EVALUATION OF THE FINNISH GENE-CONSERVATION STRATEGY FOR NORWAY MAPLE (*ACER PLATANOIDES* L.) IN THE LIGHT OF ALLOZYME VARIATION

Mari Rusanen[1], Pekka Vakkari[1] & Anu Blom[2]

[1)] Finnish Forest Research Institute, Box 18, FIN–01301 Vantaa, Finland, mari.rusanen@metla.fi
[2)] Trondheimsgatan 46, SE–16430 Kista, Sweden

Received March 14, 2000, accepted June 6, 2000

ABSTRACT

Allozyme variation at 14 loci was estimated in 29 populations of Norway maple, covering the whole distribution area of the species in Finland. The results were used to evaluate the Finnish gene-conservation program for Norway maple. The average value for H_e over all populations was 0.128; the population estimates ranged from 0.042 to 0.207. There was no statistically significant correlation between population size and within-population variability. The average genetic differentiation (F_{st}) between populations was 0.120. The average genetic distance between populations was 0.034; the correlation between genetic distances and geographic distances was non-significant. In a hierarchical analysis, the differentiation between populations within geographic groups (F_{pop} = 0.163) clearly exceeded the differentiation between geographic groups (F_{reg} = 0.030). In light of the present results, the genetic conservation program, which is based on *ex situ*-collections, seems to be justified.

Keywords: *Acer platanoides*, gene-conservation, allozymes, genetic variability

INTRODUCTION

The value of forest genetic resources was first acknowledged internationally in the 1992 Convention on Biological Diversity, after which several countries started to build up or enforce their national strategies for genetic conservation of forest trees. During a follow-up meeting to Strasbourg Resolution 2, noble hardwoods were identified as a priority group of species (TUROK *et al.* 1996). Noble hardwoods have high value for meeting human needs; many of them produce valuable timber, but several are multipurpose trees and the whole group contributes to the ecological stability of the forests and to attractive landscapes.

The gene resources of noble hardwoods have special value in northern Scandinavia, where these species grow at the northern margin of their distribution in a harsh climate, forming small, fragmented populations. The challenges for gene conservation at the margin are both to safeguard the specific adaptedness that already exists and to capture enough variation to maintain adaptibility for future evolution. An effective strategy is especially hard to constract when the basic knowledge of genetic structure is scarce or even non-existent. One way to overcome this problem is to use generalisations based on literature reviews and correlations that have been estimated for some life-history traits and genetic structure of species (HAMRICK *et al.* 1992).

When conservation of noble hardwoods started in Finland in 1993, establishment of *ex situ*-collections was chosen as the main approach. The purpose of this study is to evaluate the strategy used for gene conservation by studying the amount of genetic variation within and between Finnish populations of Norway maple at allozyme loci. While this approach may give a limited perspective for gene conservation of the traits under selection (HAMRICK & GODT, MILLAR & WESTFALL 1992), it will provide information on the isolation level of the stands and the possible role of genetic drift and migration in the evolution of the populations in question.

NORWAY MAPLE AND ITS GENETIC CONSERVATION IN FINLAND

In Finland Norway maple grows in small populations, which are seldom pure but are typically mixed with other species such as Norway spruce or birch and other broad-leaved species. Maple is monoecious and outcrossing, and the flowers are functionally unisexual (DE JONG 1976). It is mainly insect-pollinated but may also be partly wind-pollinated like sugar maple (GABRIEL & GARRETT 1984). The seeds are relatively heavy but the structure of maple samaras uses the microgeographical winds effectively for dissemination (GREENE & JOHNSON 1992). Maple regenerates easily; in good light

conditions seed crops are often abundant. In wild populations the generations are typically overlapping and the seed crop is often unevenly distributed among trees. The seeds germinate readily in rich forest soil, but the seedlings are relatively poor competitors with respect to other species, e.g. Norway spruce. The distribution of maple in Finland has been largest in the Atlantic period 5000–8000 years ago; since that time the northern margin has withdrawn southward (ALHO 1990). The distribution has become fragmented for two main reasons: maple has been suppressed by Norway spruce that spread vigorously from the east, and later on forest management practices have been unfavourable to maple, due to its low economic value in marginal areas.

For gene conservation of Norway maple, the main approach is to establish few but intensively managed *ex situ* collections, which consist of material from several natural populations (RUSANEN 1996). The goal is to collect seed from 50 populations, altogether 290 randomly selected families (seed trees) (see Figure 1). By the end of 1999, roughly two thirds of the seed collections were finished. Ten individuals per family will be planted into two intensively managed collections (south-western and eastern Finland). The collections are composed according to the geographic origin of the material. After thinning, 5 trees per family will remain. An area of one hectare for each collection will be sufficient for this need. Another option for *ex situ* conservation would be to use tissue cultures or long-term seed storage, but the equipment and expertise needed would make this choice relatively expensive in the situation where the economic value of the species does not attract funding.

As a supplementary action, two *in situ* stands have been selected to be managed strictly for purposes of gene conservation (Figure 1). This program is based on voluntary agreements, where forest owners commit themselves to reserve large indigenous stands to be regenerated naturally (for more details see KOSKI 1995). This simple procedure of conservation has turned out to

