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ORIGINAL ARTICLE

Isozyme polymorphism and seed and cone variability of Scots pine (*Pinus sylvestris* L.) in relation to local environments in Poland

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Abstract

Evolutionary processes lead to the survival of individuals best adapted to local environment. This gives rise to allele polymorphism and genetic diversity of populations. Isoenzyme proteins, which are the product of gene expression, are an effective tool for tracking these changes. On the other hand, the reproductive potential of a given population can be assessed based on its ability to produce viable and efficiently germinating seeds. The present results combine molecular analyses of isoenzyme proteins with anatomical and morphological studies of Scots pine seeds (*Pinus sylvestris* L.). The study was conducted in 6 populations that are characteristic of this species occurrence range in the country. The results confirm the correlation between seed weight and embryo size. They also show a population from northeastern Poland had a higher effective number of alleles and seed with lower germinative energy and capacity. There was genetic homogeneity in all except for the population from Woziwoda, which was significantly different based on the F_{st} test. The genetic characteristics of Scots pine from Woziwoda may be associated with the lower levels of rainfall that occur there during the growing season. The results improve our knowledge of Scots pine variability and contribute to the discussion of the impact of local environment on genetic variability.

Introduction

Genetic variability is the sum of the differences in phenotypes and genotypes amongst individuals, populations or species (Sztuba-Solińska 2005). Indices of genetic variation in forestry are becoming more widespread, with the knowledge gained used in breeding forest trees and forest protection. Maintaining a high level of genetic variation within and between populations is a basis for sustainable forestry (Food and Agriculture Organization of the United Nations 2014). Genetic variability is one of the most important attributes of any population, because it determines its stability in the face of possible changes in environmental condi-

tions (Reed and Frankham 2003). Intensive studies have been conducted in forest science to analyse the correlation of selected molecular markers with phenotypic characteristics. Some studies show that genetic markers are not a good means of assessing plasticity and adaptability of trees. For example, Hedrick and Miller (1992) claimed that molecular markers are selectively neutral and the data obtained from genetic analyses describe only a small part of the genome and, therefore, cannot be a good indicator of adaptive genetic differences. Reed and Frankham (2001) added that the loss of genetic diversity does not necessarily have to reduce adaptive potential. Despite these arguments, many studies indicate the usefulness of genetic markers, particularly isoenzymes, to evaluate the adaptive potential of forest trees. So far, research in this area has been conducted for species of the genus *Pinus* (Blumenröther et al. 2001) and *Picea* (Seifert and Müller-Starck 2009; Masternak 2015).

Populations of forest trees adapt to the environment in which they live, which, as a result, results in phenotypic and genetic variabilities. For pine, adaptive variability has a unique significance because the species occurs in a diverse set of ecosystems, whereas provenance-based studies show the adaptation of certain subpopulations to environment (Blumenröther et al. 2001).

In order to obtain the information on the genetic basis of adaptation, research is conducted at various stages of tree and stand development (Müller-Starck 1993; Starcke et al. 1996). Genetic markers have been studied in relation to survivability (Bergmann and Scholz 1989), growth (Durel et al. 1996; Wang 1996; Furnier et al. 1991), wood properties (Wang et al. 2008; Xiaet al. 2008), date of bud break and the resulting loss of resistance to late frosts (Masternak 2015), resistance to drought (Eckert et al. 2010) and fungi and insects (Quesada et al. 2010), as well as resistance to environmental pollution (Bergmann and Scholz 1985; Konert 1993; Brus 1996; Müller-Starck 1989; Prus-Głowacki et al. 2003).

For Scots pine clones, the correlation was observed between the date of flowering and frequency at the locus *Sdh-A* (Prus-Głowacki et al. 2015). An analysis of growth characteristics in common beech found a clear correlation between breast height diameter (DBH) and variation at loci *Mdh-C*, *6Pgdh-B* and *Aat-C* (Müller-Starck et al. 2005). This was confirmed by previous research on Scots pine, which showed that heterozygosity at specified loci is related to smaller tree size (Blumenröther et al. 2001). However, amongst spruce trees, variation at specified loci was more closely related to the ability of trees to bear seeds (Seifert and Müller-Starck 2009).

