Genetica (2006) 127:231–241
DOI 10.1007/s10709-005-4014-7

Genetic variability of fragmented stands of pedunculate oak (*Quercus robur*) in Finland

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Received 1 November 2004; Accepted 11 October 2005

Key words: colonization, differentiation, fragmentation, genetic variability, *Quercus*, Wahlund effect

Abstract

The genetic structure of 33 natural *Quercus robur* stands in Finland was studied using 13 allozyme loci to analyze the effects of fragmentation in a wind-pollinated tree species. The present fragmented and discontinuous distribution of oak is a result of both short-term human impact and long-term climatic and geological change, including post-glacial land uplift. In accordance with general expectations, genetic diversity in small populations was lower than that in large populations, and differentiation among small populations was higher than that among large populations. Heterozygote deficiency was more pronounced in large populations, which is proposed to be a Wahlund effect created by either spatial sub-structuring or the existence of synchronized flowering lineages. Also genetic differentiation was higher and diversity lower in Finland than the estimates reported for Central Europe. There were differences in the genetic structure on sites of different geological age. We suggest that on most geologically old sites drift has a prominent effect whereas on younger sites also founder effects may be important.

Introduction

Neutral genetic variation in a population or in a set of populations, is affected e.g., by local genetic drift, population bottlenecks, founder effects and gene flow between populations. According to the standard population genetics theory, random drift decreases variability within a population and increases differentiation among populations, whereas gene flow prevents differentiation. The effect of colonization on genetic differentiation is more complicated and depends on the details of the process, e.g., number and origin of colonizers (Whitlock & McCauley, 1990; Pannell & Charlesworth, 2000). Habitat fragmentation, namely splitting of continuous habitat into smaller and at least partially isolated units, changes the balance between gene flow and random drift. As a result, less variability within and more differentiation among the populations in fragmented than in non-fragmented areas is expected (e.g., Young, Boyle & Brown, 1996).

Forest fragmentation, influencing both the forest trees and other forest-bound organisms, is among the major concerns in conservation biology. The studies on genetic effects of fragmentation on tree populations are few and mostly on tropical species. Results have been variable: e.g., Collevatti, Grattapaglia and Hay (2001) did not find any fragmentation effect on genetic variability in *Caryocar brasiliense*, whereas White, Boshier and Powell (1999) detected loss of low-frequency alleles in fragmented populations of *Swietenia humilis*. As an example of historical fragmentation, Billington (1991) reported a correlation between population size and amount of genetic variability

in a wind pollinated shrub *Halocarpus bidwillii*. In a meta-analysis including many plant and animal species, population size as such was shown to have a positive correlation with genetic variability in a significant majority of studies (Frankham, 1996).

Colonization studies on forest trees have focused on the long-term process of post-glacial recolonization of northern parts of Europe after the last glaciation. Particularly, studies using maternally inherited chloroplast-DNA of two closely related oak species (*Quercus robur* and *Q. petraea*) have shown that the refugia and colonization routes can still be tracked and there are sometimes very narrow transition zones, where expansions from different refugia have met (Ferris et al., 1998; Jensen et al., 2002; Petit et al., 2002). The expansion reached the northern limit of oak distribution in Finland about 8000 years BP (before present) from both east and west, timing indicated by pollen data (Huntley & Birks, 1983) and directions by chloroplast haplotype data (Ferris et al., 1998; Jensen et al., 2002). Nuclear genes (isozymes and microsatellites) show much less differentiation among oak populations, probably as a consequence of efficient gene flow via pollen dispersal (e.g., Zanetto, Roussel & Kremer, 1994; Gömöry et al., 2001; Kremer et al., 2002). Indeed, microsatellite studies have shown that the proportion of immigrating pollen in France at the stand level is very high (Streiff et al., 1998). Genetic effects of colonization on a smaller geographic and shorter time scale have received attention in the context of metapopulation theory (e.g., Harrison and Hastings, 1996) and have been studied mostly on animals and also herbaceous plant species. The basic work on *Silene dioica* (Giles and Goudet, 1997) demonstrates that colonization dynamics increase genetic variance among newly founded populations.