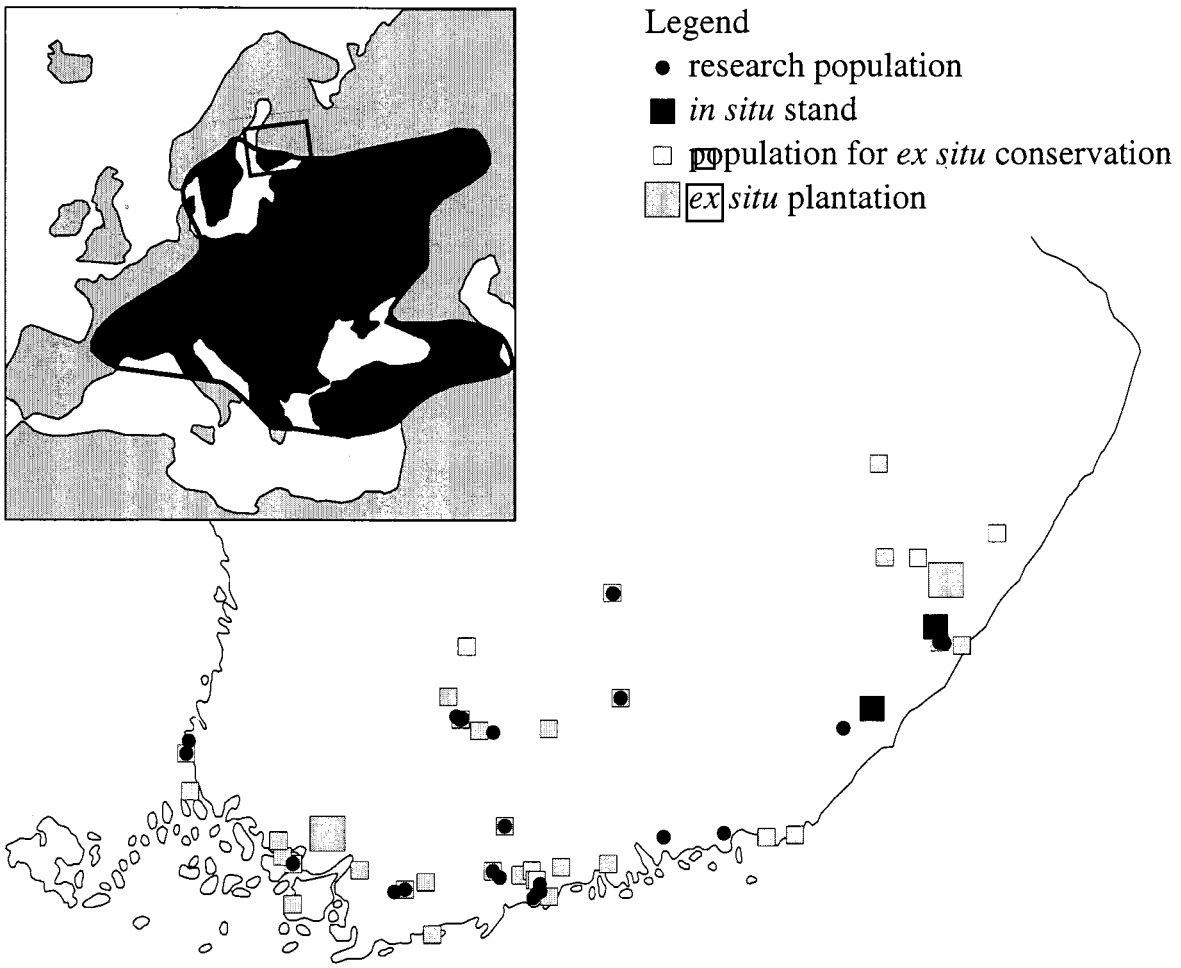

Figure 1. The distribution of research populations, stands presently in the gene conservation collections and gene reserve forest for *Acer platanoides* in Finland.

be easy to manage both operationally and economically, and works well with species like Scots pine or Norway spruce. For maple, however, this approach is feasible only in rare cases since most of the forests within the distribution of noble hardwoods are owned by private persons who refuse to make long-term commitments.

MATERIAL AND METHODS

Investigated populations and sampling

Allozyme variation was estimated in 29 populations of Norway maple, covering the whole distribution area of the species in Finland (Figure 1); of these populations the natural ones are included in the conservation scheme. Originally, subpopulations were distinguished in two populations (no. 5 and no. 19), where the shortest distance between the trees in separate subpopulations varied from 50 to 500 m. However, the genetic variability among subpopulations was so high that eventually they were analysed as separate populations, but the coding remained to remind of the short geographic distance between these samples (Table 1). The longest and shortest distances between sampled populations were 440 km and 1 km, respectively. Only 18 of the populations were clearly natural, 10 were half-urban with some uncertainty about the past history and one was clearly urban (artificially regenerated park). The isolation status of each population was defined based on distances to the nearest maples outside the population, on topography and on the density of the surrounding forest. Population sizes in Table 1 are defined as the number of individuals that potentially take part in reproduction, based on their heights. In addition, there are often many smaller trees, which normally represent a continuum of age-classes. A minimum of 40 mature trees were sampled from each population, with the exception of very small stands, where all mature trees were sampled. The sample size in Table 1 refers to the successfully analysed trees.

Extraction of enzymes

Table 1. Geographic location and region, general description, estimated population size and realized sample size of the studied populations.

Pop.	Latitude	Longitude	Region	Isolated	Description	Estimated pop. size	Sample size
1	61°05'	24°07'	Center	not	half-urban	30	24
2	60°13'	25°00'		not	half-urban	200	51
3	61°34'	29°11'	East	yes	natural	9	9
4	60°15'	25°00'		not	urban	30	31
5a	60°18'	24°30'	South	yes	natural	80	82
5b			South	yes	natural	20	17
5c			South	yes	natural	50	47
6	60°16'	24°34'	South	not	natural	80	73
7	61°04'	24°06'	Center	not	natural	50	50
8	61°05'	24°04'	Center	not	natural	50	50
9	60°11'	23°29'		yes	natural	50	45
10	60°48'	21°14'	West	yes	natural	50	48
11	61°45'	25°42'		yes	natural	40	39
12	61°13'	25°48'		not	natural	40	41
13	60°12'	24°57'		not	half-urban	300	50
14	60°11'	24°56'		not	half-urban	300	39
15	60°32'	24°37'		yes	natural	20	17
16	61°05'	24°03'	Center	not	natural	50	48
17	61°01'	24°27'		not	half-urban	100	50
18	61°03'	28°10'		not	half-urban	100	50
19a	60°52'	21°15'	West	not	natural	50	35
19b			West	not	natural	20	17
20	60°12'	23°36'		yes	half-urban	60	60
21	60°17'	22°26'		not	half-urban	20	21
22	61°05'	24°03'	Center	not	half-urban	100	102
23	60°30'	26°16'		not	natural	4	4
24	60°31'	26°54'		not	half-urban	60	39
25	61°28'	29°16'	East	not	natural	10	11
26	61°29'	29°13'	East	yes	natural	30	24

Winter buds were collected during February-April and stored at 20 °C until analysed. The buds were descaled on ice and the bright green growing point was ground in ca 150 µl of 0.12 M extraction buffer pH 7.5, together with a few grains of quartz sand. The extraction buffer (pH 7.5) was modified from (BOUSQUET *et al.* 1987) and consisted of: 0.12 M Tris-HCl, 0.2 M Sucrose, 0.3 mM NADP, 0.4 mM NAD, 0.5 mM EDTA, 5 mM Dithiothreitol, 12 mM Cysteine-HCl, 25 mM Ascorbic acid, 0.1% Bovine serum albumin, 2% Tween-80, 5% PVP.