The main objective of the present study was to evaluate the genetic variability of pine populations with isoenzyme markers. The anatomical and morphological characteristics of seeds were also analysed. Evidence was evaluated for the relation of phenotypic and genotypic differences amongst populations to be the result of adaptation to environmental conditions.

Table 1. Geographical location of the populations, their estimated age in 2016, area of the stand and habitat, species composition of the analysed population with the height and width of studied trees

Location	Latitude (N)	Longitude (E)	Area (ha)	Species composition	Age in 2016	DBH (cm)	Height (m)
Międzyzdroje (Mi)	53°55′44″	14°54′40″	7.36	Pine 70%, oak 30%	137	47.4 ± 7.7	31.6 ± 3.5
Strzałowo (St)	53°41′	21°26′	19.6	Pine 80%, spruce 20%	112	43.4 ± 6.7	36.0 ± 2.5
Białowieża (Bi)	53°15′21″	23°39′16″	10.54	Pine 50%, spruce 50%	158	52.9 ± 7.3	40.1 ± 2.1
Woziwoda (Wi)	53°39′85″	17°55′37″	13.34	Pine 100%	149	45.3 ± 6.6	30.7 ± 3.0
Bolesławiec (Bo)	51°20′20″	15°42′1″	12.01	Pine 100%	165	47.0 ± 6.1	29.1 ± 2.2
Józefów (Jz)	50°57′38″	22°54′49″	5.96	Pine 100%	125	42.2 ± 3.8	30.8 ± 3.0

MATERIAL AND METHODS

Tree material

The study was conducted using six Scots pine populations from different seed zones covering the range of the species in Poland (Tab. 1). Selected locations differed in the amount of precipitation during the growing season (Fig. 1).

From each selected tree, 10 kg of cones per populations was collected, which, after seed extraction, was used to evaluate the yield and quality of seeds. At the same time as cones were collected, shoots with dormant winter buds were sampled from standing trees for laboratory analyses.
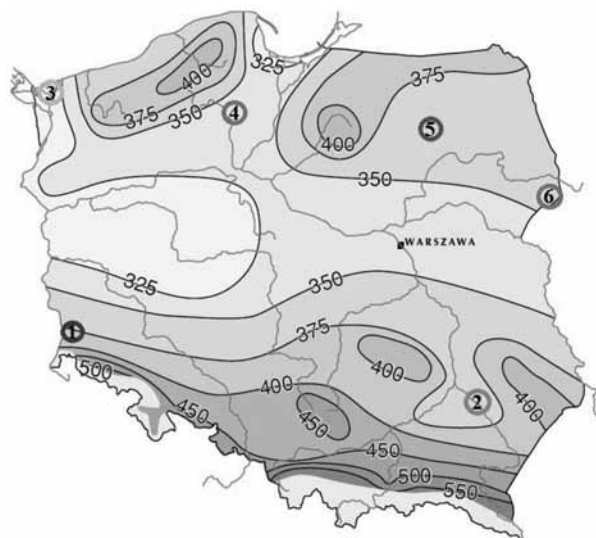

Figure 1. Geographical distribution of the population with regard to the precipitation gradient during the vegetation period (April–September). Location: 1, Bolesławiec; 2, Józefów; 3, Międzyzdroje; 4, Woziwoda; 5, Strzałowo; 6, Białowieża

Cone and seed assessment

Cones harvested from each tree were weighed, and 1,000 seeds from each population were subsampled to evaluate the average cone weight, total yield of seeds from cones, average number of seeds per cone and the mass of 1,000 seeds. A sample of seed was dissected to determine the length and width of the embryo and length and width of the endosperm. Germinative energy and capacity of seeds were determined in accordance with international seed evaluation standards (ISTA 2013).

