and hence for every i-th individual $\sum_{j\neq i} w_{ij}^{(h)} = c^{(h)}$, resulting in:

$$r_S^{(h)} = \frac{\frac{1}{\sum_i c^{(h)}} \sum_i \sum_{j\neq i} w_{ij}^{(h)} \sum_a (p_{ia} - \bar{p}_a)(p_{ja} - \bar{p}_a)}{\frac{1}{N} \sum_i \sum_a (p_{ja} - \bar{p}_a)^2} =$$

$$\frac{\sum_i \sum_{j\neq i} w_{ij}^{(h)} \sum_a (p_{iu} - \bar{p}_u)(p_{ja} - \bar{p}_a)}{\sum_i c^{(h)} \sum_a (p_{ja} - \bar{p}_a)^2} = r_{S\&P}^{(h)}.$$

In practice, given moderate to large sample sizes (say 100 individuals or more), an approximately uniform distribution of individuals and a regular shape of the habitat (e.g. circle, square, etc.), one can expect that $r_S \approx r_{S\&P}$. Violating these conditions, on the other hand, can cause that the relatedness (i.e. likeness) between a given pair of genotypes estimated with $r_{S\&P}$ can differ substantially depending on the distance between individuals. However, this does not apply to r_S coefficient.

It is also worth noting that the relatedness coefficient introduced by Streiff and co-authors (1998) reduces to well known Moran's I coefficient for bi-allelic locus. Additionally, r_S coefficient can be easily transformed into the kinship coefficient as given by LOISELLE et al. (1995). For this purpose one can note that for any number of alleles at a locus the measure of variance (i.e. the denominator of r_S), is equal to:

$$\frac{1}{N}\sum_a \sum_{ia} (p_{ia} - p_a)^2 = \sum_a p_a(1-p_a) - \frac{1}{4N}\left(\sum_a \sum_{b\neq a} n_{ab}\right) = H_e - H_o/2,$$

where n_{ab} stands for the observed number of heterozygotes made of the a-th and the b-th allele, and H_e and H_o is expected and observed heterozygosity, respectively. Because the kinship coefficient proposed by LOISELLE et al. (1995), and r_S differ only in their denominators, the ratio of the coefficients is actually equal to the ratio of their denominators, and equal to:

$$\frac{f}{r_S} = \frac{H_e - H_o/2}{H_e} = \frac{1+F}{2},$$

where F is the within-population inbreeding coefficient. The above property is a desired relation between kinship and relatedness measures, as discussed previously for bi-allelic locus (HARDY and VEKEMANS, 1999). In the case of $r_{S\&P}$ coefficient, the above relationships holds only asymptotically, under conditions mentioned above.

Inbreeding Depression in the Full-sib Offspring of *Populus nigra* L.

By V. BENETKA*), M. POSPÍŠKOVÁ, F. VRÁTNÝ and M. TKACZYKOVÁ

Silva Tarouca Research Institute for Landscape and Ornamental Gardening Průhonice, Publ. Res. Inst.CZ – 25243, Czech Republic

(Received 25th January 2007)

Abstract

Controlled pollination was carried out in the species *Populus nigra* L. in a greenhouse on isolated branches between sisters and a brother – inbreeding (S x B). Female trees (sisters) were also exposed to open pollination (OP) in the neighbourhood of a male tree (brother) and other *Populus nigra* trees in the vicinity. The analysis of 11 microsatellites was done in the offspring from the inbreeding (S x B) and from the OP. In OP offspring was found 20–76% of viable individuals that were coming from pollination with brother's pollen (spontaneous inbreeding). These individuals were separated from the offspring. In a randomised field trial the offspring were evaluated for two years. Fitness decreased in S x B offspring, traits of plant height, trunk diameter, height increment and resistance to *Melampsora larici-populina* Kleb. were lower in comparison with those of OP offspring. A coefficient of inbreeding depression (δ) ranged from 0.373 to 0.034. The significance of differences between the offspring from S x B and OP of the particular sisters was proved.

About 30% of homozygous microsatellite loci were identified in inbred S x B offspring, which was more than in OP offspring. This difference was significant in the offspring of three sisters; it was not significant in the offspring of one sister. This trend corresponded to the results of growth traits.

Key words: inbreeding depression, open pollination, spontaneous inbreeding, microsatellites, *Populus nigra* L.

Introduction

Populus nigra is a species whose populations are more and more fragmented. As a dioecious outbreeding species, a certain level of genetic load is expected which could make it sensitive to a sudden increase of inbreeding (HEINZE and LEFÈVRE, 1999). Inbreeding is a fertilization system which involves the breeding together of individuals more closely related. In diminishing subpopulation, the probability of the fusion of recessive alleles bearing possible deleterious mutations increases. Basic

*) Corresponding author: VOJTĚCH BENETKA. Tel.: +420 296 528 253, Fax: +420 267 750 440. E-mail address: benetka@vukoz.cz

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

population genetic theory tells that in small populations, genetic diversity erodes with time unless there are connections through gene flow to other populations (GOODMAN, 1987).

