Effects of Discontinuous Marginal Habitats on the Genetic Structure of Common ash (*Fraxinus excelsior* L.)

By A. M. Höltken[1*], J. Tähtinen[2] and A. Pappinen[1]

Abstract

Common ash (*Fraxinus excelsior* L.) has, in comparison to other tree species such as spruce (*Picea*), beech (*Fagus*) and oak (*Quercus*), poor competitive ability and is rarely dominant under natural conditions. This study, performed at the northern limit of the species distribution on islands in Southern Finland, investigates the genetic structure of four populations of ash which differ in their degree of isolation. Our hypothesis was that the patchy distribution of suitable habitats in terms of presence of islands and suitable soils leads to low genetic diversity within and strong genetic differentiation between populations. This study using nuclear microsatellites provides first information that increasing isolation goes along with decreasing genetic variability, an almost total interruption of gene flow and increasing population genetic differentiation. Furthermore the rarity of suitable ecological niches for *F. excelsior* seem to have fundamental effects on the mode of colonisation.

Key words: Common ash (*Fraxinus excelsior* L.), species northern margins, discontinuous habitats, microsatellites, genetic structures.

Introduction

Common ash (*Fraxinus excelsior* L.) is one of the European hardwood tree species which is native in Finland. Its distribution in this country is restricted to the southern parts and its presence here represents the northern limit of its natural distribution (*Figure 1*). Using cpDNA (chloroplast microsatellites), VENDRAMIN et al. (Conference Fontainebleau (2001) on the phylogeography of European forest trees and shrubs) showed that post glacial migration of *F. excelsior* to northeast Europe could have started from the Balkan peninsula, whereas most of the populations in western Europe may have originated in the Iberian and Italian peninsulas, as well as in the Alps.

F. excelsior is a wind pollinated tree species with a complex mating system. Flowers may be functionally male, female or hermaphroditic and individual trees may have inflorescences of one or several combinations of the three flower types, so that the individual sex expression shows a continuum of gender from purely male to purely female flowers (TAPPER, 1992). The most effective mechanism of seed dispersal within the Finnish Archipelago area is transport by wind over the wintry ice surface (HULDÉN, 1941). Further it was reported that seeds of *F. excelsior* float for three days (RIDLEY, 1990), which may also be an important dispersal mechanism within archipelagos.

There is a correlation between the occurrence of ash-rich forests and the calcium-rich deposits of limestone bedrock which are sparsely distributed in Southern Finland (HINNERI, 1988). This lack of suitable habitats in these regions has resulted in a patchy distribution of ash populations. Man has increased the degree of isolation by clearing woodlands from these base rich soils to provide land for agriculture.

The patchy occurrence of *F. excelsior* in the Finnish Archipelago provides an ideal opportunity to investigate the effects of various levels of isolation on the genetic structure of forest tree populations. *Figure 1* shows the natural distribution of *F. excelsior* in South Finland and the area where we performed our studies. *Figure 2* gives detailed information about the locations of our study populations and the very rare occurence of further ash stands. The clear decline in population density from the west to the east is due, in part, to the more severe, continental climate experienced in the east of the study area. In addition, a geological process which causes the land in this region to rise at a constant rate of $0.2–0.4 \text{ cm} \cdot \text{a}^{-1}$ results in the creation of new islands. In the west, many of these new islands have a high content of limestone bedrock and are therefore suitable habitats for colonisation by *F. excelsior*. According to HINNERI (1988), founding events are described for this species on islands with a minimal height of 120 cm above sea level and which already possess a vegetation layer with an overstory of *Alnus glutinosa* L. (European alder).

In this study we give first descriptive results about the effects of the scattered distribution of suitable habitats within the Southern Finnish Archipelago (in terms of presence of islands and suitable soils) on the population genetic structures of *F. excelsior*. Moving from the west to the east within our study area, there are increasing distances between suitable ecological niches for *F. excelsior*, many of them probably almost beyond the colonising distance of the nearest population. Our hypothesis is that higher degrees of isolation result in lower genetic diversity and higher population genetic differentiation as a consequence of founder effects and restricted gene flow. This study shall form a basis for further investigations on gene flow and calculations of effective population sizes of *F. excelsior*.
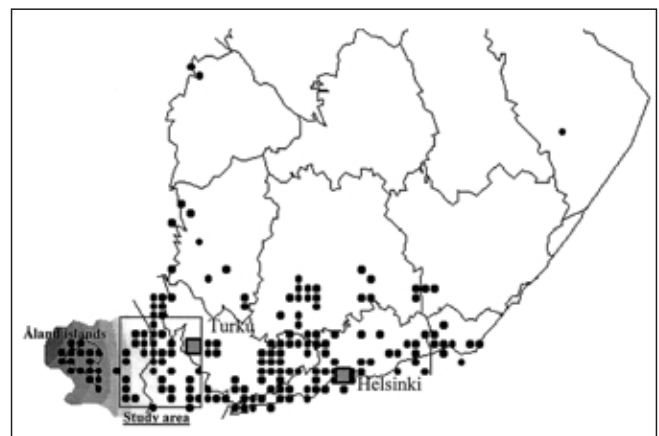

