

Reproductive Biology and Population Genetics
of Common Ash (*Fraxinus excelsior* L.)

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The Ash Yggdrasil

Friedrich Wilhelm Heine

Wagner, W.(1886). *Asgard and the gods*. London: Swan Sonnenschein, Le Bas & Lowrey. Page 27

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Abstract

Common Ash, *Fraxinus excelsior* L. (Oleaceae) is a wind-pollinated, wind-dispersed, deciduous tree, commonly occurring throughout Central and Northern Europe. The thesis addresses several questions concerning reproductive biology and factors influencing the genetic richness of ash stands. The breeding system is characterised in morphological terms as polygamous. However, individuals can be described as male, or seed producing hermaphrodites and females. The gender-related differences in vegetative growth were investigated on the branch level using dendrochronological techniques. The radial growth of male and female individuals turned out to be similar in a long-term perspective, but males invested more in building up their crown biomass. Genders responded also to different climatic variables. To determine the conditions for male gametophyte competition, a series of controlled pollinations were conducted and the pollen tube growth in naturally pollinated pistils was observed. As many other temperate trees, fertilization is delayed in *Fraxinus* and the pollen tube growth is arrested in the style. The results showed that the trait does not play any role in prezygotic selection in this species. The order of pollen grain arrival and, thus, flowering phenology was more important for paternity success than pollen tube growth performance. Furthermore, the functional breeding system of *F. excelsior* was examined. Hermaphrodite individuals were shown to have very low male fecundity and so could be classified as functionally female.

To test the effect of maternal flowering phenology on mating distances and genetic variability of their progeny nuclear microsatellite markers were employed on early and late flowering individuals. Additionally the influence of genetic population structure on the level of biparental inbreeding was discussed. Microsatellite markers were used also to examine the level of inbreeding and allelic richness in the commercial stock of ash seed available for reforestation. Results from both works confirmed that neutral genetic variation in *Fraxinus excelsior* is high and that the level of inbreeding is very low the allelic diversity of an individual seed family may vary depending on flowering synchrony of neighbouring trees. Collection of seed from limited number of trees should be avoided; also the genetic diversity and structure of seed sources should be assessed when obtaining planting material for forest restoration.

Keywords: *Fraxinus excelsior*; delayed fertilization, functional dioecy, gender-related growth difference, gene-flow, inbreeding, non random mating, seed bank, tree-rings, wind-pollination

List of papers

The thesis is based on the following publications and manuscripts. They are referred to in the text by the corresponding Roman numbers.

I. Bochenek G. M., Eriksen B. (2010). Annual growth of male and female individuals of the Common Ash (*Fraxinus excelsior* L.). *Plant Ecology & Diversity* 3:47 – 57.

II. Bochenek G. M., Eriksen B. (2011) First come, first served – delayed fertilization does not enhance pollen competition in a wind-pollinated tree, *Fraxinus excelsior* L. (*Oleaceae*). *International Journal of Plant Sciences* 172: 60-69.

III. Wallander E., Rendell S., Eriksen B., **Bochenek G.M.** and Boshier D. (manuscript) Paternal success and inbreeding depression in *Fraxinus excelsior* L. (*Oleaceae*).

IV. Bochenek G. M., Wallander E, Eriksen B. (manuscript) Seasonal variation in genetic composition of seeds in Common Ash (*Fraxinus excelsior* L.).

V. Bochenek G. M., Granberg M., Wallander E., Eriksen B. (manuscript) Genetic variation in Swedish seed bank of Common ash (*Fraxinus excelsior* L) compared to the population of seed origin.

Moim rodzicom

To my parents

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Author contribution to individual papers

In all papers the lead author had the main responsibility for completing and writing the paper, but all authors contributed in the writing process.

- I. I had the main responsibility for all parts of the paper.
- II. I had the main responsibility for all parts of the paper.
- III. My responsibility was to statistically analyze data and contribute to the interpretation of the results.
- IV. My responsibility was to genotype seeds, conduct the paternity analyzes, statistically analyze and interpret the results and write the paper. EW contributed the genetic analyzes of parental populations.
- V. MG collected and genotyped seed bank material and participated in data analysis. EW contributed the genetic analyzes of reference populations. I was mostly responsible for statistical work, compilation and interpretation of the data.

To all the papers BE contributed with field and laboratory work assistance and otherwise her ideas, knowledge and great engagement in the writing process.

Introduction

Consequences of a wind-pollination syndrome

Differences between biotic and abiotic pollination

No less than 10% of the angiosperms are wind-pollinated, and this mode of pollination has evolved at least 65 times from biotically pollinated ancestors (Friedman and Barrett 2009). The main characteristic of the wind pollination syndrome is presented in Table 1, although there are many species that do not conform to all of these associations. It is not always clear if a given character evolved as an adaptation to wind-pollination (anemophily) or rather promoted a transition from biotic to abiotic pollination. For example, traits such as small, unisexual flowers may have facilitated a shift towards wind-pollination (Friedman and Barrett 2008). Since Charles Darwin's times, many authors addressed questions about selective mechanisms driving the evolution of wind-pollination and evolutionary pathways that are involved in the transition (Friedman and Barrett 2009). This work presents some consequences of abiotic propagule dispersal to the biology and genetics of a wind-pollinated, European forest tree, *Fraxinus excelsior* (Oleaceae).

Table 1. Traits associated with wind and biotic pollination, modified from Culley et al. 2002.

Trait	Wind pollination	Biotic pollination
Stigmas	feathery	simple
Pollen:ovule ratio	high	low
Pollen diameter	10–50 um	highly variable (often >60 um)
Pollen ornamentation	smooth	often elaborate
Stamen filaments	long	variable
Nectaries	absent or reduced	present
Fragrance	absent or reduced	present
Perianth	absent or reduced	showy
Ovule number	few in numerous flowers	numerous in few flowers
Flower type	usually unisexual	usually bisexual
Inflorescence structure	pendulous , catkin-like	variable
Inflorescence position	held away from vegetation	variable
Surrounding vegetation	open	open to closed
Plant density	moderate to high	low to high
Phenology	synchronous flowering	variable

Short description of Common ash

Fraxinus excelsior (Oleaceae) is a wind-pollinated and wind dispersed deciduous tree distributed across Europe from the Atlantic and the northern Mediterranean coasts into



Fig. 1. Twig of *Fraxinus excelsior* with young leaves and female infructescences. Photo: B. Eriksen

European Russia and the Caucasus (roughly between 40° and 62° N). It is a post-pioneer species, growing best on nutrient- and base-rich soils, preferably fresh to moist, although it tolerates a much wider range of soil types (Wardle 1961, Kerr 1995). It can easily be recognized by its black bud-scales and compound leaves (Fig. 1). In early spring, before leafing starts, ash produces a large number of calyx- and corolla-less flowers gathered in short panicles. The syncarpous ovary contains four ovules, but usually only one is fertilized, and hence most fruits are one-seeded samaras.

Ash wood is hard and durable, and was widely used for building and wood-working. It was also a good source of fire wood. Because of its elasticity ashwood was a traditional material for spears, tools handles, various machine parts, sport equipment and early aeroplanes. Nowadays it is still praised for floor and furniture. According to the Nordic mythology, the World Tree, Yggdrasil, was an ash.

