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ANALYSIS OF THE GENETIC DIVERSITY AND POPULATION STRUCTURE OF LATVIAN ASH (*FRAXINUS EXCELSIOR* L.) STANDS USING NUCLEAR AND CHLOROPLAST SSR MARKERS

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*Common ash (*Fraxinus excelsior *L.) has a widespread distribution throughout Europe, and Latvia is almost at the north eastern edge of the distribution range. In Europe, ash is threatened by ash dieback, a disease caused by the introduced ascomycete* Hymenoscyphus fraxineus*. Chloroplast and nuclear DNA markers have been used to study the genetic diversity and population structure of ash both in a broader pan-European context as well as in more restricted regions. Some of the markers analysed in these previously published reports were also utilised in this study, enabling comparisons of the genetic parameters calculated from the nuclear SSR marker data and of the haplotypes identified with the chloroplast markers. Analysis of chloroplast markers revealed one dominant haplotype in Latvian stands, which corresponds to the haplotype previously found in Eastern Europe and Scandinavia. A second haplotype, corresponding to a previously reported central European haplotype was found in all individuals from the Ķemeri stand, indicating that this stand was naturally established from introduced germplasm, which was planted in a neighbouring park. The nuclear SSR markers revealed low levels of differentiation of Latvian* F. excelsior *stands, probably due efficient pollen flow between stands. The analysis of both chloroplast and nuclear DNA markers has revealed different aspects of the structure and provenance of Latvian* F. excelsior *populations.*

Key words: *pollen flow, population differentiation, introduced germplasm, post-glacial migration.*

INTRODUCTION

Common ash (*Fraxinus excelsior* L.) has a widespread distribution throughout Europe, with the exception of the central and southern parts of the Iberian Peninsula, south-east Turkey and northern Scandinavia (Dobrowolska *et al*., 2011, and references therein). Latvia is almost at the north eastern edge of the distribution range (Anonymous, 2009), and *F. excelsior* comprises approximately 0.5% (14 582 ha) of the total forested area (Anonymous, 2014). It is mainly found in mixed stands, together with other deciduous species: elm (*Ulmus glabra* Huds.), oak (*Quercus robur* L.), aspen (*Populus tremula* L.), birch (*Betula pendula* Roth.). In moist sites, it mostly occurs with black alder (*Alnus glutinosa* (L.) Gaertn.), as observed in other European countries (Anonymous, 2005; Dobrowolska *et al*., 2011). Ash grows on a wide range of site types but dominates mainly on fertile, biologically active, moist and seasonally excessively moist soil conditions (Laiviņš and Mangele, 2004; Anonymous, 2005). In Europe, ash is threatened by ash dieback, a disease caused by the introduced ascomycete *Hymenoscyphus fraxineus*. This fungus originates from East Asia, and was confirmed as the causal agent of European ash dieback in Poland in 2006 (Kowalski, 2006). Since the probable introduction of this fungus into Europe in the 1990s, it has spread throughout Europe, and is currently threatening common ash throughout most of its distributional range (Kowalski and Holdenrieder, 2009).

A limited number of nuclear microsatellite or simple sequence repeat (SSR) markers have been developed for *F. excelsior* (Brachet *et al*., 1999; Lefort *et al*., 1999), which have been used to study the genetic diversity and population structure of common ash both in a broader pan-European context as well as in more restricted regions. In addition, a set of conserved chloroplast SSR markers have been developed that have been applied to a wide range of angiosperm species (Weising *et al*., 1999). European *F. excelsior* populations have been investigated using nuclear SSR markers (Heuertz *et al*., 2004a) as well as chloroplast markers (Heuertz *et al*., 2004b). Nuclear SSRs have also been used to study ash populations in Bulgaria (Heuertz *et al*., 2001), France (Morand *et al*., 2002), Romania (Heuertz, 2003), Scotland (Bacles *et al*., 2005), Germany (Hebel *et al*., 2006), Italy (Ferrazzini *et al*., 2007) and Bosnia and Herzegovina (Ballian *et al*., 2008). In many of these studies, the DNA markers utilised are at least partially overlapping, enabling direct comparisons of the genetic parameters calcu-

lated from the nuclear SSR marker data and the haplotypes identified with the chloroplast markers.

