

## Genetic Variation in English Oak (*Quercus robur*) in Finland

Anu Mattila, Anne Pakkanen, Pekka Vakkari  
and Juha Raisio

---

**Mattila, A., Pakkanen, A., Vakkari, P. & Raisio, J.** 1994. Genetic variation in English oak (*Quercus robur*) in Finland. *Silva Fennica* 28(4): 251–256.

Genetic variation in 5 natural stands of *Quercus robur* in Finland was analyzed electrophoretically for 13 isozyme loci. Stands were on average polymorphic at 49.2 % of the loci, with 2.1 alleles per locus. Observed heterozygosities, ranging from 13.6 % to 16.9 %, were slightly lower than estimates reported for German stands. The majority of the species' genetic variation was found within each studied stand, and only 5.5 % was between stands. Mean genetic differentiation ( $\theta$ ) was the same as that found in the primary range of the species, but the differentiation estimates (D) for single Finnish populations were more variable.

**Keywords** *Quercus robur*, allozymes, heterozygosity, genetic differences, populations  
**Authors' addresses** *Mattila, Pakkanen & Vakkari*: Foundation for Forest Tree Breeding, Viljatie 4 A 5, FIN-00700 Helsinki, Finland; *Raisio*: Finnish Forest Research Institute, Box 18, FIN-01301 Vantaa, Finland **Fax** to *Mattila* +358 0 359 720  
**E-mail** anu.mattila@metla.fi  
**Received** October 14, 1994

---

### 1 Introduction

Forest genetic research and forest tree breeding have in Finland mainly focused on the predominating forest tree species Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and silver birch (*Betula pendula*). Species with more restricted natural distributions in Finland have gained almost no attention despite the fact that some, e.g. English oak (*Quercus robur*, syn. *Q. pedunculata*), are of minor commercial importance. Though never a predominating species in Finland, English oak forests in the coastal re-

gions of southwestern Finland were common a few hundred years ago (e.g. Skult 1965). Since then, agricultural activities have split the former more continuous forests of English oak into fragments and potentially resulted in restricted gene flow between local stands. The selective cutting of trees with straight trunks for ship building has probably also affected the gene pools of English oak.

Allozyme data show that tree species generally exhibit more genetic variation at the species level than other plants (Muona 1990). Among oaks, the range of variation is as large as that

observed in other plant genera (for a review see Kremer and Petit 1993). Oak species with a wide and continuous distribution, like sessile oak (*Q. petraea*) and red oak (*Q. rubra*), exhibit especially high levels of gene diversity (e.g. Kremer et al. 1991, Schwarzmann and Gerhold 1991, Müller-Starck et al. 1993). Genetic differentiation among oak populations is low, which is in agreement with the general trend for wind-pollinated, largely outcrossing tree species, with an extensive gene flow among populations (see Hamrick et al. 1992, Kremer and Petit 1993).

The English oak populations growing in Finland represent the northern limit of this species. Generally, marginal populations are expected to differ genetically from the populations in the primary range of the species. Data on marginal populations of tree species is largely based on allozymes and, on conifers. Some studies suggest that marginal populations may be less variable than central ones (Yeh and Layton 1979, Guries and Ledig 1982), but in many cases the variability of marginal populations is at least on the same level as that of the central populations (Yeh and El-Kassaby 1980, Yeh and O'Malley 1980, Steinhoff et al. 1983, Furnier and Adams 1986). In addition to the possible marginal effect, Finnish stands of English oak are small and isolated. Therefore the population structure of oaks in Finland is likely to be different from the predominating and more widely distributed conifers and birches of Finland, and this should be taken into consideration during reforestation practices. Moreover, information on the genetic structure allows for a more representative sample of the species' gene pool to be included in breeding and conservation programs.

In this paper, we present preliminary results of an investigation on allozyme variation within and among English oak stands located near the northern limit of the natural range of the species in Finland. The objectives of this study were to estimate the level of genetic variation within stands, and to determine whether the heterogeneity among stands is high enough to have any implications on the selection of seed sources.

