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ORIGINAL PAPER

Forecasting the effect of different thinning regimes on the genetic variability in Scots pine *Pinus sylvestris* L. stand – a simulation study

Agata Konecka[(1)]✉, Anna Tereba[(2)], Marcin Studnicki[(3)], Włodzimierz Buraczyk[(1)], Henryk Szeligowski[(1)], Bogdan Brzeziecki[(1)], Kamil Bielak[(1)]

[(1)] Department of Silviculture, Institute of Forest Sciences, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, Warsaw 02-776, Poland

[(2)] Department of Forest Ecology, Forest Research Institute, Braci Leśnej 3, 05-090 Sękocin Stary, Poland

[(3)] Department of Biometry, Agricultural Institute, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, Warsaw 02-787, Poland

ABSTRACT

We sought to determine changes in the gene pool in a stand of Scots pine *Pinus sylvestris* subject to different thinning regimes. The pine stand under study was located in central Poland. The simulations using the *ForestSimulatorBWINPro* involved six experimental variants representing five virtual thinning regimes, *i.e.* 1) low thinning of 12% intensity; 2) low thinning of 35% intensity; 3) geometric thinning; 4) selection thinning; 5) high-grading; and 6) the untreated control. We considered the impact of these regimes on stand genetic variability up to 10 years after the given treatment, by reference to eleven SSR sequences. While various changes in the level of genetic diversity of stand were forecast to arise under the different regimes, low thinning of 35% intensity was identified as the method least favourable to maintenance of the initial level of genetic variability in the stand (loss of 22 and 43% of rare alleles in nSSR and cpSSR). High-grading proved most beneficial in the short-term. The selection thinning represented a kind of 'golden mean'; it turned out to be a method that enables achieving important silvicultural goals and simultaneously only slightly distorts the stand genetic variability. The conducted experiment with the use of *ForestSimulatorBWINPro* program proved that the software can be successfully used in studied on the genetic variability of trees. Because it was only a preliminary study we suggest larger resampling the populations and genotyping using a larger number of microsatellite markers.

KEY WORDS

genetic variation, *Pinus sylvestris* L., SSR markers, stand tending, thinning simulation

Introduction

In intensively managed forest landscapes, such as those in Central Europe, the effect of human activity on forest gene pools has been evident for several centuries now. Both the current distributions of tree species in Europe and the genetic structure of the continent's forests reflect long-

✉e-mail: agata_konecka@sggw.edu.pl

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-term interaction between natural evolutionary processes and anthropogenic factors (Schaberg *et al.*, 2004).

The active safeguarding of genetic diversity in forests is important, given that: 1) it affects the vulnerability of trees to pests and short- or long-term climatic fluctuations; 2) genetic variants are important for future adaptability or breeding; and 3) potential destabilisation of entire forest ecosystems may ensue if genetic diversity is reduced too far (Rajora and Mosseler, 2001; Lefèvre, 2004). Gene conservation is therefore seen as crucial for maintaining forest productivity and adaptability (Hughes *et al.*, 2008), and has become an explicit priority action in forestry (Finkeldey and Ziehe, 2004; Matras, 2013; Brang *et al.*, 2014).

Human activity is likely to remain the key future influence on most forest ecosystems. Forests are managed to ensure utilisation, protection, conservation and regeneration, even as the main objective is to produce timber. In many stands with production of high-quality timber as key objective (Nilsson *et al.*, 2010), foresters apply silvicultural measures – particularly thinning – to favour onward growth of the highest-quality trees (Zeide, 2001).

The sequence of cuts over the decades in the life of a stand works to remove certain individuals – and genotypes – via a selection process guided phenotypically. Trees are removed both intensively and selectively to reduce competition (Finkeldey and Ziehe, 2004), and also to achieve changes in spatial structure (Brzeziecki, 2005). Changes occurring at the level of biodiversity are also an unintended by-effect of sylvicultural treatments (Müller *et al.*, 2007; Torras and Saura, 2008). Activity of this kind for example affects pollination, as conditions for gene exchange within and between populations are modified (Namkoong *et al.*, 1996; Hosius *et al.*, 2006).

Unfortunately, the typical episodes of stand tending over relatively short intervals of time may increasingly be viewed as harmful to the maintenance of diversity, not only among tree genes conferring adaptability to given environmental conditions, but also among those coding for traits of economic value (Hoffmann and Sgro, 2011; Rajendra *et al.*, 2014). This leaves as ambiguous the nature of the influence on stand genetic diversity exerted by different kinds of cutting and felling (Schaberg *et al.*, 2008; Ratnam *et al.*, 2014).

In paper sought to combine molecular studies with the use of virtually performed thinning regimes. The main research hypothesis was that, in the course of stand development, thinning of both artificial and of natural origin (self-thinning) operates to modify gene frequency – and hence overall genetic diversity – in a given tree species, even as some of the methods are obviously more or most advantageous in preserving the diversity in question. Indeed, by encompassing several different thinning regimes in our research, we sought to develop a hierarchy of methods interfering most or least in the genetic structure and diversity of the population of trees investigated. More specifically, our study has had as its partial objectives: (1) examination of a stand's genetic variability prior to treatment; (2) assessment of the impacts on parameters describing genetic diversity that may be attributed to five commonly-used thinning regimes; (3) assessment of the value of dedicated *ForestSimulatorBWINPro* software in simulating different thinning treatments in the context of genetic tests.

