quaking aspen (*Populus tremuloides*, Salicaceae). Plant Systematics and Evolution **175** (3–4): 115–123 (1991). — ROGSTAD, S. H., PATTON, J. C. and SCHAAL, B. A.: M13 repeat probe detects DNA minisatellite-like sequences in gymnosperms and angiosperms. Proc. Natl. Acad. Sci. **85**: 9176–9178 (1988). — RYSKOW, A. P., JINCHARADZE, A. G., PROSNYAK, M. I., IVANOV, P. L. and LIMBORSKA, S. A.: M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms. FEBS Letters **233** (2): 388–392 (1988). — STENLID, J., KARLSSON, J.-O. and HÖGBERG, N.: Intraspecific genetic variation in *Heterobasidium annosum* revealed by amplification of minisatellite DNA. Mycol. Res. **98** (1): 57–63 (1994). — VAHALA, T., ERIKSSON, T. and ENGSTROM, P.: Genetic variability in basket willow (*Salix viminalis*) detected by hybridization to a bacteriophage M13 DNA probe. Hereditas **115** (2): 153–161 (1991). — VASSART, G., GEORGES, M., MONSIEUR, R., BROCA, H., LEQUARRE, A. S. and CRISTOPHE, D.: A sequence on M13 phage detects hypervariable minisatellites in human and animal DNA. Science **235**: 683–684 (1987). — WEIR, B. S.: Independence of VNTR alleles defined as fixed bins. Genetics **130**: 873–887 (1992). — WELSH, J., HONEYCUTT, R. J., MCCLELLAND, M. and SOBRAL, B. W. S.: Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction. Theor. Appl. Genet. **80**: 1–4 (1991). — WILLIAMS, J. K. K., KUBELIK, A. R., KENNETH, J. L., RAFALSKI, J. A. and TINGEY, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research **18** (22): 6531–6535 (1990). — ZABEAU, M. and VOS, P.: Patent amplification number 92402629.7 in laboratories of KeyGene/Wageningen/NL. — ZIEGENHAGEN, B., GUILLEMAUT, P. and SCHOLZ, F.: A procedure for mini-preparations of genomic DNA from needles of silver fir (*Abies alba* MILL.). Plant Mol. Biol. Reptr. **11**: 117–121 (1993). — ZIEGENHAGEN, B., KORMUT'ÁK, A., SCHAUERTE, M. and SCHOLZ, F.: Restriction site polymorphism in chloroplast DNA of silver fir (*Abies alba* MILL.). Forest Genetics (1995). – ZIMMERMAN, P. A., LANG-UNNASCH, N. and CULLIS, C. A.: Polymorphic regions in plant genomes detected by an M13 probe. Genome **32**: 824–828 (1989).

Characterization and Propagation of an Adult Triploid Pedunculate Oak (Quercus robur L.)

By G. NAUJOKS, H. HERTEL and D. EWALD[1])

(Received 3rd August 1995)

Abstract

Investigations of "European oak decline" and genetic structure of more than 400 adult oaks showed that 1 tree was genetically variant because of unusual isozyme patterns. Further examination showed a variant leaf morphology and an increased stomatal length. Chromosome counts and isozyme analysis indicated that the tree is a triploid oak. The conservation of this remarkable genotype was achieved by rooted cuttings. The occurrence of flowering and fruit set of the adult tree offer opportunities for further research concerning problems of reproduction, genetics and stress resistance in oak with altered ploidy level.

Key words: isozyme markers, polyploidy, morphological markers, stomata, chromosome counts, rooted cuttings.

FDC: 165.3; 165.42; 165.44; 176.1 *Quercus robur*.

Zusammenfassung

Im Rahmen von Forschungsarbeiten zur Vitalität und genetischen Struktur an über 400 adulten Eichen fiel ein Baum aufgrund seiner ungewöhnlichen Isoenzym-Bandenmuster auf. Die Ergebnisse weiterer Untersuchungen zeigten eine abwei-

[1]) Federal Research Centre for Forestry and Forest Products, Institute for Forest Tree Breeding, Eberswalder Chaussee 3, D-15377 Waldsieversdorf, Germany

chende Blatt-Morphologie und erhöhte Stomata-Längen. Die Chromosomenzählung lieferte in Verbindung mit der Isoenzymanalyse den Beweis, daß der Baum aller Wahrscheinlichkeit nach triploid ist. Es konnten Stecklinge bewurzelt werden, um diesen besonderen Genotyp zu erhalten. Das Auftreten von Blüte und Fruchtansatz in geringem Umfang eröffnet die Möglichkeit für weitere Untersuchungen zu Problemen der Reproduktion, Vererbung und Resistenz bei Eichen mit abweichendem Ploidiegrad.