The populations of pedunculate oak (*Q. robur*) in Finland are strongly fragmented and growing at the northern margin of the species' European distribution. Pedunculate oak is the only oak species in Finland; even the closely related sessile oak (*Q. petraea*) is absent and hence introgression between the species does not affect genetic variability. Apparently only lineages from refugia in Italy and the Balkans colonized Finland and the contact zone of the haplotypes is still relatively well defined (Ferris et al., 1998; Jensen et al., 2002). *Quercus* pollen was quite common during a period of 3000 years in the southern part of the country (Huntley & Birks, 1983; Alho, 1990) and fossil pollen from the warm period 6000–4000 years BP has been recorded 500 km to the north of the present limit of the species (Donner, 1963; Brewer et al., 2002). The cooling climate and expansion of Norway spruce led to a long-lasting decline of oak populations and, starting about 300 years ago, the stands were further fragmented owing to increasing human pressure for more agricultural land. Oak populations have also been exploited as a source of valuable timber, and even destroyed on some occasions (Donner, 1926) as the trees were declared to be crown property by law and farmers consequently regarded oak trees as a burden and nuisance. At the present time, nature conservation and forestry policies promote practical management in favour oak. Consequently, many of the oak populations are now expanding, sometimes starting from a very small group of old trees. Planting of oak is not common in Finland, and the low number of haplotypes and sharpness of the contact zone support the idea that man-mediated seed transfer has not been very extensive in the past, either. The distribution type of pedunculate oak in Finland, namely distinct and often relatively small stands (ranging from 10 to 5000 mature trees), provides an opportunity to study fragmentation consequences in a typically wind-pollinated tree species.

In this study we test if small populations differ from large populations in genetic variability and in differentiation. We expect to see the effect of fragmentation, i.e. reduced genetic variation and increased differentiation, more clearly in small than in large populations. We study the effect of colonization by comparing genetic composition of populations in geologically old vs. more recent sites. We also compare the amount and distribution of genetic variability in Finnish pedunculate oak populations to those in Central European populations.

Material and methods

Study populations

Dormant winter buds were sampled from 28 natural populations of pedunculate oak (*Q. robur*) in southern Finland (Figure 1) and kept at −20°C

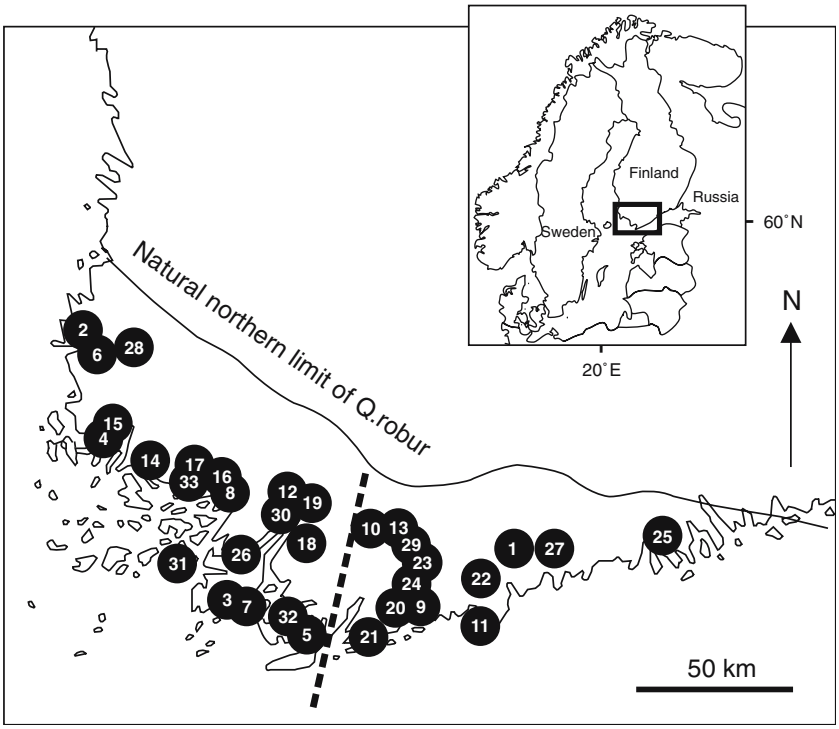

Figure 1. Location of sampled pedunculate oak stands in Finland. Dashed line indicates the contact zone of eastern and western chloroplast haplotype according to Ferris et al. (1998).

until analysis. Earlier data for five populations were obtained from Mattila et al. (1994), giving a total of 33 populations. The sampling covers the natural distribution of pedunculate oak in Finland except the Åland islands and the outer archipelago. In small populations ($N < 60$) all the mature trees were sampled, and in large populations 27–106 (on the average 55) randomly selected trees, with the exception of one very large population (number 33) where sampling was performed as 3 sub-samples (46, 40 and 44 trees) in areas of 0.5×0.5 km which were located 1–2 km apart. The approximate population size, area and distance to the nearest oak stand for each sample site is given in Table 1. The population size is not dependent on the location of the stand, e.g., small stands were sampled evenly over the study area. All stands include trees in several age classes and there is no indication that small stands would be recently colonized. The names and exact locations of the stands are available upon request from the first author.

