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How the Virtual Thinning Can Help to Control the Changing of Genetic Structure in Scots Pine Stands? †

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Abstract: The work detailed here in the context of the above Project sought to determine changes in the gene pool (at the DNA level) in a stand of Scots pine (Pinus sylvestris L.), under the influence of various kinds of forest-tending cuts. The experimental area on which the research was focused is located in Poland's Ostrów Mazowiecka FD. Genetic structure was specified using five nSSR sequences and six cpSSR loci, while the five thinning variants trialled were sanitation cutting, low thinning of 30% intensity, schematic thinning, selective thinning and destructive lumbering. The control variant was left untreated. It was virtual rather than real-life thinning that was pursued, using the *ForestSimulator BWINPro* program. Changes in the structure of the stand after a further 10 years were also simulated. The different thinning variants were shown to cause change in the gene pool and level of genetic diversity of trees in the study area. In terms of maintaining genetic variability in the stand, the least-favourable method proved to be thinning from below. Destructive selection cutting was in turn most beneficial in terms of the preservation of genetic structure, with the reduction in rare alleles being more limited than in any other analysed variant. It was with the selective thinning variant that the final number of trees, stand structure and level of genetic variation resembled the situation in the control most closely. This suggests that selective thinning provides for a rather accurate replication of processes occurring in nature.

Keywords: genetic variation; SSR markers; stand tending; Pinus sylvestris L.

1. Introduction

The size and variability of the gene pools of all forest tree species have been shaped by human activity for centuries. Both the distribution of tree species in Europe, and the genetic structure characterising its forests, reflect interaction between natural evolutionary processes and anthropogenic factors [1, 2].

The most important anthropogenic factor exerting a direct influence on the future of forest ecosystems is obviously management in the context of forestry practice. The preservation of the gene resources of forest trees in as natural a shape as possible is now an explicit priority action for Polish, European and global forestry [3, 4]. The series of cuts made over decades of the life of a stand has the effect of removing certain specimens – and genotypes – in the context of a process of selection based on phenotype-assessment criteria, and regulated via silvicultural principles. Among other things, this activity impact directly on the pollination process, as it modifies conditions for gene exchange within

and between populations [5]. Beyond that, it is reported that stand-tending measures pursued via a system in which the intervals between cuts are shorter may actually pose a major threat when it comes to maintaining a stable level of genetic differentiation among genes coding for economically-valuable traits, as well as those conferring adaptability to given environmental conditions [6-8].

The main goal of the project was to determine, based on molecular studies (analysis of organelle and nuclear DNA) changes in the gene diversity of Scots pine stands. Partial objectives of our study were: (1) to examine the genetic structure of the stand before the treatments; (2) to estimate the effect of five commonly used and historical thinning methods on the genetic diversity parameters; (3) to check whether the use of virtual thinning software (*ForestSimulator BWINPro*) is helpful in that type of scientific research.

2. Material and Methods

The area in which the trials are carried out falls within the Warsaw Regional Directorate of the State Forests and its Forest District of Ostrów Mazowiecka, approximately 95 km towards the north-east of Warsaw (52°47′16″N 21°44′58″E). The main stand thus comprises Scots pine (Pinus sylvestris), with trees 47 years old occurring at moderate density, and with discontinuous planting in some places. Plot boundaries are marked in a permanent way using paint, and each tree present is numbered individually. Breast-height diameters of all trees are measured and known, while heights are calculated using curves for the stand. Crown heights and widths are also determined, and these different data were together used classify trees on the plot from the biosocial point of view. Also determined for each tree was its location (x, y coordinates). A characterisation of the stand by reference to its main features was also made. As this was only to be a pilot study, DNA research focused in on a ca. 0.065 ha trial plot within the wider study area. The original numbering of trees there was retained as plant material from 103 trees was sampled (of wood and phloem).