Figure 1. – Natural distribution of *F. excelsior* in South Finland and the location of the study area; spots = occurrence of European ash on a scale of 10 X 10 km²; shading = frequency of *F. excelsior* populations in South Finland (LAHTI et al., 1995).

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Material and Methods

Sampling

Leaf samples of *F. excelsior* were taken in the Finnish Archipelago area and frozen in liquid nitrogen before storage in the lab in –70 °C. Four mature ash populations were selected regarding differences in the degree of isolation. The sampling area extends over the eastern Åland islands to the Archipelago area south of the Finnish city Turku (*figure 1* and *2*). From the northwest to the southeast of this sampling area there is a clear decline in the frequency of *F. excelsior* populations. Around the Åland islands we find a relatively dense occurrence of common ash populations which show distances of approximately only a few hundred meters between stands. In the southeast of the sampling area the populations are often comparatively smaller and at the same time more isolated with distances of up to 15 km. The following populations were sampled:

Västra Dommasskär (VD): This *F. excelsior* stand is located in the southeast of the study area on a small island and consists of 43 mature trees which were all sampled. This stand is strongly isolated from the other common ash occurrences. Single trees were found on islands located within distances from 6 to 10 km.

Kemiö (K): This isolated stand consists of about 90 trees and is surrounded by agricultural areas. It may be a kind of relict population of a former larger ash stand. Here we sampled 45 mature trees.

Brändö islands: Two stands (BI and BII) were selected in the western part of the study area (Åland islands) within an area of higher density of *F. excelsior* populations. Both stands consist of about 40 mature individuals and all trees were sampled. BII is surrounded by a large number of smaller ash forests whereas BI is surrounded by pine and spruce and therefore slightly more isolated.

The distances between the populations are about 12 (BI and BII) and 20 km (VD and K). The two isolated populations in the southeast are about 75 km distant from the two populations in the west. Here the populations in the order of increasing isolation: Brändö II (BII) < Brändö I (BI) < Kemiö (K) < Västra Dommasskär (VD).

DNA-extraction, PCR and silver staining

The DNA was purified with a modified CTAB (hexadecyltrimethylammonium bromide) method after LEFORT and DOUGLAS (1999) and the PCR-amplification was performed according to LEFORT et al. (1999). PCR products were resolved on 6% standard denaturing polyacrylamide gels and visualised by silver staining using the modified method of BASSAM et al. (1991). After staining, the gels were scanned and four microsatellite loci (table 1) were analysed with the Gene Ima-
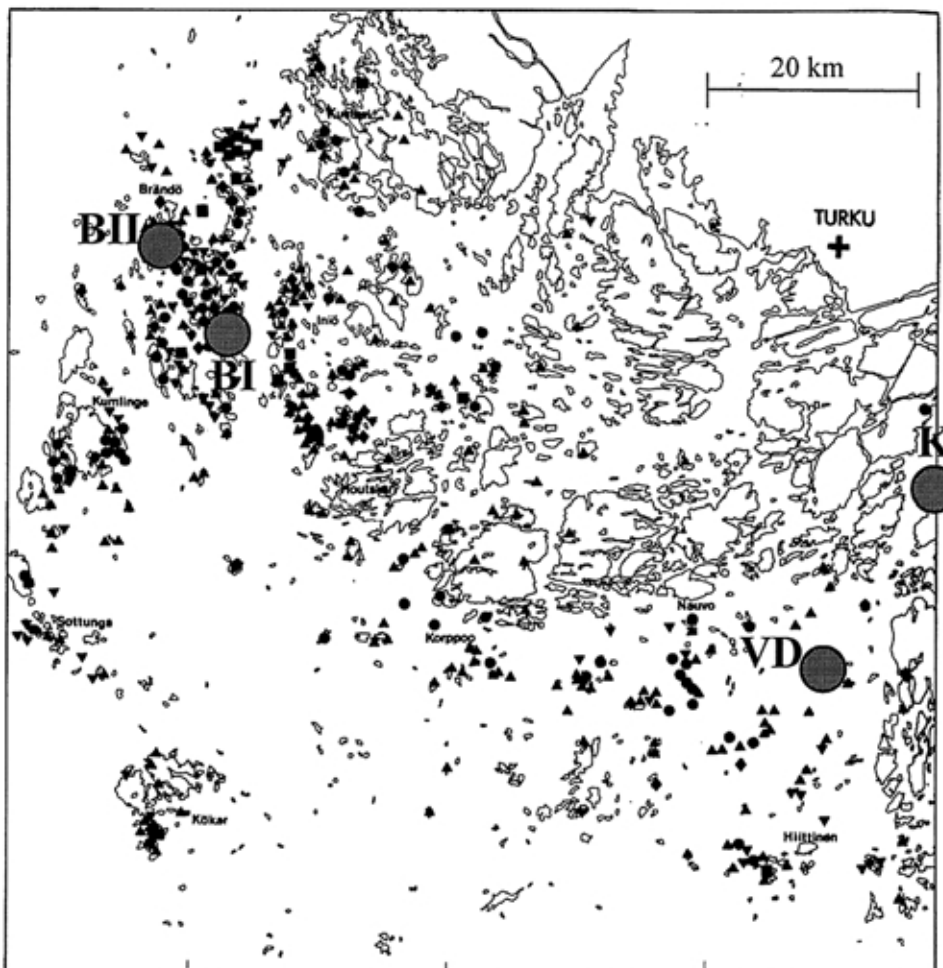