Separation and staining of the enzymes

The starch gels were prepared, electrophoresis carried out and the enzymes stained with standard methods (e.g CHELIAK & PITEL 1984). The enzyme systems investigated were: glutamate dehydrogenase (GDH), peroxidase (PER), fluorescent esterase (FEST), glutamate-oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), aconitase (ACO), alcohol dehydrogenase (ADH), shikimate dehydrogenase (SDH), menadione reductase (MNR), superoxide dismutase (SOD), phosphoglucomutase (PGM), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH) and 6-phosphogluconate dehydrogenase (6PGDH). The best results were obtained with the buffer system Ashton, pH 8.1 and two Tris-citrate buffer systems, pH 7.1 and pH 7.8 (Table 2). Substrate specificity of the enzymes was tested by staining with and without substrate to avoid background staining that could interfere with the interpretation of zymograms.

Interpretation of allozyme phenotypes

The allozyme phenotypes of 2 enzyme systems, GDH and MDH, could not be interpreted reliably in genetical terms. The slower zone of MDH is highly variable, but the complicated banding pattern could not be interpreted as genotypes, even though phenotypic classification is possible. GDH activity was too low for reliable analysis. In peroxidase, the 2 fastest zones probably represent 2 polymorphic monomeric loci, but staining intensity of the allozymes seems to depend on the developmental stage of the tree. The SOD phenotype is visible also in MNR-staining, although intensity is quite low.

The zone of activity in GOT consists of two partly overlapping loci (Figure 2). The faster locus is rather faintly stained with 2 frequent (1 and 3) and one rare (2) alleles. The structure of this allozyme is dimeric. The slower locus has also got two frequent (1,2) and one rare allele (3), and a dimeric structure. Homodimers of allele 3 of *Got–1* overlap with heterodimers of alleles 1 and 2 in *Got–2* (and heterodimers of alleles 1 and 3 of *Got–1* overlap with homodimers of allele 1 of *Got–2*). Consequently, when *Got–2* genotype is either 12 or 13, the distinction between 11 and 13 genotypes in the *Got–1* locus is based on the different staining intensities of the bands in the 4-banded joint phenotype. As the middle band (heterodimer) of dimeric allozymes is twice as strong as the homodimers, it can readily be seen that if the second slowest band (3rd) is the strongest one, the genotype in *Got–1* is 11, whereas if the two bands (2nd and 3rd) are the strongest ones, the genotype of *Got–1* is 13. The rare allele 2 of *Got–1* is not discernible when *Got–2* genotype is either 12 or 13.

The interpretation of other enzyme phenotypes was straightforward and consistent with the results for *Acer campestre* (LEINEMANN & BENDIXEN 1999). Altogether 11 enzyme systems and 14 loci were analysed. A summary of the running conditions, number of loci, sub-unit structure and number of alleles is given in Table 3.

Table 2. The buffer systems and running conditions used in the electrophoresis.

Buffer system	Gel buffer	Electrode buffer	Running cond.
Ashton pH 8.1 (A)	0.005 M LiOH 0.019 M boric acid 0.006 M citric acid 0.029 M Tris pH 7.8	0.05 M LiOH 0.019 M boric acid pH 8.1 (NaOH)	20 min. 150 V 200–260 V for cca 5.5 hrs (migration 5.8 cm)
Tris-citrate pH 7.1 (TC 7.1)	1:16 dilution of the electrode buffer	0.14 M Tris 0.06 M citric acid pH 7.1	20 min 40mA, increase to 75mA for cca 4.5 hrs (5.5 cm)
Tris-citrate pH 7.8 (TC 7.8)	1:16 dilution of the electrode buffer	0.14 M Tris 0.06 M citric acid pH 7.8 (NaOH)	20 min 40mA, increase to 75mA for cca 5 hrs (5.5 cm)

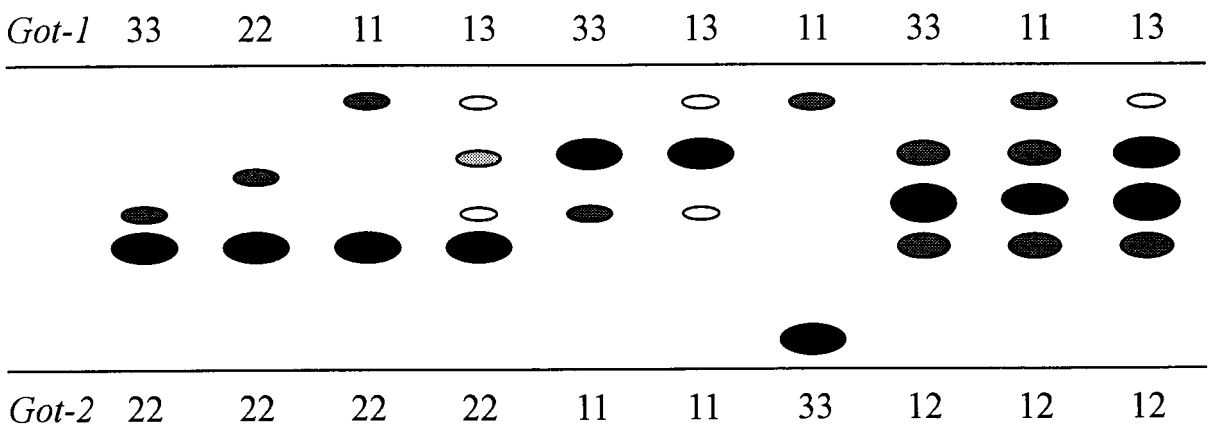

Figure 2. Schematic representation of the major GOT-phenotypes. Note the diagnostic differences in the staining intensity of the 4-banded phenotypes.