Materials and Method

Study object and collection of data. This study was conducted in a managed forest stand located in Ostrów Mazowiecka Forest District (under Regional Directorate of State Forests in Warsaw), approximately 95 km NE of Warsaw (52°47'16"N, 21°44'58"E). The Scots pine *Pinus sylvestris* L. stand was planted in 1973. The initial stand density was 12,000 trees per ha. After establishment, the stand was subject to several forms of early and late tending, and two episodes

of thinning pursued when the stand was 20 and 30 years old. At the time of study, trees present at moderate density and were 39 years old.

The trial plot of 50×50 m (0.25 ha) was established within the stand in a location regarded representative. Paint was used to mark plot boundaries permanently, and each tree present within those limits was then number-tagged. The diameters at breast height (DBH) were then determined for all trees, while heights were measured in the case of 32 specimens. To calculate height of the remaining trees, measure height of this specimens was used to derive height-DBH relationship. Each measured tree had its spatial location (x, y coordinates) determined.

As this was only a preliminary study, the work detailed in this paper has made use of the training sub-plot located centrally within the training plot, on a 0.0625 ha (25×25 m) (Fig. 1). From 103 trees growing in that sub-plot, wood was sampled for DNA analysis, at the trunk base with a Pressler increment borer.

The Virtual Thinning Treatments. Our study used the *ForestSimulatorBWINPro* program, as a basic tool permitting appropriate handling (analysis and simulation) of tree-stand data (Nagel *et al*., 2006). The core element here is *TreeGrow* – a model for growth in a stand that allows changes in basic tree and stand parameters in the course of stand development to be predicted over successive 5-year periods. The program also contains several additional algorithms and functions providing for the simulation of different silvicultural measures (including kinds of thinning), and their impact on the structure and development of a stand.

To simulate different thinning regimes it was first necessary to input measured data obtained from trees across the training plot into the *ForestSimulatorBWINPro* program. Basic

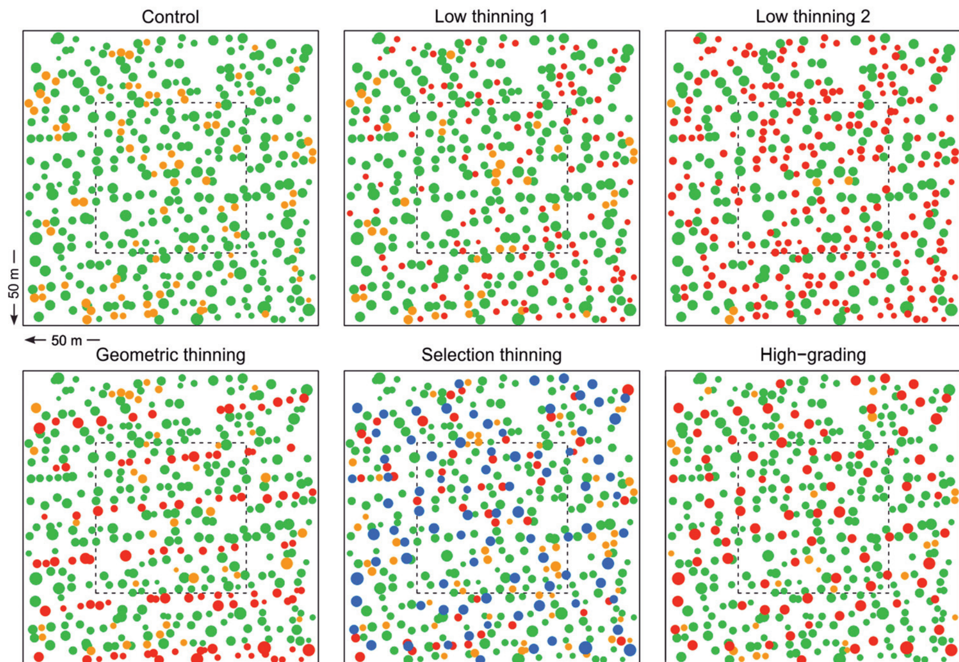

Fig. 1.

Stem maps visualising the experimental thinning treatments performed on the training plot

green – trees remaining alive at the end of the simulation period; red – trees removed under a given treatment; orange – trees dying as a result of competition and suppression; blue – crop trees (in selection thinning only). The dashed line shows the experimental subplot used in DNA analysis

calculations were then run for statistics at the level of the individual trees (height, volume) and at stand level (density, overall stand volume, mean values for DBH and height).

The five experimental thinning regimes were: 1) LT1, *i.e.* low-intensity low thinning (meaning thinning from below of around 12% of tree volume present per ha); 2) LT2 – high-intensity low thinning (35%, by tree volume); 3) GT – geometric thinning, *i.e.* the removal of every 5th row of trees. In this case neither qualitative nor quantitative traits of trees to be cut down were taken into account; 4) ST – selection thinning. Thinning designed in the field on a positive-selection basis. Carried out by selecting and marking the best-shaped trees (crop trees) and removing trees harmful that compete for living space with valuable crop trees; 5) HG, *i.e.* high-grading, testing the impact of so-called 'destructive lumbering', which assumes cutting of all best-shaped crop trees. It is not an example of either clear-cut thinning or high/crown thinning, because only trees considered best-shaped from the point of view of selection thinning are removed. Hence, HG is the 'reverse' of selection thinning adhering to the 'cut the best and leave the rest' principle. There was also a control variant (C), with no cutting, but with assumptions made as regards self-thinning through 'natural' mortality (Figs 1 and 2). In the case of low thinning, the proper algorithm selects trees for cutting, beginning with the thinnest and continuing until such time as the pool of trees selected remains below the defined threshold for tree volume to be cut. All the simulations of stand development over the assumed 10-year period were performed with an activated option allowing for 'natural' (*i.e.* competition- or suppression-related) mortality of trees. In the control variant (C) the sole sources of tree mortality were 'natural', competitively-induced processes of self-thinning.

