fraxigen

A summary of findings
from the FRAXIGEN project
EU project EVK2-CT-2001-00108

Ash species in Europe

Biological characteristics and practical guidelines for sustainable use

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To be cited as:
FRAXIGEN. 2005. Ash species in Europe: biological characteristics and practical guidelines for sustainable use. Oxford Forestry Institute, University of Oxford, UK. 128 pp.

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Published by:
Oxford Forestry Institute,
Department of Plant Sciences, University of Oxford, Oxford, UK

***ISBN**: 0 85074 163 7*

FRAXIGEN is a research project funded under the
Fifth Framework Programme (Biodiversity and Ecosystems) of the European Union
Contract no: EVK2-CT-2001-00108

Table of contents

The chapters 4, 5 and 6 outlined below provide information relating, respectively, to common ash, narrow-leaved ash and manna ash. For each species, we describe its taxonomy, ecology, silviculture and uses, and then summarise the findings of FRAXIGEN with regard to that species. Our findings relate to genetic diversity, both at a broad scale across Europe and at the level of individual populations; the ways in which this has been shaped by the species' differing reproductive biology; and the effect of local adaptation on the success of seedling survival and growth in a woodland environment.

The main findings from the research, and their practical and policy implications, particularly with regard to sound seed collection strategies and seed source selection for conservation and ecological restoration objectives

References are sorted in alphabetical order and have been assigned consecutive numbers. