

# VARIATION IN CONE AND SEED MORPHOLOGY TRAITS AMONG THE MITOCHONDRIAL DNA HAPLOTYPES OF SCOTS PINE (*PINUS SYLVESTRIS* L.)

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The objective of our study was to assess the morphological traits of cones and seeds of the two mitotypes of Scots pine, identified by DNA markers at the Nad7.1 locus of the mtDNA. We genotyped the mtDNA of 40 clones of a local origin in a seed orchard and identified two mitotypes: (a) the type A, mitotype originating from the southern refugia and (b) type B mitotype, originating from a northerly refugium west of the southern Ural Mountains (lacking the 5bp indel at the Nand7.1 locus). The following cone and seed morphology traits of these 40 clones were scored: cone length and width, seed wing length, width, seed wing length and width ratio, area, perimeter, seed wing shape, seed number per cone, weight of seed with wing. The results revealed significant differences between the mitotypes in seed wing area ( $p = 0.0004$ ), width ( $p=0.0016$ ), seed weight with wing ( $p = 0.0358$ ) and seed number per cone ( $p = 0.001$ ). There was a tendency for the type B mitotype to produce more seeds per cone. These seed size traits, however, are strongly affected by environmental variance and may represent autocorrelations with clonal properties or environmental effects. Our study indicates that the connection between the present-day cone and seed morphotype and the postglacial origin in Scots pine is uncertain and no reliable morphological markers can be observed.

Key words: Scots pine, DNA haplotype, mitotype, cone, seed morphology.

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## INTRODUCTION

Scots pine is most widespread European conifer adapted to grow over a vast range of environments (Floran et al. 2011, Govindarajulu 2014). Owing to the large distribution range, the Scots pine exhibits a complex morphology including seed and cone properties. The reproductive system of Scots pine is characterized by a very large number of seeds (Mouna et al. 1986). About 60 seeds may

be in one cone of Scots pine (Sivacioglu 2010). Seeds collected from the same tree differ in colour (Aniszewska 2006).

For organisms with long generation time such as trees, the present-day genetic structure may still show the imprints of the pattern and speed of postglacial migration (Newton et al. 1999). Researches of pollen and macrofossils showed that trees of genus *Pinus* during the last glacial

period (before 25000 – 18000 years) have not been extinct from Europe, because there were refuges in the South Europe and even Central part of the continent (Willis and van Andel 2004).

Due to their maternal mode of inheritance in conifers, mitochondrial DNA (mtDNA) markers are best suited to assess the impact of historical events on its current genetic diversity, because seeds (even if wind-dispersed) migrate over much shorter distances compared to airborne pollen (Jaramillo-Correa et al. 2003). Hence, a comparison of population genetics parameters estimated with mtDNA markers and biparentally inherited nuclear DNA markers could help to evaluate the relative contribution of postglacial migration patterns and more recent gene flow to the genetic structure of forest-trees (Gamache et al. 2003).

The maternally inherited mitochondrial DNA (mtDNA) haplotypes of Scots pine may have preserved distinct features attributable to their post glacial environments. These properties can be associated with present-day stress tolerance induced by changing climate. The problem is to find neutral morphological markers that could identify the mtDNA types (mitotypes). The objective of our study was to assess the

morphological traits of cones and seeds of the two mitotypes of Scots pine, identified by DNA markers at the Nad7.1 locus of the mtDNA.

## MATERIAL AND METHODS

A total of 715 cones were collected from 40 clones in a Jonava seed orchard located in central Lithuania in March 2016. (Table 1). Seed orchard was established in 1996. The clones were planted by 10 m between rows and 8 m within rows. The selected clones are shown in Table 1. The clones were randomly planted in the seed orchard plot. The clones originate for two locations in Lithuania: the Labanoras provenance (north-east Lithuania) and the sea-side Lithuania.

Cones from different clones were kept ad seed were extracted separately. According to Aniszewska (2013) two stages seed extraction method seeds were extracted. Cones dry in oven Triter KB-8182 at 35 °C and 50°C temperature.

Visually three classes of seed wing shapes were identified according to the mostly expressed wings features (Fig. 1). The wings of Scots pine seeds from a seed orchard were assessed according to the following classes:



Fig. 1 Seed wing classes: I - elongated, II - regularly rounded, III - irregularly rounded.