DNA Analysis. The isolation of genomic DNA was achieved with a commercially-available NucleoSpin® Plant II Kit, by adhering to the instructions from the producer (MacheryNagel®,

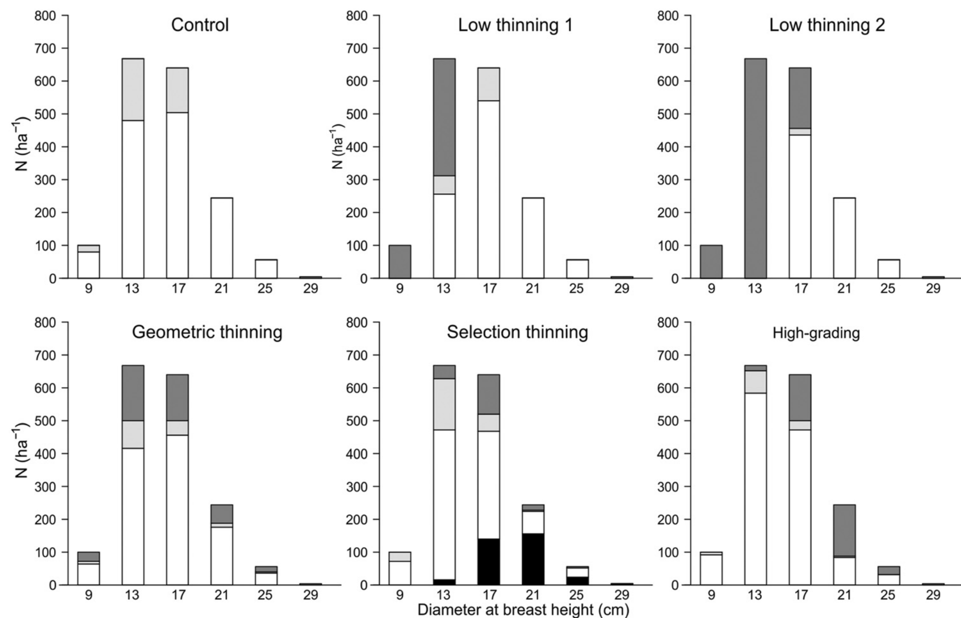

Fig. 2.

The effect of different thinning regimes on the distribution of tree diameters

white – trees still alive at the end of the simulation period; dark grey – trees removed within a given treatment; light grey – trees dying due to competition and suppression; black – crop trees (under selection thinning)

Germany). The quality and quantity of the extracted total DNA was assessed using a NanoDrop® ND-1000 spectrophotometer (ThermoScientific, Wilmington, USA).

Genetic variability in Scots pine *P. sylvestris* trees 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 (Soranzo *et al.*, 1998; Elsik and Williams, 2001; Chagné *et al.*, 2004); as well as six chloroplast DNA *loci* (cpSSR), *i.e.* PCP26106, PCP30277, PCP36567, PCP45071, PCP71987, and PCP87314 (Provan *et al.*, 1998).

Two multiplex PCRs were performed in 10 µl total volume mixture: 1 × Qiagen Multiplex PCR Master Mix (Qiagen® Multiplex PCR Kit); and 0.2 µM each of the forward primer and fluorescently labelled reverse primer; 1 µl of template DNA. PCR-multiplied fragments of DNA were electrophoresed in an ABI 3500 Genetic Analyzer (Life Technologies™, USA); which is a capillary sequencer. Fragmented material was analysed for numbers of base-pairs (bp) using version 5 of the GeneMapper™ program (Life Technologies™, USA).

Statistical analysis. Checks were made at each *locus* in the chloroplast and nuclear microsatellite DNA for the frequency of occurrence of different alleles and the numbers of rare alleles (A_{rare}). The latter were alleles of frequency by *locus* equal to or lower than 5% (based on a modified scale of Buchert *et al.*, 1997). Genetic variation before and after thinning was referred to as observed or expected numbers of alleles at the given *locus* (A, Ae), as well as observed and expected heterozygosity (Ho, He). The level of genetic diversity was described using the Nei heterozygosity index, as well as the Shannon Information Index (I). Indices of genetic similarity (I_N) were also calculated, between the control group and groups of trees remaining after different kinds of thinning had been carried out. All parameters were calculated using the GenAlEx 6.501 program (Peakall and Smouse, 2006). Common haplotype diversity indexes (A – number of haplotypes; N_e – effective number of haplotypes; R_h – haplotypic richness; He – genetic diversity and number of private haplotypes) were calculated with HAPLOTYPE ANALYSIS ver. 1.05 software (Eliades and Eliades, 2009).

The share of null alleles for each *locus* was calculated with the GENEPOP 4.0 program (Rousset, 2008). The degree of inbreeding of studied groups of trees was in turn assessed with the inbreeding coefficient (F_{IS}). The latter was tested for significance, at α=0.05, in either a positive or negative direction (respectively denoting heterozygosity in deficit or in excess). The FSTAT 2.9.4 program (Goudet, 2005) was used to assess inbreeding coefficient and allelic richness (A_R).

Tending treatments were compared for the proportional loss of A_{rare}, using the Pearson χ^2 test and Fisher's exact test for pairwise comparisons. The R 3.6.3 software was applied in this case.

The genetic variation between and within tending treatments was analysed by Analysis of Molecular Variance (AMOVA), with GenAlEx 6.501 *post-hoc* test pairwise comparisons of the genetic differentiation between tending treatments were further evaluated by calculating *PhiPT* values. Probabilities for the AMOVA indexes and *post-hoc* test were calculated on the basis of individual randomisations (999 permutations).

