Reproduction and population structure in the European aspen

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Academic dissertation

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The thesis is based on the following articles and manuscripts, which are referred to in the text by their Roman numerals:

I   Latva-Karjanmaa, T., Siitonen, J. and Penttilä, R. The demographic and spatial structure of European aspen (*Populus tremula*) populations in managed and old-growth boreal forests in eastern Finland. Submitted manuscript.

II   Suvanto L. and Latva-Karjanmaa T. (2005) Clone identification and clonal structure in European aspen (*Populus tremula* L.) Mol. Ecol. 14: 2851-2860.

III   Latva-Karjanmaa T., Suvanto L., Leinonen K. and Rita H. (2003). Emergence and survival of *Populus tremula* seedlings under varying moisture conditions. Can. J. For. Res. 33:2081-2088.

IV   Latva-Karjanmaa T., Suvanto L., Leinonen K. and Rita H. Sexual reproduction of *Populus tremula* at a prescribed burned site: the effect of moisture conditions. New Forests. In press.

V   Suvanto L., Latva-Karjanmaa T. and Pulkkinen P. The effect of elevated temperature on seed quality and quantity in crosses between European and hybrid aspens. Submitted manuscript.

CONTRIBUTIONS

The following table shows the major contributions of authors to the original articles or manuscripts

	I	II	III	IV	V
Original idea	IH, JS, MK, RP, TL-K	LS, TL-K	TL-K	TL-K	PP, LS
Study design	TL-K, JS, RP	LS, TL-K	TL-K, LS, HR, KL	TL-K, LS, HR, KL	PP
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TL-K=Tarja Latva-Karjanmaa, LS=Leena Suvanto, HR=Hannu Rita, KL=Kari Leinonen, PP=Pertti
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III   Emergence and survival of *Populus tremula* seedlings under varying moisture conditions.

IV   Sexual reproduction of *Populus tremula* at a prescribed burned site: the effect of moisture conditions.

V   The effect of elevated temperature on seed quality and quantity in crosses between European and hybrid aspens.

Summary

Introduction

The European aspen (*Populus tremula* L.) is the most widely distributed tree species in the world (Worrell 1995), while the quaking aspen (*P. tremuloides* Michx.), a closely related species, is the most widely distributed tree in North America (Jobling 1990, Fig. 1). The elevational range of the aspen is also very wide, it grows from sea level to as high as 3,350 m asl (Jones 1985). In Finland aspen occurs in the northern fringe of its geographical range and it does not usually form large stands. Only 0.3% of the forest land area in Finland is covered by aspen-dominated stands (Anon. 2005) and thus aspen represents a minority species in mixed forests. The relative abundance of aspen decreases towards northern Finland (Anon. 2005).

Even thought aspen trees represent only a small proportion of all trees in Finnish forests, aspen's importance for biodiversity is great. Large living and decaying dead trees are essential habitats or provide other resources for a wide variety of organisms, including hole-nesting birds (Angelstam and Mikusinski 1994), invertebrates (Siitonen and Martikainen 1994, Hammond 1997, Martikainen 2001), wood-rotting fungi (Martikainen et al. 2000, Junninen et al. 2006), bryophytes (Hazell et al. 1998) and lichens (Hedenäs and Ericson 2000). The nutrient rich and alkalinous litter of aspen offers significant resources for a range of soil-inhabiting organisms (Siitonen 1999). There are at least 150 specialist species that are entirely dependent on aspen in Fennoscandia (Siitonen 1999, Kouki et al. 2004).

Aspen is a broad-leaved, dioecious (female and male flowers in separate trees) pioneer tree species. Dioecy is present in about half of all angiosperm families, but it is seldom common in any family. This pattern suggests that dioecy has evolved relatively recently (Silvertown and Charlesworth 2001). Dioecy may have evolved because it is a way to avoid inbreeding and/or because resources are saved when a plant does not need to invest to both sexes. However, dioecious species may suffer from limited pollination (Reim 1929).

Figure 1. A map showing the distribution of *P. tremula* in Eurasia and *P. tremuloides* in North-America.

Box 1. Life history
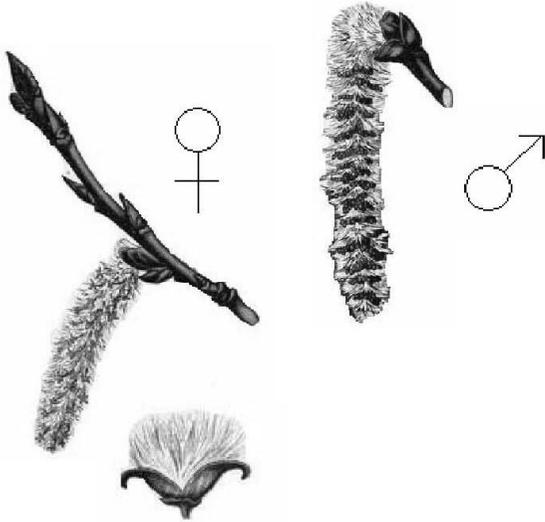
Sexual reproduction
-begins at the age of 30-40 years
-pollen and seeds dispersed by wind
-seeds are small

Seedling establishment
-relatively rare
-specific seed bed requirements

Life span
-may live at least up to 200 years
-clones may be thousands of years old

Asexual reproduction
-main form of reproduction:
 root suckers

Box 2: The costs and benefits of sexual and asexual reproduction.