Introduction

In our studies of "European oak decline" 7 adult oak stands in eastern Germany were analysed to determine the connection between vitality and the genetic structure at the individual and population level. More than 400 trees of pure pedunculate oak stands (*Quercus robur* L.), pure sessile oak stands (*Q. petraea* LIEBL.) and mixed stands originated from artificial or natural reproduction were included in this investigation.

Beside several phenotypical traits of each tree, genotypes were characterized by a set of isozyme markers.

Some altered isozyme patterns were found in 1 single tree. These isozyme patterns pointed out to the presence of more then the normal 2n = 24 chromosomes, because the effect was not restricted to one gene locus.

The investigations should clarify the nature of the peculiarity of this pedunculate oak tree.

Material and Methods

Location and plant material

Tree No. 47 was found in the forest district Chorin near Eberswalde. The stand was established by artificial reproduction approximately 90 years ago as a mixture of 70 % pedunculate oak and 30 % sessile oak. The average height of the stand was 27.8 m (MERTENS, 1994). Tree No. 47 was 27 m high with an excellent straight stem form and a diameter of 42 cm at 1.30 m. Its crown started at 18.5 m.

Electrophoresis

The following enzyme systems and gene loci were used to characterize the genetic structure: acid phosphatase (ACP-C), aminopeptidase (AP-B), aspartate aminotransferase (AAT-B), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH-B), menadione reductase (MR), NADH dehydrogenases (NDH-A, NDH-B), phosphoglucomutase (PGM-A), 6-phosphogluconate dehydrogenase (PGDH), phosphoglucose isomerase (PGI-B). The electrophoretic methods are described in detail by HERTEL et al. (1994). The mode of inheritance of allelic variants was qualitatively tested with single tree offsprings by the method of FINESCHI et al. 1990 (HERTEL, unpublished).

Stomatal measurements

The leaf undersurface of mature leaves was spread with a thin coat of clear adhesive. This peel was removed after drying and observed with a Olympus BH2-RFCA microscope at a 400-fold magnification. The length of 30 stomata was measured for each of 6 trees (the test tree and 5 control trees).

Chromosome counts

Chromosome counts were made by modifying the method developed by EIFLER (1959). In spring, young oak leaves as well as floral stalks of male flowers collected from adult trees were used. In early summer, fast growing root tips of one year old cuttings were examined. The explants were fixed in 3:1 ethanol : glacial acetic acid for 30 min and stained in acetocarmine for 2 min using a microwave (900 W).

Very small pieces (nearly 1 mm^2) of the leaf base, floral stalk or root tip were squashed in acetocarmine on microscopic slides. Methaphase chromosomes were counted at a 1000-fold magnification. The squashes were replicated 6-fold for floral stalks and leaf bases of tree No. 47 and 10-fold for floral stalks of a control tree.

Cutting propagation

Fifty leafy cuttings, 10 cm in length, were harvested in July. The 3 terminal leaves were cut in half and all other leaves were detached. Cuttings were treated with a rooting paste containing 3-indolyl butyric acid (2 g l^{-1}) and rooted in Jiffy-7 peat pellets. After placing them into a plastic greenhouse (50 cm x 20 cm x 20 cm) the cuttings were cultured under continuous red fluorescent light (35 µE m^{-2} s^{-1}, fluorescent lights LS 65 red 93; NARVA) at a constant temperature of 22 °C. After 2 months the rooted plants were counted, humidity was reduced successively over 2 weeks and the plants were potted in a 11 cm flowerpot.

Results

Genetic analyses by isozyme techniques

Two isozyme loci of 11 loci tested of the tree No. 47 revealed several bands and were assigned to be heterozygous, the remaining 9 loci with 1 single band each were homozygous. The 2 heterozygous loci exhibited patterns which differ from other heterozygous trees.