Polygamous breeding system

The breeding system of *F. excelsior* has been described as polygamous (Fig. 2). The flowers are unisexual, male with only two stamens, female with only a pistil, or bisexual (hermaphrodite) with both pistil and stamens. Four main types of inflorescences can be recognized: A, male inflorescences consisting only or mostly of staminate flowers; B, mixed male and hermaphrodite inflorescences; C, hermaphrodite inflorescences consisting mainly of flowers having fully developed both pistils and stamens; and D, female inflorescences consisting of flowers with rudimentary or no stamens (Wallander 2001b). Pure male and pure female flowers, except in very rare cases, are not seen

together in an inflorescence (Binggeli and Power 1991). The gender phenotype of a tree is described based on predominant type of inflorescences (see paper III). The gender is not likely to change during the lifetime of a tree (Larsen 1945, Picard 1982, Wallander 2001b) though fluctuations in "maleness" or "femaleness" of hermaphrodites are frequent. A clear distinction could be made between male and male-biased hermaphrodites on the one hand, and balanced hermaphrodites, female-biased hermaphrodites and females on the other hand (Tal 2006). Hermaphrodite individuals are protogynous and self-fertile (Morand-Prieur et al. 2003, FRAXIGEN 2005).

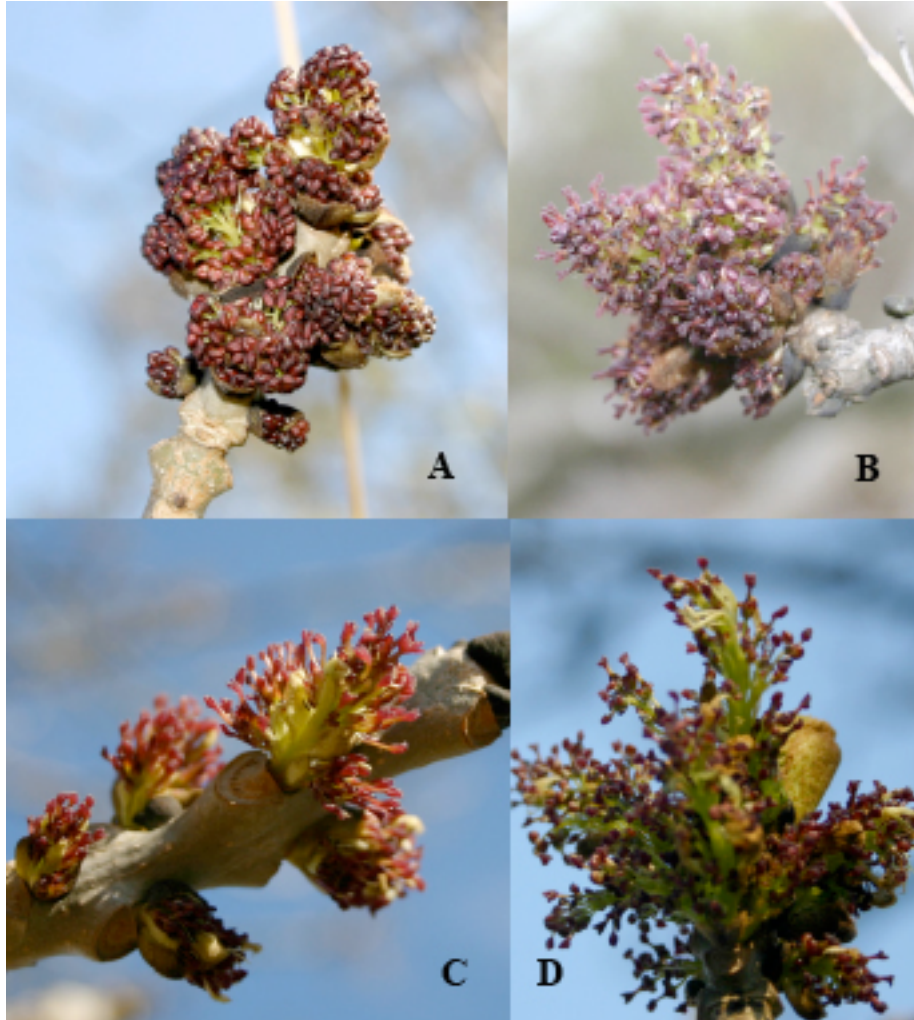


Figure 2. Inflorescences of *Fraxinus excelsior*. A, Male inflorescence with staminate flowers in dense clusters; B, D Hermaphrodite inflorescences of varying maleness; C, female inflorescence composed of pistillate flowers in loose clusters.

Breeding systems involving gender separation, especially monoecy and dioecy, are frequently found at temperate latitudes. The plant characteristics, which are most strongly correlated with gender separation, are abiotic pollination, small and inconspicuous

flowers, woody habitats and fleshly fruits (Vamosi et al. 2003). The ecological mechanism invoked to explain gender separation are for example avoidance of inbreeding, sexual selection, optimization of resource allocation, mechanism favouring massive pollen and/or fruit production etc. (Bawa 1980, Skogsmyr and Lankinen 2002, Barrett 2010).

Ancestral character states in *Fraxinus* comprise lifeform as shrubs or small trees and bisexual, entomophilous flowers. From them two main breeding systems, androdioecy (in section *Ornus*) and polygamy (in section *Fraxinus*) evolved. The transition to dioecy occurred independently at least three times (Wallander 2008). In reality however, morphologically androdioecious or polygamous species may be functionally dioecious (e.g. Verdu et al. 2004, paper III). According to Wallander (2001, 2008) a demand for increased pollen production and need of synchronization of male and female phases to facilitate pollination efficiency, may play a major role as driving mechanism for transition to anemophily. The selection pressure for these characters is particularly strong in wind pollinated species.

The mechanism of sex inheritance in *Fraxinus* is unknown. However, Tal (2006) reported that half of the *Fraxinus excelsior* trees in a stand were purely male (or had very few hermaphrodite flowers) and the other half ranged from predominantly male to purely female trees. Also FRAXIGEN (2005) studies confirmed that the proportion of male and pistillate trees is more or less equal and stable across localities. The observation may justify the speculation by Wallander (2001) that transition between a hermaphroditic and a polygamous system involves two events: loss of female function leading to differentiation of males, and gradual decrease of male function in pistillate individuals resulting in a gradient of forms varying between truly hermaphroditic to pure females. The presence of individuals with mixed gender (male and female/hermaphrodite) on different branches (although not monoecious *sensu stricto*) shows that the whole system is rather labile.

Role of hermaphrodites in the population

The hermaphrodite individuals of *Fraxinus excelsior* are self fertile (Morand et al. 2003, paper III). However the number of selfed seeds produced varies between gender morphs (Tal 2006).

Simultaneous pollination with mixed male and hermaphrodite pollen nearly always resulted in seeds sired by male fathers (Morand et al 2003, paper II). The germination rate and vigor of hermaphrodite pollen is lower when compared to that of male pollen, which also shown by *in vitro* tests.

