Thaiszia - J. Bot., Košice, 21: 167-175, 2011
http://www.bz.upjs.sk/thaiszia

THAISZIA
JOURNAL OF
BOTANY

Deviations from the Hardy-Weinberg equilibrium in selected Slovak populations of *Pinus mugo* Turra, *Pinus sylvestris* L. and their putative hybrid swarms

PETER MAŇKA[1], ANDREJ KORMUŤÁK[2] & DUŠAN GÖMÖRY[3]

[1] Arboretum Mlynany SAS, Vieska nad Zitavou 178, 951 52 Slepcany, Slovak republic; peter.manka@savba.sk

[2] Institute of Plant Genetics and Biotechnology SAS, Akademicka 2, 949 01 Nitra, Slovak republic; nrgrkorm@savba.sk

[3] Faculty of Forestry, Technical University in Zvolen, Masarykova 24, 960 53 Zvolen, Slovak republic; gomory@vsld.tuzvo.sk

---

Maňka P., Kormuťák A. & Gömöry D. (2011): Deviations from the Hardy-Weinberg equilibrium in selected Slovak populations of *Pinus mugo* Turra, *Pinus sylvestris* L. and their putative hybrid swarms. – Thaiszia – J. Bot. 21: 167-175. – ISSN 1210-0420.

Abstract: Genetic variation of two *Pinus mugo* Turra populations, one *Pinus sylvestris* population and four putative hybrid swarms of the species was studied using 12 polymorphic allozyme loci. In all populations studied the relatively high values of fixation indices were found. The causes of this phenomenon vary from population to population. The population of *P. sylvestris* from Hruštín is characterized by a small population size. We assume that this is the reason for such a high value of the fixation index of the population (0.234). The specific growth and reproductive biology (pollination occurs primarily between the nearest neighbours) of *Pinus mugo* is probably the cause of high value of fixation index in population Roháče (0.290). Putative hybrid swarms is difficult to compare. They have different population sizes and different proportions of the individuals of parent species and their hybrids. Fixation indices in the various putative hybrid swarms vary significantly (0.102–0.235). We assume that a larger portion of hybrids in hybrid swarm means a lower fixation index. Our results indicate that the highest proportion of hybrids should occur at sites Habovka and Terchová.

Keywords: *Pinus mugo, Pinus sylvestris,* putative hybrid swarm, isoenzymes, fixation index

---

Introduction

In plants hybridization and introgression are known to be important evolutionary forces (ANDERSON & STEBBINS 1954; STEBBINS 1969; GRANT 1981; ARNOLD 1992). Gene flow between species may lead to uni- or bi-directional gene exchange, may eliminate species boundaries after secondary contact generating unstable tension zone, or may initiate stable speciation events (RIESEBERG 1997). Hybrid speciation in plants through polyploidy is much more common than homoploid hybrid speciation, because the duplication in chromosome number provides the hybrid with an effective means of reproductive isolation from its parental species. Hybrid speciation without increase in ploidy must, in contrast, involve other isolation factors such as ecological and spatial isolation or some degree of chromosomal or genetic incompatibility (RIESEBERG & CARNEY 1998; BUERKLE et al. 2000).

The Scots pine (*Pinus sylvestris*) is a wide spread tree species and the main forest-forming component in Europe and Asia. This species is closely related to the dwarf mountain pine (*Pinus mugo*) that is endemic to European mountainous regions and which occurs also on some peatbogs since the postglacial period (CRITCHFIELD & LITTLE 1966). Individuals with intermediate or mixed phenotypic characters with regard to *P. sylvestris* and *P. mugo* were found in sympatric populations of the species (STASZKIEWICZ & TYSZKIEWICZ 1969, 1972; BOBOWICZ 1990; CHRISTENSEN & DAR 1997).

