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Mating patterns, genetic composition and diversity levels in two seed orchards with few clones—Impact on planting crop

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ABSTRACT

Paternity analyses using microsatellites were conducted in two conifer clonal seed orchards (CSOs). The aim was to study mating patterns and potential dysfunctions such as unbalanced male contribution, and how these factors influence genetic diversity. The effect of unequal male contribution was quantified by the status number (N_S), which reflects build up of coancestry in the seed orchard crop due to low number of clones and unequal male contribution. This approach was also used to quantify the effect of clonal differences in germination percentages on effective population sizes. Furthermore the genetic diversity of the *Abies alba* and *Larix kaempferi* CSOs was compared to other alternative seed sources via observed heterozygosity and allelic richness.

Both CSOs had a low pollen contamination rate (1.4% and 2.8%). The CSO with 12 *A. alba* clones showed a very skewed distribution of paternal contributions, where four clones (25%) sired more than 80% of the offspring, resulting in a status number of (N_S) = 4.6. The CSO with 10 *L. kaempferi* clones showed a much more equal distribution of paternal contributions, with (N_S) = 10.2. For both CSOs the paternal contributions varied significantly with sampling site within the seed orchards. The amount of selfing in the CSO seed crops was 4.7% in *A. alba* and 3.1% in *L. kaempferi* and selection against selfing could be observed. The relative self-fertility was estimated to 59% in *A. alba* and 33% in *L. kaempferi*. A study of early versus late germinating seedlings in two *L. kaempferi* clones indicated no increased proportion of selfed individuals in late germinating individuals. Clonal differences in germination percentages in the two CSOs had little impact on effective population sizes. The level of heterozygosity was not significantly different between CSOs and the alternative seed sources. Fixation indices (F_{is}) were significantly negative in the seed crops from both CSOs, indicating heterozygote excess. There were substantial differences in allelic richness between CSO seed crops and alternative seed sources. The number of alleles was 57% higher in the alternative *A. alba* seed source Mt. Gariglione and 25% higher in the alternative *L. kaempferi* seed source F410 Frøslev.

1. Introduction

Mating patterns among forest trees is of interest both in the context of genetic conservation and diversity, but also in relation to the exploitation of genetic variation through breeding (Boshier, 2000). Genetic diversity itself is often designated to be of ‘crucial’ importance (e.g. Muller-Starck, 1995) for the adaptability and long-term stability of forest ecosystems. At the same time, a large proportion of the forest planting material in boreal and temperate areas is coming from clonal seed orchards (CSOs), because these have shown to be the most cost effective way to produce genetically improved forest seed. As a rotation can last more than 100 years, the genetic diversity in seed crops from CSOs may also be relevant to consider. Consequently, much concern has been placed on this subject during recent decades, and several studies have also been conducted to compare the mating patterns and genetic diversity of clonal seed orchards with those in natural populations (e.g. Chaisurisri and Elkassaby, 1994; Stoehr and Elkassaby, 1997; Godt et al., 2001). For several of the tree species used in Danish forestry however, the CSOs available in Denmark have a fairly low number of clones, since they were established at a time when less attention was paid to genetic diversity. On the other hand, these CSOs are of well-defined origin, they may be selected specifically for Danish conditions, and alternatives may not always be at hand or the genetic constitution and origin of possible alternatives may be unclear/unknown. For those conifers,

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which are non-indigenous to Denmark, alternatives to the CSOs are mainly planted seed stands or directly imported provenances from foreign natural stands (Pers. Communication: Bjerne Ditlevsen, State Forest Tree Improvement Station, Denmark, November 2007).

1.1. Nature-based forest management and genetic diversity

In more recent years there has been an increasing interest in more 'nature-oriented' forest management in Denmark—among the main reasons is a wish for more stable forest ecosystems (Larsen and Nielsen, 2007). Nature-based forest management is based on continuous forest cover, mixed stands, uneven-aged stand structures, and extensive use of natural regeneration. In Denmark this management scheme does not only include native species, but also a continued use of a number of exotic species (Larsen and Nielsen, 2007).

In 2002 it was decided, that all state owned forests in Denmark, which comprises around 25% of the forest area, in the future should be converted to more nature-based silvicultural management (Danish Forest and Nature Agency, 2002). This decision implied that a 250 years tradition of plantation forestry with 'classical age-class forests' was about to end (Larsen and Nielsen, 2007).

The new management strategy, relying on natural regeneration, entails that the forest management from now on is bound to the genetic constitution of the stand, whether this is established by planting or builds on an existing forest of known or unknown provenance. Knowledge of the genetic diversity, and the mating patterns which influence the further faith of the initial diversity of the seed source, are therefore especially important.