In 2003 pollen from 5 males and 4 hermaphrodite trees was collected and germinated *in vitro* on microscope slides according to the procedure described in Wallander (2001). There were four replicates for each individual. After 24 hours microscope slides with pollen were fixed and the number of germinated and ungerminated grains was scored in a field of view of a binocular, in 5-8 places for each slide. Additionally 10 straight and unbroken pollen tubes were measured per slide. Average germination of male pollen was 33.7 % (SE 1.3) and for hermaphrodite 14.3 %.(SE 1.1). The difference between

phenotypic types were highly significant ($F_{1,214}=217.5$ $p<0.001$ GLM with nested design). The germinability scored by Wallander (2001) was almost twice as high but the difference between male and hermaphrodite was similar in both tests. The disparity between tests may result from different ways of distributing growth medium on the microscope slide (when the layer is thin, the medium dries a little and the concentration of nutrients increases). The average pollen tube length was 109 (SE 1.5) units for male pollen and 101 (SE 1.9) units for hermaphrodites ($F_{1,241}= 15.3$ $p<0.001$) and was not correlated with germinability ($p = 0.41$ Spearman's test). It is worth noting that pollen of one of the trees classified as "pure hermaphrodite" did not perform worse than that of male trees in neither germinability, nor pollen tube growth.

Hermaphrodite and female inflorescences include fewer flowers (80-300) than male inflorescences (200-400, but up to 700 were noted; FRAXIGEN 2005, Tal 2006). Hermaphrodite flowers are protogynous. Wallander (2001) observed that the gap between the male and hermaphrodite phase lasted about a week depending on temperature. Male trees usually started to shed pollen before hermaphrodites. Because the first pollen arriving on a stigma has an advantage over those arriving later (paper II) it is expected that the siring success of hermaphrodites in the population is very low and they can be classified as functionally female (paper III). The female function does not depend on the sexual morphotype of hermaphrodites (paper III)

The observed fecundity of hermaphroditic individuals can increase when they become frequent in a stand. In morphologically androdioecious, insect pollinated *F. ornus*, hermaphrodites sired about 30% seeds in natural populations (Verdu et al. 2006). In a mixed *F. excelsior* x *F. angustifolia* stand with very low frequency of male individuals, Gerard et al (2006) detected no effect of tree sex on male fertility. Such situation may occur in typical *F. excelsior* stand in years of low, overall flowering intensity, when the density of competing male pollen is low (FRAXIGEN 2005). The male function of hermaphrodites may also play an important role in isolated trees and in marginal habitats.

Resource allocation and secondary sexual differentiation

Individuals of sexually dimorphic plant species may exhibit gender-related differences in growth rate, survival, physiology, flowering frequency, and/or other life-history traits (reviewed by Obeso 2002; see also Wheelwright and Logan 2004; Cornelissen and Stiling 2005; Dudley 2006; Montesinos et al. 2006; Verdú et al. 2007) due to non-equal costs generated by male and female function. As a general rule, growing fruits require large quantities of energy to mature, whereas pollen production is much less expensive (Lovett Doust and Lovett Doust 1988; Delph 1999). Indeed, the majority of observations show that females of woody species tend to be smaller than males (Obeso 2002). On the other hand, it may be expected that males of wind-pollinated species invest relatively heavily in pollen production. As wind is virtually never saturated as a pollen vector, male reproductive success should be positively correlated with the amount of pollen produced. Additionally, the possible gain in fitness achieved from increased investment in seed production might be limited by saturation of the area in which seeds are dispersed. Therefore, it can be expected that the difference between male and female investment to reproduction is similar in wind-pollinated organisms, or that males invest more. The issue can be studied in two ways: directly, by comparing the biomass of male flower and fruits,

which is very difficult in full grown trees, or indirectly by measuring the decrease in growth parameters. One of the drawbacks of indirect method is the strong correlation between vegetative growth, reproduction and climate. Hence it is sometimes extremely difficult to partition a physiological answer to environmental conditions from the result of a trade-off between reproductive investments and vegetative growth (e.g. Knops et al. 2007). The second problem is related to the possible differences in physiology between genders (see e.g. Dawson and Ehleringer 1993, Xu et al. 2008) allowing individuals to compensate for high reproductive costs when water and nutrients are not limited (Dudley 2006, Nunez et al. 2008).



Fig 3. Female (at the left) and male (at the right) branches in early spring. Male trees have more robust shoots.

In a German population of *Fraxinus excelsior* Tal (2006) noticed that males differed from seed producing individuals in size (particularly height), twig morphology and flowering intensity and frequency. In contrast Wallander (2001) found no significant difference in frequency of flowering in genders in a Swedish population. The investigation of growth parameters presented in paper I (also in Sweden) showed that there is no difference in long-time radial growth between genders, but males do have more robust twigs and invest more biomass into building up their crowns (Fig. 3). Female trees suffered more from drought and frost, which indicates that they are more affected by high costs of reproduction. It is apparent that the genders have different strategies of coping with the reproductive investments but the actual quantitative differences in energy devoted to that process, particularly in the long term, remain unclear.

Masting and flowering synchrony between seasons

Wind-pollinated plants vary extensively in seed production over time (Kelly and Sork 2002). The episodic production of abundant seed crops, often synchronous over a large geographic scale, is known as masting (Silvertown 1980, Koenig and Knops 1998).

A few alternative, but not mutually exclusive, hypotheses were put forward to explain the phenomenon: synchronization by weather (Kelly and Sork 2002), predator satiation theory (Silvertown 1980, Kelly et al. 2000), higher pollination efficiency of synchronized individuals and resource budget model (e.g. Masaka and Maguchi 2001). In a recent work, Tachiki and Iwasa (2010) combined the latter three with a fourth factor, seed bank survival, and were able to explain the conditions necessary for flowering synchrony to evolve.

Flowering intensity and fruit set of *F. excelsior* shows annual fluctuations typical for masting trees although synchronization is not complete (Tapper 1992, Wallander 2001a). Autocorrelation analyzes of 33 years data series from eight forest trees (including *F. excelsior*) in southern Jutland showed a lack of any regular cyclicality of pollen accumulation rates (Nielsen et al. 2010). The Nielsen et al. (2010) analyzes and Swedish investigations of pollen series (Dahl and Wallander [Unpublished manuscript]) and seed production (Tapper 1996) proved that climatic factors play the leading role in regulating the flowering intensity in *Fraxinus excelsior*. However, observations of individual trees revealed that after a year or two of intensive fruit set some maternal trees did not flower (Tal 2006, also GB personal observation). It indicates resource limitation and inhibition of bud development triggered by reproduction (see paper I). It cannot be completely rejected that seed consumption by caterpillars and rodents has a role in stabilizing ash masting behavior (Gardner 1977, Tachiki and Iwasa 2010). *Pseudargyrotoza conwagana* (Lepidoptera, Tortricidae) has been identified as a major seed predator of Ash in Sweden (Tapper 1992; Eriksen, unpublished data), Britain (references in Harmer 1994) and Slovakia (Kelbel 1997). On average, 43% of the seeds are destroyed by the predator (Eriksen, unpublished data). This is in line with figures reported in other studies (9–66%, references in Harmer 1994).

Flowering synchrony during the flowering season

In any given *Fraxinus excelsior* population, individual variation in vegetative and reproductive phenology is observed. Based on seven years of photographic documentation of a forest canopy, four phenotypic groups of *Fraxinus excelsior* has been distinguished (Falinski and Pawlaczyk 1995). They are A, trees which develop early in spring and finish their vegetation period early in autumn; B, trees which develop early and finish their growing season late; C, trees which develop late and finish their vegetation period late; and D, trees which develop late and finish their growing season early. This behaviour seems to be repetitive and consistent over years. The degree of synchronization depends on climatic conditions and, therefore, varies between years and geographic locations (Dahl and Wallander [unpublished manuscript], FRAXIGEN 2005, Tal 2006). It is expected that individuals in a population with a low flowering synchrony may have a lower reproductive success and lower outcrossing rate (Schmitt 1983, Fox 2003). However, early flowering of some maternal trees may increase inflow of genes by receiving pollen from distant sources (paper IV).

Male flowering asynchrony and gene flow

Flowering phenology of pollen-producing individuals may determine their fecundity and the direction of gene flow in hybrid zones (Gerard et al. 2006, Salvini et al. 2009). Gerard et al. (2006) showed that in the mixing zone between two *Fraxinus* species, the late flowering trees contributed very little to the pollen cloud. On the other hand, siring success of very early individuals can be reduced by spring frost. The two factors may stabilize the flowering phenology of a stand efficiently adapting it to local climatic conditions (paper II).

Delayed fertilization –it is connected with pollination syndrome?

In contrast to most angiosperms in which fertilization occurs 1 or 2 days after pollination, there are some groups of plants in which fertilization is delayed from 4 days to more than 1 year (Benson 1894, Sogo and Tobe 2006). During the time of stigma receptivity, ovule(s) are not fully developed and, as a consequence, pollen tubes lose their directional growth in the style and/or their growth is arrested. It has been proposed that delayed fertilization increases competition among male gametophytes by providing a ‘‘fair start’’ to pollen grains deposited on the stigma at different times (Willson and Burley 1983, Dahl and Fredrikson 1996, Boavida et al. 1999, Sogo and Tobe 2006, 2008). The results presented in paper II question this explanation. Paternity tests revealed that, in *Fraxinus excelsior* pollen applied first in the experimental pollinations had an overwhelming advantage over pollen applied 24 h later although fertilization occurred no sooner than after 3 weeks.

A fraction of fruits contains two seeds (average proportion in the populations studied is 1%, Eriksen unpublished data), rarely three. Tal (2006) observed a few cases when two pollen tubes entered simultaneously into embryo sacks. Paternity analyzes conducted in this German seed orchard showed that well over 70% of the twin seeds were sired by different individuals (Hebel et al. 2007). In none of the pistils investigated in this study (paper II) such situation was detected.

The advantage of the first arriving pollen may be a trait present also in other species with delayed fertilization (for discussion see paper II).

Delayed fertilization is common among temperate, wind pollinated trees, particularly in order Fagales (reviewed by Sogo and Tobe 2006). However, in desert and parasitic plants producing a single, large flower and a number of other insect-pollinated species (e.g. Orchids, Rhododendrons) a gap between pollination and fertilization also occurs (Sogo and Tobe 2006 and references there). A preliminary investigation of ovule development in insect-pollinated *Fraxinus ornus* showed that ovules contain mature megagametophytes while the stigmas are still receptive (Wallander 2001). Also the ovule in *Olea europaea* is mature at the moment of stigma exposition, and fertilization occurs within 5 days of receptivity (Ateyyeh et al. 2000, Cuevas and Polito 2004). It is possible that delayed ovule maturation, and, consequently, fertilization is connected to cost of reproduction, particularly if flowering is short and intensive and take place in the season when photosynthetic activity is low and/or resources scarce (see paper II). This situation is particularly common among temperate, woody, wind-pollinated species. They tend to flower before leaf development in order to avoid that the canopy becomes an obstacle for pollen movement but at a time with little resources needed for growing offspring. However, it will take further investigations to securely state that this is the background for evolution of the trait.

Population genetic structure and biodiversity

Use of microsatellite markers

Microsatellites are tandem repeats of short sequence motifs widely used as genetic markers. They have comparatively high mutation rates, typically assumed to be 10^{-3} events per locus per generation, but varying between loci and species (reviewed by Balloux and Lugon-Moulin 2002). They are suitable for multiple population genetic applications as parentage analyses for quantification of gene flow, estimation of genetic differentiation within and among populations, assessment of genetic structure of populations, reconstruction of population history and phylogeography, etc. (e.g. Beaumont and Bruford 1999, Heuertz et al. 2004, Vekemans and Hardy 2004, Tollefsrud et al. 2009). One of the main problems associated with microsatellite analyses is an occurrence of null alleles, i.e., non-amplification of certain alleles, usually due to mutation within the primer sites (Beaumont and Bruford 1999, Harbourne et al. 2005). When microsatellite regions do not amplify, individuals carrying these null-alleles appear as homozygotes instead of heterozygotes. Consequently, population genetic structure is misinterpreted. Microsatellites are also considered to be selectively neutral, but there is evidence showing that they can be responsible for protein binding or in some cases linked to coding regions (Beaumont and Bruford 1999). If so, Hardy-Weinberg equilibrium should not be expected and conclusions drawn based on neutrality would prove wrong.

For *Fraxinus excelsior* a number of chloroplast and nuclear microsatellite markers have been described by Brachet et al. (1999), Lefort et al. (1999) and Harbourne et al. (2005).

Pollen and seeds dispersal

Gene flow pattern via pollen and seed is one of the most important factors that determine the spatial genetic structure of plant populations (Loveless and Hamrick 1984; Adams *et al* 1992).

The distance of seeds dispersal is usually limited, unless they are propagated by birds or mammals. Studies of the fate of marked palm seeds showed that rodents steal gathered seed from each other and, hence, distributing the plant over an area far exceeding the range of an individual animal (Xiao et al. 2006). In *Fraxinus excelsior* seed flow deduced from a model for a Romanian population was fairly restricted ($\sigma_s < 14$ m) comparing to the σ_p for pollen flow, estimated to be between 70 and 140 m. (Heuertz et al. 2003). Although patterns of seeds dispersal are both important for biodiversity and interesting from a population genetic point of view they are not concern of this work.

Gene dispersal via pollen is usually estimated to be extensive in wind pollinated trees. A number of authors reported that a high proportion of sires were coming from outside the investigated area (e.g. Dow and Ashly 1998, Heuerts et al. 2003, Hebel et al. 2006). The shape of the pollen dispersal kernel in wind-pollinated species is often described as fat-tailed or leptokurtic. In *Fraxinus mandshurica* Goto et al. (2006) detected evidence of

bipartite pollen flow, with 26% of pollen dispersing over very short distances ($r = 7.2$ m) and the remainder migrating over longer distances ($r = 209$ m).

Compared to insect-pollinated species, the correlated paternity in anemophilous species is expected to be low and effective number of fathers (N_{ep}) high due to the highly stochastic mode of pollen transport by wind. For example in insect-pollinated *Sorbus torminalis* correlated paternity among seeds belonging to the same fruit was 30% and that among seeds belonging to different fruits 14% (Oddou-Muratorio et al. 2006). In two Swedish ash stands the average value of correlated paternity within seed families seems to be similar (12.5%), but the true value is actually lower when undetermined sires are taken into account (paper IV). The effective number of fathers has not yet been estimated for *F. excelsior*, but when examples from other species are compiled a general trend can be shown (but notice that N_{ep} varies strongly depending on the method used for estimation). In insect-pollinated *F. ornus* Verdu et al. (2006) estimated the effective number of fathers per mother to be between 2.93 to 4.95. In *Prunus mahaleb*, also insect-pollinated, N_{ep} varied between 1.6 and 5.5 (Garcia et al. 2005, calculated by Nielsen's direct method). On the other hand for wind-pollinated *Quercus*, the estimation of N_{ep} oscillated between 8-10 (but up to 49 when calculated by the direct method) (e.g. Craft and Ashley 2010, Pakkad et al. 2008). In wind-pollinated *Ulmus glabra* N_{ep} of 31 and 34 was reported (Nielsen, 2010). Low numbers of full-sibs among progeny decreases the risk of biparental inbreeding, particularly if the next generation is established from seed cohorts or are spread over short distances around maternal trees.

