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# Genetic diversity and mating system of Scots pine plus trees

**Abstract:** We have investigated genetic diversity and the mating system of Scots pine plus trees from the Tuchola Forests – Poland, using allozymes as genetic markers. The studied plus trees possess high genetic diversity (expected heterozygosity $H_e = 0.427$) and low inbreeding (Wright's index $F = -0.028$), which is comparable to Scots pine trees observed in other studies in natural populations and seed orchards. The mating system analyses revealed that almost all offspring produced by the plus trees were due to outcrossing ($t_m = 0.983$), with no apparent bi-parental inbreeding. The estimate of correlation of paternity indicated that each of mother trees is pollinated on average by a relatively large number of effective males ($N_{ep} = 30.3$). The observed genetic diversity and mating system patterns indicate that the seeds produced by the studied plus trees possess high genetic variation needed in reforestation processes.

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## Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most important timber species in Eurasia. In Poland, it covers nearly 70% of forested areas, and every year large amounts of Scots pine seedlings are needed for reforestation (e.g. 363 mln seedlings in 2002; Zajączkowski 2002). This requires a large production of seeds, but fortunately, Scots pine fructifies most regularly, as compared to other forest trees, and good seed years can occur even in successive years, especially in lowland Poland (Kantorowicz 2000). Plus trees are elite superior individuals which are selected phenotypically because of their extraordinary timber production and quality. They are usually located in larger forest stands also of good quality (preferably within seed collection stands), which aims to provide large amounts of pollen, presumably of high genetic

value. Plus trees are sampled for scions when establishing clonal seed orchards, but seed samples are also collected for gene conservation purposes.

Seedlings used for reforestation should maintain superior quality inherited from their parents but they also should possess a high genetic diversity, which would enable them to adapt to changing environments (Muona 1989). Genetic diversity of offspring derived from plus trees is primarily determined by the diversity of the sampled mother trees, but also by mating patterns. High outcrossing coupled with extensive pollen dispersal may promote high genetic variation of offspring. Pines, and conifers in general, are known for the relatively high proportions of progeny resulted from outcrossing (Burczyk 1998b; Boshier 2000). For Scots pine most authors reported high outcrossing rates (over 0.95) both in seed orchards (Kärkkäinen and Savolainen 1993; Burczyk

1998a) and natural stands (Muona and Harju 1989; ; Longauer et al. 1992; Robledo-Arnuncio et al. 2004). However, low outcrossing rates are occasionally reported in pines (Changtragoon and Finkeldey 1995; Lewandowski and Burczyk 2000; Robledo-Arnuncio and Gil 2005).

The mating system of plus trees may differ slightly from an average estimate for the species. Plus trees usually exhibit high growth rates and often large crowns. Large crown of a tree may cause that the proportion of itself pollen may be considerable as compared to pollen received from other trees, increasing the probability of self-pollination (Burczyk 1998a). However, even if outcrossing is high, matings may occur with a limited number of males or the contribution of a few males may be responsible for majority of matings, leading to the reduced effective number of males and thus decreased genetic diversity (Burczyk et al. 1996; Robledo-Arnuncio and Gil 2005). Therefore, the outcrossing levels and effective numbers of males should be of major concern when predicting genetic diversity of seeds derived from plus trees.

In this paper we investigated genetic structure and mating system of several plus trees of Scots pine using allozymes as genetic markers. We were particularity interested in the levels of genetic diversity, outcrossing and the effective number of males mating with the sampled mother trees. Our results are discussed in the context of preserving forest genetic resources during reforestation.

## Material and methods

The plus trees investigated in this study grow in the Forest Disctrict Woziwoda, in a larger forest complex of the Tuchola Forests, Poland. This region is an important source of seeds utilized for reforestation purposes. In Woziwoda, Scots pine is a dominant tree species covering nearly 96% of forested area. All together there are 115 plus trees, 372 ha of commercial seed stands, and 107 ha of qualified seed stands. Several plus trees were sampled for grafts when establishing regional clonal seed orchards (e.g. Gniewkowo seed orchard, Burczyk 1998a).

Cones were collected from 40 plus trees by Woziwoda Forest District personnel in the winter of 2000. Seeds were extracted from cones and stored in 4°C until analyses in 2003. The megagametophyte and embryo tissues of 10 seeds per plus tree were subjected to standard starch gel protein electrophoresis procedures. Genetic variation was assessed based on 10 unlinked allozyme loci: *Fest* (fluorescent esterase), *Gdh* (glutamate dehydrogenase), *Got-1*, *Got-2*, *Got-3* (glutamate oxalo-acetate transaminase), *Mdh-1*, *Mdh-3* (malate dehydrogenase), *6Pgdh-1*, *6Pgdh-2* (6-phosphogluconate dehydrogenase), *Pgi* (phosphoglucose isomerase), *Skdh-1* (shikimate dehydrogenase) (Niebling et al. 1987, Szmidt and Muona 1989).