Results

Stand Parameters. Trees in different diameter classes were removed under a given thinning regimes (Fig. 2). Most trees per ha were harvested under high-intensity low thinning (LT2) (which entailed 35% by volume being removed, or 952 trees/ha) (Table 1). The most limited removal (of just 180 trees/ha) in turn characterised the selection thinning variant. The greatest volume of timber (99.1 $m^3 \cdot ha^{-1}$) was harvested with LT2, while the second-greatest volume (85.1 $m^3 \cdot ha^{-1}$)

Table 1.

Changes in stand parameters reflecting different thinning treatments

Thinning regime	Immediately after treatment				After 10 years of simulated stand development			
	N	V	DBH	H	N	V	DBH	H
C	1,712	279.7	16.2	17.5	1,368	369.9	20.2	18.4
LT1	1,256	245.9	17.5	17.8	1,100	355.6	22.0	18.8
LT2	760	180.6	19.1	18.2	740	295.9	24.3	19.1
GT	1,300	211.4	16.1	17.5	1,148	307.3	20.1	18.4
ST	1,532	248.1	16.1	17.5	1,296	346.1	20.1	18.4
HG	1,372	194.6	15.2	17.2	1,264	300.7	19.1	18.2

N – number of trees per hectare; V – stand volume [m^3ha^{-1}]; DBH and H – mean DBH [cm] and mean height [m]; C – control variant (no thinning), LT1 – low-intensity low thinning, LT2 – high-intensity low thinning, GT – geometric thinning, ST – selection thinning, HG – high-grading

was removed by high-grading (HG). In contrast, the smallest loss of volume (31.6 $m^3 \cdot ha^{-1}$) was observed after selection thinning.

Ten years on from the simulated treatments, overall stand volume was greater in all variants, despite 'natural' mortality taking place among trees throughout the simulation period. As could be expected, 'natural' mortality was highest in the control variant – at 344 deaths per ha, while it was lowest where LT2 was the treatment applied – at 20 deaths per ha. While prior to treatment average tree volume was ca. 0.16 m^3, 10 years of simulated stand development allowed this parameter to increase most (to almost 0.40 m^3) under the regime of LT2. In contrast, the smallest increase in mean volume (to 0.24 m^3) characterised HG variant. Experimental thinning regimes also differed in their effects on mean stand diameter (DBH) and height (H). In this regard, the most similar variants were the geometric (GT) and selection (ST) thinning regimes. The results obtained for these two variants were also most similar to those recorded in the control, both immediately after treatment and 10 years later. On the other hand, in comparison with the control, the values for mean stand diameter and height were higher for both the low thinning regimes, as well as lower with HG variant.

Statistics for loci. The SPAC 11.6 *locus* proved the most polymorphic and thus highly variable marker where nSSR was concerned, in all the experimental variants, both immediately after application of the different thinning treatments and 10 years later. At this *locus*, the number of alleles was in the range 26-31, with numbers of base pairs (bp) between 121 and 181. The smallest number of alleles (7-8) in turn characterised marker PtTX3107, with 150-174 bp present.

The presence of null alleles was noted for all the *loci* for nSSR under study. The estimated frequency of occurrence of null alleles was in the 0-0.19 range, so there was no reason for disqualification of any of the nSSR markers used (Belletti *et al.*, 2012).

The numbers of alleles at given cpSSR *loci* ranged from 2 to 6, with the most variable *locus* being PCP30277, which was always characterised by the presence of 6 alleles (of between 132 and 137 bp), irrespective of the treatment variant and the passage of the 10 years. A constant number of alleles (3, 3 and 4 respectively) was also present at *loci* PCP26106, PCP45071 and PCP87314. *Loci* PCP36567 and PCP71987 differed in terms of allele number, in line with the thinning regime considered.

Genetic differentiation following thinning treatments. Apparently, the number of trees remaining after different treatments with combination of selection type had the greatest effect on mean numbers of different alleles (A) and allelic richness (A_R), with the lowest value observed for the LT2 (Table 2). On the other hand, A_R was greater in HG than in the control, both imme-

Table 2.

Mean values for parameters describing genetic variability for all tested variants of thinning regimes and the control variant, based on SSR

Thinning regime	nSSR											cpSSR					
	N	A	Ae	A_R	I	Ho	He	*h* Nei	Fis	I_N	*PhiPT***	A	Ae	I	uh	I_N	*PhiPT***
Immediately after treatment																	
C	103	17.40	9.57	15.3	2.263	0.676	0.837	0.841	0.197*	–	–	10.78	5.84	0.848	0.444	–	–
LT1	83	16.80	9.62	15.2	2.267	0.676	0.842	0.847	0.203*	0.997	0.023	10.48	5.86	0.832	0.436	0.999	0.017
LT2	47	14.80	9.38	14.7	2.198	0.664	0.833	0.842	0.213*	0.985	0.033	9.23	5.71	0.816	0.440	0.998	0.023
GT	82	16.60	9.20	14.9	2.227	0.663	0.833	0.839	0.210*	0.997	0.021	10.22	5.65	0.815	0.431	0.999	0.017
ST	90	17.00	9.49	15.2	2.254	0.666	0.837	0.841	0.210*	0.999	0.020	10.58	5.79	0.850	0.445	0.999	0.018
HG	82	17.20	9.78	15.6	2.279	0.677	0.838	0.844	0.198*	0.996	0.021	10.68	5.95	0.860	0.454	0.999	0.018
After 10 years of simulated stand development																	
C	83	17.20	9.97	15.4	2.279	0.682	0.841	0.846	0.195*	0.997	–	10.60	6.04	0.846	0.444	0.999	–
LT1	71	16.20	9.68	15.0	2.262	0.675	0.842	0.848	0.205*	0.994	0.028	10.18	5.90	0.843	0.440	0.998	0.021
LT2	45	14.80	9.40	14.8	2.193	0.662	0.832	0.841	0.215*	0.982	0.036	9.23	5.73	0.820	0.441	0.998	0.028
GT	72	16.40	9.26	15.0	2.236	0.664	0.835	0.841	0.212*	0.995	0.027	10.03	5.68	0.814	0.435	0.999	0.019
ST	77	16.60	9.50	15.1	2.245	0.670	0.836	0.841	0.205*	0.995	0.029	10.30	5.79	0.842	0.444	0.999	0.020
HG	79	17.20	9.81	15.7	2.280	0.680	0.838	0.843	0.194*	0.995	0.022	10.68	5.97	0.870	0.460	0.999	0.019