Table 1. Clonal distribution scheme in the Jonava seed orchard. The clones selected for our study are marked in dark. The numbers indicate clone id., where the same id shows ramets of single clone

No	Clone number																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		
28																												
27	12	9	35	33	27	551	624	461	629	633	2		551															
26		8	461	20	26	2	8	14	20	26	633		37															
25	36	461	37	34	25	1	7	37	34	551	36	12	38															
24	39	12	38	24	30	36	12	38	24	30	39	11	633															
23	31	12	17	23	29	39	11	17	23	32	31	10	16															
22	3	624	16	461	28	31	10	16	22	28	3	9	35															
21	12	9	35	629	461	3	9	35	33	27	2	8	14															
20	1	8	14	20	26	2	8	14	513	26	1	7	37															
19	36	7	37	34	25	1	7	37	34	629	36	12	38															
18	39	12	38	24	30	36	12	38	24	30	39	11	17															
17	31	11	461	23	29	39	11	17	23	29	31	10	16	22	28	31												
16	3	10	16	22	28	31	10	16	22	28	3	9	35	33	27	3	9	35	33	17								
15	2	9	35	33	27	624	9	35	33	27	2	8	14	20	26	2	633	14	20	26	2	629						
14	1	8	14	20	26	2	8	14	633	26	1	7	37		633	1	7	37	34	25	1	7	37	1	551	629		
13	6	7	37	461	25	1	7	37	34	25	6	12	38	24	30	36	633	38	24	30	461	461	461	24	30	36		
12	5	12	38	513	30	6	12	38	24	30	5	633	17	23	29	39	11	17	23	29	39	11	17	23	29	39		
11	31	11	17	23	29	5	11	17	23	29	31	461	16	22	28	31	10	16	22	28	31	10	16	22	40	31		
10	3	10	16	22	28	31	10	16	22	28	3	9	35	21	27	3	9	35	21	27	3	9	35	12	17	24		
9	624	9	15	21	513	3	9	35	551	27	2	571	14	20	26	2	8	14	20	12	633	629	14	20	36	2		
8	1	8	14	513	26	2	8	14	461	26	633	7	13	34	25	633	7	13	34	25	1	7	13	34	25	1		
7	6	551	13	19	25	1	7	13	19	25	6	633	18	24	30	40	12	18	24	30	551	12	18	24	30	3		
6	5	12	18	24	30	461	12	18	461	30	461	629	17	23	29	5	11	17	23	29	5	11	17	23	29	20		
5	31	11	17	624	29	513	11	461	461	29	31	10	16	22	28	31	10	16	22	28	633	10	16	22	516	513		
4	3	10	16	22	28	513	10	16	461	28	3	9	15	21	27	3	9	15	21	27	3	9	15	21	12	551		
3	2	9	461	21	27	513	9	461	21	27	461	8	14	20	26	2	8	14	20	26	2	461	14	20	26	31		
2	512			14	20	26	2	8	14	461	26	629	7	13	633	25	1	7	13	19	25	1	7	13	19	571	22	
1	571			629	633	629	1	7	13	19	633	119	93	151	68	95	96	137	90	97	83	74	95	62	119	106		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26		

- I - elongated: the seed wing is elongated, narrow;
- II - regularly rounded: the wing is oval, widest near the seed, the wing gradually peaks;;
- III - irregularly rounded: the seed wing is wide, the place of widening is stretched, the wing peaks sharply.

20 seeds were selected randomly from each clone (800 seeds at all). The seeds were scanned by SAMSUNG SCX-3400 SERIES and pictures were processed by SmartGrain.

Scanned images of 20 seeds were used to identify seed wing class for each seed. This identification was done visually. Dominant class of the clone consists not less than 40 % of all seeds (n=20).

Evaluated measures of seeds:

1. wing length (mm);
2. wing width (in the widest point of wing) (mm);
3. wing area (mm<sup>2</sup>);
4. wing perimeter (mm).

Seed wing length and width ratio was calculated later.

Seed with wings was weighted with PS 600/C/2.

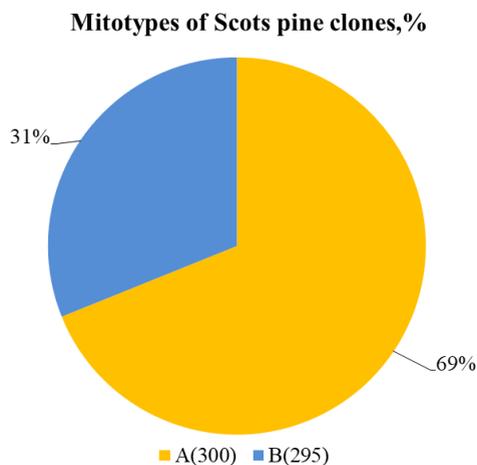


Fig. 2 Percentage of clones with A and B mitotype found in the Jonava seed orchard. The numbers at the letters A and B in the legend show the allele sizes in bp.

Four times 100 seeds with wings were weighted and then average of 100 seeds was calculated. After that mass of 1000 seeds with wings was calculated.