## 2 Material and methods

### 2.1 Stands and Plant Material

A total of 270 bud samples from five stands of English oak were collected in January and February 1994 and kept at  $-20^{\circ}\text{C}$  until analysis. On average, samples from 54 randomly chosen overstorey trees per stand were taken. The age of these trees ranged from 50 to 250 years. The study populations are located in the southwestern part of Finland (Fig. 1), within the present main distribution area of English oak in Finland. All stands are regarded as natural, but we can not totally exclude the possibility of past acorn transfers. The stands can be roughly divided into three categories according to their size: (1) Ruissalo, by far the biggest oak dominated forest in Finland covering about 90 hectares; (2) Tenhola and Halikko, ca. 10 hectares; and (3) Taivassalo and Nynäinen, 2–5 hectares. In Ruissalo, sampling was restricted to a pure oak stand in the central part of the larger oak dominated forest. In other populations sampling covered at least half of the stand area. Distances between the populations ranged from ca. 20 km to 100 km.

### 2.2 Electrophoresis

Bud scales were removed and tissue from three or four buds per tree was homogenized in 80–100  $\mu\text{l}$  of 0.1 M Tris-HCl extraction buffer,



**Fig. 1.** Locations of sampled English oak stands in southwestern Finland: Taivassalo (1), Nynäinen (2), Ruissalo (3), Halikko (4) and Tenhola (5). The dashed line indicates the northern limit of the species natural distribution.

pH 7.5 (Bousquet et al. 1987), modified by omitting polyethylene glycol. Samples were assayed for 11 enzymes by standard starch gel electrophoresis (12% Sigma Hydrolyzed Starch) and three buffer systems. Buffersystem A pH 8.1 (Ashton and Braden 1961) was used to resolve alcohol dehydrogenase (ADH, E.C.1.1.1.1), fluorescent esterase (FEST, E.C.3.1.1.1), glutamate dehydrogenase (GDH, E.C.1.4.1.3), glutamate-oxaloacetate transaminase (GOT, E.C.2.6.1.1) and phosphoglucose isomerase (PGI, E.C.5.3.1.9), buffersystem B Tris-citrate pH 7.1 (Shaw and Prasad 1970) for diaphorase (DIA, E.C.1.6.4.3) and shikimic acid dehydrogenase (SDH, E.C.1.1.1.25), and buffersystem C Tris-citrate pH 7.8 (Shaw and Prasad 1970) for isocitrate dehydrogenase (IDH, E.C.1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), 6-phosphogluconate dehydrogenase (6PGD, E.C.1.1.1.44) and phosphoglucomutase (PGM, E.C.2.7.5.1). The enzyme activity staining protocols were according to Cheliak and Pitel (1984) with slight modifications. Genetic interpretation of banding patterns was based on their subunit structure, assumed Mendelian inheritance and codominance (Kephart 1990, Müller-Starck and Hattemer 1990, Bacilieri et al. 1994). 13 loci were scored: Adh, Dia-1, Fest-3, Gdh, Got-1, Idh-2, Mdh-1, Mdh-2, 6Pgd-2, Pgi-1, Pgi-2, Pgm-2 and Sdh.

### 2.3 Genetic Analysis

For each population, and at the species level (all populations pooled), the mean number of alleles per locus and the proportion of polymorphic loci (i.e. frequency of the most common allele  $\leq 0.95$ ) and variable loci (i.e. more than one allele was observed) were calculated. Mean observed heterozygosity and Hardy-Weinberg expected heterozygosity (Nei's 1978 unbiased method),  $H_o$  and  $H_e$ , respectively, were calculated for each population over all loci. Genetic differentiation was estimated by  $D$ - and  $\partial$ -statistics (Gregorius and Roberds 1986, Hattemer 1991), where  $D$  describes the difference in the genetic composition between a population and the total gene pool, and  $\partial$  the mean differentiation among populations.

## 3 Results

### 3.1 Polymorphic Loci

Two out of 13 loci examined (Mdh-1 and Pgi-1) were monomorphic in all individuals. Populations were, on average, monomorphic for 3.8 loci: 3 loci in Ruissalo, Halikko and Nynäinen, 4 in Taivassalo and 6 in Tenhola. The mean percentage of polymorphic loci was 49.2%, ranging from 30.8 % in Tenhola to 61.5 % in Taivassalo and Nynäinen (Table 1). The percentage of variable loci ranged from 53.8 % in Tenhola to 76.9 % in Ruissalo, Halikko and Nynäinen, with a grand mean of 70.7%. Ca. 70 % of the variable loci proved to be polymorphic. At the species level the percentage of variable loci was 84.6.