Inbreeding depression is the decline in the value of a trait as a direct consequence of inbreeding (WRIGHT, 1977). The most common estimates of inbreeding depression involve traits that are closely related to fitness, or metric traits indirectly associated with fitness (e.g. plant height, stem diameter, disease susceptibility).

Inbreeding depression arises because inbreeding increases the probability that an individual will be: a) homozygous for segregating deleterious recessive alleles and b) homozygous for loci exhibiting overdominance (FALCONER, 1989; LYNCH and WALSH, 1998). According to CHARLESWORTH and CHARLESWORTH (1999) deleterious recessive alleles are the main cause of inbreeding depression.

The most straightforward approaches utilise the inbreeding coefficient *F* defined as the probability that two alleles at a locus are identical by descent (WRIGHT, 1922). Inbreeding depression is then inferred by regressing phenotype on the inbreeding coefficient (LYNCH and WALSH, 1998).

The determination of the inbreeding coefficient is often very problematic. This is the reason why the relations between heterozygosity determined by molecular markers and the phenotype value of studied traits are currently searched for.

Many studies report that individual heterozygosity at apparently neutral microsatellite markers is correlated with key components of individual fitness such as survival (COULSON et al., 1999) whereas studies reporting negative results seem to be rare (DUARTE et al., 2003).

But there exist mechanisms termed in summary incompatibility which defend the fertilization of genetically related specimens. As incompatibility of plants is ment that the plants have functional gametes but are not able to produce seeds. This phenomenon can be observed in self pollination but also after xenogamy with genetically related specimens (FRANKEL and GALUN, 1977).

We examined whether spontaneous inbreeding could occur in isolated populations of *Populus nigra* with a limited number of individuals and what the impacts of such pollination on a successive generation would be. To answer these questions we established a trial with a full sib pollination (of the sister x brother type) in controlled conditions, and at the same time these female trees (sisters) were left to open pollination in the presence of the male tree (brother) and other *Populus nigra* trees in the vicinity.

Analyses of the plant material complemented by microsatellite analyses were used for a more detailed evaluation of inbreed depression and for parentage analysis of OP offspring.

Material

Four plus trees of *Populus nigra* (*Table 1*) originating from two localities in Bohemia and one locality in Moravia were used in the experiment. The trees No. 880030, 880032 and 880061 have been from collections in the nature and they are assumed to have grown spontaneously in the locality of origin. The tree No. 880027 was also a seedling, but it has originated in a planting. All these trees were selected on the basis of morphological evaluation and subsequent analysis of isoenzymes (BENETKA et al., 1999) with the aim of eliminating potential interspecific hybrids.

From the above-mentioned trees in 1992 two parental pairs were crossed: 880030 x 880032 (I) and 880027 x 880061 (II). Trees from parental combination I have been located at a distance of about 500 m in the original locality and both of them are assumed to originate from a single population. The trees of the parental combination II are individuals from quite different localities. The offspring of the above mentioned crosses were planted in a row of 1 m spacing. At a distance of about 40 m there was another poplar row constituted by different black poplar genotypes that could be a sources of pollen for open pollination (*Table 2*).

Table 2. – Male trees of the genus *Populus* that could have taken part in open pollination of the female trees studied.

Species	Clone	Number of trees
P. nigra	880036	1
P. nigra	880044	1
P. nigra	880046	1
P. nigra	880062	1
P. nigra ´italica´		1
P. × canadensis	´Serotina´	1
P. trichocarpa	43/54	2
P. nigra	880032	3
P. nigra ´italica´	182/66	3

Table 1. – Plus trees of the species *Populus nigra* used in the trial.

Plus tree	Abbr.	Sex	Locality	Latitude	Longitude	Height above sea level [m]	Age (years)
880027	27	♀	Hrušky	48°48' N	16°57' E	193	50
880030	30	♀	Prague – Slovanský ostrov	50°05' N	15°25' E	183	100
880032	32	♂	Prague – Kampa	50°05' N	15°24' E	182	110
880061	61	♂	Starý Kolín	50°01' N	15°16' E	198	140
006/70	B1	♂	Prague – Střelecký ostrov	50°05' N	15°25' E	185	100
021/70	B14	♂	Přerov nad L.	50°11' N	14°50' E	173	100

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

For the next generation of crossing in 2002 three female trees (I/2, I/3 and I/5, sisters) and one male tree (I/4, brother) from the offspring of parental pair I (880030 x 880032) were used. The trees B1 and B14 were used as sources of pollen of unrelated fathers for controlled pollination with a mixture of pollen (MI).