Figure 2. – Distribution of *F. excelsior* in the study area (from HINNERI, 1988) and locations of the analysed populations Brändö I (BI), Brändö II (BII), Kemiö (K) and Västra Dommasskär (VD);
▲ a scanty occurrence, old record
▼ as above, but newly recorded by HINNERI (1988)
■ pure stand or ash-dominated mixed forest, old records
● as above, but new occurrences recorded by HINNERI (1988).

gIRTM, version 3.52 (Scananalytics) fragment analysis software.

Analysis of population genetic data

Allelic variation: Statistical analyses were carried out using the GSED program of GILLET (1994). The following parameters were estimated to describe allelic variation: The total number of alleles found at each locus, the average number of alleles per locus in each population and the genetic diversity or the effective number of alleles at each locus. According to GREGORIUS (1978), the genetic diversity measures the effective number of genetic types taking into account their frequencies in a population. In the version $v_2 = (\Sigma a_i^2)^{-1}$ it equals the effective number of alleles of CROW and KIMURA (1979, p. 324).

Genetic distance d_0 between two populations (GREGORIUS, 1974): This parameter measures the proportion of genetic types not shared by both of the populations. It equals half of the sum of the absolute differences of the frequencies of genetic types in populations X and Y:

$$d_0 = 0{,}5 \cdot \sum_{i=1}^{n_k} |x_i - y_i|,$$

where x_i and y_i denotes the frequency of the i-th genetic type in population X and Y. This distance parameter reaches its maximum value 1, if the two populations have no genetic types in common and its minimum value 0 if the two populations have identical genetic structures.

Genetic differentiation δ among populations Dj (GREGORIUS and ROBERDS, 1986): This parameter is measured by the genetic distance between every population and its complement (= the union of all other populations). The differentiation of the j-th population is

$$D_j = 0{,}5 \cdot \sum_{i=1}^{n_j} |u_i^{(j)} - \bar{u}_i^{(j)}|,$$

where $u_i^{(j)}$ are the allele frequencies in the j-th population and $\bar{u}_i^{(j)}$ are those in the whole complement. The average differentiation δ of all populations is the mean of all.

Fixation indices: F_{ST} is not an absolute measure of the degree of differentiation. It measures relative genetic differences in the sense of the extent to which the process of fixation has gone toward completion and not in the sense implied in the extreme case by absence of any common allele. Therefore F_{ST} reaches its maximum value 1 only if all populations are monomorphic but not fixed for the same allele (WRIGHT, 1978, pp 82–84). According to the F-statistics of WRIGHT

$$F_{ST} = \frac{F_{IT} - F_{IS}}{1 - F_{IS}},$$

where F_{IT} is the fixation index of the whole pool of populations based on mean allele frequencies and F_{IS} is the mean fixation index of the populations based on allele frequencies within populations. While δ measures differentiation irrespective of the number of alleles present within the individual population, F_{ST}

quantifies the loss of variation within populations that is brought about by genetic drift. We calculated locus-specific and overall values for the inbreeding coefficient as described by WEIR and COCKERHAM (1984) using the programme FSTAT (GOUDET, 1995). We used the 1000 permutation procedure in FSTAT to test whether F_{IS}, F_{IT} and F_{ST} are significantly different from zero. The null hypothesis (F = 0) was compared with the observed F with a 95% confidence interval.