Most of the present South Finland was submerged when the ice receded at the end of the last ice age about 10,000 years ago. This area has been, and still is, subject to a rapid land uplift (e.g., Miettinen, 2004). Colonization of the newly emerged ground in the south and simultaneous decline in the north has made the whole distribution of oak in Finland to shift towards south and west. In a way the process could be seen as the last phase of the post-glacial colonization of Europe by oaks, only now towards south.

The age of the study populations is not known, but rough geological age of a population site can be estimated from the elevation of each site using local uplift rate (Kääriäinen, 1966) and by comparing the elevation to shore displacement curves, where dating is radiocarbon-based (Eronen et al., 1995; Hyvärinen, 1999; Miettinen, Eronen & Hyvärinen, 1999). The uplift rate-based estimation was applied up to the elevation of 15 m, for the higher elevations dating was based on the shore displacement curves. On the slope sites, the elevation of highest-growing old trees was taken as the site elevation. Estimated ages of the sites are presented in Table 1. In order to study the effect of colonization, the populations were classified in one of the three site

Table 1. Sample size (n), population size (N), stand area in hectares (Area), site age in 1000 years (Age), map distance to the nearest other stand (Dist, km), chloroplast lineage (Cp, from Ferris et al., 1998, T = western and C = eastern), percentage of polymorphic loci at 95% (*P95*), number of alleles (A), number of alleles in polymorphic loci (AP), allelic richness at sample size 15 (AR), expected heterozygosity (H_e), observed heterozygosity (H_o), fixation index (F_{is}) and average genetic distance from other stands (D) for each population

Pop	n	N	Area	Dist	Age	Cp	*P95*	A	AP	AR	H_e	H_o	F_{is}	D
1	21	21	0.5	6.9	9.5	–	0.62	1.85	2.13	1.80	0.204	0.155	0.073	0.029
2	24	24	0.5	12.7	1.6	T	0.39	1.54	2.40	1.49	0.132	0.102	0.229	0.030
3	22	22	1	5.2	1.6	–	0.54	1.62	2.14	1.61	0.146	0.162	−0.113	0.028
4	26	26	1	3.9	1.0	–	0.54	1.69	2.14	1.62	0.170	0.188	−0.111	0.026
5	25	25	1	2.8	1.9	T	0.23	1.23	2.00	1.23	0.100	0.094	0.065	0.031
6	24	24	1	12.7	1.9	T	0.46	1.62	2.33	1.61	0.164	0.174	−0.064	0.014
7	16	16	1	5.2	1.7	–	0.54	1.69	2.29	1.68	0.148	0.137	0.078	0.024
8	40	40	2	2.3	6.3	–	0.46	1.92	2.50	1.65	0.134	0.137	−0.021	0.014
9	33	33	2	2.7	7	–	0.31	1.54	2.25	1.45	0.136	0.116	0.150	0.017
10	52	52	3	10.9	9.5	C	0.39	1.62	2.00	1.47	0.129	0.142	−0.103	0.024
11	28	28	3	9.9	1.5	–	0.62	1.77	2.13	1.73	0.218	0.200	0.084	0.019
12	37	120	4	2.6	8	T	0.54	2.00	2.43	1.76	0.179	0.186	−0.040	0.012
13	64	150	5	9.3	10	C	0.69	2.15	2.56	1.83	0.213	0.197	0.075	0.025
14	49	500	5	1.9	1.9	T	0.62	2.23	2.75	1.90	0.189	0.186	0.019	0.013
15	49	500	5	3.9	2.9	T	0.54	1.92	2.43	1.74	0.180	0.159	0.118	0.012
16	47	1000	6	3.3	3.6	T	0.39	2.00	2.80	1.76	0.163	0.170	−0.045	0.009
17	58	200	6	1.9	2.8	–	0.46	2.00	2.83	1.71	0.182	0.174	0.049	0.011
18	25	25	7	1.4	6	T	0.77	1.92	2.20	1.89	0.208	0.183	0.123	0.008
19	48	125	7	5.8	9	–	0.62	2.00	2.50	1.84	0.188	0.175	0.073	0.015
20	40	40	8	1.1	8.2	–	0.39	1.54	2.00	1.47	0.121	0.119	0.014	0.013
21	57	125	8	2	1.9	–	0.46	1.92	2.33	1.67	0.197	0.190	0.035	0.028
22	80	100	8	9	6.5	–	0.46	1.77	2.17	1.56	0.150	0.153	−0.021	0.014
23	51	75	8	8.8	10	–	0.46	2.15	2.33	1.70	0.141	0.138	0.023	0.010
24	65	150	11	2.7	7.5	–	0.54	2.08	2.43	1.81	0.175	0.155	0.114	0.010
25	49	100	11	11.5	5	C	0.39	1.85	2.20	1.60	0.130	0.123	0.055	0.012
26	45	200	14	5	3.0	–	0.54	1.85	2.14	1.71	0.142	0.125	0.120	0.014
27	51	150	15	6.9	5.7	C	0.39	1.69	2.60	1.64	0.166	0.142	0.146	0.017
28	49	100	16	16.5	2.8	T	0.54	1.92	2.43	1.78	0.194	0.145	0.255	0.015
29	82	250	26	9.3	7.5	C	0.62	2.39	2.75	1.92	0.153	0.129	0.158	0.011
30	69	250	29	1.8	3.3	–	0.39	1.92	2.20	1.52	0.136	0.120	0.118	0.011
31	27	2000	49	3.2	2.0	T	0.46	1.85	2.33	1.74	0.166	0.108	0.351	0.016
32	106	250	50	5.1	3.5	T	0.39	1.92	2.40	1.57	0.118	0.100	0.154	0.012
33	130	5000	350	1.9	2.7	T	0.46	2.54	3.00	1.87	0.179	0.154	0.140	0.009
Mean	48	355	20	5.8	4.8		0.49	1.87	2.37	1.68	0.162	0.150	0.070	0.017

