ASSESSMENT OF GENETIC DIVERSITY IN LATVIAN SILVER BIRCH *BETULA PENDULA* ROTH POPULATIONS

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Silver birch (*Betula pendula* Roth), is the most common broadleaf tree found in Latvia. With respect to birch provenances, Latvia is divided into three agro-climatic zones: Kurzeme (western), Vidzeme (northern) and Latgale (southern). The genetic diversity of *B. pendula* is high, and the population differentiation is low, as can be expected from an outcrossing, wind-pollinated, long-lived forest tree species. While these results do not indicate population differentiation between Latvian populations, this does not preclude the possibility of differentiation in adaptive traits. The high intra-population variation indicates that these natural populations should be able to adapt to changing climatic conditions as well as providing locally-adapted genotypes for selection programs.

Keywords: *Betula pendula* Roth, silver birch, simple sequence repeats, genetic diversity, population structure. We utilised SSR markers to examine the genetic diversity and population structure of Latvian *B. pendula* populations as well as comparing them to *B. pendula* populations from neighbouring countries.

INTRODUCTION

Silver birch (*Betula pendula* Roth), is the most common broadleaf tree found in Latvia. *B. pendula* is a diploid species (2n=2x=28). Together with downy birch (*B. pubescens* Ehrh.), these species comprise approximately 30% of the total forest area in Latvia (http://www.vmd.gov.lv/doc_upl/sugas.jpg). While these two species are very similar in appearance and wood qualities, there are slight differences in preferred habitats, with *B. pendula* found preferentially on drier, sandier soils, and *B. pubescens* more common on wet, poorly drained sites. Birch is found throughout Latvia, in all soil types and ecosystems.

B. pendula is distributed throughout Eurasia, from Western Europe to the Sea of Okhotsk and the Sea of Japan in the east. Within Europe, it ranges from the mountainous regions of Spain and Italy in the south and in the north to approximately 65ºN (Hamet-Ahti 1963, Atkinson 1992). Birch species are wind pollinated, and birch pollen can remain airbourne for 9-20 hours (Hjelmroos 1991), aiding long distance dispersal of this species. In addition, the prevailing winds in Europe are westerly, contributing to long range pollen transfer from western Europe. Birch is also an outcrossing species which exhibits self-incompatibility via retarded growth of the pollen tube (Hagman 1971).

With respect to birch provenances, Latvia is divided into three agro-climatic zones: Kurzeme (western) (Aizputes, Bauskas, Dobeles, Dundagas, Jelgavas, Jūrmalas, Kuldīgas, Liepājas, Saldus, Talsu, Tukuma, Ugāles and Ventspils VVM), Vidzeme (northern) (Alūksnes, Cēsu, Cesvaines, Gulbenes, Inčukalna, Kokneses, Limbažu, Ogres, Smiltenes, Strenču, Valmieras, Žīguru VVM, Gaujas NP, MPS Kalsnava) and Latgale (southern) (Daugavpils, Jaunjelgavas, Jēkabpils, Krāslavas,

Līvānu, Ludzas and Rēzeknes VVM) (Fig. 1.). These zones are used as guidelines for renewal of birch stands, seed transferral and breeding programmes.