Sexual reproduction	Asexual reproduction
BENEFITS	BENEFITS
• genetic variability-> capability to adapt to environmental changes • lower frequency of deleterious mutations • favourable for colonising newly disturbed areas	• higher growth rate • lower cost • rapid lateral expansion-> local dominance • lower risk that a local disturbance would kill the entire plant • no need for pollination • avoids hazards of recruitment • advantageous both under low disturbance regime and after disturbance
COSTS	COSTS
• high cost • lower survival of the seedlings • lower growth rate • rare alleles that increase fitness might not be transmitted to the offspring • beneficial gene combinations may break down in meiosis • risk of insufficient pollination	• unable to adapt to changes under selective pressure • deleterious mutations may accumulate • more prone to diseases • may lead to local crowding

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Green, R. F. and Noakes, D. L. (1995). Is a little bit of sex as good as a lot? Journal of Theoretical Biology 174:87-96.

Jaffe, K. (2004). Sex promotes gamete selection: A quantitative comparative study of features favoring the evolution of sex. Complexity 9: 43-51.

Silvertown, J. and Charlesworth, D. (2001). Plant population biology. 4th edition. Blackwell Science. pp.347

In aspen the number of male and female trees are unequal, male trees being more common (2:1) in Finland (Reim 1929, Blumenthal 1942). Several dioecious species have variation in sex ratio according to environmental conditions. Female individuals generally predominate in moist areas (Freeman et al. 1976). In North America the primary sex ratio of *P. tremuloides* is 1:1, but it varies along elevational gradients: at low elevations females are more common than males (Grant and Mitton 1979). Mitton and Grant (1996) suggest that sex determination in aspen is strictly genetic and that the observed environmental gradients are caused by differences in establishment and survival. The prevailing understanding is that aspen has no sex chromosomes (Charlesworth 2002), but the sex is determined by gene interactions.

Aspen can reproduce both sexually and asexually (Boxes 1 and 2). Having clonal maintenance and expansion aspen can be competitive in all kind of forests with low or high intensities of disturbance. With wide-ranging seed dispersal aspen has selective advantage at sites subjected to temporally unpredictable disturbance (Barnes et al. 1998). Combining these two life-history features may maximise fitness (Fenner and Thompson 2005) and thus be critical for the dynamics of aspen in boreal forests. Even a rare occasion of sexual reproduction may be highly beneficial to a mostly clonally reproducing species (Green and Noakes 1995, Box 2).

An aspen stand begins to flower usually at the age of 30-40 years, but stands located in open areas may flower already at the age of 20 years, suckers even at the age of 10 years (Kalela 1945). Aspen produces seeds almost every year, though the seed yield may vary much between the years (Reim 1929, El-Ghazaly et al. 1993, Barnes et al. 1998). Aspen exhibits masting that is synchronised reproduction of individuals (Houle 1999, Shibata et al. 2002), which leads to great year-to-year variation in seed production. Masting has several possible advantages including seed predator satiation, pollination efficiency and maximisation of resources such as precipitation, hours of sunshine, and nutrients in a given year (Kelly 1994). The seed production of aspen is potentially enormous: one catkin may hold 2 000 seeds and seed yield can be as high as 400-500 million seeds per hectare (Johnsson 1942, Reim 1929). Abundance of flowering is affected by the number of degree-days in the previous growing season. Pollination is affected by weather as well as by proximity and number of male trees. Age distribution of the stand affects the seed yield, because the seed yield of a tree correlates with the dimensions of the tree crown.

Allocation of resources to reproduction does not vary much between species, and thus there is a trade-off between seed number and seed size (Shipley and Dion 1992, Jakobsson and Eriksson 2000). In the case of aspen, seeds are very small and light, and thus they disperse effectively. The thousand-grain weight of seeds may vary from 0.06 to 0.14 g (Lagerberg 1922). Variation in seed size is mostly explained by phylogenetic, life history, and habitat differences between species (Silvertown and Charlesworth 2001), and seed size is one of the least variable traits in plants (Marshall et al 1986). There is evidence that at least some of the variation in seed size is phenotypic. Temperature, parental nutrient level, drought, competition, position of the seed in the parent plant and pollination success are known to affect seed size (Fenner and Thompson 2005). In *P. tremula*, capsules with fewer seeds have heavier seeds than capsules with a greater number of seeds (with equal pollination), and seeds in trees younger than 15 years were lighter than seeds in older trees (Reim 1929).

Aspen seeds are dispersed by wind soon after they have ripened (Reim 1929). Dispersal of seeds by wind depends on the height of release, terminal velocity of the seed, and the speed and turbulence of the wind between the ground and the point of release. Aspen seeds are known to disperse at least 400-500 meters from the mother tree (Reim 1929). Despite the potentially wide-ranging dispersal by wind, most seeds are shed relatively close to the mother tree. However, the tail of the dispersion is important for dispersal (Silvertown and Charlesworth 2001).

Seed viability in aspen usually exceeds 90% (Reim 1929, Børset 1954). The clone (Fechner and Burr 1981) and the mother tree (Børset 1955) affect seed viability. When seeds occur in an environment with sufficiently high temperature and moisture they germinate in the course of two days (Børset 1954, Børset 1955). Aspen does not have a seed bank. In natural conditions, temperature is rarely the limiting factor for germination (McDonough 1979). Aspen seeds germinate well even at the temperature of 12ºC. However, sufficient moisture is of crucial importance for germination (Fechner and Burr 1981). Alternate periods of wetting and drying lower the viability of seeds. Inhibition of germination by wet-dry cycles depends upon the duration of the phases and the number of cycles (McDonough 1979).