### 3.2 Allelic Diversity

At the species level 2.7 alleles per locus were observed. In the populations mean allelic diversity per locus ranged from 1.8 to 2.3 with a mean of 2.1 alleles per locus (Table 1). If monomorphic loci were excluded, mean number of alleles per locus would be 2.3. Two alleles encoded by Fest-3 and 6Pgd-2 were unique to Nynäinen. One allele was present only in Tenhola and Taivassalo (in Dia-1), and another in Ruissalo and Taivassalo (in Mdh-2). Their allelic frequencies in the populations were below 0.03, except for Dia-1 in Tenhola, where the frequency was 0.13.

### 3.3 Heterozygosity

The proportion of observed heterozygous individuals ( $H_o$ ) in the population samples ranged from 0.136 in Tenhola to 0.169 in Nynäinen, whereas the range for expected heterozygosity ( $H_e$ ) was from 0.148 in Tenhola to 0.218 in Nynäinen (Table 1). Standard errors of  $H_o$  ranged from 0.044 to 0.057, and of  $H_e$  from 0.058 to 0.061. The observed heterozygosities were slightly lower than those expected, but the differences were not significant. The grand mean over all populations was 0.155 for  $H_o$  and 0.188 for  $H_e$ .

**Table 1.** Mean sample size, mean no. of alleles per locus, percentage of loci polymorphic, observed and expected heterozygosities per population. Population differentiation (D) per population and in the total sample ( $\partial$ ). Standard errors are given in parentheses.

Population	Sample size	Mean no. of alleles per locus <sup>1)</sup>	Percentage of loci polymorphic <sup>2)</sup>	Mean heterozygosity		D
				observed	expected <sup>3)</sup>	
Ruissalo	51	2.2 (0.3)	53.9	0.152 (0.044)	0.191 (0.059)	0.031
Halikko	53	2.1 (0.2)	38.5	0.156 (0.051)	0.174 (0.058)	0.041
Tenhola	67	1.8 (0.3)	30.8	0.136 (0.057)	0.148 (0.061)	0.103
Taivassalo	50	2.1 (0.4)	61.5	0.164 (0.046)	0.210 (0.059)	0.052
Nyynäinen	49	2.3 (0.3)	61.5	0.169 (0.055)	0.218 (0.059)	0.048
Grand mean	54	2.1	49.2	0.155	0.188	$\partial=0.055$

<sup>1)</sup> including monomorphic and variable loci; <sup>2)</sup> 0.95 criterion; <sup>3)</sup> based on Hardy-Weinberg equation, unbiased estimate (see Nei 1978).

### 3.4 Population Differentiation

Genetic differentiation (D) between the populations and the total gene pool ranged from 3.1% in Ruissalo to 10.3% in Tenhola (Table 1). The mean population differentiation (D) computed over loci indicates that the only substantially differentiated stand, Tenhola, diverges from the remainder of the populations at 10.3% of the effective number of alleles at these loci. As measured by  $\partial$ -statistics genetic differentiation between the populations was 5.5%.

## 4 Discussion

The proportion of polymorphic loci in our results appears to be consistent with those of other long-lived woody angiosperms (59.5%) reported by Hamrick et al. (1992). The occurrence of one predominant and a few rare alleles is common in English oak populations (Kremer et al. 1991, Müller-Starck et al. 1993, see the allelic frequency data of Bacilieri et al. 1993). Similarly, in this study only ca. 70% of the variable loci

were polymorphic reflecting a substantial frequency of rare alleles. At the species level, the proportion of variable loci, 73%, in the seed samples from French stands of English oak (Kremer et al. 1991) is comparable to our 84.6% in mature trees.

The number of alleles per locus, 2.1, was in our study equal to the value reported for other angiosperm long-lived woody species (see Hamrick et al. 1992). Compared to other European deciduous tree species (see Müller-Starck et al. 1992), our stands of English oak had fewer alleles per locus than any oak (*Quercus*; 2.8–3.2), beech (*Fagus*; 2.2–4.0) or fig-tree species (*Ficus*; 2.7). The mean number of alleles within all oak species is 2.4, but for European oak species the estimate is as high as 3.8 (Kremer and Petit 1993). Our species level value was 2.7. Hence, the allelic diversity in Finnish stands seems to be slightly lower than that in central populations, but more populations throughout the natural distribution area of the species should be analyzed to confirm the possible difference.