Landscape fragmentation can greatly influence the pattern of pollen flow and effective number of sires. In a fragmented population of Oaks Sork et al. (2002) detected very low number of effective fathers and high risk of genetic isolation. On the other hand, Bacles and Ennos (2008) showed that deforestation (opening) of the landscape may increase long distance pollen flow in *Fraxinus excelsior*. Their spatial analysis suggested that 85% of detected pollination occurred within 100 m, and 15% spread between 300 and 1900m from the source.

Factors influencing population genetic diversity and structure of wind pollinated tree species

Genetic variation of a population consists of the sum of all genetic variation among individuals within a population, and is measured by parameters such as allelic richness (A) and gene diversity (H_e), referred to also as expected heterozygosity (Young et al. 1996). Under the neutral theory, genetic variation depends on fundamental factors such as random genetic drift, migration, effective population size, and mutation rates. Populations of the same effective size can radically differ in level of genetic variation because founder effects or bottle necks in the past affected the sample of alleles. Effective population size can be reduced by spatial clustering resulting from environmental heterogeneity, manner of seed dispersal or due to assortative mating. In wind pollinated trees important reasons of non-random mating may be flowering asynchrony (Gerard et al. 2006, Salvini et al. 2009, see also paper IV), although even a leptokurtic mode of pollen dispersal can introduce significant isolation of mates by distance (e.g. Dutech et al. 2005, Gerard et al. 2006, de-Lucas et al. 2008). Spatial or temporal subdivisions of population do not influence the allelic frequency (in the population as whole) but enhances biparental inbreeding and leads to decreased heterozygosity (e.g. Weis et al.

2005). However, genetic drift in smaller subunits is higher and, therefore, allelic richness may also be affected in future generations. Erosion of both heterozygosity and allelic richness can be counteracted by gene flow from outside a deme/population (immigration) and, in this respect, wind pollinated species are extreme because of long distance pollen travel.

Among other factors that may affect gene flow and, hence, influence population diversity and genetic structure of wind-pollinated trees, are selfing rate, differences in male fertility and size of individual trees. For example de-Lucas et al. (2008) found high correlation between mating system parameters and tree diameter, height and crown shape in maritime pine (*Pinus pinaster*). Population density does not only influence the number of possible mates and the diversity of the pollen cloud, but may also influence dispersal of propagules. Particularly presence of different tree species in the canopy may restrict pollen dispersal and cause partial reproductive isolation, converting the woodland into a series of subpopulations (Bacilieri et al. 1994). At a larger scale, also wind direction can play a role. In a contact zone between two previously isolated populations of Ponderosa pine gene exchange via pollen and seeds was larger with the dominating wind direction when against it (Latta and Mitton 1999). Prevailing westerly winds inhibiting movement of pollen towards the west was proposed by Sutherland et al. (2010) as a cause of an observed geographical gradient in allelic richness in a British ash populations.

Important factors, that admittedly do not influence genetic diversity as such, but have great effect on obtained results, and, therefore, should be taken into consideration are the spatial and temporal scales of an investigation (e.g. Anderson et al. 2010) and sample size (e.g. Miyamoto et al. 2008).

Genetic structure of ash populations

Fraxinus excelsior shows high level of variation within populations and low differentiation between stands (Heuertz et al. 2001, Morand et al. 2002, Heuertz et al. 2004, Hebel et al. 2006, Ferrazzini et al. 2007, Sutherland et al. 2010), a structure typical for wind pollinated forest trees (Dow and Ashley 1998, Smouse and Sork 2004, Vekemans and Hardy 2004). Usually more than 12 alleles on average can be found in a small sample (N=18-30 individuals) per locus and stand (12.4 for six loci in Bulgaria (Heuertz et al. 2001); 12.2 for a corresponding sample in northern Italy, (Ferrazzini et al. 2007); 12.35 for five loci in western and central Europe, (Heuertz et al. 2004); 12.8 for four loci (least polymorphic locus F16 not included) in Sweden, FRAXIGEN 2005, see also paper V). Average estimated H_e varies from 0.731 in Bulgarian stands (Heuertz et al. 2001) to 0.840 in northern Italy (Ferrazzini et al. 2007). However, estimates for individual localities can deviate from those values, particularly for small and isolated stands.

Fraxinus excelsior in Europe forms a single, large deme extending throughout central and western Europe and the British Isles, whereas populations from south-eastern Europe shows more differentiation (Heuertz et al. 2004). The patterns reflect routes of post-glacial recolonization and extensive gene flow between forests in Central and Western Europe. In Sweden the situation is less clear, with populations showing more similarity to

those in southeastern Europe than those in Western and Central Europe. However, mixing of western and south-eastern gene pools seems to have occurred in southern Scandinavia and can be detected in some stands in southern Sweden (FRAXIGEN). A steady decrease of allelic richness and heterozygosity was observed from east to west and with latitude in Britain (Sutherland et al.). Genetic diversity is reduced also in isolated habitats on the northern margins of the species' distribution in Scandinavia (Holtken et al. 2003, FRAXIGEN 2005).

Several studies detected a low but consistent level of inbreeding within ash stands. For example Heuertz et al. (2001) found mean $F_{IS} = 0.014 \pm 0.045$, Sutherland et al. (2010) $F_{IS} = 0.182$ for British populations, and Morand et al. (2002) estimated F_{IS} to be as high as 0.292 in some French stands. The observed values were explained by high null allele frequency, assortative mating and/or Wahlund effect, i.e., deficit of average heterozygosity resulting from obscure population subdivision.

The presence of non-amplifying alleles is inferred by high variation between F_{IS} estimations for different loci. In this study the relatively high proportion of null alleles was confirmed for locus F4 and F16 (paper IV) by the presence of "homozygote" loci among seed genotypes not agreeing with maternal "homozygote" loci. Non-Mendelian segregation was reported also for other loci, e.g., FEMSATL5 (Bacles and Ennos 2008). Therefore F_{IS} values calculated by Heuertz et al. (2004), after excluding locus F16, ranging from 0.034 on average for western sites to 0.006 in south-eastern Europe, are more realistic. The F_{IS} estimated with simultaneous correction for null alleles (see table 2 in paper IV) was 0.01 ± 0.007 and 0.005 ± 0.005 for two *F. excelsior* stands near Göteborg, Sweden. The inbreeding coefficient calculated by for three different Swedish ash stands was also low and indicated rather an excess of heterozygotes ($F_{IS} = -0.001 \pm 0.033$, Heuertz et al. 2004).