At the aminopeptidase locus, the tree No. 47 produced a pattern with 3 bands corresponding to 3 of the 4 common alleles B2, B4 and B7 (AP-B. *Figure 1*, above). At the isocitrate dehydrogenase locus, this individual produced a pattern with 3 bands similar to that of other heterozygous trees with the common genotype B4B6, but with a remarkable higher staining intensity of the band corresponding to allele B4 (IDH-B, *Figure 1*, below).

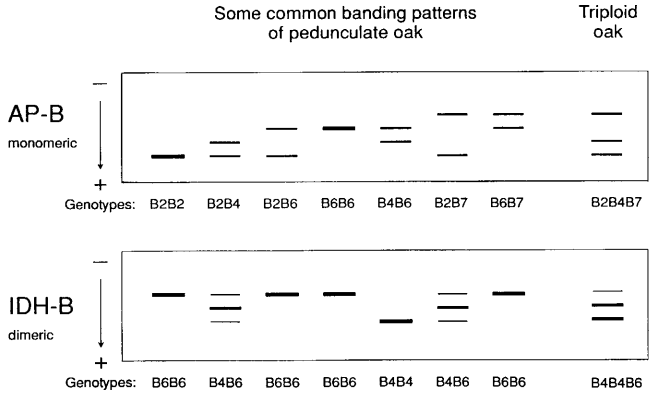

Figure 1. – Scheme of isozyme patterns of 1 triploid oak and some common patterns of diploid oaks at the loci AP-B and IDH-B.

Stomatal measurements

When the samples were taken from the crown of the trees differences in the type of leaves were obvious. Tree No. 47 had thick, nearly stiff, leather-like leaves whereas the other trees possessed leaves of normal thickness and smoothness. The average stomata length for tree No. 47 was 24.5 µm, significantly differing from the other 5 trees with a stomata length in the range from 18.2 µm to 20.1 µm. The average stomata length determined for each tree is recorded in *figure 3*.

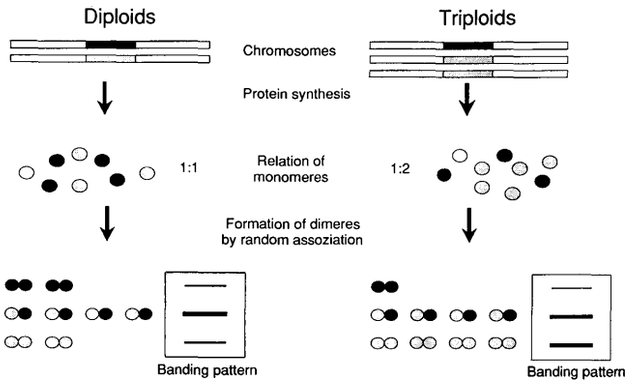

Figure 2. – Gene-dose-effect in isozyme patterns of dimeric enzymes for diploid and triploid heterozygotes.

Chromosome counts

The most useful material for chromosome counts were male floral stalks. The base of young, soft leaves in some cases became recalcitrant against the squashing procedure after fixing in ethanol : glacial acetic acid. The tree No. 47 was compared with 1 control tree (No. 28) in the frequency of counted chromosome numbers (see *Figure 4*). It is evident that tree No. 47 was not diploid. Most frequently chromosome numbers in the range of 33 to 35 were counted. The distribution of frequencies is significantly different between the 2 trees. Tree No. 47 is in all probability triploid. *Figure 5* shows cells of floral stalks of the investigated trees with a diploid (A: tree No. 28) and a triploid (B: tree No. 47) chromosome set.

Cutting propagation

Beginning with 50 cuttings, only 2 cuttings (4 %) formed roots after 2 months. The rooted plants entered into dormancy during the winter period but started to sprout in the following spring. Also the root growth and branching started anew.

It was interesting to note that the plant material harvested from the crown showed flower and fruit formation.

Discussion

Isozyme gene markers are a useful tool to describe the genetic structure of individuals and populations of forest tree species. Proteins as translation products of genes were separated by electrophoresis. Variants of genes (alleles) are visible by their banding patterns after an enzyme specific staining, if the electrophoretic mobility of enzyme proteins differ caused by different amino acid sequences. The interpretation of the banding patterns allow conclusions about gene loci and alleles. In addition to the presence or absence of bands the staining intensity can supply information about genetic structure in some cases.