Despite the abundant crop of viable seeds, successful sexual reproduction appears to be relatively rare in Europe (Worrel 1995) and in North America (Moss 1938, Kemperman and Barnes 1976). Therefore, in practice aspen reproduces mainly asexually. However, there have been well-documented occasions of successful sexual reproduction, mainly after forest fire (Williams and Johnston 1984, Kay 1993, Romme et al. 1997). Thus lack of forest fires may decrease the quantity of safe sites (Grubb 1977). In addition adequate moisture is crucial for seedling emergence (III, IV).

Root suckers are produced from shallow roots, mostly from thin barked roots (ø 0.5-2 cm). These roots are usually located less than 4 cm below the soil surface (Børset 1956, Børset 1960, Worrel

1995). Root suckers arise mainly within 20 m (Bärring 1988), but occasionally as far as 30-40 m from the parent tree (Jobling 1990). Suckers remain dependent on the root system of the parent tree for many years. The decay of the connecting parts produces a population of physiologically and morphologically independent ramets.

Auxin flow (apical dominance) is the main agent suppressing suckering in the roots (Farmer 1962, Eliasson 1971). When auxin flow decreases due to disturbance (e. g. fire or logging), the production of suckers begins. High cytosine/ auxin -ratio (Peterson and Peterson 1995) as well as other hormones also affect suckering (Schier et al. 1985). In addition to hormones and disturbance environmental conditions (temperature, nutrients, light), competition, pre-disturbance stand conditions and clonal differences have been suggested to affect suckering (Frey et al. 2003). For example, shading of residual aspen, which reduces light intensity to 50% of full sunlight, can reduce suckering density tenfold (Peterson and Peterson 1995).

One parent tree may produce hundreds of suckers (Worrell 1995). Sucker density after clear-cutting mature aspens can be up to 50,000-100,000 suckers per hectare (Børset 1985). According to Krasny and Johnson (1992), over 98% of the suckers of the quaking aspen are established within the first growing season after the felling. Apical dominance limits asexual reproduction, because growing suckers produce auxin that inhibits new shoots (Schier 1972). Aspen roots can survive in stands dominated by other species for many years, even decades with the help of a few small shoots, which live only for a short time and are replaced with new ones (Zahner and Debyle 1965, Bärring 1988). Accordingly, when disturbance occurs, aspen may become established in an area where it has not been seen for a long time. A clone may be very old, even thousands of years, while the age of individual ramets can be analysed by coring and it may be even 200 years (I).

Since aspen reproduces mainly asexually, it forms clones (Box 3). A clone is a common growth structure of aspens throughout their global range (Barnes 1966). Aspen clones are often tightly packed groups of ramets, which is called the phalanx strategy (Lovett Doust 1981). Such a clone excludes other plants effectively from its area. The phalanx growth form together with physiological integration among ramets spreads the risk of failure and buffers the effects of selection over time and space (Jelinski and Cheliak 1992). In addition, aspen often has a remnant population structure in forest landscape (Jelinski and Cheliak 1992), which is typical for long-lived plants with clonal reproduction. In this situation local populations may persist over long periods of time during unfavourable periods (Eriksson 1996). Size of the clones is affected by the number of seedlings established and disturbance after establishment (Kemperman & Barnes 1976). Large clones are probably a result of the establishment of few seedlings with little between-genet competition, periodic fires that promote suckering, and spatial expansion over a long period of time, whereas small clones are due to establishment of many seedlings per unit area, competition between clones and other more shade-tolerant vegetation, and the relatively young age of the clones.

The level of genetic variation in plants has been found to correlate with several life history traits and environmental factors (Loveless and Hamrick 1984). Generally widely distributed outcrossing woody species have more genetic variation within species and populations but less variation among populations than other woody species (Hamrick et al. 1992). Because plants are sessile, most of pollen and seed dispersal is relatively local, and it is thus likely that reproduction occurs mostly between neighbours, which produces a spatially aggregated genetic population structure.

In order to study and detect gene flow and genetic structure, genetic markers are needed. During the last two decades appropriate methods have been developed. For instance, RAPDs (Random amplified polymorphic DNA, Sanchez et al. 1998) and allozymes (Rajora and Dancik 1992) have been used to study *Populus tremula* and microsatellites have been developed for *Populus tremuloides* (Dayanandan et al. 1998, Rahman et al. 2000) and can be used for *P. tremula* as well.

Box 3. What is a clone?

A clone (**genet**) is originally established by sexual reproduction, and it is divided into a group of genetically identical ramets (in aspen an individual tree) that have emerged through asexual reproduction.
A ramet is a clonally produced part of a clone. Ramets have root connection to "parent plant" but they may become independent as they grow up. In most clonal species with physical connection, genet behaves as physiologically integrated unit. Translocation of carbohydrates, water and nutrients can take place. Division of labour between ramets may occur in some species if there are differences between access to resources.