A possible explanation for the relatively high inbreeding rate observed in western European populations might be non-random mating due to phenological asynchrony inside stands. Time of flowering, its intensity and duration depends on thermal conditions (Remphrey 1989, Dahl and Wallander [unpublished manuscript]). Generally, mild and cool weather, not interrupted by frost, prolongs flowering, and trees start their anthesis one by one and there will typically be a time lag of a couple of days in between extreme phenotypes. On the other hand, when spring is late and warm, flowering is short and trees are more synchronized (Tal 2006). In 2002 and 2003, female and male phases showed much less overlap at the British and Romanian sites when compared to Swedish sites (FRAXIGEN). Also flowering duration recorded in Germany by Tal (2006) was longer than usually observed in the Göteborg area, Sweden (Wallander 2001, FRAXIGEN 2005). Separation of male and female phases enhances reception of pollen from distant sources, but may also promote biparental inbreeding, particularly when maternal trees flowering in the local peak of pollen shedding mate with a limited number of co-flowering neighbouring males (see paper IV).

Another explanation is that F_{IS} might be underestimated when a population consists of related individuals (cohorts of half-siblings). In such cases calculation of a corrected value of expected heterozygosity requires *a priori* knowledge of sample relatedness (DeGiorgio and Rosenberg 2009). Higher relatedness of sampled offspring (i.e. limited number of maternal families sampled) explained the lower estimation of inbreeding in

seedlings when compared to that of adult trees in the study by Morand et al. (2002) in a French *Fraxinus excelsior* population.

Adaptive variation

Microsatellite allele counts are often used to estimate genetic diversity for conservation purposes. The main drawback is that neutral markers indicate the historical and presents patterns of gene flow by seeds and pollen but do not necessary reflect variation in adaptive traits. It can be expected that in species with an extensive gene flow, spatial diversity of selective markers is comparably low. Yet, if progeny sired by distant individuals is not well adapted to local conditions it may be outcompeted by local phenotypes. Under strong selection the ecotypic or clinal variation across spatially heterogeneous environments can still evolve despite of extensive gene exchange, as it was observed for example in phenological traits of *Populus tremula* (Hall et al. 2007). Artificial selection for enhanced growth may result in loss of other traits and adaptations and consequently bring damage to individual trees when adverse conditions occur (Rehfeldt 1992). For example frost resistance in pine seems to be associated with fewer internodes produced and weak shoot elongation (Chuine et al. 2006). Still, little is known about environmental adaptations in *Fraxinus*. Most data available in the literature about responses to edaphic conditions in this species was obtained from seedlings or juvenile trees (e.g. Cundall et al. 2003). Some observed patterns are also unclear. Rüdinger et al. (2008) investigated the distribution of alcohol dehydrogenase allozymes among several ash stands. The authors did not find consistency in the correlation between allele distribution and the environmental gradient. They concluded that the main shaping processes are strong human pressure and seed transport by the river rather than selection forces.

Main threats for biodiversity

Timing of developmental events and their synchronization with seasonal conditions is critical for plant growth, survival and reproduction. Vegetative phenology and flowering time is, in many plants, determined by integration of multiple environmentally sensitive gene pathways. Novel combinations of important seasonal cues in projected future climates may alter plant responses with important consequences for plant life history (Wilczek et al. 2010). Besides vital consequences for timing and synchronization of flowering period, climate change confers one immediate danger for *Fraxinus excelsior*. The species has comparatively low drought tolerance. Particularly seed-producing individuals require good water availability to replenish nutrients acquired by developing seeds and maintain high growth (paper I). Repetitive early summer draughts may therefore weaken the trees and open pathways for parasitic infestations.

During recent years a decline of common ash populations has been observed, mainly due to infection by the ascomycete *Hymenoscyphus pseudoalbidus* or rather its anamorphic state *Chalara fraxinea* (Kowalski 2006, Bakys et al. 2009) and other fungal diseases. Symptoms include wilting and blackish discoloration of leaves, dieback of shoot, twig and branch and bark necrosis. Skovsgaard et al.(2010) noticed that dieback was more frequent on trees of average or below-average size, suggesting that individual tree

resistance decreased with decreasing growth potential or tree vigor. As the history of Dutch elm disease show, the spread of the pathogen may be the mayor threat to the demographic and genetic diversity of ash populations.



Fig. 4. Natural regrowth next to the certified seed stand at Säter, Västergötland. Photo: B. Eriksen.

Most of *Fraxinus excelsior* rejuvenation in Sweden is based on spontaneous regeneration and if seeds are used they are from natural local sources (Fig. 4). Currently genetic erosion is not much of a threat due to silviculture practices. However analyses of seed available on the Swedish market revealed that more extensive use of traded planting material can result in decreased allelic richness if planted in deforested areas with no local source of ash pollen (paper V). There are no clear guidelines for the method of seed harvesting. Therefore, an owner of a seed stand may collect the seed from a limited number of maternal trees while thinning the stand. The high relatedness of planting material may trigger bi-parental inbreeding in the next generation. There is also no practice to assess the genetic structure and diversity of seed sources. Choice of seed sources based only on growth performance does not assure that the collected planting material preserves genetic variability to the extent required for conservation purposes.

Little is know about adaptive diversity of deciduous trees and the extent of provenances for them. Some attempt to establish and asses the provenance system for ash was done for example by Hebel et al (2007) in Germany and Sutherland et al. (2010) for Great Britain. Both studies were based on neutral genetic variability and presence of private alleles. Yet, the use of foreign planting material is still customary; e.g. seed present on Swedish marked may originate from neighboring countries (paper V). An additional problem is

created by a common practice of planting seedlings at low stock densities, and other routines which maximize seed germination and seedling survival (see e.g. Madsen and Löf 2005). Such practices are not only costly but also reduce selection forces allowing for weaker or not optimally adapted individuals to reach reproductive age.

Thesis part

Background

In 2003 when I was introduced to work on *Fraxinus excelsior* research in the framework of the European project FRAXIGEN was already operating. As is often the case scientific work produces as many questions as answers. And it became my job to find answers some of those generated. Four main projects were formulated concerning: difference between trade-offs in growth and reproductive investment in pollen and seed bearing individuals, competitive ability of pollen produced by hermaphrodites, how far pollen can travel and the effect of phenotypic variation in flowering phenology of maternal trees. Some of these projects (as long distance pollen travel) met technical difficulties and had to be abandoned, other developed in quite unexpected directions.

The biggest benefit from FRAXIGEN studies for this work was an access to the genotype data of two ash stands, mapped and well studied in terms of gender distribution phenology, Delsjön and Eklanda (Göteborg, west coast of Sweden). Some flowers for paper II were collected from a third Göteborg stand, Masthugget, investigated previously by Wallander (2001). Part of the data for paper I was obtained from Västerås area. We used the fact that the flowering season in central and eastern Sweden starts later than in Göteborg area, to extend the time needed for the field work.

Because of the variety of topics covering different areas of the ecology of Common Ash, diverse techniques were applied. In all cases I was trying to find a simple approach to detect trends and to assess ongoing processes with lowest possible bias. The direct approach, in contrast to modelling techniques, usually lacks the error assessment and does not allow for parameter testing. Therefore drawing conclusion sometimes becomes “an educated guess”. On the other hand, solid data, without the bias due to model simplification, shows much better the complexity and details of observed phenomena. For description of the methods used, see individual papers.