Table 3. Summary of the enzymes, buffer (table 2) and observed electrophoretic phenotypes. Columns 4.-6. refer to the number of activity zones, their interpretation as genetic loci and the sub-unit structure of isozymes observed in this investigation. In the column 6, the number of sub-units is followed by the number of respective zone of activity in parentheses, starting from the fastest zone.

Abbreviation	E.C. no.	Buffer	Zones	Loci	Sub-units	Alleles
GDH	1.4.1.3	A	1	-	-	-
PER	1.11.1.7	A	3	2?	1 (1,2)	3, 3 [1)]
FEST	3.1.1.1	A	3	2	1 (1), 2 (3)	?, 3 [2)]
GOT	2.6.1.1	A	1	2	both 2	3, 3
LAP	3.4.11.1	A	2	2	1 (1,2)	3, ? [2)]
PGI	5.3.1.9	A	2	2	2 (1,2)	3,2
ACO	4.2.1.3	TC 7.1	1	1	- (no variation)	1
ADH	1.1.1.1	TC 7.1	1	1	- (no variation)	1
SDH	1.1.1.25	TC 7.1	1	1	1	3
MNR	1.6.99.2	TC 7.1	1	1	4	4
SOD	1.15.1.1	TC 7.1	2	1	2 (1)	2
PGM	5.4.2.2	TC 7.8	2	2	1 (1,2)	3, 1
MDH	1.1.1.37	TC 7.8	2	-	-	-
IDH	1.1.1.41	TC 7.8	1	1	- (no variation)	1
6PGDH	1.1.1.44	TC 7.8	2	1	- (no variation)	1

[1)] variable, probably 3 alleles but reliable scoring not possible
[2)] variable

Statistical analysis

Allele frequencies were calculated for each population. Observed heterozygosities, Nei's unbiased heterozygosities (NEI 1978) and the percent polymorphic loci using 95 % criteria (P_{95}) were calculated for each population using all 14 scored loci. The average number of alleles per loci was calculated using only polymorphic loci (*AP*). Correlations between *AP*, P_{95} and H_e with logarithm of population size were calculated. Deviations from random mating were assessed by means of F-statistics based on the estimators of WEIR and COCKERHAM (1984). The correlation between genetic and geographic distances was tested using Mantel's test. A hierarchical F-analysis was performed, joining all populations separated by less than 10 km to form a region. The southernmost region in the Helsinki area was excluded from this analysis because human influence on these populations is markedly greater there than in the other regions. Populations which couldn't be included in any one of the regions were omitted. This made 4 regions consisting of 3 to 5 populations within each region (Table 1). *Nm* values within each region were calculated from the *F*-values. All data were analysed with Mark Miller's public software TFPGA (http://herb.bio. nau.edu /~miller).

Table 4. Allele frequencies in each population and at the species level in Finland.

Pop.	*Lap*		*Mnr*			*Got-1*			*Got-2*			*Pgi-1*			*Pgi-2*		*Sdh*			*Pgm-1*			*Fest*		
	1	2	1	2	3	1	2	3	1	2	3	1	2	3	1	2	1	2	3	1	2	3	1	2	3
1	1.0		.04	.96		*			.04	.92	.04	.69	.31		.98	.02	.91	.09			1.0			1.0	
2	1.0		.14	.86		.45		.55	.14	.58	.28	.63	.37		.94	.06	.91	.09			1.0			1.0	
3	1.0		.11	.89		*			.22	.78		.89	.11		.83	.17	.67	.33			1.0		.39	.61	
4	1.0		.05	.95		*			.02	.98		.93	.07		.97	.03	.87	.13			1.0		.02	.98	
5a	1.0		.02	.98		.37		.63	.02	.67	.31	.54	.45		.95	.05	.51	.49			.99	.01		1.0	
5b	1.0			1.0		*			.03	.94	.03	.29	.71		.88	.12	.44	.56			1.0			1.0	
5c	1.0			1.0		.04		.96		1.0		*			.61	.39	.66	.34			.93	.07		.95	.05
6	1.0		.01	.99		.30		.70	.24	.73	.03	.35	.65		.87	.13	.93	.07			.99	.01		1.0	
7	1.0		.12	.88		.30		.70	.02	.89	.09	.82	.18		.72	.28	.68	.32			1.0		.27	.72	.01
8	1.0		.25	.75		.04		.96	.01	.95	.04	.60	.40		.92	.08	.53	.47			1.0		.03	.97	
9	1.0		.01	.99		.28		.73	.04	.87	.09	.71	.29		.92	.08	.61	.39			.99	.01		.82	.18
10	.99	.01	.01	.99		.10		.90	.51	.44	.05	.87	.13		.64	.36	.93	.07			1.0		.01	.99	
11	1.0			1.0		.33		.67	.05	.90	.05	.82	.18		.94	.06	.70	.30			.97	.03		.96	.04
12	.96	.04	.09	.91		.28		.72	.05	.82	.13	.54	.43	.04	.93	.07	.77	.23			1.0			1.0	
13	1.0		.06	.94		.29		.71	.09	.91		.55	.45		.88	.12	.69	.31		*				.98	.02
14	.92	.08	.14	.81	.05	.17		.83	.05	.83	.12	.54	.46		.92	.08	.68	.32		*				.99	.01
15	1.0		.03	.97		.28		.72		1.0		.32	.68		.91	.09	.50	.50			1.0		.09	.76	.15
16	.96	.04	.21	.79		.13		.87	.02	.89	.09	.33	.67		.85	.15	.46	.54			.97	.03		1.0	
17	1.0		.11	.89		0.07		.93	.02	.93	.05	.59	.41		.85	.15	.77	.23		.11	.84	.05	.05	.92	.03
18	1.0		.06	.92	.02	.15		.85	.05	.83	.12	.57	.43		.99	.01	.79	.20	.01		.95	.05	.03	.89	.08
19a	.96	.04		1.0		.20		.80		.88	.12	.88	.12		.74	.26	.61	.39			1.0			.82	.18
19b	1.0			1.0		.29		.71	.06	.82	.12	.88	.12		.71	.29	.74	.26			1.0		.10	.90	
20	1.0		.13	.87		.30		.70	.04	.93	.03	.89	.11		.84	.16	.92	.08		.01	.99		.01	.89	.10
21	1.0		.57	.43		.52		.48	.07	.93		.42	.58		.79	.21	.75	.25		.21	.67	.12		1.0	
22	1.0		.04	.96		.35		.65	.01	.97	.02	.93	.07		1.0	.00	.79	.21			1.0			.17	.83
23	1.0			1.0		.13		.87		1.0		.63	.38		.88	.13	.62	.38			1.0		.13	.87	
24	.96	.04	.15	.85		.09		.91		1.0		.95	.05		.91	.09	.78	.22			1.0		.03	.97	
25	1.0		.36	.63		.15	.30	.55		.95	.05	.59	.41		.77	.23	.68	.32			1.0		.17	.55	.28
26	.93	.07	.04	.96		.20		.80		.91	.09	*			.67	.33	.83	.17			.95	.05	.17	.83	
	.99	.01	.08	.91	.00	.23	.00	.77	.07	.85	.08	.66	.33	.00	.87	.13	.73	.27	.00	.01	.98	.01	.05	.92	.03