The natural hybridization between *Pinus mugo* and *Pinus sylvestris* was verified by several authors using morphological and anatomical characters (BOBOWICZ 1990; STASZKIEWICZ 1996), analysis of proteins by serological techniques (PRUS-GŁOWACKI et al. 1978, 1981; PRUS-GŁOWACKI & SZWEYKOWSKI 1980), analysis of chloroplast DNA (FILLPULA et al. 1992; KORMUŤÁK et al. 2005, 2006, 2008; WACHOWIAK et al. 2006; WACHOWIAK & PRUS-GŁOWACKI 2008) and analysis of isoenzyme polymorphism (WACHOWIAK & PRUS-GŁOWACKI 2008). However, estimates of hybridization intensity in individual studies vary from creation of a small number of hybrids (NEET-SARQUEDA et al. 1988; CHRISTENSEN & DAR 1997) to the formation of complex hybrid swarms involving parental species, F_1-generation hybrid and their back-crosses (STASZKIEWICZ & TYSZKIEWICZ 1969; BOBOWICZ 1990).

By means of a simultaneous analysis of the species-specific chloroplast DNA markers in the embryo and in the corresponding mother tree KORMUŤÁK et al. (2008) have illustrated natural hybridization between *P. mugo* and *P. sylvestris* at several locations in northern Slovakia. However, the portion of hybrid seeds from different localities varied considerably. This finding may be explained by the flowering phenology (only a small number of individuals of the parent species is phenologicaly synchronized) and the genetic causes (for locations where introgressants are present, they may be more prone to hybridization). Identification of hybrid trees would require the use of species-specific markers for mitochondrial DNA (KORMUŤÁK et al*.* 2008).

STEBBINS (1950) suggests that consequence of an introgressive hybridization is the change in the variability pattern of the hybrid populations. Hybrids are characterized by an increase variability compared with the parent species. Therefore, we have made an attemp to compare the observed heterozygosity and fixation index within populations of *P. sylvestris, P. mugo* and their putative hybrid swarms at analysed isoenzyme loci.

Material and methods

Altogether, we have studied 7 populations, including 2 *P. mugo* populations, 1 *P. sylvestris* population and 4 putative hybrid swarms with intermediate forms between *P. mugo* and *P. sylvestris*. The geographic origin of investigated populations is given in Tab. 1.

Tab. 1. Populations of *P. mugo*, *P. sylvestris* and their putative hybrid swarms used in the analysis.

No.	Population	Species	Region or mountains	Latitude	Longitude	Altitude	Sub-soil
1	Hruštín	*P. sylvestris*	Orava	49°19´18´´	19°21´39´´	700	mineral
2	Roháče	*P. mugo*	Západné Tatry	49°12´33´´	19°45´05´´	1450	mineral
3	Vrátna	*P. mugo*	Malá Fatra	49°11´16´´	19°01´52´´	1230	mineral
4	Terchová	Hybrid swarm	Malá Fatra	49°15´20´´	19°01´59´´	1171	mineral
5	Tisovnica	Hybrid swarm	Orava	49°34´09´´	19°23´54´´	815	peatbog
6	Habovka	Hybrid swarm	Orava	49°16´25´´	19°36´25´´	815	peatbog
7	Suchá Hora	Hybrid swarm	Orava	49°23´20´´	19°47´11´´	765	peatbog

Bud samples were collected from 42–70 individuals (depending on size of the population). The following 6 enzyme systems encoded by 12 loci were studied (Enzyme Commission number and locus abbreviations in parentheses): leucin aminopeptidase (LAP; E.C. 3.4.1.1.1.), fluorescent esterase (F-EST; E.C. 3.1.1.1.), isocitrate dehydrogenase (IDH; E.C. 1.1.1.42.), shikimate dehydrogenase (SkDH; E.C. 1.1.1.25.), malate dehydrogenase (MDH; E.C. 1.1.1.37.), 6-phosphogluconate dehydrogenase (6 PGDH; E.C. 1.1.1.44.). The separation of isoenzymes in 12% starch gels was performed as described by Concle et al. (1982). LAP and F-EST were separated in Ashton buffer, pH 8,1. IDH, SkDH, MDH and 6 PGDH were separated in Tris-histidine buffer, pH 7,0. Detecting mixtures were prepared according to the recipes by Cheliak & Pitel (1984, 1985). Expected and observed heterozygosity and fixation index were calculated using BIOSYS program (Swofford & Selander 1981).

Results

Observed heterozygosity values are the basis for evaluation of the deviations from Hardy-Weinberg equilibrium. Our results revealed that expected heterozygosity varies between loci and between populations (Tab. 2).