DNA was extracted from Scots pine needles according adjusted ATMB DNA extraction model (Dumolin et al. 1995). Two mitotypes of Scots pine were identified by DNA markers at the Nad7.1 locus of the mtDNA (Fig. 2): the type A, mitotype originating from the southern refugia and (b) type B mitotype, originating from a northerly refugium west of the southern Ural Mountains (lacking the 5bp indel at the Nand7.1 locus).

To assess the mitotype effect, we run ANOVA on individual cone level with mitotype as the classification variable. We used correlation analysis to assess the relationships between measured characters. Variation coefficient among clones ( $VC_c$ ) and among seed wing classes ( $VC_{cl}$ ) in percent was calculated as the ratio of standard deviation of clones/seed wing class to the arithmetic means.

## RESULTS AND DISCUSSION

As expected we found the two mitotypes among the clones: (a) the type A, mitotype originating from the southern refugia (type A) and (b) type B mitotype, originating from a northerly refugium west of the southern Ural Mountains (lacking the 5bp indel at the Nand7.1 locus). Most of the clones from a seed orchard are type A mitotype – 69 % (Fig. 3).

There was no significant difference between the mitotypes in cone length ( $p = 0.6515$ ), cone width ( $p = 0.8245$ ), cone and width ratio ( $p = 0.4623$ ) (Table 2).

The results revealed significant differences between the mitotypes in an average seed number per cone ( $p = 0.003$ ) (Table 2). There was a tendency for the type B mitotype to produce more seeds per cone (at similar cone size) (Fig. 3 and 4). In addition to this, in type B mitotype, seeds

Table 2. Results of ANOVA analysis on the effects of mitotype for the cone traits

TRAIT	SOURCE	DF	F	PROB
Cone length, cm	Mitotype	1	0.2043	0.65153
Cone length, cm	ERROR	355	.	.
Length and width ratio	Mitotype	1	0.5416	0.46226
Length and width ratio	ERROR	355	.	.
Cone width, cm	Mitotype	1	0.0492	0.8245
Cone width, cm	ERROR	355	.	.
Seeds number per cone	MITO	1	9.1242	0.00271
Seeds number per cone	ERROR	355	.	.

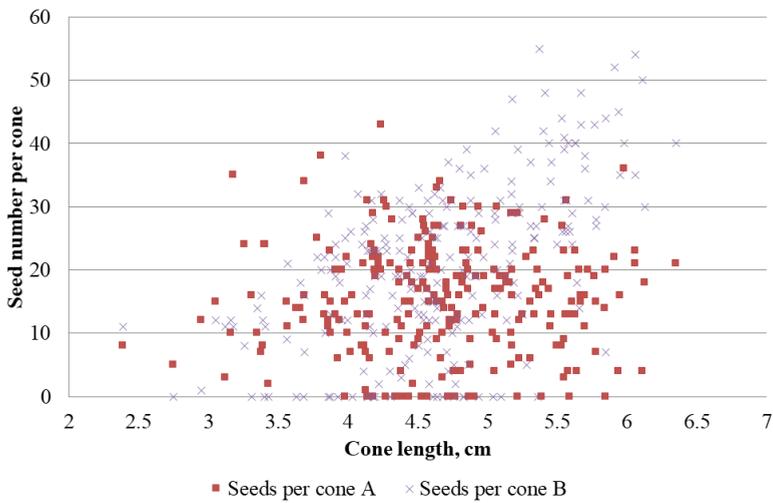


Fig. 3. Dependence of seed number per cone on cone length for the type A and type B mitotypes of Scots pine. Individual cone values are shown.

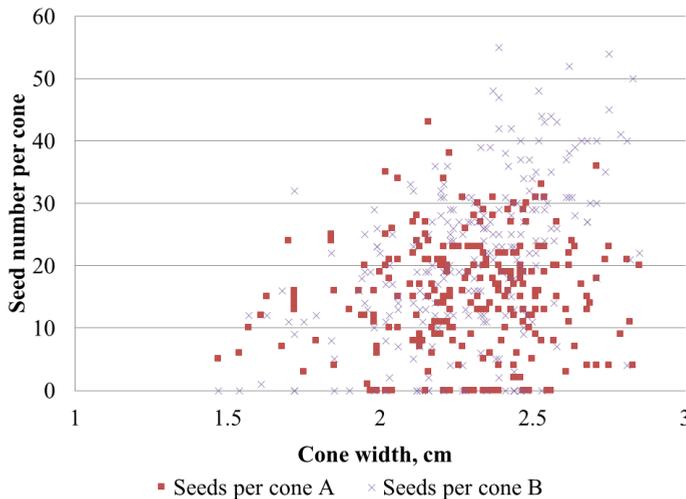


Fig. 4. Dependence of seed number per cone on cone width for the type A and type B mitotypes of Scots pine. Individual cone values are shown.