age classes (< 4000 years, 4000–8000 years, > 8000 years). The first class (young) consists of sites that have been colonized during the general decline of oak, second (intermediate) of the sites that have been available during the most favourable climatic conditions and third class (old) consists of sites that have been above surface already at the time when colonization supposedly started.

Chloroplast haplotype classification, namely T (western) and C (eastern), was obtained from Ferris et al. (1998) for 17 populations (Table 1). Note that the naming of oak cytotypes is not well established and the types T and C in Ferris et al. (1998) correspond to C and A, respectively, in Jensen et al. (2002) and Kremer et al. (2002). The populations of known chloroplast type were used

in ANOVA to test the possible differentiation of the nuclear genome of the two haplotype lineages.

Electrophoresis

Bud scales were removed and tissue from three or four buds per tree was homogenized in 3–4 drops of 0.1 M Tris–HCl extraction buffer pH 7.5 (Bousquet, Cheliak and Lalaonde, 1987), modified by omitting polyethylene glycol. Allozyme variation in the populations was analyzed at 13 loci: LAP3 (leucine aminopeptidase, E.C. 3.4.11.1), GOT (glutamate-oxaloacetate transaminase E.C. 2.6.1.1), ADH (alcohol dehydrogenase E.C. 1.1.1.1), PGI1 and PGI2 (phosphoglucoisomerase E.C. 5.3.1.9), SDH (shikimate dehydrogenase E.C. 1.1.1.25), MNR (menadione reductase E.C. 1.6.99.2), IDH2 (isocitrate dehydrogenase E.C. 1.1.1.41), FEST3 (fluorescent esterase E.C. 3.1.1.1), GDH (glutamate dehydrogenase E.C. 1.4.1.3), 6-PGD (6-phosphogluconate dehydrogenase E.C.1.1.1.44), MDH1 and MDH2 (malate dehydrogenase E.C. 1.1.1.37). This notation follows Mattila et al. (1994) and in most cases can be translated into the codes used by Zanetto, Roussel and Kremer (1994). MNR and GDH are the same as in Zanetto, and PGI1, PGI2, MDH1 and MDH2 are the same loci as in Zanetto, if A is substituted for 1 and B for 2. Furthermore, GOT, SDH and 6PGD in this paper are equal to GOT-B, SDH-B and 6PGD-A in Zanetto.

Standard starch gel electrophoresis (12% Sigma Hydrolyzed Starch) and three buffer systems were used: Ashton pH 8.1 (Ashton and Braden, 1961) and Tris–citrate pH 7.1 and pH 7.8 (Shaw and Prasad, 1970). Enzyme activity staining protocols were according to Cheliak and Pitel (1984) with slight modifications. Genetic interpretation of the isozymes and alleles was based on their sub-unit structure, and on assumed Mendelian inheritance and codominance (Kephart, 1990; Bacilieri, Labbe and Kremer, 1994; Müller-Starck et al., 1996).