Results

Genetic variation

The four microsatellite loci analysed in our study clearly show differences in their variability (table 1). At the loci FEMSATL4 and FEMSATL10 we detected high degrees of polymorphisms with 22 and 20 alleles respectively. The other two loci revealed intermediate (FEMSATL11 with 11 alleles) and lower variability (FEMSATL16 with 6 alleles). Bulgarian populations showed two to three times more alleles for three of the same loci (HEUERTZ et al., 2001).

The observed allele frequencies for all microsatellite loci of the four study populations are presented in table 2. It turned out that the most isolated population VD possesses one or two prevalent alleles {frequencies of p ≥ 0.4} but no rare alleles {frequencies of p ≤ 0.05}. In the larger population of K we found more alleles, especially more alleles with intermediate frequencies {p = 0.05 - 0.4}. Looking at the two populations of the Åland islands (BI and II) there are many alleles with frequencies less than p = 0.05. An exception is microsatellite locus FEMSATL16 which is less variable, and where one allele is predominant in all populations (allele no. 1 with p = 0.51–0.79).

It appeared, that there is a clear decline in the number of alleles per locus with increasing isolation of the F. excelsior populations (table 3). In the most isolated population VD we found the lowest diversity values, whereas the population BII located on the Åland islands showed the highest number of effective alleles per locus. We also found a quite high diversity value for the Kemiö population. Here a high number of alleles with intermediate frequencies was identified, which results in higher diversity values.

Population differentiation

The results shown in figure 3 demonstrate that there is strong differentiation between the studied populations and their respective complements. The gene pool differentiation over all four microsatellite loci ranges from 0.329 (BII) to 0.527 (VD) with an average of δ = 0.437. That means that the stands share about 56.3% of the allelic variants. It was calculated that BII is the most representative ash stand concerning the whole complement of populations. It is followed by the population BI (D_j = 0.374) and K (D_j = 0.519). The most isolated population VD is, compared to all the other populations, the most differentiated one (D_j = 0.527).

Looking more closely at each of the four microsatellite loci we found quite different results concerning this parameter. The highest differentiation could be observed at FEMSATL10 (average δ = 0.662). This high differentiation value reflects the fact that there are extreme deviations in the allelic frequency distributions between the populations. Within the most isolated population VD (D_j = 0.958) we detected the alleles no. 10 and 12 in high frequencies (p > 0.4) whereas these alleles are rare or even absent in the other populations (table 2). A similar situation holds for population K at locus FEMSATL4 (D_j = 0.708). Alleles no. 4, 5, 10, 13, 19 and 20 are rare or even missing in the other populations, but occur at intermediate frequencies in

Table 1. – Analysed microsatellite loci and GenBank Accession numbers; number of found alleles and the range of their sizes (in bp) in Finnish F. excelsior populations and in three commonly analysed loci in Bulgarian populations.

Locus	GenBank Accession no.	Bulgarian ash populations		Finnish ash populations	
		No. of alleles	Range of sizes	No. of allels	Range of sizes
FEMSATL4	AF023473	50	158-251 bp	22	160-234 bp
FEMSATL10	AF020396	not scored	not scored	20	164-264(450*)
FEMSATL11	AF029882	32	176-266 bp	11	188-214 bp
FEMSATL16	AF029880	10	176-204 bp	6	186-210 bp

Table 2. – Frequencies of all found alleles at the four analysed microsatellite loci in the four study populations (Västra Dommasskär [VD], Kemiö [K], Brändö I [BI] and Brändö II [BII]).

FEMSATL4

Allele no.	VD	K	BI	BII
1				0.013
2		0.011	0.050	0.103
3	0.663	0.167	0.488	0.205
4		0.189	0.212	0.013
5		0.089		0.026
6			0.013	0.013
7				0.038
8			0.013	0.231
9				0.013
10		0.211		
11				0.013
12	0.256	0.022	0.075	0.167
13		0.133	0.013	
14	0.081			0.026
15				0.026
16			0.038	0.013
17			0.038	
18			0.050	
19		0.078		
20		0.100		0.013
21				0.077
22			0.013	0.013