The main indicator of deviations from the population equilibrium is the fixation index. Our results show that significant differences in fixation index values exist between loci and between populations (Tab. 3, 4). Statistically significant positive fixation index value can be expected in the nearly monomorphic loci (Skdh-B, Mdh-A, Lap-B). Surprisingly, statistically significant positive fixation index values have been recorded also for other loci. Statistically significant negative fixation index was recorded in populations of *P. mugo* (Roháče).

Tab. 2. Observed heterozygosity (H_o) at different loci in different populations (underline + bold - *P. sylvestris*, simple font - *P. mugo*, bold - putative hybrid swarms).

locus	**Hruštín**	Roháče	Vrátna	**Tisovnica**	**Habovka**	**Suchá Hora**	**Terchová**
IDH A	0.024	0.016	0.083	0.094	0.000	0.017	0.000
SkDH A	0.095	0.290	0.400	0.448	0.358	0.383	0.322
SkDH B	0.190	0.097	0.100	0.156	0.132	0.117	0.067
MDH A	0.071	0.000	0.017	0.031	0.057	0.183	0.100
MDH B	0.262	0.194	0.367	0.375	0.453	0.400	0.367
MDH C	0.333	0.177	0.317	0.354	0.434	0.333	0.333
6 PGDH	0.429	0.452	0.467	0.542	0.245	0.583	0.722
LAP A	0.000	0.258	0.233	0.323	0.170	0.117	0.256
LAP B	0.071	0.129	0.117	0.219	0.113	0.050	0.267
EST A	0.119	0.355	0.467	0.417	0.245	0.233	0.478
EST B	0.524	0.581	0.683	0.396	0.472	0.283	0.489
EST C	0.286	0.177	0.317	0.240	0.245	0.233	0.378

Tab. 3. Fixation index (FIS)at different loci in different populations (underline + bold - *P. sylvestris*, simple font - *P. mugo*, bold - putative hybrid swarms).

locus	**Hruštín**	Roháče	Vrátna	**Tisovnica**	**Habovka**	**Suchá Hora**	**Terchová**
IDH A	0.000	0.000	–0.024	–0.033	-	0.000	-
SkDH A	0.288	0.337**	0.175	0.085	0.128	0.212	0.155
SkDH B	–0.091	0.416**	0.211	–0.061	–0.047	–0.044	0.418**
MDH A	0.544**	-	0.000	0.000	–0.018	–0.063	–0.031
MDH B	0.196	0.472**	0.104	0.173	0.075	0.144	0.184
MDH C	0.023	0.506**	0.261*	0.239*	0.118	0.272*	0.251*
6 PGDH	0.268	0.215	–0.237	0.160	0.377**	–0.002	–0.139
LAP A	0.988**	0.121	–0.119	0.302**	–0.082	–0.054	0.217*
LAP B	0.708**	0.428**	0.163	0.595**	–0.046	–0.020	0.120
EST A	–0.043	0.312**	0.119	0.168	0.136	0.380**	0.076
EST B	–0.152	–0.266*	–0.147	0.005	–0.102	0.371**	0.000
EST C	0.341*	0.519**	0.326**	0.441**	0.139	0.488**	0.259**

Tab. 4. Comparison of average values expected heterozygosity (H_e), observed heterozygosity (H_o) and fixation index (F_{IS}) in different populations and species (*n* - number of analyzed loci).

Population	Species	*n*	H_e	H_o	F_{is}
Hruštín	*P. sylvestr*is	12	0.258	0.200	0.234
Terchová	Hybrid swarm	12	0.356	0.315	0.120
Tisovnica	Hybrid swarm	12	0.389	0.299	0.235
Habovka	Hybrid swarm	12	0.269	0.244	0.102
Suchá Hora	Hybrid swarm	12	0.312	0.244	0.224
Average	Hybrid swarms	12	0.332	0.276	0.170
Roháče	*P. mugo*	12	0.317	0.227	0.290
Vrátna	*P. mugo*	12	0.323	0.297	0.088
Average	*P. mugo*	12	0.320	0.262	0.189

Discussion

Only a few papers have appeared referring to observed heterozygosity in populations of *P. mugo* and *P. sylvestris*. Practically it is possible to make comparison with the literature data only for some loci (Lap-A, Lap-B, Mdh-A, Mdh-B).