Statistical analysis

To describe genetic variability within and differentiation among populations, basic population genetic parameters, i.e. the proportion of polymorphic loci at the 95% level (*P95*), average number of alleles per locus (*A*), average number of alleles per polymorphic loci (AP), average allelic richness per locus (AR), (Petit, El Mousadik and Pons, 1998), expected (H_e) and observed (H_o) heterozygosity, genetic distance (Nei, 1978), total and averaged within-population genetic diversity (H_T and H_S) and F-statistics (F_{st}, F_{is}) according to Weir and Cockerham (1984), were calculated using GDA software (Lewis and Zaykin, 2001) and FSTAT ver 2.9.3.2 (Goudet, 2002) for all populations using 13 loci. Confidence intervals for F_{st} estimates were obtained by bootstrapping over the loci (Weir, 1996). For the sub-samples of the largest population (population 33), a separate F-analysis was performed to estimate differentiation among sub-samples (F_{st}) and fixation index (F_{is}). Population differentiation (F_{st}) and mean values of *P95*, AR, H_e, H_o and F_{is} were calculated also for the groups of populations assigned to eastern or western haplotypes in Ferris et al. (1998), (see Table 1) and for the site age classes. The equality of means was tested with ANOVA using software package Systat®10. The effect of population size on *P95*, AR, H_e, H_o and F_{is} was tested with regression analysis using Systat®10. As the regression of AR on population size is inherently heteroscedastic, the dependence was tested also with Spearman rank correlation.

To test for isolation by distance (distance-dependent gene flow), correlation between pairwise genetic distances and pairwise map distances was estimated and tested with Mantels test (10000 randomizations) using FSTAT ver 2.9.3.2 (Goudet, 2002). To analyze the effect of population size, and hence local random processes, on the population differentiation, correlation between pairwise genetic distances and average size of population pairs was estimated and tested with Mantels test (10000 randomizations) using FSTAT. These analyses were performed separately for the three site age classes.

Results

The allele frequencies are available upon request from the first author (P. Vakkari). One locus, *PGI-1*, was monomorphic in all the populations. Overall genetic variability was moderately high, $H_e = 0.162$ and AR = 1.678. Sample size and basic genetic parameters, i.e. the proportion of

Table 2. Regression analysis of genetic parameters vs. population size (log-transformed) in 33 Finnish *Quercus robur* populations

Genetic parameters	*F*-ratio	*R*	*P*
P95	0.028	0.030	0.868
AR	7.213	0.434	0.012
*H*e	0.788	0.157	0.381
*H*o	0.001	0.007	0.971
F_{is}	4.471	0.355	0.043
F_{is} vs. area	7.969	0.452	0.008

Regression of F_{is} vs. stand area (log-transformed) is shown on the bottom line. *F*-ratios, correlation coefficient (*r*) and *P*-values are shown.

polymorphic loci at the 95% level, alleles per locus, alleles per polymorphic loci, allelic richness, expected and observed heterozygosity and F_{is}, are given in Table 1.

Genetic variability, measured by AR, was higher in the large populations ($r = 0.434$, $P = 0.012$; Spearman $r = 0.409$, $P = 0.018$), whereas regression of *P95*, H_e or H_o on population size was not significant, although positive (Table 2, Figure 2a). The fixation index F_{is} was also significantly higher for the large (by both census number and stand area) populations (Table 2, Figure 2b). None of the correlations between the genetic parameters and the distance to the nearest oak stand were statistically significant.

Genetic differentiation (F_{st}) among all populations was 0.066 and statistically significant (Table 3). Correlation between genetic and geographic distance over all populations was positive but non-significant ($r = 0.075$, $P = 0.078$ by Mantel's test), genetic distances having fairly high and even dispersion over the range of geographic distances. Correlation between population size and pair-wise genetic distance was negative and significant, ($r = -0.245$, $P = 0.0001$ by Mantels test).

For each of the three site age classes (young, intermediate, old) the estimated population differentiation was significant ($F_{st} > 0$) (Table 4). The differentiation estimates are higher in the young and old age class, but F_{st}-estimates did not differ significantly from each other in pairwise permutation tests. The average genetic distances were higher in young and old sites compared to the intermediate sites, creating a U-shaped scatter plot (Figure 3). As there is no theoretical prediction for