The isolation of genomic DNA was achieved with a commercially-available NucleoSpin® Plant II Kit, by adhering to the instructions from the producer (MacheryNagel®, Germany). Genetic structure in pines present on the plot was studied by analysing polymorphism characterising five sequences of nuclear microsatellite DNA (nSSR), i.e. SPAG 7.14, SPAC 11.4, SPAC 11.6, PtTX3107 and SsrPt_ctg4363 [9-11]; as well as 6 chloroplast DNA loci (cpSSR), i.e. PCP26106, PCP30277, PCP36567, PCP45071, PCP71987 and PCP87314 [12]. Two multiplex PCR was designed. At each locus in the chloroplast and nuclear microsatellite DNA, the frequency of occurrence of different alleles was checked for, as were numbers of rare alleles (RA). RA was the count by locus of alleles with frequency lower than or equal 5%. Genetic variation before and after thinning was depicted by reference to main genetic parameters. The above parameters were calculated using the GenAlEx 6.501 program [13].

The achievement of virtual thinning required the use of *ForestSimulator BWINPro* ver. 7 programming (J. Nagel, Georg-August-Universität Göttingen, Germany). This allows changes in main incremental growth parameters at the level of each tree and the whole stand to be observed without any actual trees in the field needing to be cut. Five thinning variants were organised: sanitation cutting (meaning thinning from below of around 10% of the mass present per ha); thinning from below (otherwise known as low thinning) of 30% intensity; schematic thinning, i.e. the removal of every 5th row of trees; selective thinning with marking and encouragement of the best-shaped trees on a positive-selection basis; and destructive lumbering – denoting the cutting of all the best-shaped trees. The control variant on the plot denoted a lack of cutting. Use of the BWINPRo program allowed the measures to be checked for their influence on incremental growth of trees and the stand; as well as on genetic structure – including 10 years on from each measure – in comparison with the control situation. Thanks to appropriate design of the algorithms, mortality across the study area could be simulated by the programming.

3. Results

Table 1 and 2 describe mean genetic parameters for nSSR and cpSSR loci.

Low thinning (thinning from below) emerged as the least-favourable measure when it came to maintaining the level of genetic variation in the stand. The level of genetic differentiation following

this measure was constant (given a higher degree of similarity after a further 10 years). Likewise, the degree to which the stand could be regarded as inbred proved greatest with the same low-thinning variant (in comparison with all others), and that situation also remained the same 10 years on. Such impacts can be seen as a straightforward reflection of the way that the above kind of thinning entails the removal of the most trees from the secondary stand.

Destructive lumbering was the means of stand tending that favoured the retention of genetic structure most. In none of the variants was the loss of rare alleles so limited. A slight increase in genetic differentiation was even noted in the case of both nSSRs and cpSSRs. That said, the idea that stands of this kind are to act as genetic reservoirs serving the generations to come must be set against the fact the present reference to genetic variation relates to non-coding fragments of DNA. In this connection, there is no excluding the possibility that such a cutting variant may have led to the removal of the most desirable (highest-quality) genes – whose impact may indeed be reflected in high-quality wood, and high vitality and a good state of health among crop trees.

Analysis of the sanitation-cutting variant shows this measure's moderate influence in modifying genetic structure in the stand. Schematic thinning, characterised by entirely random selection of trees, also reduced genetic differentiation to only a limited degree. In this case, a marked reduction in rare alleles with cpDNA is most probably associated with the rather large number of trees removed, plus the occurrence of subsequent losses of trees from the stand.

In the variant with selective thinning, the numbers of trees and stand structure 10 years on are very similar to the numbers present after natural self-thinning under control conditions. This variant only modified stand genetic structure to a limited degree, meaning that selective thinning as carried out here may indeed provide a fairly effective imitation of processes ongoing in nature.

Table 1. Genetic parameters of nSSR loci.