C, LT1, LT2, GT, ST, HG – description of experimental variants as in Table 1; N – number of genotyped trees; A – mean number of different alleles; Ae – mean effective allele number; A_R – allelic richness; I – Shannon information index; Ho – observed heterozygosity; He – expected heterozygosity; *h* Nei – Nei heterozygosity index; Fis – fixation index (*statistically significant at α=0.05); uh – unbiased allelic diversity; I_N – Nei genetic identity compared with the control; ** *PhiPT* values obtained for pairwise comparison of a given variant with the control, or with control following 10 years of simulated stand development

diately after treatment and 10 years later. Other indices, such as the Shannon diversity index (I) (for both types of SSR) and Nei heterozygosity (*h* Nei) (for nSSR) showed only a slight decline in genetic variation compared with the control. This was above all true for LT2 (Table 2). Mean values for observed heterozygosity (Ho) were below the expected levels (He) by 20% or so, attesting to a somewhat lower share of heterozygotes in all the variants studied than could be calculated on a theoretical basis by reference to the Hardy-Weinberg (H-W) equilibrium for the treatment variants. Although reductions in numbers of trees occurred with each treatment variant analysed, they did not lead to significant alteration of the initial proportions of homozygotes and heterozygotes. A more major fall in observed heterozygosity in comparison with the control was noted after LT2, as well as after the GT and ST variants; both immediately after these treatments and 10 years later. It should be noted that the SSR markers and size of sample used may not have captured presence all of the heterozygotes. The F_{IS} coefficient was used to assess differences (in relation to the control) in the extent to which the groups of trees studied were inbred. The control and all tested variants could be seen as featuring excesses of homozygotes (statistically significant values for $F_{IS}>0$)compared with the H-W situation. The most marked increase in inbreeding occurred after LT2, both immediately after this treatment and 10 years later. Reference to the I_N value showed that the trees most similar genetically to those in the control were the ones subject to ST. The greatest genetic distance in turn characterised stands following LT2 (directly after treatment and 10 years later, only for nSSR).

Finally, low *PhiPT* values sustain a conclusion regarding no statistically significant differentiation in variability of allele frequency between the examined thinning regimes and the control variant, both immediately after treatment and 10 years later (Table 2).

The latter finding was also supported by the AMOVA analysis. Results obtained for both nSSR and cpSSR revealed no significant differences between the applied thinning regimes. The impact of treatments on variability of *loci* among the analysed markers also failed to register as statistically significant, both immediately after thinning and 10 years later (Table 3). However, the opposite was true for the given treatment when the effect was considered at the level of individual trees.

Comparison of genetic diversity indexes for cpSSR revealed the highest number of haplotypes, haplotypic richness, effective number of haplotypes and haplotypic genetic diversity for ST variant directly after treatment and 10 years later (in this case excluding index A) (Table 4).

Table 3.
Analysis of molecular variance (AMOVA) for SSR markers

Source of variation	nSSR			cpSSR		
	variance	[%]	P-value	variance	[%]	P-value
Immediately after thinning						
Variations between different thinning regimes	0.7716	0.1678	0.1123	0.2328	0.1493	0.1231
Variations between samples within given treatment	3.8281	0.8322	0.0402*	1.3257	0.8507	0.0421*
After 10 years of simulated stand development						
Variations between different thinning regimes	0.6931	0.1548	0.1236	0.2413	0.1532	0.1143
Variations between samples within given treatment	3.7843	0.8452	0.0398*	1.3333	0.8468	0.0379*

* statistical significant at α=0.05

The presence of private haplotypes was not found in any of analysed groups of trees after tested thinning regimes or in control.

The number of trees remaining after treatment was smallest with LT2, with only 47 trees left (out of an original 103) (Table 5). After 10 years of simulated stand development, that number was further reduced by 2 trees. In contrast, the most limited decline in numbers of trees occurred with ST – with only 12 trees removed virtually at the start, with an additional 13 in the context of 'natural' self-thinning.

Differential effects of simulated thinning regimes in reducing numbers of alleles at the nDNA and cpDNA levels related solely to the loss of rare alleles (A_{rare}) (Table 5). The control variant originally had 57 A_{rare} in the case of nSSR and 7 in the case of cpSSR.