Analysis of the European populations of *F. excelsior* using chloroplast DNA markers suggest post-glacial recolonization of Europe from refugia located in Iberia, Italy, the Alps and the Balkan Peninsula, as suggested by the allelic richness in these southern populations. In addition, strong signatures of the post-glacial migration routes remained in the current northern European populations, which showed high levels of differentiation of chloroplast haplotypes, indicating only a small overlap of recolonising maternal lineages (Heuertz *et al*., 2004b). In contrast, analysis of European populations of *F. excelsior* with nuclear SSR markers revealed very little differentiation of populations ranging from the British Isles over central Europe to Lithuania and Latvia (Heuertz *et al*., 2004a). The discrepancy between the high levels of population differentiation identified by the chloroplast analyses, and the low levels revealed by nuclear SSR marker analyses has been suggested to be due to efficient postglacial pollen flow (Heuertz *et al*., 2004b).

Smaller scale analyses of *F. excelsior* populations using nuclear SSR markers have revealed patterns of genetic diversity and population differentiation which are in agreement with the pan-European studies. In general, southern populations have high genetic diversity (e.g Heuertz *et al*., 2001; Ferrazzini *et al*., 2007; Ballian *et al*., 2008), while the northern populations have lower levels of genetic diversity (Bacles *et al*., 2005). The previous studies also reported low levels of population differentiation, even between fragmented populations at the northern boundary of the distribution range of *F. excelsior* (Bacles *et al*., 2005). In addition, many of the previous studies reported high inbreeding coefficients (Fis) detected with the nuclear SSR markers (e.g. Bacles *et al*., 2005; Ferrazzini *et al*., 2007; Ballian *et al*., 2008). These increased Fis values could be due to biological factors and population structure (i.e. assortative mating or a Wahlund effect) or to the presence of null alleles. Null alleles are caused by mutations leading to the non-amplification of specific alleles by PCR, leading to an apparent excess of homozygosity. This can have an influence on the estimation of population genetic parameters (Chapuis and Estoup, 2007). However, five nuclear SSR markers (M2-30, Femsatl4, Femsatl5, Femsatl11, Femsatl19) were used to genotype the progeny of controlled (test) crosses, which allows the Mendelian segregation of alleles to be observed, thus enabling detection of null alleles. Potential null alleles were only detected at one locus (Femsatl5), with the other loci conforming to the expected segregation ratios (Morand *et al*., 2002). In addition, the Fis values were not significantly positive in all studies utilising these nuclear SSR markers (e.g. Heuertz *et al*., 2003), and therefore the high inbreeding coefficients are probably due to biological factors and/or structure within the previously analysed *F. excelsior* populations.

The aim of this study was to initiate a survey of the genetic diversity and population structure of ash (*Fraxinus excelsior* L.) in Latvia. Knowledge of the distribution of genetic diversity within and between populations is required for optimal management of forest genetic resources. In addition, the analysis of haplotypes using chloroplast markers enables the post glacial recolonisation pathways to be determined and also the identification of possible introductions of germplasm. The results obtained from this study will form a basis for further studies of *F. excelsior* in Latvia, and allow comparison of the obtained results with previously published studies of *F. excelsior* populations in other European regions.

MATERIALS AND METHODS

Six nuclear and six chloroplast SSR markers were utilised to analyse 16 naturally regenerated ash stands located throughout the territory of Latvia. The markers utilised in this study were also utilised in previous studies of European *F. excelsior* populations, thus enabling comparisons of genetic diversity with these previously obtained results. Leaves were collected from approximately 24 young ash individuals (1–2 metres in height) from 16 naturally regenerated *Fraxinus excelsior* stands (Fig. 1, Table 1). The 'Skrīveri' and 'Bērvircava' stands have been designated as *F. excelsior* genetic resource stands. A total of 372 *Fraxinus excelsior* individuals were analysed with the nuclear and chloroplast SSR markers.

DNA from leaves was isolated using a CTAB-based method (Porebski *et al*., 1997). Genotyping was done using six nuclear SSR markers — Femsatl4, Femsatl10, Femsatl11, Femsatl16, Femsatl19 (Lefort *et al*. 1999) and M2-30 (Brachet *et al*. 1999) and six angiosperm chloroplast markers ccmp2, ccmp 4, ccmp6, ccmp10, ccmp7 and ccmp3 (Weising *et al*., 1999). Each forward primer was labelled with a different fluorophore (6-FAM, HEX or TMR) to facilitate visualisation using capillary electrophoresis. The PCR reactions for the nuclear SSR markers were carried out in a 20 µl solution containing, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1.5 µl DNA solution, 1x Taq buffer and 1U of recombinant Taq DNA polymerase (Thermo Scientific). PCR cycling conditions consisted of an initial denaturation step of 95 °C for 4 min; 35 cycles of 94 °C for 30 s, 52 °C for 45 s (56 °C for M2-30), and 72 °C for 60 s; followed by a final extension step of 72 °C for 10 min. The PCR reactions for