Publications in the field of isozyme analyses of polyploid material often describe examples for tetraploids or offsprings of tetraploids at the species level (i. e. BOUSQUET et al., 1987; MACHON et al., 1995; BEAVER et al., 1995).

In contrast to these results, our findings refer to only 1 individual in a tree species which is normally diploid. The 2 heterozygous loci AP-B and IDH-B with atypical isozyme patterns indicated the occurrence of additional genes.

Aminopeptidases are enzymes with a monomeric structure. The locus AP-B possesses 4 common (B2, B6, B4 and B7) and some rare alleles in oak trees and is the most variable locus in this tree species. Homozygous individuals show one band and dipoid heterozygous individuals show 2 bands. In case of tree No. 47 we observed the genotype B2B4B7 with 3 different alleles.

The isocitrate dehydrogenase with a dimeric structure consists of two subunits. In homozygous individuals the subunits are identical and the enzyme produces 1 band after electrophoresis. Heterozygous individuals possess 3 different kinds of dimeric enzymes: 2 identical subunits from the first or from the second allele or 2 different subunits each from 1 of the 2 alleles. The electrophoretic mobility of this "hybrid enzyme" is intermediate between the 2 enzymes with identical subunits. The staining intensity of the bands reflects the frequency of the respective dimetric enzyme. In diploid individuals the intensity of the hybrid band in the middle should be double in comparison with the outer bands (*Figure 2*). The common alleles at the locus IDH-B in pedunculate oak trees are B6 and B4. The genotype of tree No. 47 was designed as B4B4B6 which was concluded from the gene-dose-effect. This effect is also known from triploid offsprings from crossing experiments with diploid and tetraploid birch clones (NAUJOKS et al., 1994).

Although the possibility of duplication of at least 2 genes could not be excluded in this stage of the studies we assumed

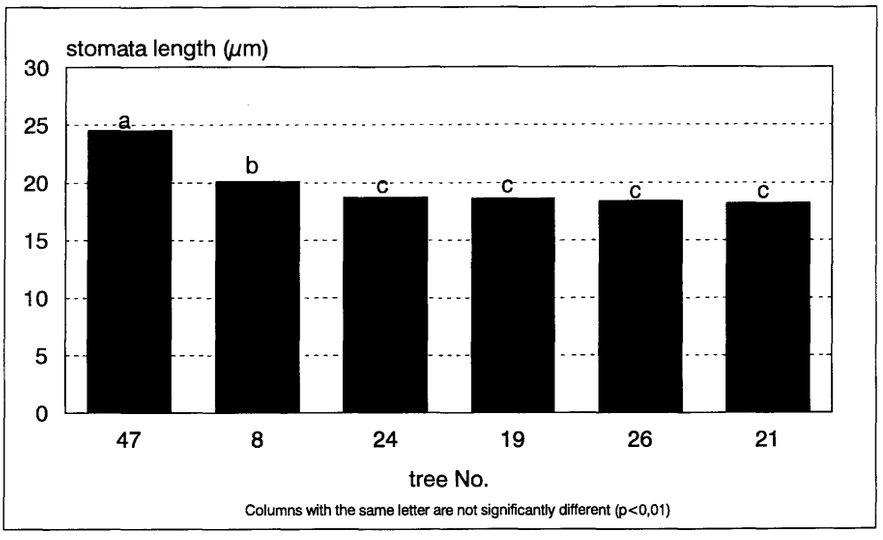

Figure 3. – Average stomata length of 6 pedunculate oaks.