This figure shows the clone structure of aspen in southern (Kotinen) and eastern Finland (Kuhmo) (II). Different colours refer to different clones. Black dots represent genotypes that had only one ramet.
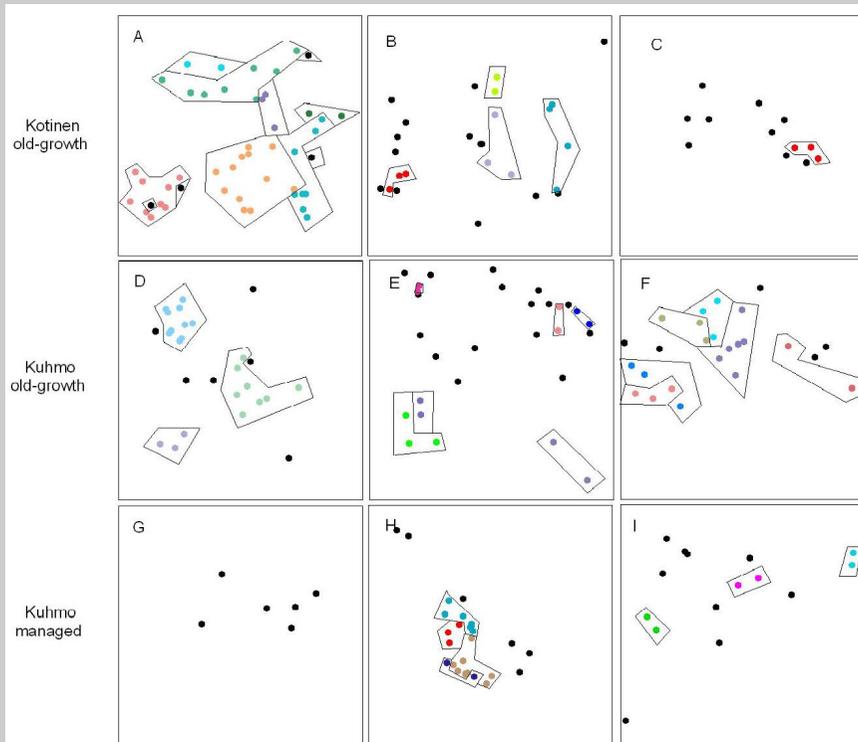
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Marshall, C. (1990). Source-sink Relations of Interconnected Ramets. Clonal Growth in Plants. SPB Academic, The Hague.
Peterson, C. J. and Jones, R.. H. (1997). Clonality in woody plants: A review and comparison with clonal herbs. in The ecology and evolution of clonal plants, pp. 263-289. edited by H. de Kroon and J. van Groenendael. Backhuys Publisher. Leiden, The Netherlands.

Before the wide-spread use of molecular methods, clone identification was based on morphological and phenological features.

Boreal forests are prone to periodic disturbances by fire and wind, which may either replace entire stands or create gaps of various sizes. Disturbance creates opportunities for recruitment (Pickett and White 1985). Forest fires are an important factor in boreal ecosystems and an essential part of aspen's ecology. A burned forest soil is suitable ground for suckers as well as for aspen seedlings (Ericsson 1992). After a stand replacing fire the first trees to become established are mainly broad-leaved hardwood trees. The pioneer tree species are replaced later on by

conifers. Nowadays, when forest fires are being suppressed, the importance of gaps created by for example winds, pathogenic fungi, and insects is greater than before.

In addition to the suppression of fires, modern forestry has affected greatly the dynamics and structure of forests for several centuries (Esseen et al. 1997, Östlund et al. 1997). In Finland, attitudes towards aspen have changed over time. Traditionally, aspen was used on small scale for various purposes. Aspen has been used for making matches from the 1850's, but the markets for aspen timber decreased in the 1970's. Aspen became an unwanted species in forestry, because it is a host of *Melampsora pinitorqua*, a rust disease of young pine stands (Kurkela 1973). Other reasons for the dislike of aspen were its low economic value and growth habit with aspen root suckers competing with other, more valuable tree species in clear-cut areas. From the 1960's until the 1980's, aspen was controlled using herbicides and mechanical clearing on clear-cut sapling stands, and by notching and girdling in mature forests before clear-cutting. In addition to forestry, high density of moose populations has been suggested to be an important reason for unsuccessful recruitment of aspen in old-growth forests in Finland (Kouki et al. 2004) and North America (Kay 1997). To study how management and changed disturbance regime have affected aspen populations, one should document the size and age structure of populations. The size structure of a tree population is some measure of its future, for example size distribution elucidates the ongoing regeneration process (Linder et al. 1997). The age structure can help in tracing past history (Harper 1977).

Today, aspen has again become a valuable tree species in forestry. The hybrid aspen (*P. tremula x P. tremuloides*), which is a man-made hybrid between the European aspen and the North American quaking aspen, is being planted to serve as raw material for high-quality paper industry. The hybrid aspen has been planted in Fennoscandia since the 1940's (Rytter and Stener 2005). Aspen, and especially the hybrid aspen, have superior fibre qualities (Ranua 2002) and

fast growth rate (Yu and Pulkkinen 2003). However, hybrid aspen is an alien species, and may have unfavourable consequences for native species. The hybrid aspen can cross with the European aspen, which means that *P. tremuloides* genes can invade the gene pool of the European aspen.