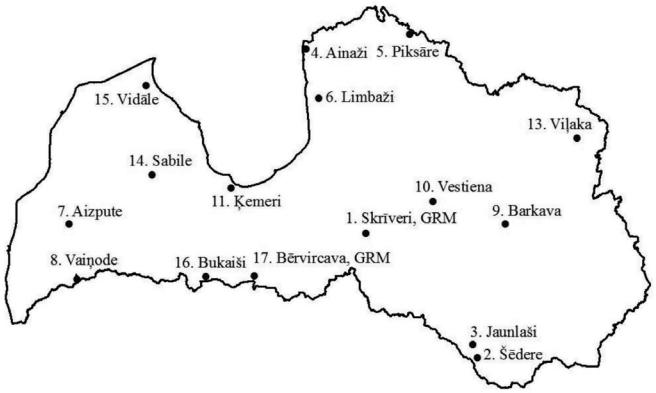

Fig. 1. Location of the sampled Latvian *Fraxinus excelsior* stands.

Table 1

COORDINATES AND ELEVATION OF THE SAMPLED LATVIAN *FRAXINUS EXCELSIOR* STANDS

Region	Stand	Coordinates		Elevation (m above sea level)
		X	Y	
Coastal lowland	4. Ainaži	523032	6409092	9
	15. Vidāle	411202	6383595	24
	11. Ķemeri	470181	6312350	8
Western Kurzeme	7. Aizpute	357289	6287409	75
	14. Sabile	415074	6321517	80
	8. Vaiņode	362909	6248835	110
Western Zemgale	17. Bērvircava	486569	6251149	34
	16. Bukaiši	452675	6250481	66
Augšzeme	3. Jaunlaši	639266	6203264	130
	2. Šēdere	642444	6194070	171
Northern Vidzeme	6. Limbaži	531633	6375057	52
	5. Piksāre	595114	6419336	96
Southern Vidzeme	1. Skrīveri	564760	6280660	90
Vidzeme highlands	10. Vestiena	611348	6302999	202
Aiviekste	9. Barkava	661933	6287151	94
	13. Viļaka	712240	6346960	122

the chloroplast SSR were similar, but with the addition of a final concentration of 1% BSA to the reaction. The PCR profile consisted of an initial denaturation at 96 °C for 5 min followed by 25 cycles of denaturation at 94 °C for 60 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s. Final extension of amplified DNA occurred at 72 °C for 10 min. All PCR reactions were carried out in an Eppendorf Mastercycler ep *gradient* thermal cycler. Amplification fragments were separated on an ABI Prism 3130*xl* Genetic Analyzer (Applied Biosystems) and visualised with GeneMapper 3.5.

The chloroplast marker data was combined into haplotypes. Analysis of nuclear SSR data was done using Fstat version 2.9.3.2 (Goudet, 2001) and GenAlEx 6.5 (Peakall and Smouse, 2012). Dendrograms were constructed using the software MEGA 5.2 (Tamura *et al*., 2011). A Bayesian clustering approach, implemented using the software STRUCTURE version 2.1 (Pritchard *et al*., 2000), was used to estimate the most likely number of clusters (K) into which the nSSR genotypes were assigned with certain likelihood. The population priors were not used, and a burn-in period of 50 000 iterations followed by 50 000 iterations was used. K was set from 1 to 16, and each run was replicated 20 times. The most likely number of clusters was identified by the delta K criterion (Evanno *et al*., 2005) with the STRUCTURE HARVESTER Web version 0.6.93 software (Earl and vonHoldt, 2012).