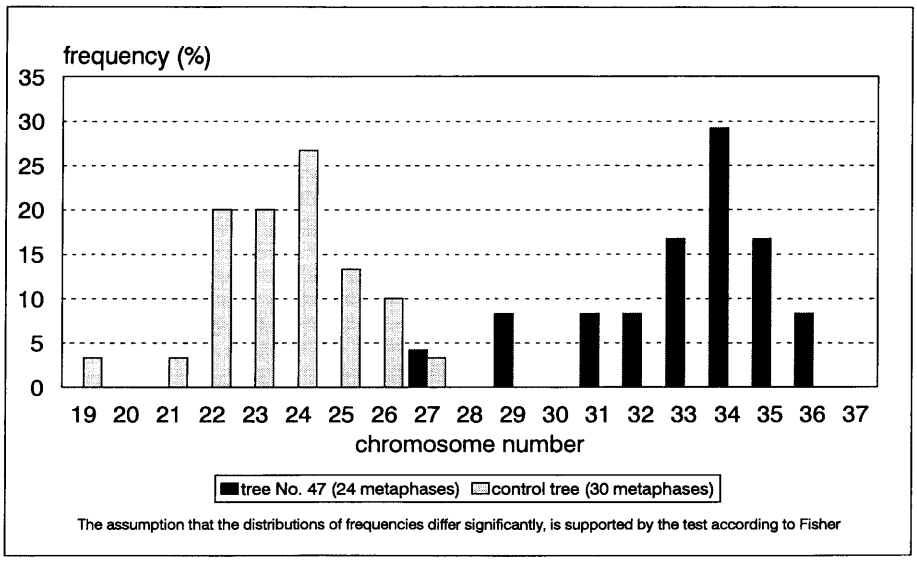

Figure 4. – Frequency of chromosome numbers counted in leaf base and floral stalks of 2 pedunculate oaks.

the existence of a further spontaneous triploid oak, since BUTORINA et al. (1983) reported about such a tree in Russia.

The real evidence for the triploid state was only given by the chromosome counts.

The use of morphological markers to characterize trees with an abnormal ploidy level is known from several publications. EIFLER (1955) described that a number of birch plants derived from colchicine treated seeds showed morphological characteristics differing from untreated seedlings. After chromosome counts it became evident that the birch trees with larger, dark green and leather-like leaves, a waved leaf surface and a strongly carved margin were tetraploids. Their stomata were significantly larger than in diploid birch plants. BRADSHAW and STETTLER (1993) worked with controlled crossings of *Populus trichocarpa* and *P. deltoides* and pointed out the increased cell size in the progeny as a marker for higher ploidy levels. KIM and LEE (1973) reported about a tetraploid Robinia tree showing very high increase in height, unusually large and dark-green leaves and very long wood fibres. Our results confirmed the applicability of morphological markers as indication for deviant chromosome numbers.

Considering the age of the donor tree, a direct formation of viable plants via cuttings without intermediate grafting steps was possible only with a very low frequency. The percentage root formation observed (4 %) was similar to results described by other authors (SPETHMANN, 1986). Nevertheless it showed that a conservation and propagation of those rare genotypes for further breeding purposes is possible also from very old oak trees. The formation, growth and branching of roots formed offers now possibilities for an improved chromosome counting and analysis.

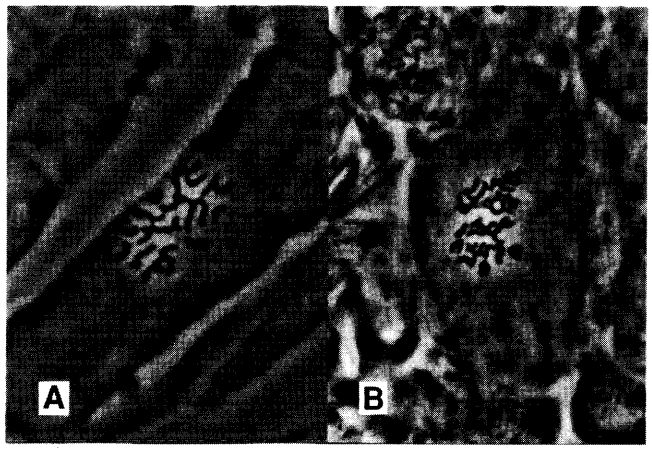

Figure 5. – Cells of floral stalks of the investigated trees with diploid (A: tree No. 28) and triploid (B: tree No. 47) chromosome set.