Aims of the thesis

In this work, I have studied the structure of aspen populations in terms of number, size, clonal and demographic properties. Additionally, I have studied the emergence and survival of seedlings and seed quantity and quality in both within European aspen and in crosses between the European aspen and the hybrid aspen. More specifically, I wanted to answer the following questions:

1) What is the structure of living and dead aspen populations in old-growth and managed forests now and in the future? Are mature aspens disappearing from old-growth forests in conservation areas? What is the demographic structure of aspen populations in old-growth and in managed forest? (I, II) What is the role of moose and hare browsing in the regeneration of aspen? (I)

2) What kind of clonal structure does the European aspen have? How big are the clones? Does aspen have a spatially aggregated genetic structure? Does clonal structure differ between managed and old-growth forests and between geographical regions? Are genetic markers more reliable than morphological traits in clone identification? (II)

3) How do environmental conditions affect seedling emergence and survival on mineral soil, (at burned and unburned sites) and on coarse woody debris and on humus? How long does it take for the majority of the seedlings to emerge? How do short-term weather conditions affect emergence? (III, IV)

4) What is the quantity and quality of seeds in crosses between the European and hybrid

aspens? Do the crosses respond differently to elevated temperature? (V)

Material and methods

Study areas

The study areas were situated in Kuhmo, eastern Finland (I-IV) and in Kotinen nature reserve in Lammi, southern Finland (I-II). Both areas belong to the middle boreal vegetation zone (Ahti et al. 1968). Kuhmo study area is a mosaic of mires and forests and includes a continuum from relatively large, continuous old-growth forests to intensively managed forests on mainly state-owned land. The dominant tree species in Kuhmo are Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.), which are present in all stands along with silver birch (*Betula pendula* Roth.) and European aspen. In Kotinen, the dominant tree species is Norway spruce. Crosses between the European aspen and the hybrid aspen were made in the Finnish Forest Research Institute's breeding station in Läyliäinen in southern Finland (V).

Mapping

Mature, living and dead aspen trees with the minimum diameter at breast height (DBH) of 15 or 20 cm were mapped within an area of 11,400 ha in Kuhmo (I). A total of 36,514 living and dead mature aspens were recorded within the mapping area. In addition, the decay class (range 1-5, 1 is recently dead and 5 most decayed) and the quality (intact or broken standing, fallen or cut trunks) of dead trees were recorded. To compare the amount and quality of mature aspen trees, the productive forest land (mean annual growth ≥ 1 m³/ha) with a total area of 6,533 ha within the mapping area was divided into four different categories: continuous old-growth forest, fragmented old-growth forest, semi-natural managed forest and ordinary managed forest. The classification was based on stand age and signs of previous management. The effect of site type on the amount of mature aspen was explored within the continuous old-growth forest. Stand data were obtained from the Forest and Park Service of Finland (Metsähallitus). To study aspen regeneration, the occurrence of all aspens with DBH ≥ 1 cm was assessed on one-hectare plots

(10 plots in the continuous old-growth forest and 26 plots in managed forest). Observations on browsing by moose and hare were also gathered.

Clonal and spatial structure

To study the clonal structure of aspen (II), nine 1-ha plots were randomly sampled in old-growth (3 ha in Kuhmo and 3 ha in Kotinen) and in managed forests (3 ha in Kuhmo). First, all trees were morphotyped according to their stem form, branching habit, bark colour and patterning and especially in terms of their spring foliage colour. Second, clones were identified using nine microsatellite loci originally developed for *P. tremuloides* by Dayanandan et al. (1998) and Rahman et al. (2000). In the old-growth forest study plots, all aspens greater than DBH of 15 cm, and in managed forests trees greater than DBH of 5 cm, were sampled. Altogether we sampled 219 trees, of which 52 were in managed and 81 in old-growth forests in Kuhmo and 86 were in old-growth forest in Kotinen. Spatial genetic structure was studied using two parameters: a relationship coefficient computed as Moran's *I* (Hardy and Vekemans 1999) and a kinship coefficient computed as a correlation between allelic states (ρ_{ij}, Loiselle et al. 1995, II).

To study spatial structure in continuous old-growth forest, spatial autocorrelation was examined with semivariance of deviance residuals from two models. First only the intercept was included. Secondly independent variables were added, and thus the effect of site type was removed. The patch structure was studied in the accuracy of one hectare (I).

Age determination

To determine the age of mature aspen trees (I) and to analyze the relationship between age and size of trees (I) trees were cored. Trees were selected randomly for this purpose in 1-ha plots within continuous old-growth forest (81 trees) and in managed forest (155 trees) in Kuhmo study area and in old-growth (65 trees) in Kotinen.

Sowing experiments

To study seedling emergence and survival two sowing experiments with randomised factorial design were conducted in Kuhmo. The first

experiment (III) was done on mineral soil and on coarse woody debris on clear-cut areas. The second experiment (IV) was conducted on prescribed burned area, where the seeds were sown on humus and on mineral soil. There were altogether 10 blocks (1m x 1m) in the first experiment and 15 in the second, each containing 16 microsites ($\varnothing$ 7 cm) and three treatments (sowing time, watering, sowing shelter) replicated twice in each block. We sowed 0.1 g seed material at each microsite containing on average 60 seeds capable to germinate. Seedling emergence and mortality were assessed 2 - 3 times/week in the beginning of the experiment and once a week at the end of the growing season. The number of survived seedlings was counted after the winter. Weather data was obtained from the Finnish Meteorological Institute.

Crossings
Intra- and interspecific crosses of European and hybrid aspens were studied in terms of seed quantity and quality in 2003 and 2004 (V). Four European aspen mothers and four fathers and six hybrid aspen mothers and six fathers were crossed with each other. All fathers and mothers were different clones. In the second year (2004) the crosses were done in different temperatures.