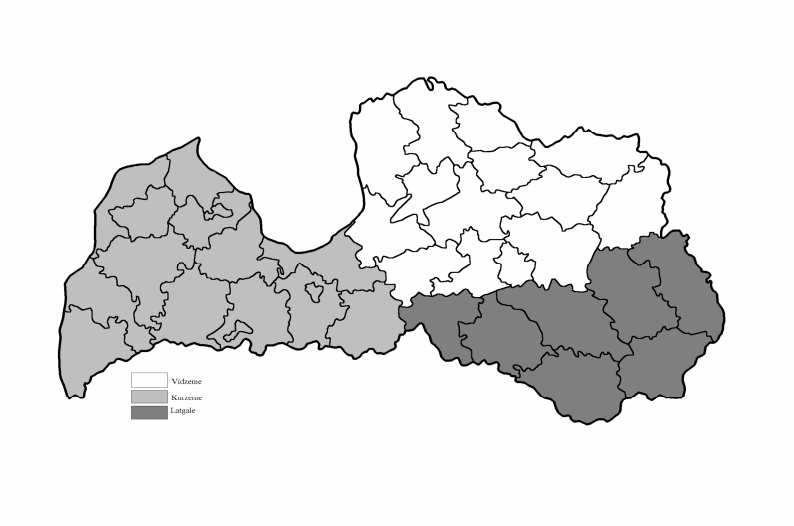

Figure 1. Birch agro-climatic seed provenance zones

The use of molecular markers is becoming more common in applied forestry studies, due to the decrease of the cost of genotyping, and more widespread access to genotyping facilities. To date, the majority of markers used are neutral genetic markers, which are not linked to genes controlling adaptive traits, and therefore can be used to describe the "background" genetic diversity and population structure, rather than assaying variation in adaptive traits which are commonly used by breeders and ecologists. Simple Sequence Repeat (SSR) markers in particular are very useful markers for clone identification as well as population and diversity studies as they are highly polymorphic, while being reproducible and easy to use. The major drawback of SSR markers is that the polymerase chain reaction (PCR) primers need to be developed for each species, which requires knowledge of the DNA sequence and involves considerable time and expense. Fortunately, SSR markers have been developed for *B. pendula* (Kulju et al, 2004). We utilised 15 of these SSR markers to examine the genetic diversity and population structure of Latvian *B. pendula* populations as well as comparing them to *B. pendula* populations from neighbouring countries.

MATERIALS AND METHODS

Plant material: Birch trees were sampled from the birch provenance trial established in Rembates parish, Ogres region. From the southern provenance region 54 individuals from the Ābeļi, Dagda, Koknese, Svente, Viļāni, and Zilupe populations were collected. From the northern provenance region 56 individuals from the Cesvaine, Dauksti, Naukšēni and Medņi populations were collected. From the western provenance region 65 individuals from the Andumi, Bauska, Blīdene, Garoza, Īle and Priekule populations were collected. In addition, 28 Estonian *B. pendula* individuals were collected from a *B. pendula* provenance trial near Tartu in Estonia, 24 Lithuanian *B. pendula* individuals were collected from a *B. pendula* provenance trial near Šiauliai in Lithuania, and 30 Polish *B. pendula* samples from a provenance trial established in Kalsnava, Latvia

DNA extraction: DNA was extracted from leaves using the Genomic DNA Purification Kit K0512 (Fermentas) with minor modifications.

SSR markers: The 15 SSR markers tested were L7.8, L7.4, L1.10, L5.1, L3.1, L2.7, L4.4, L2.3, L2.2, L3.4, L7.3, L5.5, L5.4, L022 and L13.1 (Kulju *et al* 2004) The forward primer was synthesised with a 6-FAM, HEX or NED fluorescent label to allow visualisation of amplification products on a fluorescent sequencer. PCR conditions were as follows: 95 ^{0}C for 5 min, 35 cycles of 95 ^{0}C for 30 sec, 50 ^{0}C – 30 sec, 72 ^{0}C – 30 sec; 72 ^{0}C - 7 min; in a total volume of reaction 20 µl containing 50 ng template DNA, 1,5x PCR buffer, 2 mM $MgCl_2$, 0,2 mM dNTP mix, 0,5 U *Taq* polymerase (*Fermentas*), 0,5 µM of forward (labelled) and reverse primers (*Applied Biosystems*). 1,0 µL of each PCR product was mixed with 5µL formamide and 0,7µL GS350 size standard. After denaturation, the samples were run on an ABI 3100*xl* capillary sequencer, and genotyped using GeneMapper software.

Genetic analysis: SSR markers were scored as diploid co-dominant markers, and analysed using GenAlEx software (Peakall and Smouse 2006). Latvian populations were grouped into the 3 agro-climatic zones (northern (N), southern (S) and western (W)) and samples from each of the neighbouring countries were grouped as separate populations (Estonian (Es), Lithuanian (Lt), Polish (Po)).

RESULTS

Of the 15 SSR markers tested, 9 were used for further analysis (L1.10, L5.1, L3.1, L2.7, L2.3, L2.2, L5.4, L022 and L13.1). These 9 primers amplified clearly interpretable DNA fragments. The remaining markers either amplified multiple fragments which could not be genetically interpreted, or the amplification was poor, resulting in a large number of failed reactions.