The shift from rotation forestry to nature-based forestry means that any genetic bottleneck effects introduced during the propagation in the CSO can influence the future generations.

1.2. The two species in the study

Abies alba Mill., common name silver fir, is an ecologically valuable indigenous tree species in many European mountain forests (Konnert and Bergmann, 1995). *A. alba* is not indigenous to Denmark, and has presently only a limited use in its forestry. However, in light of the changing forest management scheme, *A. alba* is becoming more interesting for Danish forestry. Especially important is its higher potential for natural regeneration than the conifers often used in Danish plantation forestry (e.g. *Picea abies* and *Picea sitchensis*) (Henriksen, 1988, p. 181). Another advantage is its high shade tolerance, which makes it suitable for the mixed stand forestry that in many instances will be a result of the new silvicultural scheme.

The non-indigenous *Larix kaempferi* (Lamb.), common name Japanese Larch, which originates from the mountainous regions of central Honshu Island in Japan (Isoda and Watanabe, 2006) has also a relatively limited use in Danish forestry today. This is mostly due to the extended use of the hybrid larch (*Larix x eurolepis*), which has shown superior growth characteristics compared to the pure parental species—*Larix decidua* and *L. kaempferi* (Keiding, 1980). *Larix x eurolepis* seed is normally produced in CSOs with very few clones of each parental species, and by natural regeneration the offspring (=F2 generation) may have very low genetic diversity. Furthermore, the F2 generation may have an unpredictable phenotype due to segregation to the parental species. Because of these potential problems related to *Larix x eurolepis*, *L. kaempferi* is considered an important tree species in the new forest management system, where it is expected to replace the hybrid larch.

1.3. Objectives of the study

The present study explores the mating systems of *A. alba* and *L. kaempferi* through a paternity analysis in two few clone seed orchards. Using the same data, the genetic diversity of these two CSOs is estimated and compared to the most likely alternatives as seed source for these species in Danish forestry. For *A. alba*, this is performed with the clones in another CSO containing more clones, and with an imported Italian provenance (natural stand). In *L. kaempferi* the comparison is performed with a planted Danish approved seed stand.

2. Materials and methods

2.1. *A. alba* seed orchard FP621 with few clones—not approved

The clonal seed orchard FP621 is situated in the C.E. Flensborg Plantation, in the north-western part of Jutland, Denmark. It includes 12 clones originating from the Romanian provenance Lapus, collected in the western Carpatians at 47°38N, 23°50E—700 m a.s.l. The 12 clones were selected in a Danish provenance trial which no longer exists (Løfting, 1978); the intensity of selection was weak due to a limited number of trees in the trial. By establishment in 1968, 389 ramets were planted at approximately 5 m × 4.5 m. By seed harvest for the present study in 2005 the number of ramets had decreased to 263 through thinnings and natural mortality (see Fig. 1 (panel A) for distribution of ramets).

2.2. Approved *A. alba* seed orchard FP242—but not in production

The seed orchard FP242 contains 25 clones, which have been selected from one provenance, Mt. Gariglione, Sila di Catanzanro, in Calabria, Italy (1600 m a.s.l.), which was part of a series of provenance field trials (Larsen, 1983). The starting point for selection was only a few trees, so the effect of plus tree selection is considered minimal, and the CSO should more be seen as an exploitation of a provenance which have shown good performance in Denmark (Larsen, 1983). Based on these results, the CSO was approved by the Danish seed authorities in 2006, but it will be around 4–5 years before it starts to produce seeds in commercial quantities.

2.3. Imported *A. alba* seed source Mt. Gariglione

The imported seed source was from Mt. Gariglione in Calabria (1600 m a.s.l.), Italy. Presently, this is the most obvious choice for a Danish forester who is intending to plant *A. alba* (Larsen and Møller, 1997).

2.4. *L. kaempferi* seed orchard FP601 with few clones—approved

FP601 is also situated in the C.E. Flensborg Plantation. Originally it included 13 clones selected in a wind exposed stand at Kongenhus Heath, west of Viborg. The stand was presumably progeny of seed directly imported from Hokkaido, Japan, and the purpose of the selection and establishment of the seed orchard was to produce a *L. kaempferi* especially suited to the western part of Denmark with windy conditions (Brandt, 1968). The seed orchard was established from 1955 to 1962 on an area of 2.2 ha, and the clones were planted at approximately 4.5 m × 3 m with only one clone per row. In 1968, three clones were removed through thinnings, due to a stem form assessment of clones as well as progeny of these (Brandt, 1968). Today the 10 remaining clones are represented by a total of 381 ramets (see Fig. 2 (panel A) for distribution of ramets among the clones).