Table 3 Scots pine mitotypes differences in seed traits

TRAIT	SOURCE	TYPE	DF	F	PROB
Seed wing class	MITO	SS3	1	0.25	0.61284
Seed wing class	ERROR	ERROR	778	.	.
Wing length, mm	MITO	SS3	1	3.42	0.06469
Wing length, mm	ERROR	ERROR	778	.	.
Perimeter, mm	MITO	SS3	1	2.687	0.10157
Perimeter, mm	ERROR	ERROR	778	.	.
Wing area, mm <sup>2</sup>	MITO	SS3	1	12.888	0.00035
Wing area, mm <sup>2</sup>	ERROR	ERROR	778	.	.
Wing width, mm	MITO	SS3	1	9.9977	0.00163
Wing width, mm	ERROR	ERROR	778	.	.
Length and width ratio	MITO	SS3	1	1.0252	0.31161
Length and width ratio	ERROR	ERROR	778	.	.

### Seed wing classes in A and B mitotypes; %, units

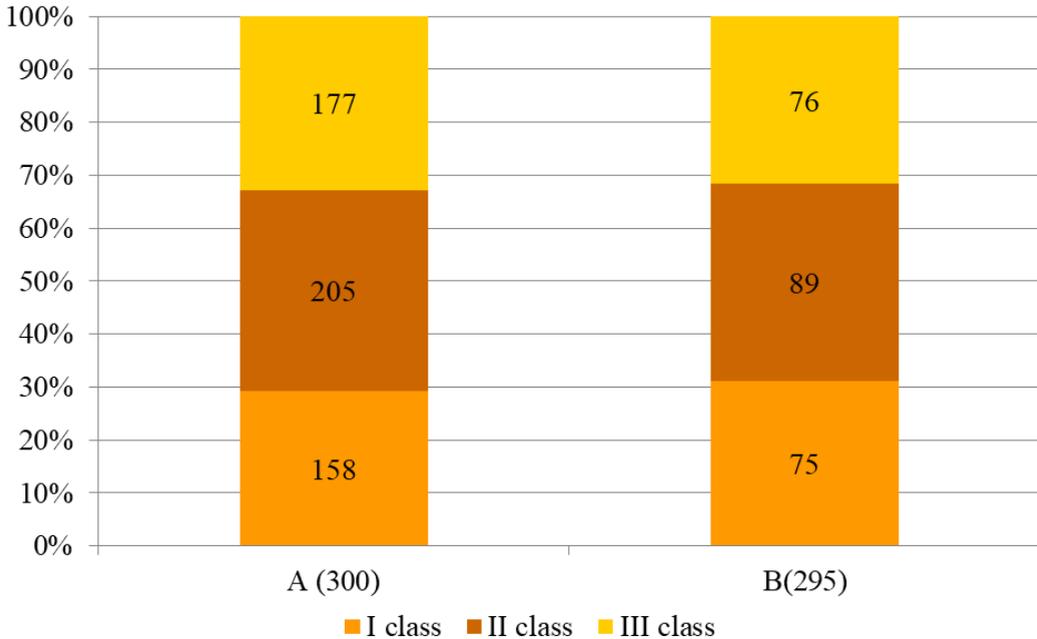


Fig. 5. Distribution of the seed wing shape (% , units) between and within Scots pine mitotypes.

number per cone depends on cone length ( $R^2=0.30$ ) and cone width ( $R^2=0.22$ ). The comparison of the seed traits revealed significant differences between the mitotypes in seed wing area ( $p=0.0004$ ), width ( $p=0.0016$ ) and seeds with wing weight ( $p=0.0358$ ) (Table 3).

Types of mitotypes difference in seed wing classes was not statistically significant ( $P=0.6128$ ) (Table 3). The distribution of seed wing classes between and within mitotypes was quite equal – each class consists about 30 % (Fig. 5).

Hertel and Kohlstock (1994) identified two morphological types of Scots pine (according to crown shape and branches thickness) was identified. Morphotype with narrow crown and finer branches had a higher level of heterozygosity based on a higher number of rare alleles compared with the other morphotype (with a wide crown and thicker branches) (Hertel and Kohlstock 1994). This research shows that morphological differences may be used as a marker for genetic differentiation. However, the cone and seed size traits are strongly affected by environment and may represent autocorrelations with clonal properties or even environmental effects.

## CONCLUSION

Our study indicates that the connection between the present-day cone and seed morphotype and the postglacial origin in Scots pine is uncertain and no reliable morphological markers can be observed.

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