These nine SSR markers revealed a large amount of polymorphism in the birch populations surveyed (Table 1, Figure 2). The total number of alleles detected ranged from 106 in the North Latvian population to 54 in the Lithuanian

population. The mean number of alleles (averaged over the 9 loci) also followed the same pattern, with 11.778 in the North Latvian population, and 6.000 in the Lithuanian population. However, the number of alleles with a frequency over 5%, and the number of effective alleles per population did not show such a large discrepancy, with 49 alleles over 5% frequency in the Estonian population, and 37 in the Polish population. The Lithuanian population had 38 alleles of over 5% frequency. The expected heterozygosity of the SSR markers were also fairly high, ranging from 0,732-0,769 for the Latvian and Estonian populations and 0,634 and 0,620 for the Polish and Lithuanian populations.

Table 1.

Allele number, effective allele number, private allele number and expected heterozygosity as measured with 9 SSR loci over all population

Population	S	W	N	Es	Po	Lt
Total no. of alleles	97	104	106	92	73	54
No. of alleles (mean)	10,778	11,556	11,778	10,222	8,111	6,000
Total no. of alleles with freq. ≥ 5%	43,000	46,000	43,000	49,000	37,000	38,000
No. of alleles with freq. ≥ 5% (mean)	4,778	5,111	4,778	5,444	4,111	4,222
No. of effective alleles (mean)	4,639	5,325	4,930	4,850	3,653	3,234
No. Private Alleles (mean)	0,556	0,778	1,000	0,000	0,444	0,222
Expected heterozygosity (mean)	0,732	0,769	0,740	0,733	0,634	0,620

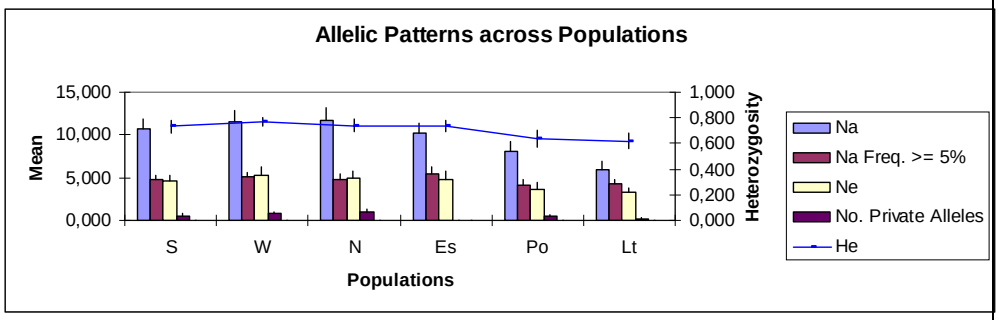

Figure 2. Mean number of alleles (Na), mean number of alleles with a frequency ≥ 5% (Na Freq. ≥ 5%), mean number of effective alleles (Ne), mean number of private alleles (No. Private alleles) and Expected heterozygosity (He) across all populations

In general, the total number of alleles detected by the markers was high, and included a large amount of rare alleles, as shown by the lower number of effective alleles and the number of alleles with a frequency over 5%. The Latvian

populations were the most polymorphic, and the least polymorphic was the Lithuanian population. However, this is probably due to the sample sizes and the origin of the samples. The number of common alleles (frequency greater than 5%) and the number of effective alleles is similar across all populations. Also the number of unique alleles was highest in the Latvian populations, but these were all relatively low frequency (only one allele had a frequency >5%).

Population differentiation was low between the Latvian populations, with AMOVA analyses showing that all variation was within populations, and no difference between populations. Comparisons with the foreign populations indicated a larger amount of differentiation, with the Estonian population closer to the Latvian populations, while the Lithuanian and Polish were more distinct. Pairwise Fst values range from 0.005 to 0.066, indicating low population differentiation (Table 2). The lowest values were between Latvian populations, and the largest values were between the Lithuanian population and all other populations.

Table 2.