RESULTS

Of the six analysed chloroplast SSR markers, only three were polymorphic (ccmp6, ccmp7, ccmp10). The genotypes of these three loci were combined into two haplotypes, of which one was found in all individuals from all stands except for the 'Ķemeri' stand, where all individuals had the second identified haplotype. While the sizes of all the chloroplast alleles amplified in this study were not identical to the values reported previously, it was possible to unambiguously match the identified haplotypes to previously reported European *F. excelsior* haplotypes (Heuertz *et al*., 2004b). The most common haplotype in this study, which was found in all stands except 'Ķemeri' was the haplotype H01, which is widespread in Eastern Europe and Scandinavia. The allele sizes for this haplotype were as follows (with the previously reported sizes in brackets): ccmp6 – 97 (97); ccmp7 – 115 (118); ccmp10 – 104 (103). The haplotype found exclusively in the 'Ķemeri' stand corresponds to H02, which is the most common haplotype found in central Europe. The allele sizes for this haplotype were as follows (with the previously reported sizes in brackets): ccmp6 – 99 (99); ccmp7 – 114 (117); ccmp10 – 105 (104). The H01 and H02 alleles were the most common alleles in European *F. excelsior* populations, together representing 68% of the analysed individuals (Heuertz *et al*., 2004b). In addition, the other previously reported haplotypes were able to be excluded from comparisons with the results from this study, given the size differences of the polymorphic alleles. The differences in allele sizing are probably due to the use of differing genotyping platforms, protocols and reagents (Pasqualotto *et al*., 2007).

The total number of alleles identified by each nuclear SSR markers ranged from 10 (Femsatl16) to 37 (M2-30) (mean 23.17 alleles). The number of effective alleles was considerably lower, ranging from 1.96 (Femsatl16) to 20.88 (M2-30) (mean 9.55 alleles), indicating the high proportion of low frequency alleles. The expected heterozygosity ranged from 0.49 (Femsatl16) to 0.95 (M2-30) (mean 0.82), while the observed heterozygosity was lower, ranging from 0.37 (Femsatl16) to 0.85 (Femsatl19) (mean 0.64). As a result, the fixation index (F) was positive for all loci, ranging from 0.06 (Femsatl19) to 0.43 (Femsatl4) (mean 0.23). These were all significantly higher than zero, with the exception of the locus Femsatl19 ($p < 0.001$) (Table 2).

The mean number of alleles found in each population over all the analysed nuclear SSR loci was similar, ranging from

Table 2

GENETIC DIVERSITY STATISTICS OF THE ANALYSED NUCLEAR SSR MARKERS

Locus	N	Na	Ne	Ho	He	F
Femsatl4	364	23	5.23	0.46	0.81	0.43
Femsatl10	348	33	11.68	0.54	0.91	0.41
Femsatl11	370	16	6.79	0.78	0.85	0.09
Femsatl16	369	10	1.96	0.37	0.49	0.25
Femsatl19	368	20	10.77	0.85	0.91	0.06
M2-30	363	37	20.88	0.83	0.95	0.12

N – number of individuals, Na – number of alleles, Ne – number of effective alleles, Ho – observed heterozygosity, He – expected heterozygosity, F – inbreeding coefficient.

Table 3

GENETIC DIVERSITY STATISTICS OF THE ANALYSED LATVIAN *F. EXCELSIOR* STANDS

Population	Skrīveri	Šēdere	Jaunlaši	Ainaži	Piksāre	Limbaži	Aizpute	Vaiņode	Barkava	Vestiena	Ķemeri	Viļaka	Abava	Vidāle	Bukaiši	Bērvircava	Mean
Na	12.33	10.67	11.00	10.17	10.50	11.17	12.00	9.00	11.83	9.83	10.50	8.17	11.00	11.83	11.00	11.33	10.77
Na (freq. ≥ 0.05)	5.33	5.67	4.67	6.33	5.33	5.50	6.67	4.50	4.83	5.00	5.00	4.50	4.50	6.17	5.33	5.67	5.31
Ne	7.32	5.71	5.91	6.85	5.57	6.57	8.28	4.72	6.38	5.08	6.04	4.00	5.84	7.07	6.95	7.11	6.21
No. of private alleles	0.33	0.67	0.33	0.00	0.17	0.33	0.17	0.17	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.15
He	0.80	0.76	0.75	0.77	0.77	0.76	0.82	0.74	0.77	0.73	0.78	0.67	0.76	0.78	0.80	0.83	0.77

Na – number of alleles, Ne – number of effective alleles, He – expected heterozygosity.

8.17 (Viļaka) to 12.33 (Skrīveri) (mean 10.77). The number of alleles with a frequency of over 0.05 was also similar, ranging from 4.50 (Vaiņode) to 6.67 (Aizpute) (mean 5.31). A small number of private or unique alleles (found in only one stand) were only found in eight stands (Skrīveri, Šēdere, Jaunlaši, Piksāre, Limbaži, Aizpute, Vaiņode, Vidāle) (ranging from a mean over all loci of 0.17-0.67 alleles) (Table 3). A total of 14 private alleles were identified with five nuclear SSR markers (Femsatl4, Femsatl10, Femsatl16, Femsatl19, M2-30), of which only three had a frequency above 0.05 in their respective stand (Table 4).