Results and discussion

Structure of aspen populations
The average volume of mature (here: DBH$\geq$ 20 cm) aspen was greatest in continuous old-growth (5.3 m³/ha) and lowest in managed forest (0.8 m³/ha). Vegetation class explained a part of the variation in the abundance of aspen, but in forests not differing in vegetation class the difference in mature aspen volume between old-growth and managed forest was from four to six-fold and in living aspen even 14-fold. Moreover, in continuous old-growth forest, the volumes of living and dead aspens were correlated, whereas in managed forest particular one-hectare plots with high aspen volume had mostly either dead or living trees. The volume of living trees was 75% in continuous old-growth and 26% in managed forest of the total volume of aspen. The proportion of dead aspens killed by notching or

girdling was 43% of the total volume of dead trees in managed forests but nearly 0% in old-growth forests. There were no major differences in the distribution of dead trees qualities among the forest categories. The majority of dead aspens were on the ground. Decay classes 2 and 3 accounted for most of the CWD volume in all forest categories. The proportion of decay class 1 varied from 1.0 to 2.5% which represent the approximate mortality rates of mature trees during the previous year (I).

Aspen populations in old-growth forests largely lacked young age classes. The diameter distribution in old-growth forest was normal, but in managed forest resembled a reversed J-shape. On average, there were only 3.7 living saplings (DBH < 15 cm) per ha in old-growth forest and their volume accounted only less than 1% of the total volume. The number of saplings was greater in managed forest, but these forests largely lacked mature trees. Browsing was more common in managed than in old-growth forests. In old-growth forest, browsing by hare was almost as common as browsing by moose in the case of small saplings (I).

On the basis of the present and previous results, the volume of 5 m³/ha of aspen seems to be typical for spruce-dominated, mesic old-growth forests in Fennoscandia (Siitonen et al. 2000, Kouki et al. 2004). Aspen regeneration was poor in old-growth forests. The very low number of saplings clearly shows that the aspen populations are not viable. Half of the mature trees that are alive at present will die in the next 50 yrs and the last ones after some 100 yrs. If the size distribution of mature trees is interpreted as a survival distribution, the number of living trees smaller than DBH of 20 cm is not sufficient to replace trees in the larger diameter classes in old-growth forests in the long term. However, browsing does not seem to be the primary factor hindering regeneration in old-growth, where aspen suckers appear to escape browsing once having passed the regeneration stage. The role of hare browsing on reproduction of aspen has not been studied and it seems to deserve more attention. The primary reason for the poor regeneration success seems to be the absence of sufficiently large disturbances that
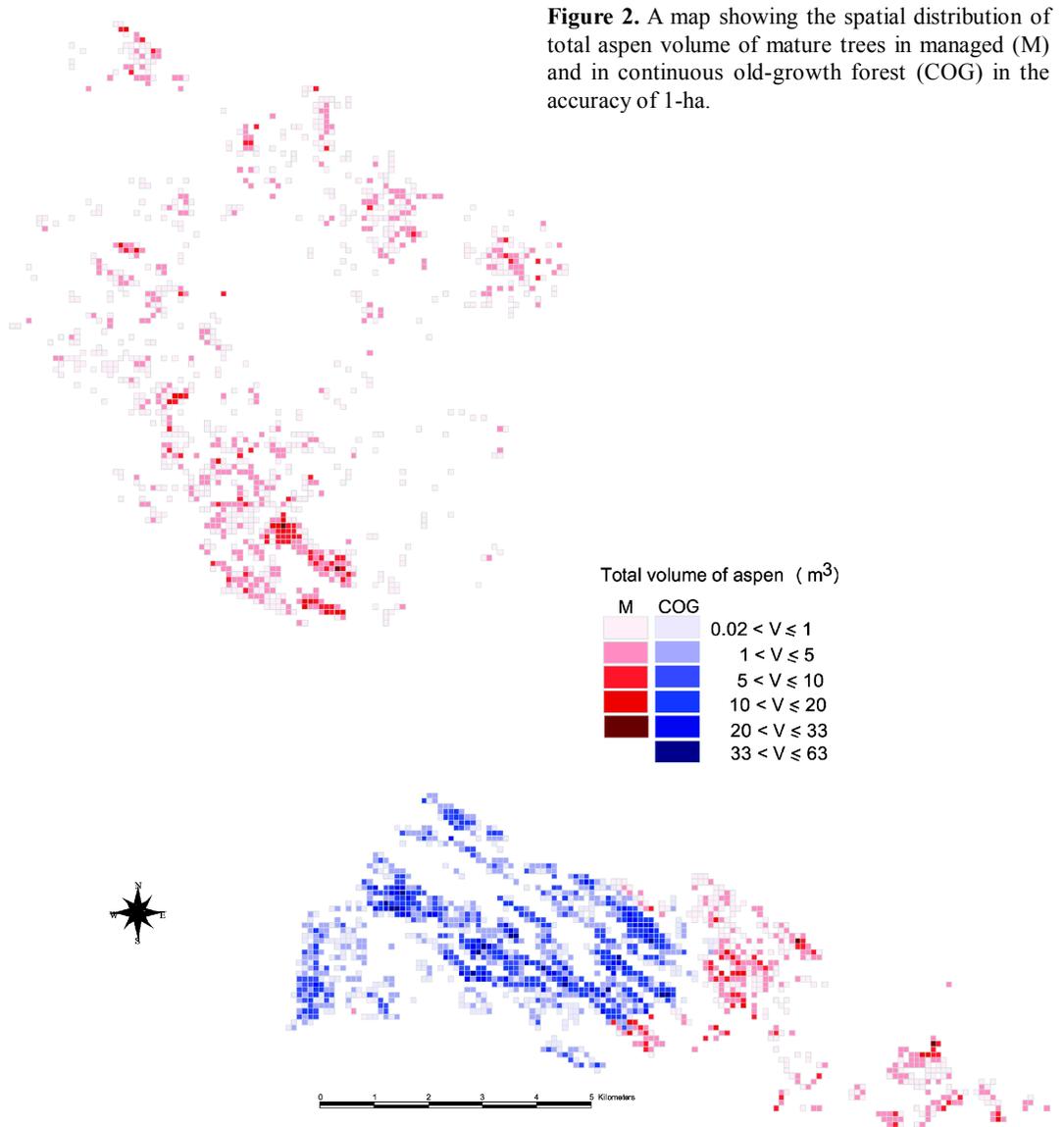
would create opportunities for aspen regeneration and recruitment.