Genetic diversity of adult population was investigated through mean ($n_a$) and effective ($n_e$) allele numbers, expected heterozygosity ($H_e$) and Wright's fixation index ($F$), which all were calculated using POPGENE computer program (Yeh et al. 1999). The population-wide single-locus ($t_s$) and multilocus ($t_m$) estimates of outcrossing rate, as well as several other parameters, including correlation of paternity ($r_p$) and correlation of outcrossing ($r_t$), were calculated based on a mixed-mating model, using maximum-likelihood procedures implemented in MLTR for Windows computer program (Ritland 2002).

## Results

### Genetic diversity

Two of the loci (*Got-1* and *Mdh-1*) exhibited low variation and were excluded from genetic diversity analyses, however, they were utilized while estimating multilocus outcrossing rates. Genetic diversity of the investigated plus trees was found to be high and comparable to earlier studies on natural stands and seed orchards of Scots pine. The number of alleles per locus ranged from 2 to 4, while the effective numbers of alleles were 1.393 to 1.979, with a mean of 1.796 (Table 1). The expected heterozygosity (a measure of genetic diversity) was high and ranged from 0.282 for *Pgi* to 0.495 for *Got-2*, with a mean of 0.427. However, it should be remembered that in this study we deliberately utilized rather polymorphic loci, which are preferred for mating system studies. The Wright's fixation index ($F$) varied considerably among loci (from –0.209 to 0.183), however none of them exibited significant departure of genotypic frequencies, compared to the Hardy-Weinberg expectations. The mean $F$ was slightly negative (-0.027) indicating excess of heterozygosity.

### Mating system

The multilocus estimate of outcrossing was high ($t_m = 0.983$) and not significantly different from unity (Table 2). This means that the proportion of selfed offspring ($s = 1 – t_m = 0.017$) was meaningless. The single-locus $t$ estimates ranged from 0.862 to 1.119, with a mean of 0.964, and were not significantly heterogeneous across loci. The apparent difference between single and multi-locus outcrossing rates ($t_m – t_s$) may indicate that some proportion of inbreeding additional to selfing (i.e., biparental inbreeding) may be present among offspring (Shaw et al. 1992, Ritland 2002). However, we have found the difference insignificant. The correlation of outcrossed paternity within progeny arrays (or the probability that a randomly chosen pair of progeny from the same array are

Table 1. Genetic diversity measures and single-locus outcrossing rates of Scots pine plus trees ($n_a$ – observed number of alleles; $n_e$ – effective number of alleles; $H_e$ – expected heterozygosity; $F$ – Wright's fixation index; $t_s$ – single-locus outcrossing rate, SD in parentheses)

| Locus | Genetic structure | | | | Mating system |
| | $n_a$ | $n_e$ | $H_e$ | $F$ | $t_s$ |
| --- | --- | --- | --- | --- | --- |
| Fest | 4 | 1.853 | 0.460 | 0.022 | 1.007 (0.081) |
| Gdh | 2 | 1.835 | 0.455 | –0.209 | 1.119 (0.075) |
| Got-2 | 3 | 1.979 | 0.495 | –0.061 | 0.976 (0.060) |
| Got-3 | 3 | 1.915 | 0.478 | –0.046 | 0.905 (0.052) |
| Mdh-3 | 2 | 1.600 | 0.375 | –0.200 | 0.954 (0.080) |
| 6Pgdh-1 | 3 | 1.960 | 0.490 | 0.183 | 0.862 (0.094) |
| 6Pgdh-2 | 2 | 1.882 | 0.469 | 0.060 | 1.060 (0.055) |
| Pgi | 3 | 1.393 | 0.282 | –0.182 | 0.917 (0.045) |
| Skdh | 4 | 1.507 | 0.336 | 0.182 | 0.922 (0.046) |
| Mean | 2.889 (0.782) | 1.769 (0.214) | 0.427 (0.076) | –0.028 | 0.964 (0.015)[a] |

a – minimum variance mean

Table 2. Summary of the mating system parameters of Scots pine plus trees ($s$ – self-fertilization = $1 - t_m$; $t_m$ – multilocus outcrossing rate; $t_s$ – single-locus outcrossing rates; $r_t$ – correlation of outcrossing; $r_p$ – correlation of paternity; $N_{ep}$ – effective number of pollen parents; SD in parentheses)

| Mating system parameters | | | | | |
| $s$ | $t_m$ | $t_s$ | $t_m - t_s$ | $r_p$ | $N_e$ |
| --- | --- | --- | --- | --- | --- |
| 0.017 | 0.983 (0.013) | 0.964 (0.015) | 0.018 (0.013) | 0.033 (0.014) | 30.3 |

full sibs, Ritland, 2002) was very low ($r_p$ = 0.033), although significant (p=0.018), indicating that on average, the effective number of outcrossing males mating with a given mother tree was relatively high ($N_{ep}$ = $1/r_p$ = 30.3).