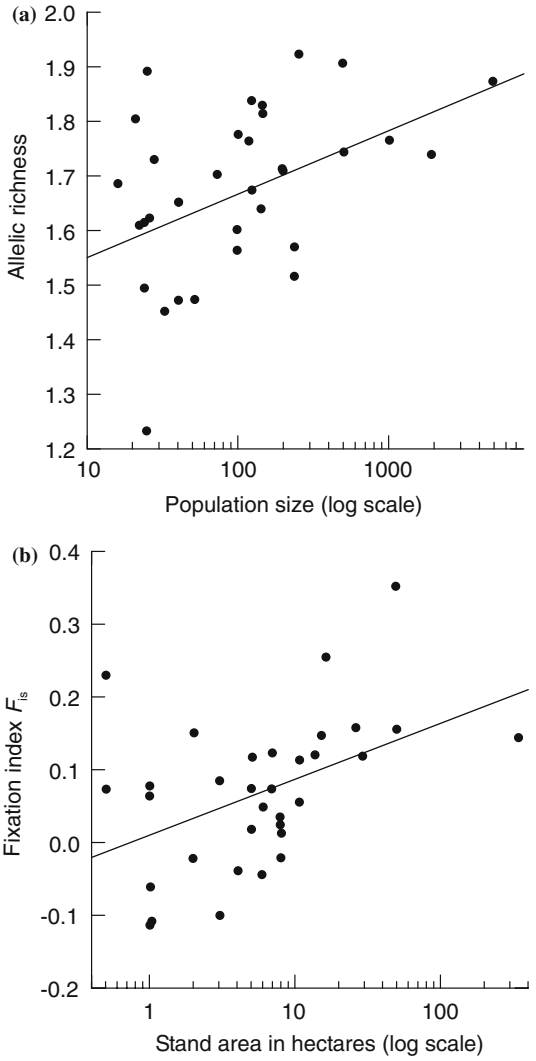

Figure 2. The relationship between population size and the allelic richness (AR) (a) and between population area and the inbreeding coefficient (F_{is}) (b) for 33 populations of *Quercus robur* in Finland.

this relationship, a simple correlation analysis was made by splitting the data in two parts at the age of 4000 years and performing separate Mantel tests between Nei's genetic distance and pairwise average of site ages. Both correlations were significant, coefficient was negative in the young class ($r = -0.58$, $P = 0.0001$) and positive in the old class ($r = 0.38$, $P = 0.0001$), indicating that the pattern is not only a random one. Correlation between genetic and geographic distance was positive and statistically significant for the young sites, whereas for intermediate and old sites the correlation was

Table 3. Total genetic diversity (H_T), averaged diversity within populations (H_S), average fixation index within populations (F_{is}) and genetic differentiation among populations (F_{st}) at 12 polymorphic loci

Locus	H_T	H_S	F_{is}	F_{st}
GOT	0.085	0.081	−0.049	0.048
IDH-2	0.444	0.421	−0.004	0.038
GDH	0.362	0.318	−0.006	0.103
PGI-2	0.084	0.077	−0.100	0.097
ADH	0.088	0.081	0.041	0.065
MNR	0.522	0.477	0.177	0.067
6-PGD	0.081	0.076	0.148	0.071
FEST-3	0.157	0.147	−0.011	0.085
MDH-1	0.004	0.004	−0.036	0.035
MDH-2	0.009	0.009	0.136	0.011
LAP-3	0.318	0.296	0.187	0.050
SDH	0.137	0.125	0.193	0.068
Over loci	0.191	0.176	0.079	0.066
Cl 95% upper	0.302	0.278	0.146	0.084
Lower	0.080	0.072	0.002	0.051

F_{st} is estimated according to Weir and Cockerham (1984), and the confidence interval (95%) is obtained by bootstrapping over the loci (Weir, 1996).

not statistically significant (Table 4). Correlation between population size and genetic distance was negative and statistically significant for the young sites, whereas for intermediate and old sites the correlation was not statistically significant (Table 4).

Between the regions of the eastern and western haplotype, hierarchical F-analysis did not indicate any differentiation ($F_{st} = 0$), nor were the means for allele numbers, allelic richness (AR), heterozygosities or F_{is} significantly different (Table 5). Results in Table 5 are from an ANOVA, where only populations of known chloroplast haplotype are included; the outcome is the same (no significant differences) if all the study populations are grouped as eastern or western based on their location in relation to the contact zone of haplotypes (dashed line in Figure 1). In the largest population (33) the three subpopulations did not differ from each other ($F_{st} = 0.005$), and the F_{is} estimates within subpopulations were positive although variable (F_{is}: 0.08; 0.20; 0.12).

Discussion

Population fragmentation is expected to lead to higher genetic differentiation among fragments and lower genetic diversity within each fragment. The genetic erosion should first be seen in the smallest remaining populations, where random drift is efficient. In support of this expectation, we found that the genetic differentiation was higher ($F_{st} = 0.066$) and variability lower ($H_e = 0.164$) in Finland than in Central Europe ($F_{st} = 0.024$, $H_e = 0.252$, Zanetto, Roussel and Kremer, 1994). It is reasonable to assume that the higher differentiation in Finland is attributed to the small populations since the pair-wise genetic distances correlated negatively with population size. The lack of correlation between genetic and geographic distance (estimated over all populations) further stresses the importance of random processes relative to distance-dependent gene flow. In addition, the small oak populations in Finland had lower allelic richness than the large ones, whereas correlations between population size and other genetic diversity estimates were not statistically significant. This observation is in concordance with the result that number of alleles is more sensitive than heterozygosity to the reduction in population size (e.g., Kuittinen and Savolainen, 2000). This observed genetic structure might be a