The level of population differentiation detected by the analysed nuclear SSR markers was low, but significant (Fst = 0.045, $p < 0.001$), as calculated by AMOVA (999 permutations). The maximum pairwise population Fst value was 0.151 between Vaiņode and Viļaka, and these two stands were the most differentiated from each other and also from the other analysed stands. The pairwise Fst are shown in Table 5. The most genetically differentiated stands are Viļaka, Šēdere, Vestiena and Vaiņode, with pairwise Fst values above 0.05. These stands were also differentiated from each other, with all pairwise Fst values between these four stands being higher than 0.075.

The pairwise Nei genetic distances between stands ranged from 0.10 (Jaunlaši-Ainaži) to 0.75 (Vaiņode-Viļaka) (mean 0.28). The pairwise Nei genetic distances were used to construct a neighbour-joining dendrogram (Fig. 2). As can be seen from the branch lengths, the most differentiated stands were Viļaka, Vaiņode, Vestiena and Šēdere, as previously shown by the pairwise Fst results. The clustering of the populations did not show any geographical or other structure, which was expected due to the low level of population differentiation as previously reported by the AMOVA.

The Bayesian clustering approach, implemented using the software STRUCTURE version 2.1, identified that the most likely number of clusters was three (Fig. 3). However, there were additional peaks in the delta K plot corresponding to a larger number of clusters. These clusters did not correspond to any geographic grouping, and differentiated individual stands with a higher proportion of membership of a particular cluster. In the case of three clusters, three stands (Šēdere, Limbaži, Vestiena) had a relatively high proportion (over

Table 4

FREQUENCY OF PRIVATE ALLELES ACROSS LOCI

Population	Locus	Allele size (bp)	Frequency
Skrīveri	Femsatl4	208	0.042
Skrīveri	M2-30	210	0.021
Šēdere	Femsatl4	210	0.042
Šēdere	Femsatl10	261	0.021
Šēdere	Femsatl16	173	0.063
Šēdere	M2-30	188	0.021
Jaunlaši	Femsatl19	165	0.021
Jaunlaši	M2-30	267	0.021
Piksāre	Femsatl10	161	0.043
Limbaži	Femsatl10	267	0.042
Limbaži	Femsatl16	185	0.063
Aizpute	Femsatl16	194	0.100
Vaiņode	M2-30	225	0.022
Vidāle	Femsatl16	204	0.021

bp – base pairs.

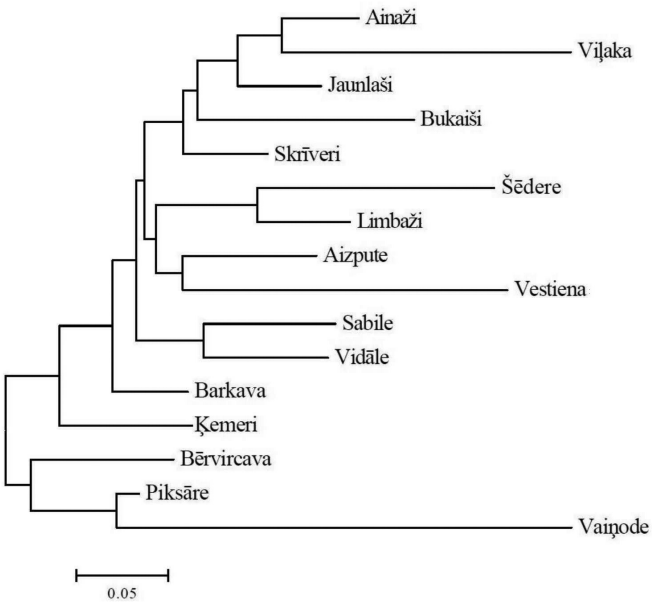

Fig. 2. Neighbour-joining dendrogram of pairwise Nei genetic distances between the analysed Latvian *F. excelsior* stands.