* no observations in this group

RESULTS

Allele frequencies for polymorphic loci in each population are presented in Table 4. The monomorphic loci were *6pgdh*, *Aco*, *Idh*, *Adh* and *Pgm–2*. Percentage of polymorphic loci (P_{95}), mean number of alleles per polymorphic loci (*AP*), expected heterozygosities using Nei's unbiased estimate (H_e), observed heterozygosities (*H*) and F_{is}-values are presented in Table 5. Overall expected and observed heterozygosity values at species level were 0.147 and 0.131, respectively. Nine of the 14 loci (64%) were polymorphic in at least one population (95% criteria). The average value for H_e over all populations was 0.128; the population estimates ranged from 0.042 to 0.207. The average H_e over natural populations (n = 18) was 0.133 and for half-urban populations (n = 11) 0.120. In a t-test (P = 0.4) there was no statistically significant difference between these two groups. We found no statistically significant correlation between population size and within-population variability: the correlations between logN and *AP*, P_{95} and H_e were 0.366, 0.089 and 0.110 respectively. On average, the F_{is}-values did not deviate from zero.

The average genetic differentiation (F_{st}) between populations (n = 29), was 0.120. Nei's unbiased genetic distances between populations varied from –0.010 to 0.159, the average being 0.034. The correlation between Nei's unbiased genetic distances and geographic distances, tested with Mantel's test, was non-significant (r = 0.0678, P = 0.2730).

When part of the data was used to study the regional differentiation between the four geographic groups, the differentiation between populations within geographic groups (F_{pop} = 0.163) clearly exceeded the differentiation between geographic groups (F_{reg} = 0.030) (Table 6). UPGMA diagram for this regional analysis is

Table 5. Population size (N), sample size (n), number of alleles per polymorphic loci (AP), the percentage of polymorphic loci (P_{95}), expected heterozygosity (H_e), observed heterozygosity (H) and F_{is} -values for each population.

Population	N	n	AP	P_{95}	H_e	H	F_{is}
1	30	24	1.83	28.5	0.063	0.066	–0.048
2	200	51	1.83	42.9	0.148	0.120	0.189
3	9	9	1.83	50.0	0.147	0.143	0.027
4	30	31	1.83	21.4	0.042	0.040	0.048
5a	80	82	1.83	35.7	0.151	0.123	0.185
5b	20	17	1.67	35.7	0.090	0.097	–0.078
5c	50	47	1.50	35.7	0.090	0.085	0.056
6	80	73	1.83	35.7	0.120	0.116	0.033
7	50	50	2.17	50.0	0.172	0.179	–0.041
8	50	50	2.00	35.7	0.125	0.117	0.064
9	50	45	2.00	42.9	0.145	0.141	0.028
10	50	48	2.17	35.7	0.116	0.160	–0.379
11	40	39	1.83	42.9	0.116	0.104	0.103
12	40	41	2.00	50.0	0.142	0.153	–0.077
13	300	50	1.83	57.1	0.134	0.129	0.037
14	300	39	2.33	64.3	0.154	0.158	–0.026
15	20	17	1.83	64.3	0.143	0.166	–0.161
16	50	48	2.00	78.6	0.151	0.142	0.060
17	100	50	2.17	85.7	0.144	0.150	–0.042
18	100	50	2.50	71.4	0.132	0.129	0.023
19a	50	35	1.83	71.4	0.144	0.149	–0.035
19b	20	17	1.83	71.4	0.142	0.159	–0.120
20	60	60	2.17	78.6	0.116	0.115	0.009
21	20	21	1.67	85.7	0.207	0.238	–0.150
22	100	102	2.00	64.3	0.095	0.098	–0.032
23	4	4	1.50	71.4	0.130	0.125	0.038
24	60	39	1.83	42.9	0.084	0.085	–0.012
25	10	11	2.00	57.1	0.131	0.128	0.023
26	30	24	2.00	57.1	0.131	0.128	0.023
Mean		41	1.92	53.9	0.128	0.129	–0.012

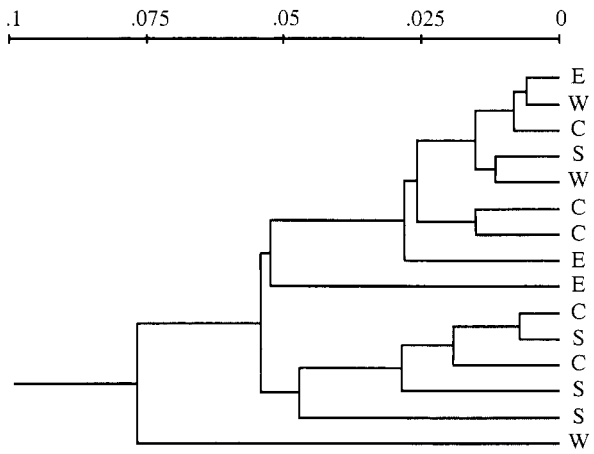

Figure 3. UPGMA dendrogram of regionally classified populations (Table 1). Legend: E – east, C – center, S – south and W – west.

presented in Figure 3 and shows no regional structure. The estimate of Nm, the effective number of migrants exchanged between regions, was 8.08; whereas for populations within regions, the average Nm was 1.28. F_{st} estimates for populations within each region are presented in Table 7.