In 2003 two female trees (II/1 and II/5) and one male tree (II/6) from the offspring of the parental pair II (880027 x 880061) were used for crossing while the open pollination variant was derived from seeds randomly collected from several branches of the female plants.

Scheme of pollination processes.

	pollination process I				pollination process II		
P	880030 × 880032				880027 × 880061		
	↓				↓		
F_1	I/2	I/3	I/5	I/4	II/1	II/5	II/6
F_2	I/2 × I/4	I/3 × I/4	I/5 × I/4		II/1 × II/6	II/5 × II/6	
OP	I/2	I/3	I/5		II/1	II/5	

The number of seedlings analysed by microsatellite-analysis from each crossing combination was (S x B/OP): I/2 – 33/20; I/3 – 33/7; I/5 – 77/25; II/5 – 25/25.

Methods

Pollination process

The variants of pollination used to study inbreeding depression:

I) in offspring from the year 2002
1) controlled pollination of the sister x brother type (S x B) - inbreeding
2) open pollination (OP)
3) controlled pollination with a mixture of pollen of unrelated male trees B1 + B14 (MI)

II) in offspring from the year 2003
1) controlled pollination of the sister x brother type (S x B) – inbreeding
2) open pollination (OP)

The controlled pollination was realised on flower-bearing branches taken from the respective trees. Female branches were placed into bottles with water in a greenhouse. Pollen was collected from flowering branches that were placed separately in rooms at room temperature. The collected pollen was stored at 4 °C after drying. Female flower-bearing branches with developing buds were isolated with paper bags. After the buds started to open, pollen was blown from a rubber balloon through a hole into the isolation bag. The hole was sealed afterwards. The pollination was carried out on two successive days. The isolation bags were removed after three or four days. When the capsules started to ripen, gauze bags were put on branches to capture mature seeds. Collected seeds with hairs were sown in special boxes designed for this purpose. Germinated seedlings were transplanted onto beds after they had formed several true leaves.

Seeds from the open pollination variant were harvested from the respective mothers by collecting several branches randomly. The branches were put into bottles filled with water in a greenhouse and immature infructescences were isolated with gauze bags in the same way as described above. In the OP variant were left only those seedlings in which spontaneous inbreeding had been excluded by means of the microsatellite analysis.

Field trial

In the next year the plants were set out on an experimental plot in a randomised block design in 4 replications by 10 plants. The growth traits (plant height; stem diameter at a height of 0.4 m) and susceptibility to *Melampsora larici-populina* Kleb. were evaluated. The susceptibility to the rust was evaluated under field conditions by a 5-point scale: 0 – no signs of rust infection on leaves; 1 – small patches covered by rust sori on a half of the leaves; 2 – small patches covered by rust sori on most leaves; 3 – larger patches of rust up to continuous coverage on all leaves; 4 – rust coverage of the whole leaves, incipient leaf necrosis; 5 – all leaves necrotised or shed.

The offspring from full sib (S x B) inbreeding were compared with the offspring from OP of the respective mother and/or with the offspring of this mother pollinated with a mixture of pollen of two unrelated males. The following traits were evaluated: plant height in two successive years; stem diameter; year-on-year height increment and susceptibility to *Melampsora larici-populina* Kleb. at the age of two years.

Evaluation of results

The data were processed by one-way analysis of variance (ANOVA) and multiple range test (Multifactor ANOVA) of homogeneous groups using the programme Statgraphics Plus for Windows 1.4 (1994-1995 by Statistical Graphics Corporation). Differences are significant on a significance level $\alpha < 0.05$.

The coefficient of inbreeding depression δ was calculated from the equation

$\delta = (W_o - W_i) / W_o$ (LANDE and SCHEMSKE, 1985)

W_o is the set of individuals from outbreeding

W_i is the set of individuals from inbreeding

Analysis of DNA – microsatellites

Total DNA was extracted from single leaves using a DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Eleven polymorphic loci with high quality of banding patterns were chosen among the nuclear microsatellites described for poplar by VAN DER SCHOOT et al. (2000) and SMULDERS et al. (2001) and listed in Poplar Molecular Genetics Cooperative SSR Database (PMGC): WPMS03, WPMS04, WPMS05, WPMS07, WPMS09, WPMS12, WPMS14, WPMS16, WPMS18, WPMS20 and PMGC14. Amplification reactions and electrophoretic separation of products followed the procedure described by VAN DER SCHOOT et al. (2000). For the PMGC14 locus from the PMGC database, the

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

primers were 5´-TTCAGAATGTGCATGATGG-3´, and 5´-GTGATGATCTCACCGTTTG-3´, and PCR was performed in the same way as for the WPMS12. The products were visualized by silver staining according to the Promega Silver Sequence DNA Sequencing System. The sizes of the PCR products were determined by comparison to an accompanying sequence reaction using pGEM®-3Zf(+) control DNA (Promega).