The only published data on mature English oak is based on one stand examined by Bacilieri et al. (1993, 1994). From their allelic frequency-

data (Bacilieri et al. 1993) it can be concluded that at least three alleles per locus were present in that stand, many of them with a frequency below 0.05. In 2-year old German juvenile populations the mean number of alleles per locus was 3.2 (Müller-Starck and Ziehe 1991, Müller-Starck et al. 1993). In our stands, allelic diversity was lower, only 2.1. However, Müller-Starck and Ziehe (1991) and Müller-Starck et al. (1993) scored genotypes only for polymorphic loci, and the published data of Bacilieri et al. (1993) is also based solely on polymorphic loci. Furthermore, a value of 2.7 has been reported for English oak (see Kremer and Petit 1993), where, apparently, also the monomorphic loci have been taken into account. If the monomorphic loci are excluded from our study, the number of alleles per locus only increases by 0.2 to 2.3, and is still below that previously reported for the species.

Mean observed heterozygosity ( $H_o=0.155$ ) was in our study lower than for other European tree species (review by Müller-Starck et al. 1992). In our data, based on mature trees,  $H_o$  was lower than the previously reported values for English oak,  $H_o=0.264$  for seeds (Kremer et al. 1991) and  $H_o=0.213$  for juveniles (Müller-Starck et al. 1993). The low heterozygosity in Finnish stands might indicate a marginal effect, and is contradictory to the suggestion of Müller-Starck et al. (1993) that the relatively low heterozygosity in English oak compared to several other tree species might characterize juvenile oak populations but not the succeeding life stages. However, more data is needed on heterozygosity in English oak, both from mature trees in central populations and Finnish juveniles.

In oaks, the level of population differentiation is on average 6%, with extreme values of 1 to 17% (see Kremer and Petit 1993). Middle-European oak stands are only slightly differentiated as indicated by the  $G_{st}$ -value of 0.02 for English oak (Kremer and Petit 1993). In our study the estimated mean differentiation of  $\partial=0.055$  is the same as in German stands of English oak and only slightly higher than in European beech (Müller-Starck and Ziehe 1991). However, in Finnish stands the range of population differentiation estimates ( $D=0.031$ – $0.103$ ) is wider than in German stands ( $D=0.050$ – $0.062$ ) (Müller-Starck and Ziehe 1991). Considering the signifi-

cantly smaller area covered by our study this may indicate a lower level of gene flow between Finnish stands compared to German ones.

Our results indicate that Finnish stands of English oak are not only more differentiated than e.g. northern Scots pine stands (Muona and Szmidt 1985), but they also have a number of rare alleles. Nevertheless, the majority of the species' variation is found within each studied stand, as is usual for a wind-pollinated tree species (e.g. see Hamrick et al. 1992). Therefore each stand, per se, could be used for seed collection for reforestation purposes. However, all the studied stands are protected by law, and as productive seed orchards are lacking in Finland, only a few cultivated stands are used for reforestation purposes. We have no data on their genetic structure which may differ substantially from that of natural stands.

Furthermore, these results have two additional implications for practical operations. First, if the genetic resources of English oak are to be conserved e.g. in clonal archives, the presence of rare alleles in natural stands must be considered and the archives should be established with material from as many stands as possible. Second, the potentially lower overall variability in Finnish stands must be taken into consideration when breeding populations are established.

## Acknowledgements

We thank Dr. Heikki Toivonen from the University of Turku, Finland, for valuable information on the localities of the natural populations, and sampling of the stands Ruissalo, Nyynäinen and Taivassalo.

## References

- Ashton, D. G. & Braden, A.W.H. 1961. Serum  $\beta$ -globulin polymorphism in mice. Australian Journal of Biological Sciences 14: 248–254.
- Bacilieri, R., Roussel, G. & Ducousso, A. 1993. Hybridization and mating system in a mixed stand of sessile and pedunculate oak. Annales des Sciences Forestières 50, Suppl. 1: 122–127.