Analysis of genetic markers

Proteins were extracted from buds in a state of winter dormancy. The buds, with bud scales removed, were ground, and then extraction was carried out with 150 µL of extraction buffer (100 mM of Tris-HCl with pH 7.5, with the addition of 10 of mM 2-mercaptoethanol and 3 g of PVP K 25 in 100 mL of buffer) (Odrzykoski and Gottlieb 1984). Whatman filter paper strips (31ET 4 mm x 11 mm) were soaked with the resultant solution and stored at -80°C before analysis.

Electrophoretic separation was performed in 13% starch gel (Starch-Art) using two buffer systems, A and C, whose composition was described by Odrzykoski and Gottlieb (1984) and Cieślewicz (2008). After electrophoresis, the gel was cut into 1.5-mm thick layers and each layer was used to visualise isoenzymatic proteins. The location of proteins in the gel was carried out according to the procedure described by Conkl et al. (1982) and modified by applying an 'agar overlay' for excluding loci: *Got-A*, *Got-B* and *Got-C* (Manchenko 1994). The most variable enzyme systems within the *Pinus* species were selected (Opracowanie szczegółowych wymagań 2003). The list of examined loci is shown in Table 2.

Table 2. List of enzymes with buffer systems used to separate them

Buffer system	Enzyme	E.C. number	locus
A	Glutamic oxaloacetic transaminase	2.6.1.1	Got-A Got-B Got-C
	Diaphoresis	1.8.1.4	Dia-C
	Glutamate dehydrogenase	1.4.1.2	Gdh-A
	6-phosphogluconate dehydrogenase	1.1.1.44	Pgd-B
C	Shikimate dehydrogenase	1.1.1.25	Sdh-A Sdh-B
	Malate dehydrogenase of NAD-dependent	1.1.1.37	Mdh-A Mdh-C

Statistical analysis

Cone and seed measurements were evaluated using the Shapiro–Wilk test to determine those with a normal distribution. Homogeneity of variance was verified with the Leven test. For features meeting the assumptions of normality and homogeneity of variance, parametric ANOVA tests were performed, followed by Tukey's test applied post hoc to identify significant differences amongst populations. In other cases, the non-parametric Kruskal–Wallis test was used to differentiate populations.

The relationships between cone and seed attributes were evaluated using the Pearson correlation method. Relationships between geographic coordinates and genetic variability of populations, such as germinative energy and capacity and seed production and seed quality features, were determined using the non-parametric Spearman correlation method. Calculations were performed using Statistica ver. 9.0 (Stat Soft 2010).

The variation of isoenzymatic loci was analysed using the GeneAlex 6.5 program (Peakall and Smouse 2006). Allele frequencies, the percentage of polymorphic loci, the average number of alleles at the locus (N_i) and the observed heterozygosity (H_o) were calculated (Wright 1969; Bergmann 1989). The effective number of alleles at a locus (N_e) was calculated according to the formula given by Wright (1969): $N_e = 1/1 - H_e$, where H_e is the expected heterozygosity.

The genetic diversity of the consolidation coefficient (F_{st}) (Wright 1987), expressed by the formula $F_{st} = (H_T - H_s)/H_T$, was also calculated, where H_s is the ratio of heterozygous genotypes assuming random mating in subpopulations and H_T is the proportion of heterozygous genotypes for the particular gene, assuming genetic equilibrium in the entire population. For F_{st}, PCoA analysis was performed.

F_{st} is related to the inbreeding coefficient (F_{is}), which determines the proportional reduction of het-erozygosity because of inbreeding, compared to the population as a whole, and is expressed by the formula: $F_{is} = (H_s - H_I)/H_s$, where H_I is the probability that a gene in an inbred individual is heterozygous.

The statistical significance of differences in the level of deviation from Hardy-Weinberg equilibrium was tested using the chi-square test (χ^2).

Differences in aspects of genetic variation depending on origin were determined with the Kruskal–Wallis test using Statistica ver. 9.0 (Stat Soft 2010).