Mean number of alleles per locus (A) and allele frequencies were determined in the offspring. Parentage analysis was performed to examine true fathers of the seedlings from open pollination. We used the technique of exclusion given by DOW and ASHLEY (1996). The genotypes of adult trees were compared to the offspring's genotypes and were eliminated as parents if a mismatch occurred at one or more loci. If a seedling matched two or more adults, the matching genotypes were compared to determine whether there were two "complementary" genotypes. Genotypes are complementary if all the seedling's alleles are present in the two adult genotypes. These two adults are the presumed parents of the seedling. The process of exclusion is based on simple codominant inheritance of alleles that is typical for microsatellite loci in poplar (TABBENER and COTTRELL, 2003; Vanden BROECK et al., 2004). Because of very high sensitivity of this technique to genotype errors that would exclude the true parents, we also used a supplementary likelihood-based approach. This method involves calculating a logarithm of the likelihood ratio (LOD score) by determining the likelihood of a pair of individuals being the parents of a given offspring divided by the likelihood of these individuals being unrelated. Offspring are assigned to the parental pair with the highest LOD score. An analysis was performed using the software FAMOZ (GERBER et al., 2003) to check that the parents identified by complete exclusion are also the most probable, if genotyping error had occurred.

Results

Proportion of inbreeding in the offspring from open pollination

The offspring from OP originating by the mothers I/2; I/3; I/5 and II/5 were analysed by using the microsatellite analysis. All the 11 analysed microsatellite loci were highly polymorphic (the mean number of alleles per locus was 9.1), displaying from 6 up to 13 alleles in F_2 (*Table 3*). Alleles inherited from 4 original parents 80030, 80032, 80027 and 80061 were alleles with the highest frequencies. The other alleles descended from unknown fathers from open pollination and were much less frequent.

Although the female trees could have been pollinated with pollen from unrelated fathers that were present at different distances, they also received the pollen of their own brother. The percentage of viable offspring originated in such type of pollination (S x B) ranged from 20 to 76% (*Table 4*).

Decrease in fitness in the offspring of full-sib (sister x brother)

1) Growth traits and susceptibility to *Melampsora larici-populina* Kleb.

The influence of a pollination method on growth traits and susceptibility to *Melampsora larici-populina* Kleb. in the offspring derived from the combination 80030 x 80032 is shown in *Table 5*.

Table 3. – Allelic frequency for eleven microsatellite loci in F_2. Alleles inherited from 4 original parents are typed in bold.

Locus / Allele	WPMS 03	WPMS 04	WPMS 05	WPMS 07	WPMS 09	WPMS 12	WPMS 14	WPMS 16	WPMS 18	WPMS 20	PMGC 14
A	0.0016	**0.2672**	**0.0067**	**0.0017**	**0.0698**	**0.6170**	**0.0241**	**0.4775**	**0.0321**	**0.3673**	**0.0693**
B	**0.0825**	**0.2951**	**0.1107**	**0.1689**	**0.1883**	**0.0208**	**0.0659**	**0.0338**	**0.5224**	**0.0194**	**0.2690**
C	**0.4029**	**0.2164**	**0.0302**	**0.2007**	**0.3571**	**0.0641**	**0.3569**	**0.0466**	**0.3942**	**0.2751**	**0.3086**
D	**0.3867**	**0.0295**	**0.3406**	**0.0619**	**0.1705**	**0.2885**	0.0129	**0.4325**	**0.0288**	**0.3333**	**0.2376**
E	**0.0453**	**0.1049**	**0.0369**	**0.4766**	**0.2013**	0.0048	**0.1463**	0.0080	0.0080	0.0032	**0.1056**
F	0.0113	**0.0246**	**0.3574**	**0.0719**	0.0081	0.0032	**0.3376**	0.0016	0.0128	0.0016	0.0066
G	**0.0631**	0.0016	**0.0302**	**0.0050**	0.0016	0.0016	**0.0048**		0.0016		0.0017
H	0.0016	0.0148	0.0671	0.0017	0.0032		0.0032				0.0017
I	0.0016	0.0016	**0.0185**	0.0033			0.0032				
J	0.0016	0.0016	0.0017	0.0017			0.0016				
K	0.0016	0.0033		0.0033			0.0016				
L		**0.0393**		0.0017			**0.0418**				
M				0.0017							

Table 4. – Proportion of inbreeding in the offspring from open pollination (OP).