DISCUSSION

Genetic structure of Norway maple

The present study provides ample information on the genetic diversity and population differentiation of Norway maple populations in Finland, based on allozyme markers. Allozyme variability is used to evaluate the evolutionary potential of individual stands and to estimate the effect of genetic drift on the genetic structure. As a practical application, the results can be used to assess the value of individual stands as seed sources. The genetic diversity (H_e) at species level in Finland was moderate, 0.147; it is comparable to the average for long-lived woody perennial species (0.149; HAMRICK *et al.* 1991) and the average for angiosperms (0.183;

Table 6. Wright's F-statistics for four regional clusters. F_{pop} is the F_{st} value for populations within regions and F_{reg} is the F_{st} value among regions. The upper and lower values are 95% C.I. from bootstrapping across loci.

Locus	F_{it}	F_{reg}	F_{pop}	F_{is}
Lap	0.221	0.012	0.031	0.197
Mnr	0.078	0.048	0.138	–0.070
Got–1	0.057	–0.040	0.079	–0.023
Got–2	0.016	0.042	0.203	–0.234
Pgi–1	0.436	0.123	0.268	0.229
Pgi–2	0.089	0.037	0.136	–0.054
Sdh	0.099	–0.040	0.124	–0.029
Pgm–1	–0.008	–0.005	0.029	–0.038
Fest	0.166	0.043	0.156	0.012
overall	0.157	0.030	0.163	0.006
upper	0.288	0.081	0.216	0.103
lower	0.058	–0.021	0.109	–0.112

HAMRICK *et al.* 1992). Even within angiosperm trees, the heterozygosity levels vary from one species to another. VILLANI *et al.* (1991) have estimated the heterozygosity values for *Castanea sativa* in Italy and Turkey to be 0.25 and 0.34, respectively. Diversity in insect-pollinated *Sorbus aucuparia* has been reported to be high both at the species level (H_e = 0.229) and within populations (H_e = 0.212) (RASPÉ & JACQUEMART 1998). For wind-pollinated *Quercus robur*, the estimated heterozygosity level was 0.16 in Finland (MATTILA & VAKKARI 1997) and 0.252 over a wide range in Europe (ZANETTO *et al.* 1994). Most of the studies on maples have been conducted in North America on sugar maple (*Acer saccharum*). PERRY and KNOWLES (1989) estimated the average H_e over five populations to be only 0.11, whereas FORÉ and VANKAT (1992) estimated the H_e of seven isolated populations to range from 0.12 to 0.24.

Allelic richness, the number of alleles per polymorphic loci, reflects the ability of a species to cope with future environmental changes. In the present study the Finnish maple populations had about the same, or a little less, variation than in the above-mentioned studies. The average number was 1.9; compared to 2.1 for oak in Finland (MATTILA *et al.* 1994) and for sugar maple 2.1 (YOUNG *et al.* 1993), 1.95 (PERRY & KNOWLES 1989) or between 1.1–2.5 (FORÉ *et al.* 1992). However, the estimated number of alleles is very dependent on e.g. sample size and is thus inconvenient to use for comparisons.

With values between 0.04 and 0.21, the range of expected heterozygosity in the populations was quite wide. Both of the extremes were found in half-urban populations, but the mean H_e values for natural and half-urban populations were not significantly different (0.13 and 0.12, respectively). Although the semi-urban populations are unlikely to be of natural origin, their regeneration is still natural, even if it is supported by tending. In fact, one could expect the semi-urban populations to contain more variation than natural ones, since in urban areas management generally favours maple over spruce, whereas in forests the practice has been the opposite.

Table7. Estimates of population differentiation (F_{st}) and gene flow between populations within regions (*Nm*). C.I. for F_{st} is presented in parenthesis.

Region	No of populations	F_{st}	*Nm*
south	4	0.145 (0.09–0.19)	1.47
west	3	0.129 (0.02–0.24)	1.70
center	5	0.142 (0.09–0.20)	1.54
east	3	0.088 (0.04–0.13)	2.61

In our study the amount of expected heterozygosity did not correlate with population size, although standard population genetics theory predicts such dependence, especially for neutral markers such as allozymes. FRANKHAM (1996) found a positive correlation between allozyme genetic variation and the logarithm of population size in 22 of 23 cases studied, including both animals and plants. A possible explanation for our lack of correlation is that, in terms of generation time, the population sizes in our data may have decreased rather recently. Furthermore, the mechanisms that affect genetic variation are complicated and it is becoming clear that not all fragmentation events lead to genetic losses (YOUNG *et al.* 1996). In sugar maple, YOUNG *et al.* (1993) compared allozyme variation in eight fragmented patches with variation in samples from continuous forest and found no reduced variation in patches.

The genetic differentiation among the sampled 29 maple populations (F_{st} = 0.12) was higher than that reported in Finland for oak (*Fst* 0.05, MATTILA & VAKKARI 1997) or in Canada for sugar maple (*Fst* 0.03, PERRY & KNOWLES 1989). In our data the differentiation was high among populations within geographic regions (F_{pop} =0.16), compared to that between geographic regions (F_{reg} = 0.03). Our results are in accordance with some recent results on annual plants. For example, MCCUE *et al.* (1996) studied the hierarchical structure of *Clarkia springervillensis* and found that, on average, the subpopulations were more differentiated from each other than were populations. GILES *et al.* (1998) found that for *Silene dioica* the differentiation among subpopulations within islands (F_{PL} = 0.080) was twice as high as among islands (F_{LT} = 0.048). NYBOM

and ROGSTAD (1990) studied the geographic and genetic pattern of *Acer negundo* with minisatellite markers and found an association between DNA variation and sampling distances, although a later study on the same species (NYBOM *et al.* 1992) did not confirm this pattern.