What the thesis adds to the story

Paper I

The main objective was to compare male and female vegetative growth. Tree ring series revealed that there is no significant disparity in long time radial growth of different genders. But there are differences in year-to-year growth patterns, which could be related to climatic factors, as well as to flowering intensity. A trade-off between reproductive investment and vegetative growth was observed at the branch level in both genders. However, the costs were reduced due to the association of flower buds with the internodes and the number of previous-year leaves. Male individuals invested more of the

biomass into building up their crown; and they had more robust and longer shoots. These observations indicate that the tree architecture may be associated with a gender-specific strategy of coping with reproductive investments. Furthermore, it is plausible that male and seed-bearing individuals differ in physiological traits in *F. excelsior*.

Paper II

When starting the project we knew that hermaphrodite pollen has a lower germination rate and produces shorter pollen tubes *in vitro* than males. Soon it became apparent from studies by Morand et al. (2003) and from our pollination tests that hermaphrodite microgametophytes were completely outcompeted when they were applied on stigmas simultaneously with male pollen. We then turned our attention to what would happen if they were applied first. In *Fraxinus excelsior*, as in many other wind-pollinated temperate trees, ovule development is postponed and starts after stigmas have withered. It was proposed that this mechanism evolved to facilitate pollen tube competition by giving all arriving pollen a “fair start”. The stigma is receptive usually for 1-3 weeks (Wallander 2001, FRAXIGEN 2005) and pollen grains deposited early would otherwise get a head start. By postponing fertilization, the most vigorous microgametophyte should sire the seed regardless of its time of arrival. The paternity analyses showed however that the pollen applied 24 hours earlier had an overwhelming advantage over pollen applied later. Even if the first applied pollen was from a hermaphroditic tree. Microscopic observations confirmed that fertilization is performed by the first arriving pollen. Paternity analyses in other species, e.g., in a *Quercus* hybrid stand (Salvini et al. 2009) indicate that the phenomenon is not restricted to *Fraxinus* and that male gametophyte competition, postulated to occur based on pistil micrographs (e.g. Boavida et al. 1999), is restricted to early arriving pollen. The results call for revising the role of delayed fertilization.

Paper III (manuscript)

The breeding system of ash has been characterised in morphological terms as polygamous. Yet later pollen dispersal, lower pollen fertility and fewer flowers per inflorescence in hermaphrodites compared to males, as well as their lower germinability *in vitro* are factors speaking against a male function of hermaphrodites. Although hermaphrodite trees have been shown to be self-fertile, the extent of possible inbreeding depression was unknown. Controlled pollinations were carried out to produce outcrossed (sired by male or hermaphrodite pollen) and selfed (sired by hermaphrodite pollen) offspring. The resulting seeds were germinated and seedlings grown for one season in a nursery. Paternal success of hermaphrodites was about half that of males at the seed set stage and very low for selfing. The seeds produced, however, had similar weights and germinated equally well in all treatments and there were no differences in seedling growth after the first season either. Inbreeding depression was found to be very high at the first critical stage, during fertilisation and embryo development, but very low in the following stages (germination and first year seedling growth). Because of very low male fecundity of hermaphrodites they could be classified as functionally female.

Paper IV (manuscript)

Several authors (see *Genetic structure of Ash populations* in introduction) found a significant level of inbreeding in *Fraxinus excelsior* stands. They attribute the observed positive F_{IS} values to the Wahlund effect, alternatively to non-random mating. This work presents the results of a paternity investigation and estimation of allelic richness and relatedness of seeds produced by “early” and “late” flowering phenotypes. The overall genetic diversity of seeds was usually not departing from that which might be observed by chance. The increased number of seed sired by the close neighbours and consequently higher relatedness among seeds was observed in mothers starting to flower during the peak of pollen-shedding (the “late” trees) in one of the stands when the time lag between the phenotypes was largest. The lack of detectable patterns in the other stand could be partly explained by a relatively large number of seeds with unresolved paternity. There was no significantly larger inbreeding of the progeny in the stand having a strong genetic substructure than in the more homogenous stand, probably due to high pollen flow between groups of individuals within the population and from outside “clusters”. Yet the proportion of undetected sires did not have a significant effect on their genetic variation or relatedness. It appeared that factors such as relative position of an individual and flowering synchrony of nearby neighbours play a major role in shaping the genetic composition of progeny. The biparental inbreeding observed in a few individual cases results rather from temporal disassortative mating than the Wahlund effect *per se*, although the degree of relatedness of close neighbours does play a role in genetic seed composition. Additionally the high ratio of null alleles for two microsatellite loci (F4 and F16) was corroborated.

Paper V (manuscript)

This study was initiated as an attempt to investigate the genetic variation in the Swedish commercial seed-bank of *Fraxinus excelsior*. It turned out that only a few traders had ash seed in stock and that a limited number of available certified seed sources were exploited, one of which consisting of a single tree. This fact reflects the low interest in deciduous species compared to that given to a highly commercial species such as spruce. It also indicates that rejuvenation of ash is currently mostly relying on natural regeneration. Yet, posing that the situation was to change in the future and the seed bank were to be used, exploitation of resources as well as sampling techniques could be of importance for maintaining genetic diversity. The average allelic richness of seed samples was lower than that of mature trees in their parental population. However only 30 to 60% of the alleles were shared between seeds and adult trees indicating that the potential number of alleles is much higher, and the number of maternal trees used for seed harvesting should be higher than the twenty selected for this study. The results are not totally unexpected or very novel. However they signify that the whole system of seed sources certification and seed collection has to be redefined and based on a more scientific approach, or otherwise simply abandoned as not functional in the present shape.

Conclusions and future aims

Under evolutionary forces different traits become complex networks and feedback systems making a group of related individuals distinguishable as a “species”. Particularly a species’ pollination syndrome and mating system together with the mode of seed dispersal have great influence on all aspects of its ecology and genetic diversity. The results of this work contribute to a better understanding of these associations in *Fraxinus excelsior*.

Wind pollination favour high investment in pollen production. Hence differentiation in vegetative development between male and female individuals does not necessarily directly reflect differences in reproductive input, particularly long-term investment. An individual trade-off between vegetative growth and reproduction reduces input to the second one, when resources are scarce. However, seed producing individuals are affected when adverse conditions coincide with seed-nursing. Reduced resources can be replenished only in the following vegetative season. Therefore, the difference in vegetative growth among genders can be a consequence of disparities in resource requirement, and result in different responses to climatic and edaphic factors (I).

It is demonstrated that pollen arriving first on a stigma has an advantage, which indicates that the conditions for pollen tube competition are limited in Ash. It is possible that a few grains arrive at the same time, or that the first arriving pollen is defective, and in such cases the fastest growing one has a chance of siring success. In most cases, however, the flowering phenology of the trees will determine paternity. Consequently early-flowering males are expected to have a higher fecundity than late ones, unless their flowers are destroyed by frost. The phenomenon may determine the direction of gene flow, particularly in stands where flowering synchrony among individuals is low. The main function of delayed ovule maturation may be postponing the reproductive costs of developing embryos. If the hypothesis is correct, delayed fertilization should occur in species which flower before full leaf development or during a draught season, alternatively produce costly flower(s) during a short flowering season. It should, on the other hand, not be observed in species having flowers that emerge one by one during a lengthy flowering period (II).