Mother	Number of analysed individuals	Number of identified inbred individuals	% of spontaneous inbreeding	Year of pollination
I/2	25	5	20	2002
I/3	38	29	76	2002
I/5	31	23	74	2002
II/5	25	6	24	2003

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

Table 5. – Pollination method and its influence on growth traits and susceptibility to *Melampsora larici-populina* Kleb. in the offspring of combination 80030 x 80032 (mean values given).

mother I/2

Pollination method	n	Height 2005		Stem diameter 2005		Increment of height 04/05		Rust susceptibility 2005		Rust susceptibility 2006	
		cm		mm		cm		points		points	
S × B	37	340	a	28.5	a	157	a	1.568	a	1.611	a
OP	25	364	a	32.7	b	167	a	1.188	b	1.237	b
MI	26	406	b	37.5	c	187	b	1.231	b	1.204	b

mother I/3

Pollination method	n	Height 2005		Stem diameter		Increment of height 04/05		Rust susceptibility 2005		Rust susceptibility 2006	
		cm		mm		cm		points		points	
S × B	39	349	a	31.4	a	169	a	1.210	a	1.236	a
OP	11	393	b	40.4	b	192	b	0.909	a	0.900	b

mother I/5

Pollination method	n	Height 2005		Stem diameter 2005		Increment of height 04/05		Rust susceptibility 2005		Rust susceptibility 2006	
		cm		mm		cm		points		points	
S × B	81	367	a	32.4	a	167	a	1.147	a	1.630	a
OP	22	384	a	37.3	a	179	a	1.141	a	1.425	ab
MI	8	370	a	33.2	a	173	a	1.312	a	1.125	b

S x B sister x brother pollination
OP open pollination
MI pollinated with a mixture of B1 and B14 pollen
The values which are tagged with the same letter are not significantly different at significance level $\alpha = 0.05$.
(Point evaluation of the rust susceptibility: 0 – infection-free, 5 – maximum infection).

The values of growth traits in the variant of S x B pollination were lower in all cases than in the variant from OP and/or in the variant from pollination with a mixture of pollen of unrelated males (MI). In the mother I/2 offspring differences were significant between S x B variant and MI variant in all traits, between S x B variant and OP only in the trait of stem diameter. In the mother I/3 offspring differences were significant in all traits. In the mother I/5 offspring differences between the variants were not significant.

Susceptibility to *Melampsora larici-populina* was evaluated by the degree of susceptibility to infection caused by the studied pathogen; decreased viability is therefore expressed by a higher value of the given trait. The variant of S x B pollination showed decreased viability (expressed by a higher susceptibility to the rust) compared to the variant of OP and/or MI pollination. In the mother I/2 differences were significant in all cases. In the offspring of the mothers I/3 and I/5 differences were not significant in one year (2005) and significant in the next year (2006).

The influence of a pollination method on growth traits and susceptibility to *Melampsora larici-populina* Kleb. in the offspring derived from the combination 80027 x 80061 is shown in *Table 6*.

The only evaluated growth trait was plant height in 2005. The measured values were lower in the S x B variant compared to the OP variant. These differences were significant in the offspring of both mothers (II/1; II/5). The offspring of the mother II/1 from the S x B variant showed significantly increased susceptibility to *Melampsora larici-populina* and thereby also decreased viability compared to the OP variant. No differences in susceptibility to the rust were observed in the offspring of the mother II/5.

2) Coefficient of inbreeding depression

Coefficients of inbreeding depression (δ) are shown in *Table 7*. They were calculated from the results shown in table 5. Their values were in the range from 0.034 to 0.373 (significance level $\alpha = 0.05$).

Specimens without fitness reduction after inbreeding

Table 8 shows the evaluation of specimens of S x B offspring from mothers I/2 and I/3, in which no inbreeding depression occurred. The height of particular plants is indicated and compared with the average height of the whole offspring of the relevant mother (I/2; I/3) after open pollination. Also the numbers of homozygous loci of 11 microsatellites detected in the plants are indicated in the table.

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

Table 6. – Pollination method and its influence on plant height and susceptibility to *Melampsora larici-populina* Kleb. in the offspring of combination 80027 x 80061 (mean values given).

Pollination method	n	Plant height 2005 cm		n	Rust susceptibility 2006 points	
II/1 × II/6 (S × B)	21	163	a	10	2.450	a
II/1 - OP	33	236	b	11	1.609	b
II/5 × II/6 (S × B)	51	183	a	21	2.21	a
II/5 - OP	22	216	b	9	2.22	a

S x B sister x brother pollination
OP – open pollination
The values which are tagged with the same letter are not significantly different at significance level $\alpha = 0.05$.
(Point evaluation of the rust susceptibility: 0 – infection-free, 5 – maximum infection).

Table 7. – Coefficient of inbreeding depression (δ) in the offspring of three mothers

	I/2		I/3		I/5	
Height 2004	0.06	ns	0.087	×	0.056	×
Height 2005	0.064	ns	0.112	×	0.046	ns
Height increment 04/05	0.059	ns	0.120	×	0.034	ns
Stem diameter 2005	0.127	×	0.222	×	0.111	ns
Rust susceptibility 2006	0.302	×	0.373	×	0.143	ns

x significant at significance level $\alpha = 0.05$.
ns not significant.