Gene conservation strategy

The *ex situ* conservation plan for Norway maple in Finland assumes that there is no need to define more than one ecological zone to be used in conservation. This is based on climatic descriptors and adaptive trials on other tree species. Allozyme data can neither confirm nor reject this assumption; field trials are needed for that purpose. Following a single ecological zone, the *ex situ*-collections have been established only in one place, with a smaller backup collection in the east of Finland for eastern material. With most of the material in only one collection there is the risk that a biological or technical disaster could ruin the conservation. Furthermore, in a situation where widely collected material is reproducing together, the increased gene flow might cause outbreeding depression, which would reduce the value of the collections. Although we bring together progeny from several distant populations, with the gene-flow level measured in this study (Nm = 8.08), we do not expect to encounter problems. According to ELLSTRAND and ELAM (1993) the level of $Nm < 0.5$ is low enough for outbreeding to become a problem.

In the establishment of a conservation collection sampling is a major question. In Central America, CAMCORE (Central America & Mexico Coniferous Resources Cooperative) generally samples from 10 to 75 trees per population from a large number of populations (DVORAK *et al.* 1999). For endangered species BROWN and BRIGGS (1991) suggested that a minimum of five populations and ten individuals per population be sampled in order to obtain an overall sample of 50. This absolute minimum is a realistic goal in a situation where the resources have to be divided among many endangered species. On the other hand, the strategy for forest gene conservation in Finland includes only 14 species, 6 of which need *ex situ* collections. Since the task is limited, it has been possible to set the goal of collecting 50 populations and 290 trees (families) of maple for conservation. On average, this would make a little less than 6 trees/population, which has turned out to be a realistic figure in conditions where the natural populations are small and flowering is heavily suppressed. In fact, at a stage where 35 populations have been collected, the number of trees per population varies from one to ten. Naturally there have also been years when there is no seed production in the natural populations. Since it is not possible to mark the collected trees in the stands in private areas, to avoid accidental double harvesting we collect a population only once. Moreover, our observations strongly suggest that good seed trees produce seed almost every year; some others produce only in the good conditions, and the vast majority do not flower. This means that, compared to one collection in a good flowering year, collecting a certain stand twice or three times would not add to the number of trees included.

For administrative and economic reasons (e.g. private ownership) the *in situ* conservation of maple by specific gene-reserve forests is limited. Genetically, it can be questioned whether the marginal, small populations maintain enough variation to be worth conserving. In practice, only two stands have been selected, one of which is included in the present study (population no. 3). It consists of only nine trees of reproducing size on an area of one hectare. Even so, there is considerable natural regeneration. Although the population is so small that its conservational value can be questioned, it is not genetically uniform measured by the allozyme variation. The number of alleles per polymorphic locus (AP = 1.83) and the percentage of polymorphic loci (P_{95} = 50.0) are slightly under the average; on the other hand, the expected heterozygosity is fairly high (H_e = 0.147) compared to the average over all populations (0.128). Recently the stand has been opened and, contrary to most of the populations, all of the trees produce seed. We expect that this state-owned stand, which is situated at the margin of the natural distribution, can be expanded to a proper-sized *in situ*-stand later on. This kind of approach would be effective in capturing adaptive variation and providing conditions for evolution to work on the populations. *In situ* conservation will probably be a good choice for species like maple in temperate regions where it is possible to combine efforts for several rare species.

A gene-conservation strategy that has been widely acknowledged is the multiple population breeding system (MPBS) introduced by NAMKOONG *et al.* (1980) (see also ERIKSSON *et al.* 1993). MPBS involves establishment of multiple breeding populations from diverse sources and then selecting separate trait combinations and site adaptabilities with recurrent procedures. MPBS combines breeding and conservation, and gives special emphasis on preparing for uncertain future. The system is especially suitable for situations where conservation and forestry can be combined and the use of forestation material supports gene-conservation (eg. ROTACH (1998), KLEINSCHMIT (1994)). If MPBS were used for Norway maple in Finland, sampling could follow the same lines we have started with, but more resources would be needed to increase the number of collection

sites. This would help to capture and promote a wider range of adaptations, which certainly would give insurance against unpredicted changes in the future. However, the extra input might be hard to justify in Finland, where the distribution of the species is narrow, especially when the demand for forestation material is so small that no breeding program is called for.

In conclusion, Norway maple populations in Finland express moderate genetic variability. Population differentiation is quite high but has no geographic pattern. In light of these results, the genetic conservation program applied in Finland seems to be justified. The next step in genetic research on maple will be to measure the variability of adaptive characteristics in field trials.

ACKNOWLEDGEMENTS

We greatly appreciate the technical assistance of Mr J. Raisio in identifying the populations and Ms A. Korpijaakko in the allozyme analyses. The manuscript was notably improved by the guidance Dr K. Kärkkäinen kindly offered to us. We also thank Prof. P. M. A. Tigerstedt for his helpful comments and the anonymous reviewers for their valuable suggestions. This study was supported by the FIBRE program, Academy of Finland.

REFERENCES

ALHO, P. 1990: Suomen metsittyminen jääkauden jälkeen. [The history of forest development in Finland after the last ice age.] *Silva Fennica* **24**: 9–19.

BOUSQUET, J., CHELIAK, W. M. & LALAONDE, M. 1987: Allozyme variability in natural populations of green alder (*Alnus crispa*) in Quebec. *Genome* **29**: 345–352.

BROWN, A. H. D. & BRIGGS, J. D. 1991: Sampling strategies for genetic variation in *ex situ* collections of endangered plant species. In Genetics and conservation of rare plants. (ed. D. E. Falk and K. E. Holsinger). pp. 99–119. Oxford University Press, Oxford.

CHELIAK, W. M. & PITEL, J. A. 1984: Techniques for starch gel electrophoresis of enzymes from forest tree species. *In:* Information Report. Petawawa National Forestry Institute, Canadian Forestry Service, Chalk River Ontario.

DE JONG, P. C. 1976: Flowering and sex expression in *Acer* L. A biosystematic study. *Meded. Landbouwhogeschool Waheningen* **76**: 1–201.

DVORAK, W. S., HAMRICK, J. L. & HODGE, G. R. 1999: Assessing the sampling efficiency of *ex situ* gene conservation efforts in natural pine populations in Central America. *Forest Genetics* **6**: 21–28.

ELLSTRAND, N. C. & ELAM, R. D. 1993: Population genetic consequences of small population size: Implications for plant conservation. *Ann. Rev. Ecol. Syst.* **24**: 217–242.