KORSHIKOV et al. (2002) reported of a large range of values of observed heterozygosity (0.080 to 0.360) for populations of *P. sylvestris* at locus Lap-A. The authors KORSHIKOV et al. (2007) found for this locus in the population *P. sylvestris* var. *cretacea* different values of observed heterozygosity for haploid and diploid tissue (0.172; 0.036). These results correspond well with our data. KORSHIKOV & PIRKO (2002) found in populations of *P. mugo* higher values of observed heterozygosity (average value 0.578) than our findings.

KORSHIKOV et al. (2002) detected values of observed heterozygosity ranging from 0.120 to 0.240 at locus Lap-B of *P. sylvestris* populations. Other authors (KORSHIKOV et al. 2007) found for this locus in the population *P. sylvestris* var. *cretacea* different values of observed heterozygosity for haploid and diploid tissue (0.172; 0.018). These values are similar to our results. KORSHIKOV & PIRKO (2002) found in populations of *P. mugo* higher values of observed heterozygostity averaging at 0.208. The figure is higher than our findings.

KORSHIKOV et al. (2002) found that observed heterozygosity in populations of *P. sylvestris* at the Mdh-A locus ranges from 0.040 to 0.111. Their results are similar to our results. KORSHIKOV & PIRKO (2002) found in populations of *P. mugo* at locus Mdh-A of *P. mugo* populations slightly higher values of observed heterozygosity (0.043) than in our case.

KORSHIKOV et al. (2007) found for the Mdh-B locus in the population *P. sylvestris* var. *cretacea* similar values of observed heterozygosity for haploid and diploid tissue (0.310; 0.316). However, KORSHIKOV et al. (2002) found even higher values ranging from 0.460 to 0.760. These values are quite larger than our observed results. KORSHIKOV & PIRKO (2002) found in populations of *P. mugo* at locus Mdh-B similar values of observed heterozygosity (0.220) as our results.

In the studied populations, we have found considerable variability in the fixation index values of different loci. However, statistically significant differences were detected not only in the nearly monomorphic loci (Skdh-A, Skdh-B, MDH, Lap-B), where we can expect such values, but also in the more polymorphic loci (F-Est-A, F-Est-B, F-Est-C, Skdh-A, 6 PGDH, Mdh-B, Mdh-C and LAP). Comparing our results with literature data is possible only for those loci which analyzed other authors (Mdh-A, Mdh-C, F-Est-A, F-Est-B, Lap-B, Skdh-A, Skdh-B).

Values of fixation indices for Mdh-A locus in populations of *P. mugo, P. sylvestris,* and their putative hybrid swarms vary significantly. A high value of fixation index was recorded in the population of *Pinus sylvestris*, while in populations of *P. mugo* the recorded values were lower. A similar finding also provides ODRZYKOSKI (2002). The author found for the population of *P. sylvestris* fixation index values ranging between –0.036 and –0.275, whereas for the population of *P. mugo* between –0.030 and –0.004. Our results are also consistent with the findings of other authors averaging in *P. sylvestris* populations at 0.266 DVORNYK (2001), in *P. mugo* populations at –0.033 (KORSHIKOV & PIRKO 2002). However, comparison of fixation indices at this locus is rather difficult. The reason is that this locus is almost monomorphic. Even a small random change in the number of heterozygotes may significantly affect the index value.

Higher values of fixation indices for populations of *P. mugo* than for populations of *P. sylvestris* were detected at locus Mdh-C. ODRZYKOSKI (2002) similarly found that high positive index values are more typical for populations of *P. mugo* than for populations of *P. sylvestris* (*P. sylvestris:* –0.036 – –0.069; *P. mugo*: –0.103 – –0.212). Results of other authors also support this argument as evidenced by the average fixation index of –0.173 for *P. sylvestris* (DVORNYK 2001) and average indexes of 0.252 and 0.323 for *P. mugo* (SLAVOV & ZHELEV 2004).

Highly variable values of fixation indices varying from population to population were calculated for locus F-Est-B. DVORNYK (2001) postulates for populations of *P. sylvestris* the value – 0.100, whereas ODRZYKOSKI (2002) the values –0.091 – –0.185. The values of 0.209 and –0.048 – –0.023 were calculated by SLAVOV & ZHELEV (2004) and ODRZYKOSKI (2002) for *P. mugo* populations.