Table 5

PAIRWISE FST VALUES BETWEEN ANALYSED *F. EXCELSIOR* STANDS

Skrīveri	Šēdere	Jaunlaši	Ainaži	Piksāre	Limbaži	Aizpute	Vaiņode	Barkava	Vestiena	Ķemeri	Viļaka	Abava	Vidāle	Bukaiši	Bērvircava	
	0.001	0.026	0.290	0.002	0.001	0.001	0.001	0.062	0.001	0.005	0.001	0.002	0.006	0.009	0.004	Skrīveri
0.030		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Šēdere
0.012	**0.056**		0.454	0.002	0.001	0.001	0.001	0.004	0.001	0.014	0.001	0.001	0.001	0.001	0.001	Jaunlaši
0.002	0.050	0.000		0.002	0.002	0.001	0.001	0.039	0.001	0.034	0.001	0.001	0.001	0.002	0.003	Ainaži
0.026	0.047	0.028	0.031		0.001	0.001	0.001	0.004	0.001	0.004	0.001	0.001	0.001	0.001	0.005	Piksāre
0.021	0.028	0.042	0.024	0.042		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Limbaži
0.024	**0.057**	0.048	0.034	0.047	0.049		0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	Aizpute
0.072	**0.109**	**0.084**	**0.075**	0.045	**0.106**	**0.086**		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Vaiņode
0.009	0.035	0.020	0.012	0.019	0.022	0.023	**0.067**		0.001	0.014	0.001	0.003	0.001	0.001	0.005	Barkava
0.047	**0.077**	**0.058**	0.046	**0.070**	**0.052**	0.048	**0.121**	0.043		0.001	0.001	0.001	0.001	0.001	0.001	Vestiena
0.017	**0.052**	0.015	0.013	0.020	0.030	0.038	**0.061**	0.015	0.049		0.001	0.001	0.001	0.001	0.001	Ķemeri
0.045	**0.091**	**0.054**	0.035	**0.091**	**0.063**	**0.063**	**0.151**	**0.058**	**0.107**	**0.076**		0.001	0.001	0.001	0.001	Viļaka
0.020	**0.057**	0.028	0.033	0.039	0.041	0.038	**0.090**	0.019	**0.066**	0.037	**0.072**		0.025	0.001	0.006	Abava
0.014	**0.050**	0.036	0.031	0.034	0.027	0.027	**0.084**	0.019	**0.054**	0.032	**0.067**	0.011		0.001	0.001	Vidāle
0.013	0.049	0.024	0.021	0.031	0.048	0.039	**0.082**	0.032	**0.059**	0.026	**0.069**	0.029	0.040		0.001	Bukaiši
0.017	**0.059**	0.031	0.031	0.020	0.049	0.024	0.045	0.016	**0.061**	0.022	**0.074**	0.020	0.025	0.028		Bērvircava

Fst values below diagonal, probability P (rand >= data) based on 999 permutations above diagonal. Pairwise Fst values ≥ 0.05 are highlighted.

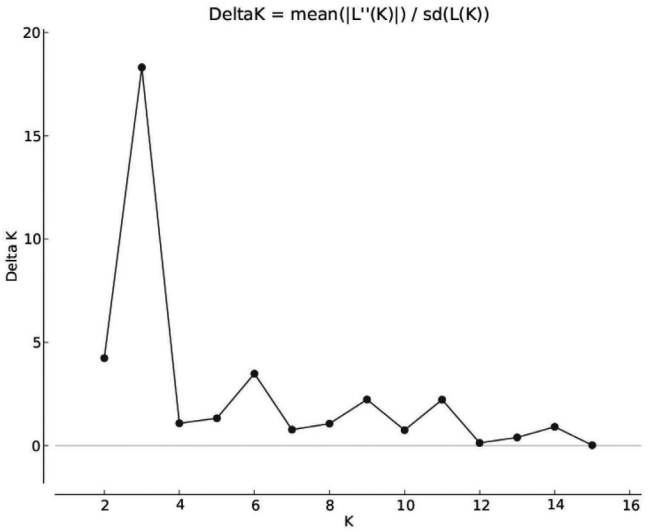

Fig. 3. Plot of delta K (the second order rate of change of the likelihood function with respect to K) vs. K (predefined number of clusters). The mode of delta K at K = 3 indicates the most probable number of clusters (3) in the analysed Latvian *F. excelsior* individuals.

0.6) of individuals assigned to one cluster; while the Vaiņode and Viļaka stands had a high proportion (over 0.7) of individuals assigned to each of the other two clusters. The remaining stands had an approximately equal proportion of individuals assigned to each of the three clusters (data not shown).