The average volume of mature aspen in managed forests was only 15% of the volume in continuous old-growth. Moreover, the proportions of living and dead mature aspens were strikingly different in continuous old-growth and managed forests. Mature living and particularly dead aspens seem to be a legacy of the past in less intensively managed forests. The ratio of living and dead mature trees in managed forests shows that the volume of dead aspen will decline to a fraction of the present amount within the next decades.

Age structure

The mean age of mature aspen trees in old-growth forest was 154 years in Kuhmo and 132 years in Kotinen. The maximum age of a single tree was over 200 years in Kuhmo old-growth. There was no correlation between the age and the size in old-growth forest, but there was a positive

Figure 2. A map showing the spatial distribution of total aspen volume of mature trees in managed (M) and in continuous old-growth forest (COG) in the accuracy of 1-ha.

Total volume of aspen （m^3）

M	COG	
		0.02 < V ≤ 1
		1 < V ≤ 5
		5 < V ≤ 10
		10 < V ≤ 20
		20 < V ≤ 33
		33 < V ≤ 63

correlation between the age and the size among trees ≤ 60 years old in managed forest (I). The age difference between the youngest and the oldest ramets within a clone ranged from 14 to 76 years in old-growth and from 2 to 10 years in managed forests (II).

It has for long been a common belief that aspen would seldom reach an age more than 100 years (Cajander 1917, Holm 2000), whereas the maximum age in the present material was over 200 years, and the average age of mature (DBH ≥ 15 cm) aspens was as high as 150 years. Moreover, it is possible that those trees for which the age could not be determined because of decay were even older than those that could be cored. Age determination of aspen is difficult (Campbell 1981) because the inner part of the trunk of old trees is frequently decayed by the polypore *Phellinus tremulae* (Wikström and Unestam 1976).

Clonal and spatial structure
The microsatellites suited well for genotyping European aspen clones. There were more clones identified by microsatellites than by morphological traits both in old-growth and in managed forests. The clones were generally small, with the average size of 2.3 ramets and most clones (70%) consisting of only one ramet (Box 3). There were 13 clones (out of 107) with at least five ramets. Spatial structure of the clones was mostly highly aggregated. The size of the clones showed no difference between managed and old-growth forests nor between north-eastern and southern Finland in terms of the number of ramets. However, in old-growth the ramets within a clone were further away from each other than ramets in managed forest. The spatial genetic structure as measured by Moran's *I* was significantly positively autocorrelated up to the distance of 10 m and the kinship coefficient showed positive autocorrelation up to the distance of 20 m (II).

The small size of the aspen clones together with relatively high number of genotypes in the studied area suggest that most of them are relatively young. Therefore, sexual reproduction may be more common than has been previously thought. Low level of co-ancestry can be explained by relatively unrestricted gene flow, the important

role of disturbance in reproduction, and/or local selection. These results for the clonal structure of the European aspen are in line with results for the *P. tremuloides* (Namroud et al. 2005). There are also previous studies reporting often the clones of the European aspen consist of only one ramet (Culot et al. 1995, Easton 1997). However, before molecular methods were applied, many studies suggested that in North America the clone size of *P. tremuloides* could be even several hectares (Grant et al. 1992).

Despite of small clone size, aspen often occurs in larger patches of trees (Fig. 2). In continuous old-growth spatial aggregation occurred up to 500 meters, of which half can be explained by site type (I).

The patchy occurrence of aspen is likely not resulting from clone structure, because of the small clone size (II). More probably it originates from past disturbance events or then biotope variation that has earlier been noticed to affect patchy existence already in seedlings of *P. tremula* (deChantal et al. 2005). Limited dispersal of pollen or seed may also effect to spatial aggregation of a species.

Seedling emergence and survival
The proportion of microsites with at least one seedling emerging was 56% (III) and 88% (IV) on mineral soil, 8% on humus (IV), and 50% on CWD. Higher proportion of microsites with at least one seedling occurred when natural precipitation was higher (III) as well as when microsites were watered (III). The quickest rate of emergence and the largest numbers of seedlings were achieved in the sowing in which the microsites were watered and covered, when ca 80% of the seedlings emerged in three days (III, IV). The average number of seedlings per microsite was 4.4 (range 0-39, III) and 12 (range 0-53, IV) on mineral soil. The number of seedlings varied significantly between blocks (III, IV) and between sowing times (more seedlings on 2nd sowing). The survival of seedlings was 10% (III) and 20% (IV) after the first growing season and 45% over winter (III, IV). Maximum survival was achieved with watering and sowing shelter (III, IV).