Results

Evaluation of seed production and seed quality

Average attributes of the seed production and seed quality from the populations are presented in Table 3. Seed performance was lowest in the Józefów population, whereas the highest performances were in those from Międzyzdroje and Woziwoda. The average mass of 1,000 seeds ranged from 5.49 to 6.60 g. The lowest weight and embryo size were observed for seeds from Białowieża, whereas the highest weight and embryo size were in the population from Woziwoda, where the mean length of the embryo was 3.04 mm and the width was 0.51 mm. Overall, the highest variation was exhibited by attributes such as the average number of full seeds

Table 3. Mean performance indicators and statistic significant of seeding and seed quality

Origin	Yield of seeds per cone (%)	The mass of a single cone (g)	The mean number of seeds in a cone	Mass of 1000 seeds (g)	Germination capacity (%)	Energy of germination (%)	Embryo length (mm)	Embryo width (mm)	Endosperm length (mm)	Endosperm width (mm)
Bi	1.30	5.28	13	5.49	95.70	94.70	2.81	0.50	3.17	1.86
Bo	1.96	5.67	19	5.88	97.90	97.40	2.91	0.50	3.27	1.88
Jz	1.12	4.55	9	5.82	97.40	96.30	2.87	0.50	3.20	1.87
Mz	1.99	6.54	21	6.23	98.40	98.30	2.97	0.51	3.32	1.90
St	1.29	6.12	13	6.05	94.80	93.50	2.92	0.51	3.28	1.88
Wi	1.89	6.71	19	6.60	98.90	98.60	3.04	0.51	3.37	1.97
Mean	1.59	5.81	16	6.01	97.18	96.47	2.92	0.51	3.27	1.89
SD	0.40	0.82	4.68	0.38	1.60	2.04	0.08	0.01	0.07	0.04
V%	24.84	14.04	29.85	6.32	1.65	2.11	2.72	1.08	2.27	2.10
P-Value	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0005	0.1599	0.0216	0.0146

Significance at the level of *** 0.001,

in a cone, the yield of seeds per cone and average cone mass. Significant (0.001–0.05) differences amongst genetic parameters such as N_e, H_o and H_e for the two origins were obtained for germinative capacity and energy.

Variability and genetic variation of populations

The mean number of alleles per locus ranged from 1.8 to 2.1. The highest mean number of alleles was found in the population from Józefów, whereas the lowest was in the population from Woziwoda. The highest effective number of alleles was observed in populations from Strzałowo and Białowieża. The lowest values were observed in the population from Woziwoda. Heterozygosity (H_o) in the populations ranged from 0.24 to 0.32, whereas the expected heterozygosity (H_e) was greatest in populations from Strzałowo and Białowieża, with the population from Józefów being the least variable despite having the highest number of alleles (Fig. 2). In the populations from Józefów, Międzyzdroje and Strzałowo, an excess of heterozygotes was demonstrated, but differences among all populations were not significant.

The highest degree of genetic variation was demonstrated between the Międzyzdroje and Woziwoda populations ($F_{st} = 0.108$). Populations from Białowieża and Strzałowo were least divergent ($F_{st} = 0.005$) (Tab. 4).

The PCoA analysis (Fig. 3) for two main components of F_{st} (84.48% variability) indicates a significant genetic distance of the Woziwoda population from all the others (Tab. 4).

Table 4. Wright's (1987) coefficient of genetic diversity (F_{st})

	Biało-wieża	Bole-sławiec	Józefów	Między-zdroje	Strza-łowo	Wozi-woda
Białowieża	0.000					
Bolesławiec	0.015	0.000				
Józefów	0.016	0.011	0.000			
Międzyzdroje	0.024	0.030	0.015	0.000		
Strzałowo	0.005	0.020	0.023	0.036	0.000	
Woziwoda	0.085	0.096	0.094	0.108	0.079	0.000

Correlations between traits

There was a significant correlation between the mass of seeds and the length of both embryo and endosperm ($r = 0.7$). The average cone weight was positively correlated with the yield, the number of seeds per cone and the mass of 1,000 seeds ($r = 0.8$). On the other hand, the mass of 1,000 seeds and the dimensions of the embryo and endosperm did not depend on the number of seeds that were in cones or the yield of seeds per cone.