FEMSATL10

Allele no.	VD	K	BI	BII
1	0.024			
2			0.025	0.064
3				0.064
4		0.167	0.112	0.051
5		0.011		0.038
6				0.013
7	0.098			
8		0.011	0.038	
9		0.533	0.312	0.346
10				0.013
11	0.402		0.025	0.051
12	0.476		0.025	0.026
13		0.111		
14	0.011			0.192
15			0.087	
16		0.038		
17			0.025	0.038
18	0.156		0.025	0.064
19			0.287	
20				0.026

FEMSATL11

allele no.	VD	K	BI	BII
1	0.122	0.011	0.162	0.171
2		0.185	0.137	0.029
3			0.013	0.043
4				0.014
5	0.405	0.098	0.062	0.171
6	0.338	0.435	0.425	0.371
7			0.038	0.029
8	0.135		0.150	0.114
9		0.011		0.014
10		0.250	0.013	0.029
11		0.011		0.014

FEMSATL16

allele no.	VD	K	BI	BII
1	0.537	0.511	0.788	0.632
2	0.280	0.298	0.087	0.236
3		0.011		
4	0.183		0.125	0.066
5		0.181		0.026
6				0.013

Table 3. – The four studied Common ash (*F. excelsior*) populations (in brackets the total population size and sample size): the number of alleles found and (in brackets) the effective number of alleles at four microsatellite loci (FEMSATL4, FEMSATL10, FEMSATL11 and FEMSATL16).

Population (total size; sample size)	FEMSATL4	FEMSATL10	FEMSATL11	FEMSATL16	mean
Västra Dommasskär (43; 43)	3 (1.96)	3 (2.51)	4 (3.21)	3 (2.50)	3.25 (2.46)
Kemiö (90; 45)	9 (6.65)	7 (2.86)	7 (3.38)	4 (2.62)	6.75 (3.39)
Brändö I (40; 40)	11 (3.37)	11 (4.84)	8 (3.96)	3 (1.55)	8.25 (2.85)
Brändö II (39; 39)	17 (6.93)	14 (5.58)	11 (4.66)	5 (2.11)	11.75 (3.95)

Kemiö. The lowest differentiation could be observed at FEM-SATL16, because all populations share the most prevalent alleles no. 1 and 2.

There is a clear indication for higher population genetic differentiation in the southeast of the study area. Although the geographical distance between the populations VD and K is only about 20 km, there are large differences in their allelic structures. At FEMSATL10 these two local populations have no alleles in common and also at the two loci FEMSATL4 and 11 we found several alleles of intermediate frequency (p = 0.07–0.20) either only in K or in VD.

The populations BI and II are located in the northwest of the study area which represents the main distribution area of this tree species in Finland. With some exceptions these two stands share many of the prevalent, intermediate and rare alleles. Both stands are, concerning the whole complement of all the other populations, less differentiated.

However, it has to be noted, that differences in the genetic structures between two populations do not necessarily indicate
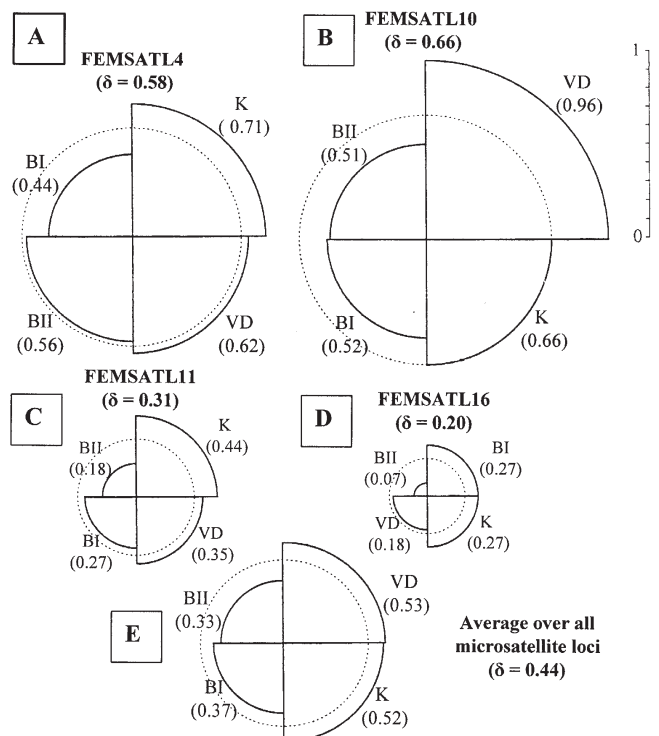