Polyploid forest trees should be used in a broader range in breeding, especially aimed at an improvement of resistance against biotic and antropogenic influences. There are several reports describing polyploid forest trees differing in their growth habit, increase in height, type of leaves and resistance behaviour from normal diploid trees. In most cases they were discovered spontaneously like 1 triploid aspen clone found by NIELSSON-EHLE (1936), 2 aspen and 2 birch trees detected as triploids by SARVAS (1958) or 2 triploid pedunculate oaks examined by BUTORINA (1983 and 1993). SHERALD et al. (1994) found a naturally occurring triploid elm hybrid which was highly resistant to *Ophiostoma ulmi* (BUIS.) NANNF. The artifical induction of tetraploid forest trees was successful in some cases (KIELLANDER, 1950; EIFLER, 1967; JOHNSSON, 1975), but the use of these plants for a production of triploids was hindered due to a low survival rate of the tetraploids, their disturbed growth habit and the lack in flowering.

Observing a fruit formation at the described tree of pedunculate oak seems to contradict the widespread opinion that triploids are often sterile. On the other hand the fertility of this tree offers new possibilities to get a broad genetic variation in the offsprings which could be a valuable tool for breeding (GEBHARDT, 1988).

The conservation and propagation of such extraordinary genotypes could create new initial positions for research, first by the production of genome mutants and the observation of performance and resistance for example, and second by improving our knowledge about chromosome effects in plants supported by modern molecular techniques now.

Acknowledgements

The authors would like to thank Mrs. I. EIFLER for fruitful discussions and her support in developing ideas concerning the utilization of polyploid forest trees.

References

BEAVER, J. A., IEZZONI, A. F. and RAMM, C. W.: Isozyme diversity in sour, sweet, and ground cherry. Theor. Appl. Genet. **90**, 847–852 (1995). — BOUSQUET, J., CHELIAK, W. M. and LALONDE, M.: Allozyme variability in natural populations of green alder (*Alnus crispa*) in Quebec. Genome **29**, 345 (1987). — BRADSHAW, D. H. and STETTLER, F. R.: Molecular genetics of growth and development in *Populus*. I. Triploidy in hybrid poplars. Theor. Appl. Genet. **86**, 301 (1993). — BUTORINA, A. K.: Cytogenetic study of diploid and spontaneous triploid oaks, *Quercus robur* L., Ann. Sci. For. **50** (Suppl 1), 144–150 (1993). — BUTORINA, A. K., IEVLEV, V. and MURAYA, L. S.: Cytogenetics of the spontaneous triploid of oak (*Quercus robur*). Genetika **19**, 647–659 (1983). — EIFLER, I.: Künstliche Polyploidie-Erzeugung bei *Picea abies* und *Betula verrucosa*. Silvae Genetica **4**, 162–166 (1955). — EIFLER, I.: Beschreibung einer Fixiermethode, die das Auszählen von Birkenchromosomen erleichtert. SD "Der Züchter" **29** (1): 57–59 (1959). — EIFLER, I.: Anwendungsmöglichkeiten der Ploidiezüchtung in der Forstwirtschaft. Arch. Forstwesen **16**, 515–528 (1967). — FINESCHI, S., GILLET, E. and MALVOLTI, E.: Genetics of sweet chestnut (*Castanea sativa* MILL.). III. Genetic analysis of zymograms of single tree offspring. Silvae Genet. **39**, 189–194 (1990). — GEBHARDT, K.: Erzeugung und Nutzung von Ploidie-Mutanten. Allg. Forstzeitg. **49**, 1351–1352 (1988). — HERTEL, H., ZASPEL, I. and EISENHAUER, D.-R.: Investigations about vitality and genetic structure in oak stands. International symposium „Environmental contrains and oaks: ecological and physiological aspects" in Nancy/France August 29 to September 1 1994. p. 153 (1994), — JOHNSSON, H.: Observations on induced polyploidy in some conifers (*Pinus sylvestris, P. contorta, Picea abies, Larix sibirica*). Silvae Genetica **24**, 62–68 (1975). — KIELLANDER, L. C.: Polyploidy in *Picea abies*. Hereditas **36**, 513–516 (1950). — KIM, C. S. and LEE, S. K.: Morphological and cytological characteristics of a spontaneous tetraploid of *Robinia pseudoacacia*. Research Report of the Institute of Forest Genetics, Korea **10**, 57–65 (1973). — MACHON, N., LEFRANC, M., BILGER, I. and HENRY, J.-P.: Isoenzymes as an aid to clarify the taxonomy of french elms. Heredity **74**, 39–47 (1995). — MERTENS, C.: Ökologisch-genetische Untersuchungen von Eichenbeständen der Oberförsterei Chorin. Diplomarbeit, Universität Göttingen (1994). — NAUJOKS, G., ZASPEL, I. und HERTEL, H.: Charakterisierung von Nachkommen tetraploider und diploider Birken. Arbeitstagung d. AG Gehölze d. Gesellsch. f. Pflanzenzüchtung in Berlin, (Poster) (1994). — NIELSSON-EHLE, H.: Über eine in der Natur gefundene Gigasform von *Populus tremula*. Hereditas **21**, 379–393 (1936). — SARVAS, R.: Two triploid aspens and two triploid birches. Comm. Inst. Forest. Fenniae **49**, 1–25 (1958). — SHERALD, J. L., SANTAMOUR, F. S., HAJELA, R. K., HAJELA. N. and STICKLEN, M. B.: A dutch elm disease resistant triploid elm. Can. J. For. Res. **24**, 647–653 (1994). — SPETHMANN, W.: Stecklingsvermehrung von Stiel- und Traubeneiche (*Quercus robur* L. und *Quercus petraea* (MATT.) LIEBL.). Schriften aus der Forstlichen Fakultät der Universität Göttingen und der Niedersächsischen Forstlichen Versuchsanstalt, Band 86. J. D. Sauerländer's Verlag, Frankfurt am Main. pp. 99 (1986).