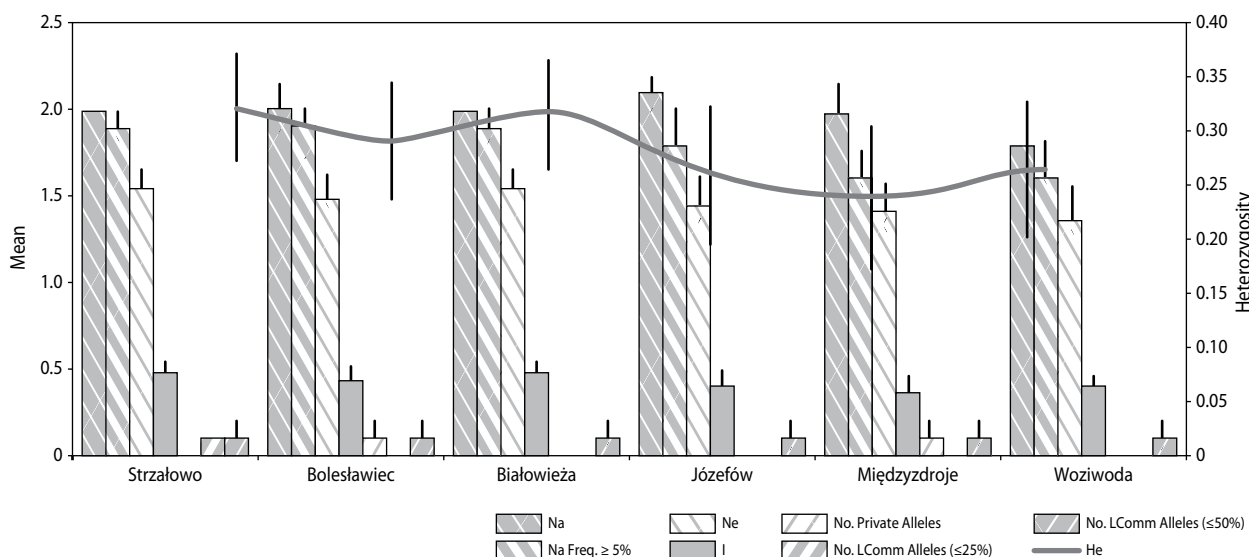Table 5 shows the Spearman correlation coefficients amongst indicators of productivity, seed produc-

Figure 2. The average number of alleles (N_a), effective number of alleles (N_e), Shannon index (I) and expected heterozygosity (H_e)

tion, seed quality and geographic origin, as well as the genetic variation of the studied Scots pine populations. There is a strong negative correlation between germinative energy and capacity and measures of genetic variation, such as the effective number of alleles at a locus, Shannon index and the observed heterozygosity.
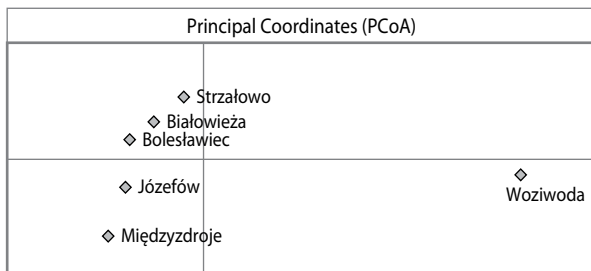

Figure 3. PCoA analysis of the variability of two main components for the F_{st} of studied populations

The mean number of alleles at a locus was negatively correlated with the average cone weight. Moreover, a negative correlation between a population's latitude of origin and seed yield per cone and the average number of seeds per cone was demonstrated. Geographic variation at the locus A of glutamate dehydrogenase was confirmed (Tab. 5).