Table 4.
The cpSSR genetic diversity indexes for all tested variants of thinning regimes and the control variant

Thinning regime	A	N_e	R_h	He
		Immediately after treatment		
C	63	41.280	33.424	0.985
LT1	53	37.238	33.293	0.985
LT2	33	25.391	30.974	0.981
GT	53	36.543	33.066	0.985
ST	61	43.356	34.615	0.988
HG	56	38.921	33.998	0.986
		After 10 years of simulated stand development		
C	56	38.769	33.853	0.986
LT1	49	35.752	33.794	0.986
LT2	33	25.000	32.000	0.982
GT	49	34.381	33.144	0.984
ST	55	40.026	34.742	0.988
HG	55	38.554	34.257	0.986
Mean	**51.333**	**36.268**	**33.430**	**0.985**

Descriptions of thinning regimes are as in Tables 1; A – number of haplotypes; N_e – effective number of haplotypes; R_h – haplotypic richness; He – genetic diversity

Table 5.
Reduction in numbers of rare alleles at nSSR and cpSSR *loci*, depending on the thinning regime, immediately and 10 years after treatment, in relation to the initial state (C)

Thinning regime	Immediately after treatment						After 10 years of simulated stand development					
	C	LT1	LT2	GT	ST	HG	C	LT1	LT2	GT	ST	HG
Tree number*	0	20	56	21	12	20	0 *19*	20 *12*	56 *2*	21 *9*	12 *13*	20 *3*
							nSSR					
A_T	87'	84	74	83	85	86	86	81	74	82	83	86
A_{rare} lost	na	3a	13b	4a	2a	1a	1a	6ab	13b	5a	4a	1a
% of A_{rare} lost	na	5	22	7	3	2	2	10	22	8	7	2
P – value**	0.0011						0.0079					
							cpSSR					
A_T	25"	25	22	23	25	25	24	25	22	22	24	25
A_{rare} lost	na		3a	2a			1a		3a	3a	1a	
% of A_{rare} lost	na		43	29			14		43	43	14	
P – value**	0.7543						0.8789					

*Numbers of trees removed (normal font) and dying as a result of self-thinning (in italics). Descriptions of thinning regimes are as in Tables 1; A_T – total number of alleles; 'including 57 A_{rare}; "including 7 A_{rare}; a, b – different letters indicate significant differences (Tukey's test, p=0.05); **for the Pearson χ^2 test used to compare % of A_{rare} lost between tending treatments; na – not applicable

The most significant loss of A_{rare} resulted from LT2. Under this regime, 22% and 43% of A_{rare} were lost to nSSR and cpSSR, respectively. These figures did not differ 10 years of simulated stand development later, even though 2 further trees had been lost to self-thinning. However, there were other thinning regimes under which natural mortality in the course of simulated stand development caused additional losses of A_{rare}. Thus, for example, with low-intensity low thinning (LT1), the death of a further 12 trees led to the loss of 3 more A_{rare} (for nSSR).By the end of the simulation period, the fewest rare alleles had been lost in the control, as well as with HG. In relative terms, the average loss of A_{rare} alleles was greater for cpSSR than for nSSR *loci*.

Statistically significant differentiation of percentage A_{rare} loss between the thinning regimes was only noted for nSSR (p=0.0011 for the situation immediately after treatment and p=0.0079 after 10 years of simulated stand development) (Table 5).

Discussion

In line with earlier studies, the genetic variability of the investigated Scots pine *P. sylvestris* stand was only slightly affected by different thinning treatments, even at a high intensity (Paffetti *et al.*, 2012; Danusevicius *et al.*, 2016). Taking into account the above analysed indices, from the point of view of preserving the initial level of genetic variability, the high-grading and the control variant would be most beneficial. On the other hand, the geometric thinning and high-intensity low thinning appeared to be least favourable. Selection thinning and low-intensity low thinning occupied intermediate positions.

These results seem to confirm the findings drawn by Konnert and Spiecker (1996) who showed that the selective removal of trees in a manner imitating natural self-thinning processes affects genetic diversity less than does the felling of trees from the point of view of timber production. On the other hand, high-grading (removed trees representing best phenotypes) resulted in greater increase in heterozygosity than any other thinning regime. The heterozygosity change observed for high-grading was similar over the next 10 years, and was closest to the value calculated for the control stand. The other thinning regimes resulted in a decrease in observed heterozygosity only by 2-3% for both nSSR and cpSSR. These results seem to confirm previous reports of 1-5% reduction in heterozygosity observed in the postharvest residual stands (Rajora *et al.*, 2000).

Another important aspect of overall genetic diversity is the reduction in numbers of rare alleles (Rajora and Mosseler, 2001), as caused by a given treatment. Looking from that perspective, in our study, the high-intensity low thinning appeared to be the measure most disadvantageous to the retention of the original gene pool. In this thinning regime, 22% of the nSSR rare alleles and 43% of the cpSSR rare alleles that had been present before the measure was taken, were finally (*i.e.*, 10 years after treatment) lost. Although Danusevicius *et al.* (2023) revealed that repeated episodes of treatments (up to the age of 60) do not appear to significantly reduce genetic diversity of the second generation of Scots pine *P. sylvestris* stands, however they confirmed that systematic tending significantly reduces the number of rare alleles and genotypic diversity.

In the low thinning regime mainly trees from small size classes (containing overtopped and/or suppressed individuals) were selected for removal. As suggested by other authors, relatively many rare alleles are related to such trees (Cheliak *et al.*, 1988; Zhong *et al.*, 2001). Similar results were also obtained by Danusevicius *et al.* (2016), in their analysis of nSSRs, in 20-year-old pine stand subject to low thinning at an intensity above 30% a majority of the rare alleles was removed. Work by Hawley *et al.* (2005) on stands of *Tsuga canadensis* L., as well as results

obtained by Schaberg *et al.* (2008), again showed that cuts selectively removing poorly-developed and small individuals from stands caused a reduction in numbers of rare alleles, and hence had a negative impact on the genetic potential that would characterise future generations. Danusevicius *et al.* (2016) also made it clear that cuts using DBH as a selection criterion did allow rare alleles to be retained. Also Buchert *et al.* (1997) showed that a 75% reduction in tree density in a mature seed stand of *Pinus strobus* L. caused an impoverishment of the gene pool through the removal of 80% of rare alleles. As the purpose of such cutting is to ensure natural regeneration, this kind of loss of multiple rare alleles prior to the establishment of a new forest generation may be viewed as an unmitigated disaster from the point of view of the preservation of genetic resources.