DISCUSSION

The chloroplast DNA markers identified one stand (Ķemeri), where all analysed individuals had the haplotype H02, in contrast to the common haplotype H01 found in all other stands and individuals. The H02 haplotype is common in central Europe, and the closest previously identified natural *F. excelsior* stands with this haplotype were in Poland (Heuertz *et al*., 2004b). The individuals that were sampled were young, naturally regenerated individuals, and the Ķemeri stand from which they were collected did not show any signs of being artificially established. Given the uniform presence of this haplotype within the Ķemeri stand, and the complete absence of it in the other analysed populations, it seems likely that this stand has been established by ash individuals that have escaped from nearby parks or other artificial plantings, where genotypes from central Europe may have been introduced. The Ķemeri stand is adjacent to the Ķemeri sanatorium, which has been in operation since 1796 (Kupcis, Lībietis, 1933/34). In the period between 1839 and 1846, the nursery owner C. H. Wagner established a park adjoining the sanatorium (Dambis *et al*., 2007), planting material (including *F. excelsior*) obtained from commercial nurseries, the majority of which was sourced from Western Europe (Zigra, 1805; Wagner, 1822). This stand and surrounding stands should be examined in more detail, including sampling mature individuals, to determine the extent of this haplotype in this area. In addition,

Table 6

COMPARISON OF ALLELE NUMBER AND INBREEDING COEFFICIENTS

	Latvia[1]		Scotland[2]		France[3]		Italy[4]		Bulgaria[5]		Bosnia and Herzegovina[6]		Romania[7]	
Locus	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F
Femsatl4	25	0.455	nd	nd	37	0.102	32	0.274	50	0.081	20	0.393	37	0.007
Femsatl10	34	0.421	nd	nd	nd	nd	76	0.332	nd	nd	55	0.371	nd	nd
Femsatl11	17	0.088	nd	nd	40	0.033	42	0.312	32	0.080	24	0.328	32	0.003
Femsatl16	10	0.274	6	0.032	nd	nd	9	0.191	10	0.124	12	0.156	10	0.165
Femsatl19	21	0.075	19	0.334	36	0.130	55	0.078	33	0.133	26	0.167	27	-0.07
M2-30	37	0.136	30	0.191	56	0.161	nd	nd	59	0.114	nd	nd	42	0.043

Na – number of alleles, F- inbreeding coefficient, nd – no data. 1 – this study, 2 – Bacles *et al.*, 2005, 3 – Morand *et al*., 2002, 4 – Ferrazzini *et al*., 2007, 5 – Heuertz *et al*., 2001, 6 – Ballian *et al*., 2008, 7 – Heuertz *et al*., 2003.

a survey of the known artificial plantings within the area would enable comparison to the naturally regenerated stands in order to determine any genetic relationships between them. Interestingly, analysis of the nuclear SSR marker results did not differentiate the Ķemeri stand from the other analysed stands. This is probably due to the efficiency of pollen flow over larger distances, which would reduce the differentiation of the naturally regenerated individual that were descended from the artificially introduced genotypes, while maintaining the maternally inherited chloroplast haplotype. Analysis of older individuals may enable a higher level of differentiation from other Latvian *F. excelsior* stands to be identified with the nuclear SSR markers, however, given that these artificial introductions have been occurring since the early 19th century, the original founder genotypes may not be present in this area, and only the chloroplast haplotype persists. In addition, populations from central Europe could be analysed with the chloroplast and nuclear markers in conjunction with the Latvian samples. This would enable a direct comparison of the obtained haplotypes, and to determine the level of differentiation identified with the nuclear markers between Latvian and central European ash populations.

The genetic diversity identified by the nuclear SSR markers utilised in this study was lower than identified in more southern populations in previous studies (Table 6). At almost all loci that were also analysed in previous studies, the number of alleles was higher in previous studies, with the exception of Scotland, where the number of alleles was consistently lower. This is in agreement with the post-glacial expansion of *F. excelsior* from southern refugia, with populations at the northernmost edge having a lower level of genetic diversity. However, the inbreeding coefficients were significantly positive for most of the loci/studies, with the exception of Romania, indicating that this could be a feature of *F. excelsior* stands, regardless of their location within the distribution areal. This could be related to the mating system of *F. excelsior*, which is polygamous, with a continuum between pure male and pure female individuals with hermaphroditic intermediates (Wallander, 2008), or to other population structure processes.