Discussion

Traits of cones and seeds

The quantity and quality of seeds produced have a significant influence on population's stability and the likelihood of breeding features being expressed. Scots pine starts yielding good cone crops from the age of 35 years and produces seeds until death (Załęski 1995). Pine seeds exhibit high germinative capacity, often up to 100% (Załęski 1995). Mast years occur every 3–4 years, although some trees bear seeds every year (Tyszkiewicz 1949). In our study, the mean individual cone mass was 5.81 g, which was lower than the mean cone mass of 6.30 g reported for Poland by Bodył and Załeski (2005). Seed mass per 1,000 seeds from Polish forests ranged from 4.0 to 8.5 g, averaging 6.2 g (Antosiewicz 1970). In the present study, 1,000-seed mass averaged 6.01 g, similar to that found for the East European Plain (Mameav 1972). The embryo dimensions in

Table 5. Spearman correlation coefficients amongst indicators of seed bearing efficiency, seed quality, location and the genetic variation of the investigated pine stands

Feature	Latitude	Longitude	Seed yield per cone	The number of seeds per cone	The mass of 1000 seeds	Germination capacity	Germination energy	Embryo length	Embryo width	Endosperm length	Endosperm width
Latitude	-	-	0.486	0.618	0.657	0.142	0.142	0.657	0.600	0.657	0.657
Longitude	-	-	-0.657	-0.657	-0.714	-0.657	-0.657	-0.714	-0.542	-0.714	-0.714
Germination capacity	0.143	-0.657	0.600	0.706	0.657	-	-	0.657	0.428	0.657	0.657
Germination energy	0.143	-0.657	0.600	0.706	0.657	-	-	0.657	0.428	0.657	0.657
N_a	-0.507	0.338	-0.845*	-0.609	-0.676	-0.507	-0.507	-0.676	-0.676	-0.676	-0.676
N_e	-0.086	0.486	-0.486	-0.500	-0.600	-0.942**	-0.942**	-0.600	-0.371	-0.600	-0.600
I	-0.200	0.600	-0.428	-0.588	-0.543	-0.886*	-0.886*	-0.543	-0.257	-0.543	-0.543
H_o	-0.314	0.371	-0.600	-0.677	-0.486	-0.828*	-0.828*	-0.486	-0.257	-0.486	-0.486
H_e	-0.028	0.485	-0.371	-0.382	-0.314	-0.714	-0.714	-0.314	-0.028	-0.314	-0.314
GA allel1	0.828*	0.028	0.371	0.088	0.257	-0.371	-0.371	0.257	0.314	0.257	0.257
GA allel2	-0.828*	-0.028	-0.371	-0.088	-0.257	0.371	0.371	-0.257	-0.31	-0.257	-0.257

Significance at the level of *** 0.001, ** 0.01 and * 0.05.

this study did not differ from mean values found for the Polish climate. The mean length of the embryo in this study was 2.92 mm, with a width of 0.51 mm and an endosperm length of 1.89 mm. In comparison, the average embryo length in another study of Scots pine from Poland is 3.02 mm, width is 0.52 mm and endosperm length is 0.94 mm (Załęski 1995).

In this study, we did not observe a significant correlation between the geographical location of stands and seed weight. This contradicts studies performed for the entire range of the species (Staszkiewicz 1993; Reich et al. 1994) that show decreased seed mass for more northerly provenances. This probably results from severe winters and low growing season temperatures. According to Cherepnin (1964) and Pravdin (1969), the weight of pine seeds may also depend on local habitat and stand density.