Figure 3. – Radius of the slices = Genetic differentiation D_j of the studied populations (VD = Västra Dommasskär, K = Kemiö, BI = Brändö I, BII = Brändö II) at four microsatellite loci (FEMSATL4, FEMSATL10, FEMSATL11 and FEMSATL16) [A–E] and the average D_j over all loci [E]; Dotted lines = average differentiation _ over all populations for each locus [A-D] and the average δ over all populations and over all loci [E].

that they also vary in their differentiation values. Therefore we included calculations of the genetic distances (d_0) between each of the populations (*table 4*): The two populations of the Brändö islands show the lowest ($d_0 = 0.385$), whereas the two more isolated populations (VD and K) show the highest value for genetic distance ($d_0 = 0.643$). The genetic distances between the two isolated and the two Brändö populations reveal intermediate distances ($d_0 = 0.473–0.495$).

Table 4. – Gene pool distances (d_0) between each of the studied populations (Västra Dommasskär, Kemiö, Brändö I and Brändö II).

	VD	Kemiö	Brändö I	Brändö II
VD	0.00			
Kemiö	0.643	0.00		
Brändö I	0.495	0.473	0.00	
Brändö II	0.479	0.476	0.385	0.00

Summarising the results, we could demonstrate that the two stands in the main area of the species Finnish distribution area (BI and BII) are, concerning the structure of the whole studied gene pool, more representative than the two more isolated ones K and VD. WRIGHT's F-statistics (*table 5*) gave variable values for F_{IS}, F_{IT} and F_{ST}. The within-population fixation values (F_{IS}) ranged from -0.116 to 0.306 with an overall average estimate of 0.041, which turned out to be significantly different from zero. That means, the average expected heterozygosity over all populations is not equal with the observed heterozygosity averaged over the whole complement of populations.

Table 5. – F-statistics for each of the four analysed microsatellite loci and over all loci in four Finnish *F. excelsior* populations; * = the F-value is significantly different from zero (95% confidence interval).

Locus	F_{IS}	F_{IT}	F_{ST}
FEMSATL4	- 0.106	0.044	0.135*
FEMSATL10	0.152*	0.324*	0.202*
FEMSATL11	- 0.116	- 0.042	0.067*
FEMSATL16	0.306*	0.344*	0.055*
average	0.041*	0.159*	0.123*

The index F_{IT} for overall inbreeding ranges from -0.042 (FEMSATL11) to relatively high values of 0.344 (FEMSATL16) with an average of 0.159 over all loci. Inbreeding was measured at three of the four studied microsatellite loci. Here the 1000 permutation test also gave the result that this value is significantly different from zero.

F_{ST} showed significant values at all studied loci ranging from 0.055 at locus FEMSATL16 to 0,202 at FEMSATL10. The average estimate for F_{ST} is 0.123.

Discussion

Most of the European hardwoods (*Prunus, Alnus, Ulmus, Tilia, Fraxinus, Sorbus sp.*) show (with some exceptions) a scattered population structure due to their limited interspecific competitive ability and narrow ecological plasticity (ROTACH, 1999; HEUERTZ et al., 2001). The effects of such a patchy distribution as well as continuous processes of colonisation and extinction of demes on their genetic structures have been of

interest for many years. Particularly the species' northern boundaries are, compared to plant populations located in more central areas of their natural range, marked by larger distances between small local populations, which consequently results in smaller effective population sizes.

According to HINNERI (1988) there are quite different growing conditions for common ash within our study area. Only 20% of the Brändö islands are covered by forests, but most of these forest patches are ash-rich (with over 30% common ash). Here we can assume higher levels of gene flow through pollen and seed due to lower levels of isolation than in the east of the study area, where, on the other hand, suitable habitat patches are very rare. Our study provides first descriptive results reflecting these differences, if the number of alleles, genetic distances and genetic differentiation are concerned.

The genetic distances between each pair of populations show, on average over all four loci, the highest value for the two more isolated populations ($d_0 = 0.643$ for VD and K) and the lowest value for the two populations of the Brändö islands ($d_0 = 0.385$ for BI and BII). Particularly, if allele frequencies of VD and K are considered, these two populations have at locus FEMSATL10 no alleles in common, which leads to a maximum value of genetic distance at this locus of $d_0 = 1.0$. Thus we can assume that the lack of suitable habitats may lead to highly restricted gene flow between local populations. Further evidence we can find in the observed values of allele numbers within populations (*table 2*). In the two more isolated populations we found a mean number of alleles per locus of 3.25 and 6.75, whereas 8.25 and 11.75 alleles per locus were detected within the two populations on the Brändö islands.