# Dicsussion

## Genetic diversity

Our results of genetic diversity are concordant with the earlier studies on genetic diversity of Scots pine using allozyme markers (Mejnartowicz and Bergmann 1985; Müller-Starck 1986; Muona et al. 1988; Muona and Harju 1989; Paule and Mrazikova 1990; Prus-Głowacki and Stephan 1994; Dzialuk and Burczyk 2002; Burczyk et al. 2000). Lower genetic variation was observed in Spain at the species distribution limits (Prus-Głowacki et al. 2003). The excess of heterozygotes (as measured by $F$ index) is often observed in adult coniferous populations, both natural stands and seed orchards (Lewandowski et al. 1991; Linhart et al. 1981; Muona 1989; Muona and Szmidt 1985). This phenomenon is caused probably by the elimination of inbred (thus more homozygous) individuals with population ageing (Brown 1975, Mitton 1998). Yazdani et al. (1985) observed that in a naturally regenerating population of Scots pine the excess of homozygotes had been removed until the population was 10 years old. The early elimination of homozygotes has been observed also in other coniferous species (Smouse and Bush 1992; Mitton 1998). However, this should be noted that Prus-Głowacki (1982) observed elimination of heterozygotes in consecutive age classes of a naturally regenerating Scots pine population.

## Mating system

Conifers are known to be predominantly outcrossing. Recent reviews of mating system studies in pines indicate that for most species the outcrossing rates higher than 0.9 were observed (Burczyk 1998b; Boshier 2000). However, a variable and sometimes significant amount of selfing has been observed in some pine populations (Changtragoon and Finkeldey 1995; Lewandowski and Burczyk 2000; Robledo-Arnuncio and Gil 2005).

Recent estimates based on allozyme markers confirm high proportions of cross-fertilization in pines. The outcrossing rate in *Pinus strobus* L. varied from 0.867 to 0.991 among 9 populations (Rajora et al. 2002). The estimates were negatively correlated with stand density but positively correlated with the proportion of filled seeds per cone. Parraguirre-Lezama et al. (2004) observed outcrossing above 0.90 in four populations of *Pinus greggii* Engelm, with no relationship to stand density or the presence of other tree species in the populations.

Our estimates of outcrossing are very similar to the estimates reported from the seed orchard consisted of clones originating from the study area (0.987; Burczyk 1998a). Muaona and Harju (1989) found in Finland higher outcrossing in seed orchards (0.977) than in natural populations (0.948). However, Siregar and Hattemer (2001) reported in *Pinus merkusii* Jungh. et de Vriese, that the $t_m$ estimates were higher in natural stand (0.98) than in a seedling seed orchard (0.87).

Apart from allozymes, microsatellites (or SSRs – *Simple Sequence Repeats*) are becoming increasingly used for mating studies in pines. The high polymorphism of SSR markers enables to use a lower number of loci maintaining or even increasing the exclusion probability of marker sets, as compared to allozymes. Lian et al. (2001) used three SSRs to investigate outcrossing rate in *Pinus densiflora* Sieb. and Zucc., which was estimated to be 0.955. Similarly, three nuclear SSR markers applied to maritime pine (*Pinus pinaster* Ait.) indicated that outcrossing was at least 0.962 (Gonzalez-Martinez et al. 2003). Finally, in Scots pine three SSR loci ($t_m$ = 0.932–0.990, Robledo-Arnuncio et al. 2004) or a combination of four chloroplast and two nuclear SSRs (Robledo-Arnuncio and Gil 2005) were sufficient to estimate outcrossing it two different populations. In the later one, unusually low outcrossing was observed (0.75), which was attributed to the nearly complete isolation and low density of the small population (Robledo-Arnuncio and Gil 2005).

## Conclusions

High genetic diversity of adults, high outcrossing with no bi-parental inbreeding, and large numbers of effective males mating with each mother three are obviously positive characteristics of plus trees. The offspring they produce should maintain high genetic diversity, inheriting at least from mother parents quantitative traits important for wood production. The high outcrossing rates and low inbreeding in later life stages observed in Scots pine are caused mainly by high levels of inbreeding depression during both early and later stages of the life cycle (Koelewijn, et al. 1999). However, the high outcrossing observed in this and other studies might be partially the result of extensive pollen flow, as observed in pines (Lindgren et al. 1995; Adams and Burczyk 2000; Hamrick and Nason 2000). Distant pollen-mediated gene flow may reduce fitness of offspring if genes come from populations maladapted to the habitat of offspring establishment. Therefore accurate estimation of contemporary levels of pollen-mediated gene flow is essential for designing seed collection stands and conservation strategies that maximize interconnectedness of populations while minimizing unwanted gene flow (Adams and Burczyk 2000).

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