Table 8. – Specimens after inbreeding (full-sib) with high fitness. Plant height (cm) and number of detected homozygous loci from11 analysed microsatellites.

Above–average specimen after inbreeding		Average height of the offspring	
height (cm)	n- homozygous loci	S × B	OP
offspring of mother I/2			
390	5	340	364
410	4		
390	4		
435	2		
420	5		
415	5		
offspring of mother I/3			
395	5	349	393
405	7		
400	6		
400	8		
405	3		

Representation of homozygous loci in three generations (P; F_1; F_2)

Homozygous loci in the 11 analysed microsatellites are illustrated in *Table 9a*. These homozygotes were determined by an analysis carried out on parental trees 80030 and 80032 (generation P) and on their four offspring I/2; I/3; I/5 and I/4 (generation F_1), which were used for further crossing. The majority of the loci were heterozygous both in the P generation and in the F_1 generation.

In F_2 generation the percentages of homozygotes are given for the offspring of three sisters from the crossing S x B and from OP that relate to the particular microsatellites. The average percentage of these homozygotes is also given. The significance of differences in the number of homozygotes between the offspring from various types of pollination was tested by t-test (significance level $\alpha = 0.05$). The theoretical percentage of homozygotes was calculated from the assumed segregation of parental alleles.

Table 9b shows these values for parental combination 80027 x 80061.

Discussion

Occurrence of inbreeding can be supposed in small populations of allogamic species. Inbreeding causes decline in genetic variability and loss of valuable alleles

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

Table 9a. – Occurrence of homozygotes in the loci of 11 microsatellites in two parental trees (80030; 80032) and their F_1 offspring (I/2; I/3; I/5; I/4). Percentage of homozygotes in S x B and OP offspring (F_2 generation) and significant differences between S x B and OP.

	Microsatellites											Actual average % of homozygotes	Theoretical average % of homozygotes
	WPMS 03	WPMS 04	WPMS 05	WPMS 07	WPMS 09	WPMS 12	WPMS 14	WPMS 16	WPMS 18	WPMS 20	PMGC 14		
P	(occurrence of homozygous loci)												
30	+					+							
32								+	+				
F_1													
I/2 ♀						+			+				
I/3 ♀													
I/5 ♀						+							
I/4 ♂													
F_2	(% of homozygotes)												
I/2 × I/4	39	26	29	43	0	35	0	55	52	16	45	30.9 x	34.1
I/2 OP	27	14	18	23	0	41	14	41	36	14	18	22,4	
I/3 × I/4	25	19	25	20	37	56	28	37	50	25	41	36.4 x	33.0
I/3 OP	0	22	22	22	22	22	11	0	0	25	0	13,3	
I/5 × I/4	46	0	22	32	0	37	55	42	56	23	0	29.5 ns	28.6
I/5 OP	43	0	29	29	0	25	25	43	43	0	0	23,8	

x significant at significance level $\alpha = 0.05$
ns not significant
OP – open pollination
S x B – sister x brother

Table 9b. – Occurrence of homozygotes in the loci of 11 microsatellites in two parental trees (80027; 80061) and their F_1 offspring (II/5; II/6). Percentage of homozygotes in S x B and OP offspring (F_2 generation) and significant differences between S x B and OP.

	Microsatellites											Actual average % of homozygotes	Theoretical average % of homozygotes
	WPMS 03	WPMS 04	WPMS 05	WPMS 07	WPMS 09	WPMS 12	WPMS 14	WPMS 16	WPMS 18	WPMS 20	PMGC 14		
P	(occurrence of homozygous loci)												
27	+					+							
61													
F_1													
II/5 ♀													
II/6 ♂													
F_2	(% of homozygotes)												
II/5 × II/6	58	0	32	40	50	27	30	0	0	52	42	30.1 x	31,8
II/5 × OP	11	0	6	0	16	26	0	26	16	26	0	11,5	

x significant at significance level $\alpha = 0.05$
OP – open pollination
S x B – sister x brother

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

of different genes (KELLER and WALLER, 2002). In our study inbreeding was performed after controlled pollination between sister and brother (full-sib) to detect the effect of inbreeding on the offspring in *Populus nigra* and whether spontaneous inbreeding can occur after open pollination (OP).

Microsatellite analysis revealed a surprisingly high percentage of S x B type of pollination in the offspring from open pollination. This indicates that the incompatibility of allogamic species is not an absolute phenomenon and can be influenced by external factors (in this case by a lack of pollen from unrelated male trees in the blossoming time of female trees). GEIGER (1982) reports non – absolute incompatibility also in another plant species. Spontaneous inbreeding was observed in pollination of all mothers both in the first and second year; that diminished the influence of year (weather influence) and also of parental genotype. Essential is that a typical allogamic, diocious species such as *Populus nigra* is able to produce viable seeds and plants with a closely related specimen. These specimens increase the risk of further possible inbreeding depression.