The overall level of genetic differentiation identified with the nuclear SSR markers between the analysed Latvian stands was low, which is in agreement with previous pan-European studies, which found little differentiation of *F. excelsior* populations stretching from the British Isles over central Europe to Lithuania and Latvia (Heuertz *et al*., 2004a). Four of the Latvian *F. excelsior* stands were slightly more differentiated (Viļaka, Šēdere, Vestiena, Vaiņode). These were not geographically clustered; however, it is interesting to note that all of these stands had an elevation above sea level of over 100 m. The remaining stands were all located at an elevation of less than 100 m above sea level, with the exception of the Jaunlaši stand, which had an elevation of 130 m above sea level. The Jaunlaši stand was geographically close to the Šēdere stand (Fig. 1); however they were not clustered together in the neighbour-joining dendrogram and the difference in elevation between the two stands was 41 m. One possibility is that the flowering time of the stands with a higher elevation is altered in comparison to the other stands, thus reducing the amount of gene flow between these stands and those at a lower elevation. Each of the elevated stands is geographically separated from each other as well, also decreasing the level of gene flow between the elevated stands, as demonstrated by the pairwise differentiation between the elevated stands as well as between the stands with a higher elevation and the stands with a lower elevation. The phenology of these stands should be further investigated to determine the extent of the role of flowering time in the differentiation of these stands, or if there are other possible factors influencing the population structure of Latvian *F. excelsior* stands.

The health status with regard to *H. fraxineus*, the causal pathogen of ash dieback, was assessed for a subset of the analysed *F. excelsior* populations (I. Pušpure, unpublished results). This revealed that the lowland coastal stands had the least favourable health status in comparison to the Eastern stands, which could be a result of more favourable environmental conditions for the pathogen. There was no discernible correlation of genetic population structure identified in this study with health status. Pliūra *et al*. (2011) found significant population differences with regard

to resistance to *H. fraxineus* in Lithuania. However, these results were obtained from progeny trials in three locations. Therefore, the resistance status of Latvian *F. excelsior* populations should be assessed in a common environment, and the results of this study could be used as guide for the selection of the most genetically diverged populations for inclusion into future *F. excelsior* progeny trials, as well as the designation of additional genetic resource stands.

CONCLUSIONS

Analysis of chloroplast markers revealed only one dominant haplotype in Latvian stands, which corresponds to the haplotype previously found in Eastern Europe and Scandinavia. In addition, the central European haplotype was found in all individuals from the Ķemeri stand, indicating that this stand has originated from introduced germplasm. The lack of differentiation by the nuclear SSR markers of the Ķemeri stand from the other analysed stands indicates efficient pollen flow and the natural regeneration of the stand over several generations since the initial introduction. The nuclear SSR markers revealed low levels of differentiation of Latvian *F. excelsior* stands, again, probably due the efficient pollen flow. The slightly higher differentiation of stand found at a higher elevation above sea level could be due to phenological differences, however further investigation is required to elucidate this. The analysis of both chloroplast and nuclear DNA markers revealed different aspects of the structure and provenence of Latvian *F. excelsior* populations.

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LATVIJAS OŠU (*FRAXINUS EXCELSIOR* L.) AUDŽU ĢENĒTISKĀS DAUDZVEIDĪBAS UN POPULĀCIJAS STRUKTŪRAS ANALĪZE, IZMANTOJOT KODOLA UN HLOROPLASTU SSR MARĶIERUS

Parastais osis (*Fraxinus excelsior* L.) ir plaši izplatīts Eiropā. Latvija ir ošu izplatības areāla ziemeļos. Eiropā novērojama ošu audžu masveida atmiršana, ko izraisa patogēnā sēne askomicēte *Hymenoscyphus fraxineus*. Ošu ģenētiskās daudzveidības un populāciju struktūru izpētei Eiropas līmenī, kā arī tās atsevišķos reģionos, ir izmantoti hloroplastu un kodola DNS marķieri. Darbā izmantotie marķieri ar tiem vismaz daļēji sakrīt, dodot iespēju salīdzināt Latvijas un citus Eiropas reģionu ošu audžu ģenētiskos parametrus. Ar hloroplastu marķieriem veiktās analīzes Latvijas audzēs uzrādīja tikai vienu dominējošo haplotipu, kas sakrita ar Austrumeiropas un Skandināvijas haplotipu. Savukārt visos Ķemeru audzes indivīdos atrasts Centrāleiropas haplotips, kas norāda, ka šī audze dabīgi izveidojusies no blakus esošajā parkā introducētajiem ošiem. Kodola SSR marķieri uzrādīja zemu Latvijas ošu audžu diferenciāciju, kas, iespējams, ir saistīts ar efektīvu putekšņu plūsmu starp audzēm. Gan ar hloroplastu, gan kodola DNS marķieriem veiktās analīzes atklāj dažādus Latvijas ošu populāciju struktūras un izcelsmes aspektus.