- , Labbe, T. & Kremer, A. 1994. Intraspecific genetic structure in a mixed population of *Quercus petraea* (Matt.) Liebl and *Q. robur* L. *Heredity* 73: 130–141.
- Bousquet, J., Cheliak, W.M. & Lalonde, M. 1987. Allozyme variability in natural populations of green alder (*Alnus crispa*) in Quebec. *Genome* 29: 345–352.
- Cheliak, W.M. & Pitel, J.A. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42 Petawawa National Forestry Institute, 49 p.
- Furnier, G.R. & Adams, W.T. 1986. Geographic patterns of allozyme variation in Jeffrey pine. *American Journal of Botany* 73: 1009–1015.
- Gregorius, H.-R. & Roberds, J.H. 1986. Measurement of genetical differentiation among subpopulations. *Theoretical and Applied Genetics* 71: 826–834.
- Guries, R.P. & Ledig, F.T. 1982. Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). *Evolution* 36: 387–402.
- Hamrick, J.L., Godt, M.J.W. & Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.
- Hattemer, H.H. 1991. Measuring genetic variation. In: Müller-Starck, G. & Ziehe, M. (eds.). Genetic variation in European populations of forest trees. Sauerländer's Verlag, Frankfurt am Main. p. 2–19.
- Kephart, S.R. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *American Journal of Botany* 77: 693–712.
- Kremer, A., Petit, R., Zanetto, A., Fougère, V., Ducouso, A., Wagner, D. & Chauvin, C. 1991. Nuclear and organelle gene diversity in *Quercus robur* and *Q. petraea*. In: Müller-Starck, G. and Ziehe, M. (eds.). Genetic variation in European populations of forest trees. Sauerländer's Verlag, Frankfurt am Main. p. 141–166.
- & Petit, R.J. 1993. Gene diversity in natural populations of oak species. *Annales des Sciences Forestières* 50, Suppl. 1: 186–202.
- Müller-Starck, G. and Hattemer, H.H. 1990. Genetics and breeding of oaks. Final Report, MA 1B-0012-D, CEC, DG XII, Brussels, Belgium. 22 pp.
- & Ziehe, M. 1991. Genetic variation in populations of *Fagus sylvatica* L., *Quercus robur* L., and *Q. petraea* Liebl. in Germany. In: Müller-Starck, G. & Ziehe, M. (eds.). Genetic variation in European populations of forest trees. Sauerländer's Verlag, Frankfurt am Main. p. 20–37.
- , Baradat, Ph. & Bergmann, F. 1992. Genetic variation within European tree species. *New Forests* 6: 23–47.
- , G., Herzog, S. & Hattemer, H.H. 1993. Intra- and interpopulational genetic variation in juvenile populations of *Quercus robur* L. and *Quercus petraea* Liebl. *Annales des Sciences Forestières* 50, Suppl. 1: 233–244.
- Muona, O. 1990. Population genetics in forest tree improvement. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L. & Weir, B.S. (eds). Plant population genetics, breeding, and genetic resources. Sinauer, Massachusetts. p. 282–298.
- & Szmídt, A.E. 1985. A multilocus study of natural populations of *Pinus sylvestris*. In: Gregorius, H.-R. (ed.). Population genetics in forest trees. Springer Verlag, Berlin. p. 226–240.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.
- Schwarzmann, J.F. & Gerhold, H.D. 1991. Genetic structure and mating system of northern Red oak (*Quercus rubra* L.) in Pennsylvania. *Forest Science* 37: 1376–1389.
- Shaw, C.R. & Prasad, R. 1970. Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochemical Genetics* 4: 297–320.
- Skult, H. 1965. *Quercus robur* L. Tammi. In: Jalas, J. (ed.). *Otavan suuri kasvikirja II*. Otava, Keuruu. p. 102–107.
- Steinhoff, R.J., Joyce, D.G. & Fins, L. 1983. Isozyme variation in *Pinus monticola*. *Canadian Journal of Forest Research* 13: 1122–1131.
- Yeh, F.C. & Layton, C. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine (*Pinus contorta* ssp. *latifolia*). *Canadian Journal of Genetics and Cytology* 21: 487–503.
- & El-Kassaby, Y.A. 1980. Enzyme variation in natural populations of Sitka spruce (*Picea sitchensis*). 1. Genetic variation patterns among trees from 10 IUFRO provenances. *Canadian Journal of Forest Research* 10: 415–422.
- & O'Malley, D. 1980. Enzyme variations in natural populations of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. 1. Genetic variation patterns in coastal populations. *Silvae Genetica* 29: 83–92.

Total of 27 references