In our study we also evaluated the influence of inbreeding on the viability of *P. nigra* individuals. Inbreeding depression occurred already in the offspring of both parental combinations after the first inbreeding - pollination S x B. Fitness was decreased not only in terms of growth traits but also in susceptibility to the rust. The values of inbreeding depression coefficient ranged from 0.373 to 0.034. Similar values for other plants species (0.331 ± 0.038) were stated by CRNOKRAK and ROFF (1999). Among the offspring of inbreeding, however, also specimens with above - average trait values were found. These specimens showed no inbreeding depression, even though they had a number of homozygous microsatellite loci comparable with those observed in the specimens with evident inbreeding depression. Moreover, differences in intensity of inbreeding depressions were noticed among the offspring of particular mothers from parental combination both I and II. Considering that in the open pollination mother trees were probably pollinated with pollen of the same fathers and that in the controlled pollination the same fathers were always employed, we can assume that differences in inbreeding depression were caused mainly by the mother genotype. Different reaction of particular genotypes on inbreeding can be expected, as demonstrated by FOX (2005) and also revealed by our study.

The results illustrated in our study could be useful for understanding population dynamics occurring nowadays in fragmented populations of *Populus nigra*. According to POSPÍŠKOVÁ and ŠÁLKOVÁ (2006), in fact, only very limited pollen flow and seed dispersion probably occur among these subpopulations. The authors have proved that trees of the same population participated for 80% in the reproduction of seedlings and only 20% of seedlings originated from parents of which one or both grew far away and have no genetic relationship with the analysed population. It can be deduced from this finding that despite the long flying range of poplar pollen and seeds, mostly local trees participate in the reproduction and regeneration of the population and therefore the inbreeding depression can occur in these fragmented populations. Our results also showed that in small populations of *Populus nigra* a relevant fitness decline can occur as a consequence of narrow genetic diversity due to the high probability of parental pollination as revealed by the high number of homozygous loci present in the offspring of the controlled pollination crosses. From the displays of fitness decline the higher susceptibility to diseases is especially warning.

In last part of our study we tried to find a correlation between occurence of homozygous microsatellite loci and expected degree of homozygosity in generations of interest. According to our expectation, occurence of homozygous loci was sporadic both in P and F_1 generation. In F_2 generation, an important result was found – that the actual average percentage of homozygous loci after inbreeding was more or less identical with the theoretical average value concluded from the segregation ratio of F_1 generation. The percentage of homozygous loci in the offspring after inbreeding was higher than in the offspring from open pollination. Moreover, significant and insignificant differences between inbred and allogamous offspring were in accordance with results from field trials, which could indicate the possibility of using the microsatellite analysis to predict inbreeding depression.

Despite the small extent of the experiments – 5 groups of offspring from 2 original parental crossing – it was proved that already in the first generation after full-sib inbreeding a significant decrease of fitness can occur. Inbreeding evidently occurs spontaneously in a high percentage of specimens. However, many specimens with a high number of homozygous loci have shown good fitness.

Acknowledgements

This study was supported by Grant No. 0002707301 of the Ministry of Environment of the Czech Republic.

References

BENETKA, V., J. MOTTL, K. VACKOVÁ, M. POSPÍŠKOVÁ and M. DUBSKÝ (1999): Estimation of the Introgression Level in *Populus nigra* L. Populations by Means of Isozyme Gene Markers. Silvae Genetica **48**: 218–223.

CHARLESWORTH, B. and D. CHARLESWORTH (1999): The genetic basis of inbreeding depression. Genetical Research **74**: 329–340.

COULSON, T., S. ALBON, J. SLATE and J. PEMBERTON (1999): Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. Evolution **53**, 1951–1960.

CRNOKRAK, P. and D. A. ROFF (1999): Inbreeding depression in the wild. Heredity **83**: 260–270.

DOW, B. D. and M. V. ASHLEY (1996): Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. Mol. Ecol. **5**: 615–627.

DUARTE, L., C. BOUTEILLER, P. FONTANILLAS, E. PETIT and N. PERRIN (2003): Inbreeding in the greater white-toothed shrew, *Crocidura russula*. Evolution **57**: 638–645.