Trees with rare alleles may be undesirable in both phenotypic and commercial terms, but may at the same time contain the genetic information capable of saving entire populations. In this regard, a question arises as to whether or not some of these should be left in the stand (Makrickiene *et al.*, 2019). It is important to stress here that today's population genetics has no unequivocal conceptual outlook on this phenomenon's ecological significance. One thesis holds that, while the alleles occurring frequently in a population account for the majority of its genetic potential, low-frequency and rare alleles represent a stand's latent genetic potential (Bergmann *et al.*, 1990). This means that some of the latter might at some point prove crucial to adaptation. On the other hand, considering that many rare alleles seem to occur in trees occupying lower strata of the stand, many might well be expected to vanish anyway, in the course of longer-term stand development (as a result of self-thinning which is quite intensive in the case of the very light-demanding Scots pine *P. sylvestris*).

In terms of rare alleles preservation, in our simulation trial, the high-grading treatment appeared to be most beneficial, with only 2% lost in the case of the nSSR rare alleles. Thus, high-grading ranked first, both in terms of preservation of general genetic variation as well as in terms of conservation of rare alleles. However, this does not mean such a thinning method should or could be advocated from the silvicultural point of view. Premature removal of trees in the high-grading treatment would inevitably hit productive potential of the whole stand, in terms of both the timber's quantity and quality, as well as eliminating valuable genotypes. The same was also suggested by Hosius *et al.* (2006) and Danusevicius *et al.* (2016) who asserted that the long-term history of overexploitation of Scots pine *P. sylvestris* stands by removal of best trees caused severe degradation and loss of genetic diversity.

Similar results as for high-grading were also obtained for the control variant (no thinning, natural mortality only). Nevertheless, it is important to note that the main purpose of any thinning method is to reduce a density in the stand and to improve the growing conditions of the trees remaining in the stand. Beside this, thinning may have a direct impact on more efficient pollen dispersion and gene exchange between populations (Rajora and Mosseler, 2001; Finkeldey and Ziehe, 2004). This phenomenon has been found to be one of the main reasons for the appearance of new alleles in descendant generations (Kosińska *et al.*, 2007; Konnert and Hosius, 2010). Thus, there is a possibility of naturally-occurring gene-flow between populations compensating for the loss that the removal of rare alleles from the stand represent. Of course, that will only happen where given alleles continue to occur in adjacent populations (Ziehe and Hattemer, 2002).

In this regard, it is worth noting that a relatively weak negative effect on stand genetic structure occurred also in the case of selection thinning. The main purpose of selection thinning is favouring the growth of best stand components (elite trees) by removing their strongest competitors. Trees playing such a role usually represent high biosocial classes, distinguished by a relatively

low probability of occurrence of rare alleles. Simultaneously, selection thinning ranked relatively high taking into account its effect on the general level of genetic diversity, as measured by several genetic indices. Considering this, we would indicate the selection thinning as a kind of 'golden mean': a method that enables achieving important silvicultural goals and only slightly distorts the stand genetic variability.

Conclusion

Use of the *ForestSimulatorBWINPro* computer program to envisage virtual thinning regimes allowed many variants to be tested across the same study area, also allowed for the forecasting of changes in the gene pool in 10 years from treatment. The presented SSR-based analysis confirms a varied impact of different thinning regimes on the genetic structure characterising stands of Scots pine *P. sylvestris*.

The presented study was only a preliminary study. We suggest larger resampling the populations and genotyping using a larger number of microsatellite markers. This information would be helpful in better understanding the long-term impacts of thinning treatments on the genetic variability of Scots pine *P. sylvestris* stands.

Authors' contributions

A.K. – conceived the study, supervised and administrated the project; A.K., A.T., K.B., W.B., H.S. and B.B. – carried out the field measurements; A.K. and A.T. – carried out genetic analyses; K.B. and A.K. – performed the virtual thinning simulation; A.K., A.T. and M.S. – carried out statistical analyses. All authors contributed to writing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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STRESZCZENIE

Prognozowanie wpływu różnych reżimów trzebieży na zmienność genetyczną drzewostanu sosnowego *Pinus sylvestris* L. – badanie symulacyjne

Działalność człowieka pozostanie prawdopodobnie kluczowym czynnikiem wpływającym na większość ekosystemów leśnych. Nawet jeśli głównym celem jest produkcja drewna, lasy są zarządzane tak, aby jednocześnie czerpać z nich korzyści, chronić je, zachowywać i regenerować. W wielu drzewostanach, w których głównym celem jest produkcja drewna wysokiej jakości, leśnicy stosują zabiegi hodowlane, takie jak trzebieże, w celu sprzyjania dalszemu wzrostowi drzew najwyższej

jakości. Trzebienie drzewostanów w relatywnie krótkich odstępach czasu może być coraz częściej postrzegane jako szkodliwe dla zachowania różnorodności – nie tylko w kontekście genów drzew warunkujących zdolności adaptacyjne do danych warunków środowiskowych, ale także genów kodujących cechy o wartości ekonomicznej. Ponieważ wpływ cięć pielęgnacyjnych na zróżnicowanie genetyczne drzewostanów jest niejednoznaczny, w pracy starano się połączyć analizy molekularne z wykorzystaniem praktycznie wykonywanych reżimów trzebieży. Cele częściowe określały: (1) zbadanie zmienności genetycznej drzewostanu przed zabiegiem; (2) ocenę wpływu 5 powszechnie stosowanych reżimów trzebieży na parametry opisujące różnorodność genetyczną; (3) ocenę przydatności oprogramowania *ForestSimulatorBWINPro* w symulacji zabiegów trzebieżowych w kontekście badań genetycznych.