Many positive correlations have been shown amongst the seed size and mass with germinative capacity, germination energy (Cicek and Tilki 2007; Singh and Sofi 2011) and also size and vigour of seedlings (Cicek and Tilki 2007; Gonzáles-Rodriguez et al. 2011). We also showed significant relationships between the seed mass and dimensions of the embryo and endosperm. This correlation did not impact the germinative energy or ability of seeds to germinate. This confirms similar studies performed using Polish pine populations (Załęski 1995). However, based on Jovanovic's observation (1960), one-year-old black pine seedlings (*Pinus nigra* Arn.) grown from larger seeds exhibited greater seedling height, root length and dry mass compared to those grown from smaller seeds. Similar results were obtained by Vojčal (1961), who examined the vigour of pine seedling growth. Consistent with Jovanovic (1960) and Vojčal (1961), Novoselce (1968) observed a correlation between the pine seedling growth and seed weight. Studies have shown that the germination of seeds less than 4 mg in weight was only 12%, but when seeds weighed 5 mg, the germination was 83.8% (Novoselceva 1968). Novoselceva (1968) pointed out that particularly large seedling size was reached when pine seeds weighed more than 8 mg. A detailed analysis of seed weight in relation to their anatomy was conducted by Wrześniewski (1982). The author showed a strong positive relationship between the weight and dimensions of the embryo and endosperm. The dry matter of the seed coat, endosperm

and embryo increased proportionally with the weight of the seeds, but their percentage contribution to the dry mass of the seeds was almost the same in all weight classes. The mean length of the embryo was smallest in seeds with the lowest weight and increased proportionally with seed weight. For heavy seeds, the length of the embryo was similar. In our research, we showed significant relationships between the seed weight and dimensions of the embryo and endosperm. Seed weight did not affect seed germinative energy or capacity in this study. Different results were obtained by Załęski and Gozdalik (1994), who observed a significant correlation between the germinative capacity and germinative energy in pines, and the length of the embryo and endosperm. Załęski and Gozdalik (1994) assumed that embryos with a width of less than 0.46 mm suffer significantly poorer germination. Owing to discrepancies in published results, additional research in this field is important, because container seedling nurseries require high seed quality. The application of the 'one seed – one seedling cell' method requires that germination be close to 100%. Most research on the viability of Scots pine seeds in Poland comes from the past century, with unfortunately no new publications known concerning seed research in this country. Studies concerning seed properties must also take into account the effects of climate change.

Genetic variability of Scots pine populations

A lower number of alleles per locus was observed in this study than that has been reported previously for Scots pine populations in Europe, for example, Prus-Głowacki and Stephan (1994) reported 2.77 alleles per locus and Scaltsoyiannes et al. (2009) reported 2.48 alleles. The highest average number of alleles per locus was reported by Kosińska et al. (2007). A lower number of alleles can occur if the sample size analysed is small, because this reduces the likelihood of detecting rare alleles. The high number of effective alleles for the two populations from northeastern Poland is noteworthy. This region of Poland is important because of the fact that Scots pine populations growing there have the best-preserved genetic structure in the country. This probably reflects the absence of planting of trees from uncontrolled trade in tree seeds in the nineteenth century, which occurred commonly in the west and south of Poland.

Genetic variability (H_e) of the populations in this study does not differ from the average for European Scots pine populations analysed using similar loci (Burczyk 1990; Prus-Głowacki et al. 1993; Prus-Głowacki and Stephan 1994; Scaltsoyiannes et al. 2009). Genetic variability in this study ranged from 0.5% to 10.8%, demonstrating that 90% of genetic variability is contained within a single population, which is compatible with the results obtained by other authors (Prus-Głowacki et al. 1993; Goncharenko et al. 1994; Prus-Głowacki and Stephan 1994; Hu and Li 2001; Kosińska et al. 2007). This is typical for wind-pollinated conifers with a wide geographic range (Loveless and Hamrick 1984). Attention should be paid to the fact that the highest genetic variability was recorded for populations located in relatively close proximity to one another, whereas populations separated by greater distance were more closely genetically related. It seems justified to suppose that it is local climate that determines a given population's genetic structure, not distance from other populations. According to our results, the crucial climate component for Scots pine is the amount of rainfall during the growing season, because other climate factors for populations tested were not sufficiently discriminative.