Table 4. Number of populations (n), population size (N), stand area in hectares (Area), map distance to the nearest other stand (Dist, km), genetic differentiation (F_{st}), lower and upper confidence limits for F_{st}, correlation between genetic and geographic distance (r_{gg}), correlation between pairwise genetic distance and average population size (r_{gs}) and the respective risk probability (P) for both correlations, by Mantel test for each site age class

Age class[a]	n	N	Area	Dist	F_{st}	Cl 95% low	Cl 95% high	r_{gg}	P	r_{gs}	P
1	18	570	30	5.5	0.065	0.052	0.074	0.225	0.006	−0.333	0.0001
2	8	106	10	5.3	0.044	0.034	0.054	−0.154	0.426	−0.165	0.398
3	7	83	5	6.5	0.083	0.040	0.143	0.350	0.120	−0.351	0.117

[a]Age classes: 1 young (< 4000 years), 2 intermediate (4000–8000 years), 3 old (8000 years and over).

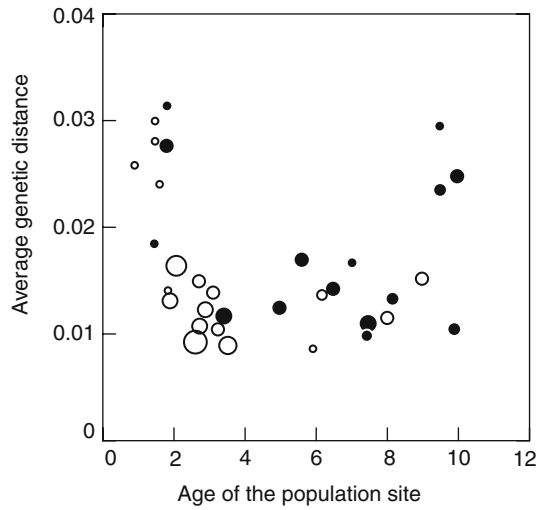

Figure 3. The relationship between average pairwise genetic distances and site age (1000 years) for 33 populations of *Quercus robur* in Finland. Solid circles indicate populations east and open circles populations west of the contact zone of eastern and western haplotype. Circle diameter is proportional to the logarithm of population size.

consequence of man-induced fragmentation, which started about 300 years (4–5 oak generations) ago, providing enough time for genetic changes of the observed magnitude to arise.

In other species, reduced variability after fragmentation has been found in small populations of *Eucalyptus albens* (Prober and Brown, 1994), as well as to some extent in *Carapa guianensis* (Dayanandan et al., 1999) and *Swietenia humilis* (White, Boshier and Powell, 1999). Also non-significant fragmentation effects on variability are reported, e.g., for *Sorbus aucuparia* (Bacles, Lowe and Ennos, 2004), *Antirrhinum subbaeticum* (Jiménez et al., 2002) and for *Silene nutans* (van Rossum et al., 1997). The consequences of fragmentation may also be just the opposite of expectations: Foré et al. (1992) reported lower differentiation after fragmentation among stands of *Acer saccharum*. They explained this observation by assuming that fragmentation exposes trees to the wind, thus leading to higher gene flow (Foré et al., 1992). This inverted relationship might be observed over relatively short distances (see threshold distance in Young, Boyle and Brown, 1996), and also when pollination is by insects or other animals that are able to adapt to a decreasing number of trees (White, Boshier & Powell, 2002; Dick, Etchelecu and Austerlitz, 2003). Apparently, fragmentation evident to a human observer may not be fragmentation at all in terms of gene flow.

In addition to fragmentation, colonization events may affect differentiation among populations. Especially, differentiation among newly colonized populations can be higher than that among older populations, excluding the oldest, declining populations (Giles and Goudet, 1997). In our data the F_{st} was lowest in the intermediate site age class, which is concordant with the observations in Giles and Goudet (1997), although the pair-wise comparisons between classes were not statistically significant. The observed pattern (Figure 3) in this study could also be explained by an action of both colonization (younger sites) and random drift (older sites). However, there are issues that have to be considered. Firstly, the oldest sites are located in the region of the eastern chloroplast type, which makes our data unbalanced in this respect. Secondly, the age of the site is the maximum possible age of the stand whereas the information on the true age of the populations is not available. Thirdly, the age of the 'young' sites is quite high, over 1500 years, and thus there has been enough time also for drift and gene flow to operate. This also implies, that the group may be heterogeneous, including populations at quite different post-colonization stages. The negative correlation between present population size and average genetic distance among young sites is an