Populus tremula seeds are reported to lose their germinability shortly after dispersal (Reim 1929, Moss 1938, Børset 1960). Results from studies III and IV indicate that germination may however happen even after two months, which is comparable to the results of Zasada et al. (1983). Since the germination process of aspen is rapid (Børset 1954), it seems likely that the weather conditions right before the sowing are as important as after it. Even a one day's difference in sowing time affected the number of seedlings, which highlightes the importance of weather. Seedling emergence was quick when there was adequate moisture. In addition to moisture, the significant effects of block and its interaction with sowing time and sowing shelter indicated that the emergence depends also on the seedbed conditions. Differences in seedling emergence among various seedbeds suggest that *P. tremula* has specific seedbed requirements as does *P. tremuloides* (Kay 1993). The great importance of biotope and substrate in seedling emergence of *P. tremula* was also noticed by deChantal et al. (2005).

Overall survival of seedlings was low, which is expected for a species that produces large numbers of small seeds (MacArthur 1962). Since the aspen seed has no endosperm (Børset 1954), the quality of the seedbed is critical, as the seedling is soon dependent of external resources. This was seen as a significant effect of block on survival. The positive effects of watering and sowing shelter to survival and the interaction between watering and sowing shelter suggest that seedbed conditions and moisture are most important for the survival of aspen seedlings. The importance of adequate moisture for survival of *P. tremuloides* seedlings has been reported also in previous studies (McDonough 1979).

The average number of seedlings was doubled and the survival of the seedlings was elevated (27% compared to 18%) when the study site was burned. This could be due to increased amount of nutrients and higher soil pH (Ahlgren and Ahlgren 1960, Wells et al. 1979, Little and Ohmann 1988, Ericsson 1992) or removal of allelopathic effects following fire (Zackrisson and Nilsson 1992).

Crossings

In both years of the study interspecific crosses produced more seeds with higher quality than crosses within the European aspen. This effect was most distinct in the crosses between hybrid aspen mother and European aspen father. The number of seeds per flower was highest in maturation temperatures of 16-17°C. The number of germinated seeds increased with elevated seed maturation temperatures. The response to elevated temperature was different in different crosses. When temperature was elevated by 3-4°C, crosses including hybrid aspen had a greater increase in germinability than the *P. tremula x P. tremula* crosses (V).

Crossing European and hybrid aspen was easy. *P. tremula* and *P. tremuloides* are close relatives and belong to the same section of the genus, and generally crosses within sections occur more easily than between sections in the genus *Populus* (Villar et al. 1987). Interspecific crosses have traditionally been considered to be less fit than their parents (reviewed by Burke and Arnold 2001). However, the outcome much depends on the species and the environment (Arnold et al. 2001, Burke and Arnold 2001, Johnston et al. 2001), and recombinant phenotypes can even outperform their parents (Vila and D'Antonio 1998, Burke and Arnold 2001). The mechanisms leading to increased fitness may include the segregation of additive genetic factors and to a lesser degree epistasis (Burke and Arnold 2001). In general, maternal effects control much of the performance at the seed stage along with an important environmental effect. For instance, seed germination is almost entirely determined by maternal effects (Roach and Wulff 1987). The fitness of hybrids is also dependent on the level of hybridisation. Kirk et al. (2005) have found that early hybrid generations do better than natural hybrids, which have experienced intercrossing and back-crossing through several generations. This could explain the higher seed viability of backcross hybrids compared to F_2 hybrids in this experiment.

Conclusions

Long-term persistence of aspen in modern stands of old-growth is not ensured due to lack of disturbance and subsequent succession. Aspen reproduces successfully in managed forests, but the current ratio of living to dead mature trees shows that the volume of large-diameter dead aspen will decline in managed forest in the future. In order to maintain aspen populations in protected areas some restoration may be needed. Depending on the area prescribed burning could be used and/or sufficiently large gaps in the canopy should be created. As the area of protected areas is small in southern Finland, less than 1% (Anon. 2000), the role of management practices is great. In managed forests aspen should be favoured in intermediate cuttings, and aspen retention trees should be left in regeneration areas. These restoration actions should be concluded especially next to the protected forests, since aspen-associated species from protected forests have a possibility to colonize and disperse to these areas (see e.g. Hanski 2000).

Sexual reproduction of aspen may be more common than has previously been thought, as the results concerning the small size of aspen clones refer. Aspen can keep its old territories with root suckers and the rarer event of seedling establishment seems to be enough for bringing out the advantages of sexual reproduction. It has been shown that even rare sexual reproduction is advantageous for species reproducing mainly asexually (Green and Noakes 1995).

Seed and pollen production in aspen is efficient although it varies yearly, and it seems that the poor survival of seedlings is the restricting factor in successful sexual reproduction. When the right seedbed conditions and adequate moisture are available, sexual reproduction may take place. These conditions have been most often available after forest fire (Kay 1993) and thus, seedling establishment might become rarer in situation where forest fires are suppressed.

There is a possibility of gene flow from hybrid aspen to the European aspen, and in the light of this study it is more likely to happen in warmer climate. Because pollen travels further than seeds, the impact of hybrid aspen pollen flow has greater importance than seed flow. Furthermore, the ability to invade can evolve, and hybridisation between species can induce such evolution (Ellstrand and Schierenbeck 2000). If the genes or gene combinations of *P. tremuloides* that invade the gene pool of the European aspen through hybrid aspen are advantageous for survival and evolution, the hybrid aspen may benefit of changing climate.

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