Highly variable values of fixation indices we calculated also for locus Lap-A. ODRZYKOSKI (2002) found in populations of *P. mugo* wider range of values of fixation indices (–0.068 – –0.262) than in populations of *P. sylvestris* (–0.003 – – 0.104). KORSHIKOV & PIRKO (2002) calculated for the population of *P. mugo* low average fixation index (–0.116), whereas SLAVOV & ZHELEV (2004) found a high average value of the kind (0.380).

Lap-B locus appears to be almost monomorphic, and the high variation in fixation index values can be expected. KORSHIKOV & PIRKO (2002) found low average fixation index for this locus (–0.024) in populations of *P. mugo,* while SLAVOV & ZHELEV(2004) show a higher average fixation index for the population of the same species (0.300).

SLAVOV & ZHELEV (2004) calculated for the locus Skdh-A in *P. mugo* positive average fixation index (0.174). ODRZYKOSKI (2002) for populations of *P. sylvestris* and *P. mugo* published a small range of values of fixation indices at this locus (0.021 – –0.186; –0.038 – –0.084).

Skdh-B locus is characterized by a high volatility of fixation indices values. ODRZYKOSKI (2002) found higher fixation index values range in populations of *P. sylvestris* (–0.076 – –0.228) than in populations of *P. mugo* (–0.062 – –0.013). However, SLAVOV & ZHELEV (2004) calculated a higher average fixation index also for the populations of *P. mugo* (0.152), which corroborate better our results.

Comparison of our results with findings in the literature (PRUS-GŁOWACKI & BERNARD 1994; PRUS-GŁOWACKI & STEPHAN 1994; DVORNYK 2001; PRUS-GŁOWACKI et al. 2003; MÁNEK & EŠNEROVÁ 2004; SLAVOV & ZHELEV 2004; EŠNEROVÁ & MÁNEK 2005; PRUS-GŁOWACKI et al. 2005; KORSHIKOV et al. 2007) indicates that total value of the fixation indices are mostly lower in populations of *P. sylvestris* as compared with populations of *P. mugo*.

Values of fixation indices in the putative hybrid swarms vary significantly. In populations studied by us were found relatively high values of fixation indices. The causes of this phenomenon vary from population to population. SLAVOV & ZHELEV (2004) suggest that high levels of fixation index in populations of *P. mugo* may be due to peculiar growth and reproductive biology of the species with pollination occurring primarily between the nearest neighbours. The population of Roháče shows a similar fixation index value as reported by these authors. The population of *P. sylvestris* from Hruštín is characterized by a small size. The reproduction process involves only a few individuals. The consequence is that intrapopulation variability decreases from generation to generation under simultaneous increase of homozygote proportion. Putative hybrid swarms have different size and different proportion of hybrid individuals and individuals of the parent species. Phenological isolation exists between the parental species. This has reduced the effective population size. We assume that hybrid individuals have greater ability to break down barriers to gene flow. For this reason, a larger portion of hybrids in hybrid swarm means lower fixation index. KORMUŤÁK et al. (2008) and DEMANKOVÁ & KORMUŤÁK (2007) found the highest proportion of hybrid seeds at Terchová and Habovka. Their findings correspond well with our results.

Acknowledgements

The work was supported by the VEGA Grant Agency, project no. 2/0076/09.

References

ANDERSON E. & STEBBINS G.L. (1954): Hybridization as an evolutionary stimulus. – Evolution, 8: 378-388.

ARNOLD M.L. (1992): Natural hybridization as an evolutionary process. – Annu. Rev. Ecol. Syst. 23: 237-261.

BOBOWICZ M.A. (1990): Mieszańce *Pinus mugo* Turra × *Pinus sylvestris* L. z rezerwatu „Bór na Czerwonem“ w kotline Nowotarskej. Wydawnictvo Naukowe Uniwersytetu im. Adama Mickiewicza w Poznaniu, Ser. Biol. 40, Poznań, 284 pp.

BUERKLE C.A., MORRIS R.J., ASMUSSEN M.A. & RIESEBERG L.H. (2000): The likelyhood of homoploid hybrid speciation. – Heredity, 84: 441-451.

CHELIAK W.M. & PITEL J.A. (1984): Techniques for starch gel electrophoresis of enzymes from forest tree species. National Forestry Institute, Petawawa, 49 pp. ISBN 0-662-13418-4.