Analysis variant	N	Na	Ne	A_R	I	Ho	He	*h* Nei	Fis	I_N
C	101	17,4	9,57	15,3	2,263	0,676	0,837	0,841	0,197*	-
SC	83	16,8	9,62	15,2	2,267	0,676	0,842	0,847	0,203*	0,997
TB	47	14,8	9,38	14,7	2,198	0,664	0,833	0,842	0,213*	0,985
ST	80	16,6	9,2	14,9	2,227	0,663	0,833	0,839	0,210*	0,997
SLT	89	17	9,49	15,2	2,254	0,666	0,837	0,841	0,210*	0,999
DL	81	17,2	9,78	15,6	2,279	0,677	0,838	0,844	0,198*	0,996
C_10	83	17,2	9,97	15,4	2,279	0,682	0,841	0,846	0,195*	0,997
SC_10	71	16,2	9,68	15	2,262	0,675	0,842	0,848	0,205*	0,994
TB_10	45	14,8	9,4	14,8	2,193	0,662	0,832	0,841	0,215*	0,982
ST_10	72	16,4	9,26	15	2,236	0,664	0,835	0,841	0,212*	0,995
SLT_10	77	16,6	9,5	15,1	2,245	0,67	0,836	0,841	0,205*	0,995
DL_10	78	17,2	9,81	15,7	2,28	0,68	0,838	0,843	0,194*	0,995

C –control variant without treatment; SC –sanitary cutting; TB –thinning from below; ST – schematic thinning; SLT – selective thinning; DL – destructive lumbering; C_10 – control variant after 10 years of natural processes; SC_10 – 10 years after sanitary cutting; TB_10 – 10 years after thinning from below; ST_10 – 10 years after schematic thinning; SLT_10 – 10 years after selective thinning; DL_10 – 10 years after destructive lumbering; N – mean number of genotyped trees; Na – mean number of different alleles; Ne – mean effective alleles number; A_R – the allelic richness; I – the Shannon's information index; Ho – the observed heterozygosity; He – the expected heterozygosity; *h* Nei – the Nei's heterozygosity index; Fis – the fixation index (* statistical significant at α=0,05); uh – unbiased allelic diversity; I_N – the Nei's genetic identity compared to control.

Table 2. Genetic parameters of cpSSR loci.

Analysis variant.	N	Na	Ne	I	uh	I_N
C	102	10,78	5,84	0,848	0,444	-

SC	83	10,48	5,86	0,832	0,436	0,999
TB	47	9,23	5,71	0,816	0,44	0,998
ST	81	10,22	5,65	0,815	0,431	0,999
SLT	90	10,58	5,79	0,85	0,445	0,999
DL	82	10,68	5,95	0,86	0,454	0,999
C_10	83	10,6	6,04	0,846	0,444	0,999
SC_10	71	10,18	5,9	0,843	0,44	0,998
TB_10	45	9,23	5,73	0,82	0,441	0,998
ST_10	72	10,03	5,68	0,814	0,435	0,999
SLT_10	77	10,3	5,79	0,842	0,444	0,999
DL_10	79	10,68	5,97	0,87	0,46	0,999

C, SC, TB, ST, SLT, DL, C_10, SC_10, TB_10, ST_10, SLT_10, DL_10 – description in the Table 1; N, Na, Ne, I, I_N – description in the Table 1; uh – unbiased allelic diversity.

In the cases of both types of DNA studied, the most major loss of rare alleles (frequency ≤ 0,05) was observed shortly after intensive low thinning had been carried out. In the case of nuclear DNA as many as 22% of rare alleles (in comparison with the control) were removed, while with chloroplast DNA the noted loss from the rare alleles pool was of 43%. This level had not changed 10 years on from the measure, so – despite the loss from the stand of 2 further trees –it was still possible to identify a loss where these same markers and alleles were concerned. It can be noted that, with nDNA, the thinning of trees in the 10-year period following sanitation felling (of 12 trees) and selective felling (of 13 trees) – as compared with numbers of trees removed in the course of these measures – influenced greater loss of rare alleles (3 and 2 more alleles lost respectively). The most limited impact upon genetic structure at the level of rare alleles was associated with destructive lumbering at the time, as well as 10 years following that form of cutting, as well as with the control plot once natural processes had run their course for a further 10 years.

4. Conclusions

Use of the *ForestSimulator BWINPRo* computer programming to make virtual (as opposed to real) cuts of the stand-tending type allowed many variants to be tested across the same study area.

Despite the created ranking of the impact of the tending cuts made in the pine stand, it should be noted that the genetic diversity and structure after virtual thinning of various types and after next 10 years of natural processes was very similar to that observed in the control.

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