The number of effective alleles per locus show a slightly different trend. Here we found in the isolated population of Kemiö, compared to the other populations, a quite high diversity value of 3.39 effective alleles per locus. This can be explained with a higher evenness of the allele frequencies, which means that alleles occur in almost equal frequencies. This appearance has an increasing effect on this diversity index. We also assume that this stand is a kind of relict population of a former larger mixed forest of hardwoods, because it is surrounded by agricultural areas and because of the high estimated age of the oldest trees.

LECORRE and KREMER (1998) could model that the genetic effects of founding events vary according to the number of founding individuals, their total genetic diversity and the counterbalancing action of gene flow. The strongest effects were obtained in a one-dimensional stepping-stone model, where migrants are drawn from only one source population. The two-dimensional island model showed the weakest founding effects, because colonists came from several surrounding source populations (see also studies on migrant and propagule pool mode of colonisation, SLATKIN (1977) and McCAULEY et al. (1995).

According to AUSTERLITZ et al. (2000) founder effects, such as the reduction of genetic diversity within populations or an increase of differentiation among populations, are much more limited for tree species than for annual plants. When tree populations are established, expansion within these new local populations is not due to reproduction, because it starts much later than in populations of annual plant species. It is because of further seed flow from surrounding source populations which allows genetic diversity to accumulate. Annual plant individuals on the other hand, arriving in new suitable habitat patches, can already reproduce in the following year. Thus their offspring have the opportunity to colonise the area in a quite short time. The consequences for annual plants are greater loss of genetic diversity and a higher level of population differentia-

tion than for tree species. Several studies on annual or short-lived perennial plant species (McCauley et al., 1995; Giles and Goudet, 1997) and on forest tree species correspond with this model. Mariette et al. (1997) found both in newly colonised and in long-established populations of *Prunus avium* L., despite strong population fragmentation, insect (vector dependent) pollination and animal dispersed seed, nearly identical values of genetic diversity and the differentiation among populations was also low (G_{ST} = 0.052). They also attributed these results to the life history of tree species and they explained their results with a large number of migrants that already have become established in new sites before reproduction has occurred. Heuertz et al. 2001 found high genetic diversity in scattered populations of *F. excelsior* in Bulgaria and differentiation among populations explained only about 8,7% of total genetic diversity.

On the Brändö islands, where we found the highest level of genetic variability within populations and the lowest interpopulation differentiation, we can assume that gene flow via pollen and seed is much more effective. It is very likely that the first colonists on new developing islands and further migrants are drawn from several surrounding source populations. This would also agree with the results from Austerlitz et al. (2000) and Mariette et al. (1997) that recently established, rare forest tree populations accumulate genetic diversity due to further migration before the first colonists reach reproductive age.

But this does not seem to hold for the populations in the southeast of the study area. The populations show high levels of genetic distance, they are the most differentiated ones and less variable concerning the number of alleles found at each locus. It seems that the colonists were drawn from a few source populations, probably only one, and gene flow between populations is almost interrupted. In addition, it appears that migration of additional material from neighbouring ash forests does not contribute to the expansion of the population after the initial period of colonisation. The population began to expand after the few colonists reached their reproductive age, so that further genetic diversity could not accumulate. Moreover, gene flow among *F. excelsior* populations through pollen, with 50–90% of the pollen dispersed less than 10–50 meters (Heuertz et al., 2001), does not seem to be an important factor.

An interesting prospect for the analysis of population genetic consequences of habitat fragmentation will be a combined application of two different parameters. Because the analysis of F_{ST} generally tend to yield low estimates, if within deme diversity is high, even if different populations have no alleles in common (Pannell and Charlesworth, 1999), we will additionally use an absolute measure of population differentiation (δ) described by Gregorius and Roberts (1986). If one population has no alleles in common with its complement, although we observed high variability within the demes, then the differentiation δ reaches its maximum value 1,0. This condition is not fulfilled by Wright's F_{ST}, which makes the interpretation in terms of differentiation more difficult. For this purpose consider the four extreme cases: *1) Both δ and F_{ST} show large values:* The populations are strongly fragmented (reproductive isolation) and the size of each population is small (genetic drift). Here the populations have no or only a few alleles in common and every population is almost fixed on a private allele. *2) Both δ and F_{ST} show small values:* The populations are almost unfragmented without spatial discontinuity and the populations are large in size. The populations show high levels of genetic diversity and they share most of the alleles with nearly identical frequencies. *3) F_{ST} shows small and δ large values:* The populations maintain high levels of genetic variation, but

they do not have many alleles in common. We can thus suggest that the population is strongly fragmented, but the populations are large in size. *4) F_{ST} shows large and δ low values:* Most of the populations are fixed on one allele and one or only a few populations are fixed on another (private) allele (= low differentiation but high fixation). Here we can suggest small populations, intermediate gene flow among most of the populations but high isolation of some others.