CHELIAK W.M. & PITEL J.A. (1985): *Techniques d´électrophorése sur gel d´amidon des enzymes d´essences d´arbres forestiers.* National Forestry Institute, Petawawa, 47 pp. ISBN 0-662-92784-2.

CHRISTENSEN K.I. & DAR G.H. (1997): A morphometric analysis of spontaneous and artificial hybrids of *Pinus mugo* × *Pinus sylvestris* (Pinaceae). – Nordic Journal of Botany, 17: 77-86.

CRITCHFIELD W.B. & LITTLE E.L. (1966): Geographical Distribution of Pines of the World. U.S.D.A. For. Serv. Misc. Public. 991. Washington D.C. 97 pp.

DEMANKOVÁ B. & KORMUŤÁK A. (2007): Introgression between *Pinus sylvestris* L. and *Pinus mugo* Turra on the locality Obšívanka near Terchová. In: Križová E. & Ujházy

K. (eds.) Dynamika, stabilita a diverzita lesných ekosystémov. Vyd. TU, Zvolen, pp. 155-159. ISBN 978-80-228-1821-6.

DVORNYK V. (2001): Genetic variability and differentiation of geographically marginal Scots pine populations from Ukraine. – Silvae Genetica, 50(2): 64-69.

EŠNEROVÁ J. & MÁNEK J. (2005): Studium genetické diverzity přirozených populací agregátu *Pinus mugo* na území NP Šumava. In: Autochtónna dendroflóra a jej uplatnenie v krajine. Vyd. TU, Zvolen, pp. 164-170. ISBN. 80-228-1481-4.

FILPPULA S., SZMIDT A.E. & SAVOLAINEN O. (1992): Genetic comparison between *Pinus sylvestris* and *P. mugo* using isozymes and chloroplast DNA. – Nordic Journal of Botany, 12: 381-386.

KORMUŤÁK A., DEMANKOVÁ B. & GÖMÖRY D. (2008): Spontaneous hybridization between *Pinus sylvestris* L. and *P. mugo* Turra in Slovakia. – Silvae Genetica, 57: 76-82.

KORMUŤÁK A., MAŇKA P. & DEMANKOVÁ B. (2006): The utilization of chloroplast DNA by analyse introgression between *Pinus sylvestris* L. and *P. mugo* Turra. – Acta fytotechnica et zootechnica, Ročník 9-Mimoriadne číslo: 210-212.

KORMUŤÁK A., VOOKOVÁ B. & FEČKOVÁ M. (2005): Molecular evidence of natural hybridization between *Pinus mugo* and *Pinus sylvestris* as revealed by a chloroplast DNA marker. – Acta Biologica Cracoviensia, Series Botanica, 47: 165-166.

KORSHIKOV I.I. & PIRKO Y.V. (2002): Genetic variation and differentiation of peat-bog and dry-meadow populations of the dwarf mountain pine *Pinus mugo* Turra in the highlands of the Ukrainian Carpathians. – Genetika, 38: 1235-1241.

KORSHIKOV I.I., PIRKO N.N., MUDRIK E.A. & PIRKO Y.A. (2007): Maintenance of genetic structure in progenies of marginal mountainous and steppe populations of three species of Pinaceae Lindl.family in Ukraine. – Silvae Genetica, 56(1): 1-10.

KORSHIKOV I.I., VELIKORIDKO T.I. & BUTILSKAYA L.A. (2002): Genetic structure and variation in *Pinus sylvestris* L. populations degrading due to pollution-induced injury. – Silvae Genetica, 51(2-3): 45-49.

MÁNEK J. & EŠNEROVÁ J. (2004): Isoenzymové rozlíšení borovic *Pinus mugo* Turra a *Pinus × pseudopumilio* (Willk.) Beck. In: Aktuality šumavského výzkumu II. Srní, 4.-7. října 2004, pp. 151-157.

NEET-SARQUEDA C., PLUMETTAZ-CLOT A.C. & BÉCHOLEY I. (1988): Mise en évidence de l´hybridation introgressive entre *Pinus sylvestris* L. et *Pinus uncinata* DC. En Valais (Suisse) par deux méthodes multivariées. – Botanica Helvetica, 98: 161-169.