The PCoA analysis performed for F_{st} provided significant separation of the populations in this study. The Woziwoda population is separated from other populations, which agrees with the hypothesis that growing season rainfall has strong effects on genetic variability. Woziwoda receives significantly lower growing season precipitation than other locations in this study. It should be noted that these results might be influenced by a history of uncontrolled seed transfer in Poland in the nineteenth century. However, evidence to support this hypothesis is lacking.

Relationship of local growing conditions to seed characteristics and genetic variation

The relationship between latitude and the frequency of occurrence of alleles at the locus A of glutamate dehydrogenase was observed. This enzyme has strong diagnostic importance for forest trees. For example, for *Picea abies*, its variability is considered because of a history of long-term isolation in refugia that had different environments. Locus *Gdh-A* in spruce is characterised by high polymorphism in northeastern Europe; however, in the southern part of the range, this locus

is practically monomorphic (Gömöry 1992; Lewandowski and Burczyk 2002). A similar result was described by Fourier and Adams (1986), who confirmed that there are differences in the frequencies of alleles at the locus A of glutamate dehydrogenase in *Pinus jefferyi*. These differences seemed to be a consequence of adaptation of *P. jefferyi* to ultramafic soils. Fourier and Adams (1986) argued, however, that the adaptation of plants to soil conditions is not associated with latitude or longitude of the geographical location, but with non-clinal features of the ecosystem. Therefore, the geographical selection of locus A of glutamate dehydrogenase depends on stand adaptation to local environmental conditions, as demonstrated for *P. abies* (Gömöry 1992; Lewandowski and Burczyk 2002). This is supported by the metabolic function of glutamate dehydrogenase, which catalyses the synthesis of glutamic acid, that incorporates ammonium ions. Damage to this mechanism or its reduced effectiveness may lead to the accumulation of excess ammonia in plant cells and, consequently, lead to the death of trees. Glutamate dehydrogenase is also closely related to the availability of zinc. In order to explain the observed genetic variation in glutamate dehydrogenase, additional studies on a larger number of populations should be conducted, which should include soil analyses.

Germinative capacity and energy decreased with increased heterozygosity of the populations. This phenomenon has been seen previously in pine (Goncharenko et al. 1994) and was described for *Pinus ponderosa* (Hu and Li 2001), *Pinus radiata* (Kosińska et al. 2007) and *Pinus sylvestris* (Loveless et al. 1984). It is possible that the reduction in the frequency of heterozygotes is related to the elimination of allele forms that are not adapted to a particular environment. Previous research, however, compared mature trees with young stands, whereas our study shows that selection occurs during seed formation. In particular, in the case of the population from Białowieża, the diverse genetic structure of the parent stand will not be passed on to subsequent generations, because of poor seed production.

These results show negative relationships between germinative energy and capacity and the effective number of alleles. In this context, we note the age of the analysed stands and the high probability that they originated from trees that had previously grown on the same area, so that selective pressure affected the gene pool.

On the other hand, selective factors favour homozygotes in specific loci, preserving positive adaptations (Whitlock 2002). The results might also be interpreted as being a consequence of the decrease of seed mass and germinative energy in pines in more northerly locations, which was not found in the present research. This phenomenon was also described by Cherepnin (1964) and Pravdin (1969).

Conclusions

Variability in seed traits and isozymes in six Scots pine populations in this study is representative for the entire range of the species in Poland. Despite significant geographical distance amongst the studied populations, the main site environmental differences are mainly the amount of rainfall received during the growing season. Published information suggests that other environmental factors in Poland do not sufficiently differ between the studied populations to exert an influence on population variability.

The results of seed analysis confirm the expected correlation between seed mass and embryo size, although size did not impact germination. Instead, the results indicated a higher effective number of alleles in northeastern populations, compared to other populations; at the same time, northeastern populations are characterised by lower germinative energy and capacity. In the present study, there was no direct link between geographical distance and genetic variability. On the other hand, based on the PCoA analysis for F_{st}, as well as climates maps, we hypothesise that rainfall during the growing season creates selective pressure affecting the genetic variability of Scots pine.

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