Although hermaphrodites are male-fertile and self-compatible, low vigour of their pollen and protogyny favours pollination by males. In direct competition with male pollen, hermaphrodites will almost always lose but in absence of other competing pollen, hermaphrodites have a paternal reproductive capacity about half that of pure males.

Seeds from outcrossing and selfing germinate equally well and there are no apparent differences in first year seedling growth. However, ongoing field trials indicate that outcrossed offspring survive better and outgrow the selfed after some years under harsher field conditions where inbreeding depression is more readily expressed.

Under natural conditions, hermaphrodites appear to produce a fraction of the offspring in the population due to lower fertility (and early acting inbreeding depression in selfed seed). It is likely that the selfed offspring they do produce will be outcompeted later in life under conditions of natural regeneration. The breeding system of *F. excelsior* maybe termed subdioecious, although the fecundity of hermaphrodites through male function is

uncertain. Maintenance of male function in hermaphrodites may be beneficial during a colonization process. Further research is needed to better understand the role of hermaphrodites in the populations and their relative reproductive contribution through male and female function (III).

The flowering phenology of maternal trees, in relation to neighbouring males, can influence the effective number of sires and the genetic diversity of progeny. Although no profound differences between seeds of extreme phenotypes were observed in the studied populations, “early” individuals tend to receive pollen from early flowering males from an extensive area, and, therefore, the diversity of seeds within a family is high. However if the nearest male tree is also early flowering its siring success will be large due to little competition from other pollen. In contrast, “late” trees experience higher probability that their stigmas will be saturated by pollen from a limited amount of close neighbours resulting in slight biparental inbreeding. The effect might become stronger when the overlap between pistillate and male trees in a population is small. Observations from a larger number of stands from different climatic region is needed to confirm the observation and better understand association between pollen cloud diversity and population structure in this species (IV).

Currently, genetic erosion of ash populations due to low genetic diversity of planting material is no threat, mostly because rejuvenation is based on natural regeneration. However, clear guidelines are needed for seed collection methods to minimise the risk of biparental inbreeding in future generations of forest trees. High allelic richness computed for neutral loci may falsely indicate that even the samples from single individuals present a high genetic variability. Yet, if the sample includes a high proportion of half-sibs the loss of genetic variation due to genetic drift in future generation is possible. Therefore seed intended for planting non-commercial forest should have its relatedness assessed, or, harvesting from multiple maternal trees should be assured. Such routine may become particularly important when local population are removed due to deforestation or dieback. There is also no practice to assess the genetic structure and diversity of seed sources. The existing system of seed stands and orchards may work well for commercial timber production, but should not be uncritically applied for nature conservation purposes (V).

Some of the results in this thesis may be useful in forecasting the species’ response to climate change. Paper I showed that gender-specific difference in performance along environmental gradients may be observed not only on spatial scales but also temporally. Especially pistillate individuals are vulnerable to water deficit and frost. Repeating drought events may, therefore, greatly reduce their vegetative growth and change the sex ratio in the population due to higher female mortality. It is more difficult to predict the effect of increased suddenness of spring to the genetic structure of population. But it might be expected that a higher frequency of warm springs following a frosty winter may diminish the temporal variation in the pollen cloud and increase the paternal success of late flowering individuals. Therefore initially a lower bi-parental inbreeding might be expected.

During the last 10 years our knowledge about *Fraxinus excelsior* biology and genetics rapidly increased due to application of microsatellite markers and more advanced

statistical modelling in a number of studies. But there are still questions that remain to be answered. I would like to see two mechanisms explained: One is described in paper I and concerns the putative physiological difference between male and female individuals. The other is the genetic basis of sex determination and inheritance, knowledge that should facilitate demographic population studies. Other remaining issues are long-term paternal effects and the problem of an adaptation to local environments. All projects require many years of experiments, sampling and growing of numerous tree-saplings. However, development of tree genomics together with more powerful statistical tools to spatially analyze creates a possibility to speed-up the process.

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Svensk sammanfattning

Ask, *Fraxinus excelsior* L. (Oleaceae) är ett vindpollinerat, vindspritt och lövfällande träd som är vanligt förekommande i Central- och Nordeuropa. Denna avhandling ställer och besvarar frågor om artens reproduktionsbiologi och faktorer som har inflytande på den genetiska mångfalden hos askbestånd.

Fortplantningssystemet hos ask kan morfologiskt sett beskrivas som polygamt med flera kön. Individer är antingen hanar, honor eller hermafroditer. De senare två kategorierna har förmåga att sätta frukt och frö. Könsskillnader i vegetativ tillväxt studerades på grennivå med hjälp av dendrokronologiska tekniker. Stamtillväxten, mätt som ökning i radien, visade sig vara likartad för hanar och fröproducerande individer i ett längre tidsperspektiv, fast hanar investerar mera i uppbyggnad av sin kronas biomassa. Könen svarar också olika på klimatfaktorer som frost och torka där honor visar sig vara mer utsatta.

Både hanar och hermafroditer producerar pollen men frågan är hur de fungerar i konkurrens med varandra? Som hos många andra träd i tempererade skogar sker befruktningen hos ask inte direkt, utan pollenslangarna slutar gro i stiftet och hangameten avvaktar ett tag innan den växer sista biten ner till fröämnet. Detta har ibland tolkats som att arten utvecklat ett system för att låta hanar tävla med varandra och därmed gynna de bästa genkombinationerna och få de mest livsdugliga avkommorna. För att ta reda på om det finns möjligheter för konkurrens mellan hanliga gametofyter hos ask gjordes en serie kontrollerade pollinationer, och pollenslangstillväxten i stiftet hos naturligt pollinerade honblommor undersöktes. Resultatet visade att egenskapen inte spelar någon roll för prezygotisk selektion (urval före befruktningen ägt rum) hos arten. Den ordning pollen kommer i till honans märke har större inflytande på faderskapet än pollenslangens duglighet. Som följd av denna slutsats kan man också konstatera att trädens fenologi, alltså tidpunkten för deras blomning, kan ha betydelse för hur väl en individ lyckas som fader. Även det funktionella fortplantningssystemet studerades och det kunde visas att hermafroditiska individer har mycket låg hanlig fortplantningsförmåga och att de kan klassificeras som funktionella honor.

För att testa effekten av honträds fenologi på avståndet till hanliga partners och den genetiska variabiliteten av deras avkomma användes såkallade nukleära mikrosatelliter som markörer hos tidigt respektive sent blommande individer. Den genetiska populationsstrukturens effekt på andelen inavel som beror av mötet mellan gameter från genetiskt närstående individer (*biparental inbreeding*) diskuteras också.

Mikrosatellit-markörer blev också använda för att undersöka graden av inavel och genetisk mångfald (allelrikedom) hos den fröbank som finns att köpa för återplantering hos kommersiella företag i Sverige. Resultatet från detta såväl som det förra arbetet bekräftar att den genetiska mångfalden när det gäller neutrala markörer (alltså sådana som inte är utsatta för naturligt urval) är stor hos ask, och att inavelsnivån är mycket låg. Mångfalden av alleler hos enskilda fröfamiljer (avkommor från samma hona) kan variera beroende av hur honans blomning är synkroniserad med omgivande hanträd. Insamling av frön från ett begränsat antal träd måste undvikas och den genetiska mångfalden hos beståndet, liksom dess struktur, måste utvärderas innan man väljer ut planteringsmaterial för återplantering och restaurering av skogar.