Badany 39-letni drzewostan sosnowy znajdował się w centralnej Polsce (RDLP Warszawa, Nadleśnictwo Ostrów Mazowiecka). Poletko doświadczalne o wymiarach 50×50 m (0,25 ha) zostało założone w obrębie drzewostanu w miejscu uznanym za reprezentatywne. Pomierzono pierśnicę wszystkich drzew znajdujących się w jego obrębie. Wysokość drzew ustalono na podstawie krzywej wysokości powstałej na bazie pomiarów wysokości reprezentatywnej grupy 32 osobników. Określono współrzędne lokalizacji (x, y) oraz pozycję biosocjalną każdego drzewa. Ponieważ były to badania wstępne, do badań genetycznych wykorzystano powierzchnię położoną centralnie w obrębie powierzchni doświadczalnej, na powierzchni 0,0625 ha (25×25 m) (ryc. 1). Do analizy DNA pobrano próbki drewna 103 drzew.

Symulacje z wykorzystaniem oprogramowania *ForestSimulatorBWINPro* obejmowały 6 wariantów eksperymentalnych: 1) LT1 – trzebież dolna o niskiej intensywności (12% miąższości); 2) LT2 – trzebież dolna o wysokiej intensywności (35%); 3) GT – cięcia schematyczne (usuwanie co 5 rzędu drzew); 4) ST – trzebież selekcyjna, projektowana w terenie; 5) HG – wycinka wszystkich drzew dorodnych, a więc „odwrotność" ST. Założono również wariant kontrolny (C), bez cięcia, ale z występowaniem samotrzebienia poprzez „naturalną" śmiertelność (ryc. 1 i 2).

W badaniach wykorzystano program *ForestSimulatorBWINPro*, jako podstawowe narzędzie pozwalające na odpowiednie opracowanie (analizę i symulację) danych drzewostanowych. Kluczowym elementem jest w nim model wzrostu drzewostanu *TreeGrow*, który pozwala przewidywać zmiany podstawowych parametrów drzew i drzewostanu w trakcie rozwoju w kolejnych 5-letnich okresach. Prognozowano wpływ testowanych trzebieży na zmienność genetyczną badanej grupy drzew tuż po wykonaniu cięć oraz 10 lat później.

Do określenia zmienności genetycznej wykorzystano 11 *loci* mikrosatelitarnego DNA jądrowego i chloroplastowego. Analizy obejmowały podstawowe parametry genetyczne, ze szczególnym uwzględnieniem rzadkich alleli (częstotliwość poniżej 5%). Zmienność genetyczna grup drzew pozostałych po symulacji reżimów trzebieży była porównywana między testowanymi wariantami oraz względem kontroli. Analizy dotyczyły efektu występującego zaraz po cięciu oraz 10 lat później, z uwzględnieniem „naturalnej" śmiertelności (również symulowanej przez oprogramowanie).

Zmiany parametrów badanego drzewostanu pod wpływem testowanych reżimów trzebieżowych scharakteryzowano w tabeli 1. Niskie wartości *PhiPT* (tab. 2) oraz AMOVA (tab. 3) wskazują na brak istotnego statystycznie zróżnicowania częstości występowania alleli w badanych *loci* nSSR i cpSSR pomiędzy reżimami trzebieży a wariantem kontrolnym, zarówno bezpośrednio po zabiegu, jak i 10 lat później. Jednak w przypadku, gdy rozpatrywano efekt na poziomie pojedynczych drzew w ramach testowanych trzebieży, istotność została wykazana (tab. 3). Największą liczbę haplotypów, bogactwo haplotypowe, efektywną liczbę haplotypów oraz haplotypowe zróżnicowanie genetyczne prezentował wariant ST bezpośrednio po zabiegu i 10 lat później (w tym przypadku

z wyłączeniem indeksu A) (tab. 4). Wykazano również statystycznie istotne zróżnicowanie procentowego ubytku alleli rzadkich w zależności od symulowanej trzebieży dla cpSSR (dla sytuacji bezpośrednio po zabiegu i po 10 latach symulowanego rozwoju drzewostanu) (tab. 5).

W konsekwencji za metodę najmniej sprzyjającą utrzymaniu początkowego poziomu zmienności genetycznej drzewostanu (utrata 22 i 43% rzadkich alleli w nSSR i cpSSR) uznano dolną trzebież o intensywności 35%. Trzebież rabunkowa okazała się najbardziej korzystna w krótkim okresie. Trzebież selekcyjna stanowiła rodzaj „złotego środka": okazała się metodą, która umożliwia osiągnięcie ważnych celów hodowlanych, a jednocześnie tylko w niewielkim stopniu zaburza zmienność genetyczną drzewostanu.

Eksperyment z wykorzystaniem oprogramowania *ForestSimulatorBWINPro* dowiódł, że może być ono z powodzeniem wykorzystywane w badaniach nad zmiennością genetyczną drzew. Ponieważ było to tylko badanie wstępne, korzystne byłoby większe ponowne próbkowanie populacji i genotypowanie przy użyciu większej liczby markerów mikrosatelitarnych.