ODRZYKOSKI I.J. (2002): Studies on genetic diversity ofdwarf mountain pine (*Pinus mugo*) using biochemical and molecular markers. Wydawnictwo Naukowe UAM, Poznaniu, 135 pp. ISBN: 83-232-1166-3.

PRUS-GŁOWACKI W., BACZKIEWICZ A. & WYSOCKA D. (2005): Clonal structure of small isolated populations of *Pinus mugo* Turra from peatbogs in the Tatra Mts. – Acta Biologica Cracoviensia, Series Botanica, 47(2): 53-59.

PRUS-GŁOWACKI W. & BERNARD E. (1994): Allozyme variation in populations of *Pinus sylvestris* L. from a 1912 provenance trial in Pullawy (Poland). – Silvae Genetica, 43(2-3): 132-138.

PRUS-GŁOWACKI W., SADOWSKI J., SZWEYKOWSKI J. & WIATROSZAK I. (1981): Quantitative and qualitative analysis of needle antigens of *Pinus sylvestris*, *Pinus mugo, Pinus uliginosa* and *Pinus nigra* and some individuals from a hybrid swarm population. – Genetica Polonica, 22(4): 447-454.

PRUS-GŁOWACKI W. & STEPHAN B.R. (1994): Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. – Silvae Genetica, 43: 7-14.

PRUS-GŁOWACKI W., STEPHAN B.R., BUJAS E., ALIA R. & MARCINIAK A. (2003): Genetic differentiation of autochthonous populations of *Pinus sylvestris* (Pinaceae) from the Iberian peninsula. – Plant Systematics and Evolution, 239: 55-66.

PRUS-GŁOWACKI W. & SZWEYKOWSKI J. (1980): Serological characteristic of some putative hybrid individuals from a *P. sylvestris* × *P. mugo* swarm population. – Acta Societatis Botanicorum Poloniae, 49(1-2): 127-142.

RIESEBERG L.H. (1997): Hybrid origins of plant species. – Annu. Rev. Ecol. Syst. 28: 359-389.

RIESEBERG L.H. & CARNEY S.E. (1998): Plant hybridization. – New. Phytol. 140: 599-624.

SLAVOV G.T. & ZHELEV P. (2004): Allozyme variation, differentiation, and inbreeding in populations of *Pinus mugo* in Bulgaria. – Canadian Journal of Forest Research, 34: 2611-2617.

STASZKIEWICZ J. (1996): Natural hybrids of *Pinus mugo* × *P. sylvestris* (Pinaceae) in Tatra Mtns. – Fragmenta Floristica et Geobotanica, Series Polonica, 3: 23-30.

STASZKIEWICZ J. & TYSZKIEWICZ M. (1969): Natural hybrids of *Pinus mugo* Turra × *Pinus sylvestris* L. in Nowy Targ Valley. – Fragmenta Floristica et Geobotanica, 15: 187-212.

STASZKIEWICZ J. & TYSZKIEWICZ M. (1972): Zmienność naturalnych mieszanców *Pinus sylvestris* L. × *P. mugo* Turra (= *P. rotundata* Link) w poludniowo-zachodnej Polsce oraz na wybranych stanowiskach Czech i Moraw. – Fragmenta Floristica et Geobotanica, 18: 173-191.

STEBBINS G.L. (1950): *Variation and Evolution in Plants*. Columbia University Press, New York, 643 pp.

STEBBINS G.L. (1969): The significance of hybridization for plant taxonomy and evolution. – Taxon, 18: 26-35.

SWOFFORD D.L. & SELANDER R.B. (1981): Biosys-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. – Journal of Heredity*,* 72(4): 281-283.

WACHOWIAK W., STEPHAN B.R., SCHULZE I., PRUS-GŁOWACKI W. & ZIEGENHAGEN B. (2006): A critical evaluation of reproductive barriers between closely related species using DNA markers – a case study in *Pinus*. – Plant Systematics and Evolution, 257: 1-8.

WACHOWIAK W. & PRUS-GŁOWACKI W. (2008): Hybridisation processes in sympatric populations of pines *Pinus sylvestris* L., *P. mugo* Turra and *P. uliginosa* Neumann. – Plant Systematics and Evolution, 271: 29-40.

Received: December 2nd 2010
Revised: October 25th 2011
Accepted: October 28th 2011