Table 5. Number of populations (n) in the eastern and western haplotype groups according to Ferris et al. (1998), mean values of percentage of polymorphic loci (*P95*), number of alleles (A), alleles per polymorphic loci (AP), allelic richness (AR), unbiased expected heterozygosity (H_e), observed heterozygosity (H_o) and fixation index (F_{is}) for both groups

Chloroplast haplotype	n	*P95*	A	AP	AR	H_e	H_o	F_{is}
East	5	0.492	1.938	2.421	1.693	0.158	0.147	0.066
West	12	0.481	1.891	2.459	1.697	0.164	0.147	0.109

None of the group means are statistically significantly different by ANOVA.

indication of drift – unless we assume that present population size is also correlated with the number of colonizers. Furthermore, there seems to be isolation-by-distance-structure among the young sites (positive correlation between genetic and map distance, Table 4). This structure could arise from short-distance colonization and/or from efficient gene flow from adjacent populations after colonization. As the isolation-by-distance-structure is not observed in the other age classes, we suggest that the short-distance colonization is the more plausible explanation. Short-distance colonizing events after primary colonization would allow also the persistence of the distinct contact zone of the chloroplast haplotypes (Ferris et al., 1998). The correlation patterns in our data are exactly the opposite of that observed by Giles and Goudet (1997), which indicates that in our data either colonization mode or gene flow or both follow a different pattern. This is not surprising considering the biological differences of an insect-pollinated herb and a wind-pollinated tree. Actually, it has been shown that longevity, long juvenile phase and wind-pollination of many trees affect the genetic effects of colonization (Austerlitz et al., 2000; Austerlitz and Garnier-Géré, 2003).

In this study, heterozygote deficiency is more pronounced in large than in small stands, an observation which can be explained by a Wahlund effect. This positive correlation between F_{is} and population size is opposite to the observations on several herbaceous species, e.g., *Swainsonia recta* (Buza, Young and Thrall, 2000) and *Succisa pratensis* (Vergeer et al., 2003), where heterozygote deficiency is significantly stronger in small populations. Positive F_{is} values, based on allozymes, have also been reported for *Quercus petraea* in Central Europe (see Kremer et al., 2002) and, using microsatellites, for several other species such as *Fraxinus excelsior* (Morand et al., 2002), *Swietenia humilis* (White, Boshier and Powell, 1999) and *Magnolia sieboldii* (Kikuchi and Isagi, 2002). The basic explanations for this include the existence of null alleles, (partial) selfing and cryptic population substructuring, resulting in a Wahlund effect (see e.g., Hartl and Clark, 1997, p.125). Null alleles in isozymes have not been reported in other European studies on oak (Zanetto, Roussel and Kremer, 1994; Müller-Starck et al., 1996; Gömöry et al., 2001), and more null alleles in the large stands is not a very likely explanation. In our study the population substructuring (either spatial or temporal or both) seems to be the most plausible explanation for the positive fixation index (F_{is}) in the large populations.

The genetic structure of Finnish oak populations is also affected by their location at the northern margin of the European distribution of the species. This position of the populations could affect their genetic structure through two factors in addition to fragmentation: the closely related *Q. petraea* is absent in Finland and long-distance pollen flow is (probably) not as efficient as in more central areas. Although some southern pollen undoubtedly reaches Finland, this seems to occur before the northern flowers are receptive, and the difference in timing is enhanced by the Baltic Sea and Gulf of Finland. In fact, in some years the temporal distribution of airborne oak pollen has two peaks, the first one almost 1 month prior to the flowering of local oaks (Anonymous, 1994). Thus the location at the margin of the species distribution enhances the effects of fragmentation. Similarly the heterozygosity estimate for Russian populations in the eastern-most part of the distribution ($H_e = 0.163$, Gömöry et al., 2001) is lower than in Central Europe.

This study demonstrates that fragmented distribution pattern in combination with range shift and colonization can affect genetic structure even in a wind-pollinated tree species. In support of this we found that differentiation among the Finnish oak populations is higher and genetic variability lower than in Central Europe, and that genetic structure of the populations is affected by population size and age of the population site. We suggest that the observed genetic structures are in young sites attributed to short-distance colonization and gene flow, and also to random drift. The older populations are characterized by low level of gene flow between adjacent populations and by drift, which removes the isolation-by-distance-structure and later increases population differentiation. Both the fragmented distribution and the location at the species' margin are suggested to facilitate the random genetic processes.

Acknowledgements

The authors thank Christoph Haag, Anni Harju, Katri Kärkkäinen, Ben Steele and anonymous referees for their valuable comments on the

manuscript. Part of the study was funded by the FIBRE-programme of the Academy of Finland.

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