Isozyme Gene Loci and Their Allelic Variation in *Pinus sylvestris* L. and *Pinus cembra* L.[1])

By F. BERGMANN and H. H. HATTEMER

Abteilung für Forstgenetik und Forstpflanzenzüchtung,
Georg-August-Universität, Büsgenweg 2, D-37077 Göttingen, Germany

(Received 3rd August 1995)

Summary

The genetic structure and variation of 5 Scots pine (*P. sylvestris* L.) and 4 Swiss stone pine (*P. cembra* L.) populations was compared using allele frequency data from the same isozyme-gene-systems. Differences between the 2 pine species belonging to the subgenera *Pinus* and *Strobus*, respectively, concern both the number of controlling gene loci and the number and frequency of alleles. Although Scots pine is characterized by larger populations and a wider natural range than Swiss stone pine, the genetic diversity values did not generally differ to a similar degree or in the direction expected. While Scots pine possesses higher diversity at several enzyme loci, Swiss stone pine reaches the same degree of diversity or even a higher degree at other loci.

Interestingly, the greatest differences in gene diversity between the 2 pine species were detected for isozyme-gene-systems that differ in the number of controlling loci, suggesting that variable allozymes at only 1 (or 2) loci and invariant isozymes at multiple loci are alternate forms of enzyme adaptation in the 2 species. The relationship between such adaptive strategies at the enzyme level and the ecological conditions and life history traits of the 2 species are discussed.

Key words: Pinus sylvestris, Pinus cembra, isozyme loci, genetic diversity, adaptive strategies.

FDC: 165.3; 165.5; 174.7 *Pinus sylvestris;* 174.7 *Pinus cembra.*

Introduction

Most European pine species differ greatly in the sizes and geographic locations of their natural ranges. Whereas Scots pine (*Pinus sylvestris*, section *Pinus*, subgenus *Pinus*) is one of the most widely distributed conifers in central, northern and eastern Europe, Swiss stone pine (*P. cembra*, section *Strobus*, subgenus *Strobus*) is restricted to the high elevations of the Austrian and Swiss Alps, the Eastern Carpathians, and the High Tatra Mountains (HOLZER, 1975). Therefore, it appears to be worthwhile to compare the types and amounts of genetic variation between these 2 pine species.

Extensive studies on the genetic diversity and differentiation of Scots pine have been carried out by, among others, GULLBERG *et al.* (1985), MEJNARTOWICZ and BERGMANN (1985), PRUS-GŁOWACKI and STEPHAN (1994) and GONCHARENKO *et al.* (1994), whereas Swiss stone pine populations have been investigated only by SZMIDT (1982). Russian research groups have compared different stone pine species of the subsection *Cembrae*, section *Strobus*, which occupy a large part of the Eurasian area of the former Soviet Union (GONCHARENKO *et al.*, 1992; KRUTOVSKII, *et al.*, 1995).

[1]) Dedicated to Prof. Dr. G. H. MELCHIOR on his 70th birthday.