Genetic differentiation of populations as measured by pairwise Fst values

	S	W	N	Es	Po	Lt
S	0,000					
W	0,009	0,000				
N	0,005	0,012	0,000			
Es	0,026	0,019	0,024	0,000		
Po	0,036	0,032	0,035	0,029	0,000	
Lt	0,048	0,053	0,049	0,066	0,050	0,000

DISCUSSION

B. pendula has a wide distribution ranging from Western Europe to Asia, is wind-pollinated and has a strong self-incompatibility mechanism. These facts are reflected in the high levels of genetic diversity, and low population differentiation found in the Latvian *B. pendula* populations using SSR markers. Previous studies in birch have also noted this high level of diversity and low population diversity using both morphological and molecular markers (Eriksson and Jonsson 1986; Kulju et al 2004). A study of postglacial re-colonisation routes in *B. pendula* using chloroplast markers indicates the possibility of multiple refuges and a rapid re-colonisation following the glacial maximum (Palme et al 2003). Interestingly, populations from southern Sweden had one of the highest levels of chloroplast diversity which may indicate a convergence of different postglacial migration routes. Unfortunately, Latvian birch samples were not included in this study, however, the close proximity of the Swedish populations to Latvia, would suggest

that the chloroplast diversity in Latvian birch populations is also high. Again, the location of Latvia at the convergence of different post-glacial re-colonisation routes would be reflected in the high levels of nuclear DNA marker polymorphism found in this study.

The Lithuanian population had a smaller number of alleles, but the number of common alleles (frequency >5%) was similar to the other populations studied. This is probably a result of the sample origins of these populations. The other populations included multiple individuals from the same population either from provenance trials or natural populations, while the Lithuanian samples were taken as a single individual from each provenance. This would have the effect of reducing the incidence of rare alleles, while preserving the number of more common alleles, as can be seen from the results.

Similarly, the Polish population showed lower genetic diversity, and again this is probably due to the origin of the samples. These samples were taken from a provenance trial of Polish birch located in Latvia, which contained individuals from 3 populations. Multiple individuals were taken from each population, however, as only 3 populations were represented, this would have the effect of reducing the total number of alleles detected. However, the number of more frequent alleles (>5%) is similar to the Lithuanian population and closer to the Latvian and Estonian populations than the total allele number. In contrast, the Latvian and Estonian populations were sampled by multiple individuals from a larger number of populations (4-6 populations were combined for the South, West and North Latvian populations.

The Latvian populations are similar to Estonian provenances investigated, while the Lithuanian and Polish provenances were more genetically distinct from Latvian populations, and also distinct from each other. The Lithuanian population was the most distinct from all other populations, even the Polish provenances. Further investigation of Latvian populations using chloroplast markers may allow greater population differentiation, and by comparison to populations further south, it may be possible to further elucidate the postglacial re-colonisation routes.

These results show that the genetic diversity of *B. pendula* is high, and the population differentiation is low, as can be expected from an outcrossing, wind-pollinated, long-lived forest tree species. While these results do not indicate population differentiation between Latvian populations, this does not preclude the possibility of differentiation in adaptive traits. Studies are underway to examine the extent of morphological and phenological differentiation between Latvian *B. pendula* populations. Given the high genetic diversity identified by neutral markers, it would be surprising this diversity did not also extend to adaptive traits. While locally adapted populations of Latvian *B. pendula* undoubtedly exist, the high intra-population variation indicates that these natural populations should be able to adapt to changing climatic conditions as well as providing locally-adapted genotypes for selection programs.

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Āra bērza *Betula pendula* Roth Latvijas populācijas ģenētiskās daudzveidības novērtējums

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Kopsavilkums

Raksturvārdi: Āra bērzs, Betula pendula, SSR marķieri, ģenētiskā daudzveidība, populācijas struktūra, Latvija.

Āra vai kārpainais bērzs (*Betula pendula* Roth) ir visizplatītākā lapu koku suga Latvijā. Latvijas bērzu populācijas tiek iedalītas trijās agroklimatiskajās zonās –Kurzemes (rietumu), Vidzemes (ziemeļu) un Latgales (dienvidu). Latvijas bērzu populāciju ģenētiskā daudzveidība ir augsta, bet to diferenciācija, kā to jau varēja sagaidīt, zema, jo *B. pendula* ir svešapputes, apputeksnēšanās notiek ar vēja palīdzību, kā arī tā ir ilglaicīgai meža koku suga. Ar izmantotiem "neitrāliem" SSR marķieriem populāciju diferenciācija netika atrasta. Augstā iekšpopulācijas daudzveidība norāda, ka Latvijas bērzu dabiskās populācijas varētu pielāgoties mainīgiem klimata apstākļiem, ka arī tās var nodrošināt selekcijas programmas ar lokāli adaptētiem genotipiem.