ERIKSSON, G., NAMKOONG, G. & ROBERDS, J. H. 1993: Dynamic gene conservation for uncertain futures. *Forest Ecology and Management* **62**: 15–37.

FORÉ, S. A., HICKEY, R. J., VANKAT, J. L., GUTTMAN, S. I. & SCHAEFER, R. L. 1992: Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum*. *Can. J. Bot.* **70**: 1659–1668.

FRANKHAM, R. 1996: Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**: 1500–1508.

GABRIEL, W. J. & GARRETT, P. W. 1984: Pollen vectors in sugar maple (*Acer saccharum*). *Can. J. Bot.* **62**: 2889 –2890.

GILES, B. E., LUNDQVIST, E. & GOUDET, J. 1998: Restricted gene flow and subpopulation differentiation in *Silene dioica*. *Heredity* **80**: 715–728.

GREENE, D. F. & JOHNSON, E. A.1992: Fruit abscission in *Acer saccharinum* with reference to seed dispersal. *Can. J. Bot* **70**: 2277–2283.

HAMRICK, J. L. & GODT, M. J.1989: Allozyme diversity in plant species. *In*: Plant Population Genetics, Breeding and Genetic Resources. (ed. A. H. D. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir). pp. 43–63 Sinauer Associates, Inc, Sunderland.

HAMRICK, J. L., GODT, M. J. W., MURAWSKI, D. A. & LOVELESS, M. D. 1991: Correlations between species traits and allozyme diversity: implications for conservation biology. *In:* Genetics and conservation of rare plants. (ed. D.A. Falk. and K.E. Holsinger). pp.75–86. Oxford University Press, New York.

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.

KLEINSCHMIT, J. 1994: Strategy for conservation of gene resources and examples for oaks, Norway spruce and Douglas fir. *In:* Conservation and manipulation of genetic resources in forestry. (ed. Z.-S. Kim and H. Hattemer). pp. 255–284. Seoul.

KOSKI, V. 1995: Conservation of Norway spruce gene resources in Finland. *In: Picea abies* network. Report of the first meeting. (ed. J. Turok, V. Koski, L. Paule and E. Frison). pp.12–15. IPGRI, Rome, Italy.

LEINEMANN, L. & BENDIXEN, K. 1999: Inheritance of isozyme variants in field maple (*Acer campestre* L.). *Forest Genetics* **6**: 73–77.

MATTILA, A., PAKKANEN, A., VAKKARI, P. & RAISIO, J. 1994: Genetic variation in English oak (*Quercus robur*) in Finland. *Silva Fennica* **28**: 251–256.

MATTILA, A., & VAKKARI, P. 1997: Genetic variation in *Quercus robur* and *Ulmus laevis* in Finland. *In:* Proc. Nord. Meet. For. Geneticists and Tree Breeders, June 3–7, 1996. (ed. M. Kurm and Ü. Tamm). pp. 63–68. Metsanduslikud Uurimused , Tartu, Estonia.

MCCUE, K. A., BUCKLER, E.S. & HOLTSWORD, T.P. 1996: A hierarchial view of genetic structure in the rare annual plant *Clarkia springvillensis*. *Conservation Biology* **10**: 1425 –1434.

MILLAR, C. I., & WESTFALL, R.D. 1992: Allozyme markers in forest genetic conservation. *New Forests* **6**: 347–371.

NAMKOONG, G., BARNES, R.D. & BURLEY, J. 1980: A philosophy of breeding strategy for tropical forest trees. Tropical Forestry Paper, Oxford University Press **16**: 67 p.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

NYBOM, H., RAMSER, J., KAEMMER, D., KAHL, G. & WEISING, K. 1992: Oligonucleotide DNA fingerprinting detects a

multiallelic locus in bow elder (*Acer negundo*). *Molecular Ecology* **1**: 65–67.

NYBOM, H. & ROGSTAD, S. 1990: DNA "fingerprints" detect genetic variation in *Acer negundo* (Aceraceae). *Pl. Syst. Evol.* **173**: 49–56.

PERRY, D. J. & KNOWLES, P. 1989: Allozyme variation in sugar maple at the northern limit of its range in Ontario, Canada. *Can. J. For. Res.* **19**: 509–514.

RASPÉ, O. & JACQUEMART, A.-L.1998: Allozyme diversity and genetic structure of European populations of *Sorbus aucuparia* L. (Rosaceae: Maloideae). *Heredity* **81**: 537 –545.

ROTACH, P. 1998: *In situ* conservation and promotion of Noble Hardwoods: silvicultural management strategies. *In:* EUFORGEN Noble Hardwoods Network, Report of the third meeting. (ed. J. Turok, J. Jensen, C. Palmberg-Lerche, M. Rusanen, K. Russell, S. de Vries and E. Lipman.) pp. 39–50. IPGRI, Rome, Italy.

RUSANEN, M. 1996: Noble Hardwoods genetic resources and their conservation in Finland. *In:* EUFORGEN Noble Hard-woods Network, first meeting. (ed. J. Turok, G. Eriksson, J. Kleinschmit and S. Canger). pp.155–158. IPGRI, Rome, Italy.

TUROK, J., ERIKSSON, G., KLEINSCHMIT, J. & CANGER, S. 1996: Noble Hardwoods Network. Report of the first meeting. IPGRI, Rome, Italy.

VILLANI, F., PIGLIUCCI, M., BENEDETTELLI, S. & CHERUBINI, M. 1991: Genetic differentiation among Turkish chestnut (*Castanea sativa* Mill) populations. *Heredity* **66**: 131 –136.

WEIR, B. S., & COCKERHAM, C. C. 1984: Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

YOUNG, A., BOYLE, T. & BROWN, T. 1996: The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* **11**: 413–418.

YOUNG, A. G., MERRIAM, H. G. & WARWICK, I. 1993: The effects of forest fragmentation on genetic variation in *Acer saccharum* Marsh. (sugar maple) populations. *Heredity* **71**: 277–289.

ZANETTO, A., ROUSSEL, G. & KREMER, A. 1994: Geographic variation of inter-specific differentiation between *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. *Forest Genetics* **1**: 111–123.