DOI:10.1515/sg-2008-0031
edited by Thünen Institute of Forest Genetics

FALCONER, D. S. (1989): Introduction to Quantitative Genetics. John Wiley and Sons, New York.
FOX, C. W. (2005): Problems in measuring among-family variation in inbreeding depression. American Journal of Botany **92**: 1929–1932.
FRANKEL, R. and E. GALUN (1977): Pollination Mechanisms, Reproduction and Plant Breeding. Springer-Verlag, Berlin, Heidelberg, New York.
GEIGER, H. H. (1982): Breeding methods in diploid rye (*Secale* L.) Tag. Ber. Akad. Landwirtsch. Wiss. DDR **198**: 305–332.
GERBER, S., M. CHABRIER and A. KREMER (2003): FaMoz: a software for parentage analysis using dominant, codominant and uniparentally inherited markers. Mol. Ecol. Notes **3**: 479–481.
GOODMAN, D. (1987): The demography of chance extinction, pp. 11–34. *In:* Viable Populations for Conservation, edited by M. E. SOULÉ, Cambridge University Press, Cambridge, UK.
HEINZE, B. and F. LEFÉVRE (1999): *In situ* restoration genetics of riparian populations of *P. nigra*, pp. 43–50. *In: Populus nigra* Network, edited by J. TUROK et al., IPGRI, Rome, Italy.
KELLER, L. F. and D. M. WALLER (2002): Inbreeding effects in wild populations. Trends in Ecology and Evolution **17(5)**: 230–241.
LANDE, R. D. and W. SCHEMSKE (1985): The evolution of self fertilization and inbreeding depression in plants. I. Genetic models, Evolution **39**, 24–40.
LYNCH, M. and B. WALSH (1998): Genetics and Analysis of Quantitative Traits. Sinauer, Sunderland/MA.
PEMBERTON, J. (2004): Measuring inbreeding depression in the wild: the old ways are the best. Trends in Ecology and Evolution **19**: 613–615.
POSPÍŠKOVÁ, M. and I. ŠÁLKOVÁ (2006): Population structure and parentage analysis of black poplar along the morava River. Canadian Journal of forest Research **36**: 1067–1076.
SMULDERS, M. J. M., J. VAN DER SCHOOT, P. ARENS and B. VOSMAN (2001): Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). Molecular Ecology Notes **1**: 188–190.
TABBENER, H. E. and J. E. COTTRELL (2003): The use of PCR based DNA markers to study the paternity of poplar seedlings. For. Ecol. Manage. **179**: 363–376.
VANDEN BROECK, A., V. STORME, J. E. COTTRELL, W. BOERJAN, E. VAN BOCKSTAELE, P. QUATAERT and J. VAN SLYCKEN (2004): Gene flow between cultivated poplars and native black poplar (*Populus nigra* L.): a case study along the river Meuse on the Dutch-Belgian border. For. Ecol. Manage. **197**: 307–310.
VAN DER SCHOOT, J., M. POSPÍŠKOVÁ, B. VOSMAN and M. J. M. SMULDERS (2000): Development and characterization of microsatellite markers in black poplar (*Populus nigra* L.). Theor. Appl. Genet. **101**: 317–322.
WEBER, E. (1978): Mathematische Grundlagen der Genetik. VEB Gustav Fischer Verlag Jena.
WRIGHT, S. (1922): Coefficients of inbreeding and relationship. American Nature **56**: 330–339.
WRIGHT, S. (1977): Experimental Results and Evolutionary Deductions, Evolution and the Genetics of Populations, vol. 3. University of Chicago Press, Chicago.

Short Note: The Genetic Correlation Between Air-dried Density and Basic Density in *Eucalyptus Nitens* Wood Cores

By M. G. HAMILTON[1),*)], C. A. RAYMOND[2)] and B. M. POTTS[1)]

(Received 5th February 2007)

Abstract

Cores extracted from trees to assess wood chemistry are generally not used to assess basic density in eucalypt pulpwood breeding programmes, as the measurement of basic density requires high temperature drying. However, both wood chemistry and air-dried density can be assessed on the same core. This study found that the inter-trait genetic correlation between core air-dried and basic density to be effectively equal to one in two Tasmanian *Eucalyptus nitens* progeny trials. This implies that selection for basic density could be undertaken using air-dried density with little or no reduction in genetic gain, thus negating the need to extract a separate core to assess basic density and wood chemistry. The adoption of this practice could considerably reduce the cost of assessing these traits in eucalypt breeding programmes.

Key words: Eucalyptus nitens, selection trait, non-destructive assessment, air-dried density, basic density, wood chemistry, pulp yield, cellulose content, near infrared spectroscopy (NIR), genetic correlation.

Introduction

Eucalypt pulpwood breeders routinely select trees according to diameter at breast height and wood core basic density (i.e. oven-dry weight divided by green volume; TAPPI, 1989). Furthermore, near infrared

1) School of Plant Science and Cooperative Research Centre for Forestry, University of Tasmania, Private Bag 55, Hobart, Tasmania, 7001 Australia.
2) School of Environmental Science and Management, Southern Cross University, PO Box 157, Lismore, NSW, 2480 Australia.
*) Corresponding author. Email: matthew.hamilton@utas.edu.au. Phone: +61 3 62262603, Fax: +61 3 62262698.

DOI:10.1515/sg-2008-0032
edited by Thünen Institute of Forest Genetics