Recall that our analysis demonstrated that decreasing population size and stronger isolation from the west to the east of the study area go along with loss of genetic variability (genetic drift) and increasing genetic distance d_0. This is corroborated by the summarising analysis of δ and F_{ST}, particularly in comparison with the results obtained from studies of Heuertz et al. (2001) on ash in Bulgaria (F_{ST} = 0.087). Our F_{ST} estimate (F_{ST} = 0.123) tends to indicate higher degrees of average fixation and, on the other hand, δ estimates are large even on an absolute scale (δ = 0.437). Of course more populations have to be analysed to obtain reliable evidence, but the distribution characteristics of our populations seem to be governed by a combination of the above cases 1 and 3, as is in accordance with our analysis.

This study shall form a basis for further investigations on gene flow and and calculations of effective population sizes of *F. excelsior*. Different degrees of isolation seem to have fundamental effects on the mode of colonisation as well as genetic diversity and differentiation which was rarely observed in forest tree populations. Particularly because of the rarity of suitable ecological niches and the fact that most seeds move short distances, unusual events leading to long-distance seed dispersal are of critical importance. Here archipelago areas such as the landscape of South Finland provide a very interesting opportunity, because whole populations or single trees can easily be mapped and – in most cases – highly variable markers are available for contemporary estimates of dispersal, e.g. parentage analyses, and long-term estimates of dispersal, e.g. F_{ST}-based, likelihood and genealogical methods (Cain et al., 2000).

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SYNCHRO: A SAS Program for Analysing the Floral Phenological Synchronisation in Seed Orchards

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Abstract

We provide a comprehensive SAS program to facilitate the analysis of the phenological synchronisation among all the genetic entries of a seed orchard. The program, intended for SAS-PC 6.12 under the Microsoft Windows, computes several phenological synchronisation indices for each male-female combination and performs the male and female phenograms as well as other simple graphics that may help in the interpretation of the phenological synchronisation parameters. An example of the phenological synchronisation study in a *Pinus pinaster* Ait. seed orchard in Northwest Spain is presented to demonstrate the use of the program and the features of the outputs.

Key words: Seed orchard, Genetic diversity, Floral phenology, Phenology synchronisation, *Pinus pinaster*, Computer program.

Introduction

The main objective of a seed orchard is to provide high genetic quality seed for reforestation. This genetic quality depends on both the genetic value and the genetic diversity of the seedlot (KANG *et al.,* 2001). The genetic diversity of the seed crop reaches its maximum when all clones in the orchard mate in equal proportions (called panmixis) and the resulting seedlot would contain equal contributions of all clones. However, panmixis is an ideal situation and clonal reproductive contributions vary with changes in many factors such as the strobili production, fecundity, distance among mates and flowering synchronisation (XIE *et al.,* 1994; BURCZYK and PRAT, 1997; GÖMÖRY *et al.,* 2000; 2003).

Flowering phenology in an orchard is probably the single most important influence on outcrossing patterns (EL KASSABY *et al.,* 1988; ERICKSON and ADAMS, 1989; BURCZYK and PRAT, 1997). Phenology affects the gene exchange among clones and the genetic compositions of the seeds derived from the seed orchard (MATZIRIS, 1994; BURCZYK and CHALUPKA, 1997). Differences in floral phenology lead to unbalanced contribution of clones, may preclude some combination crosses and, even, may remove the contribution of some clones in the seed crop. Thus, the lower the phenological synchronisation, the lower is the effective population size of the orchard seedlot. On the contrary, a high synchronisation can counteract the differences in male and female contributions among clones (BURCZYK and CHALUPKA, 1997) as well as decrease the probability of background pollination (WEBBER and PAINTER, 1996). Furthermore, the quantification of the phenology synchronisation is fundamental in making decisions about orchard roguing, supplemental mass pollination or controlled pollinations within the orchard (EL KASSABY and RITLAND, 1986; BLUSH *et al.,* 1993).

The phenological data is often presented as time lines or phenograms in which the proportion of strobili in a given phenological stage is represented by bands on a line time. However, the degree of overlapping phenology is difficult to quantify by such empirical technique even though the differences are visually apparent (ASKEW and BLUSH, 1990). In order to overcome this problem, several authors have proposed different mathematical models for quantifying the reproductive synchronisation among all possible pair of clones in the orchard.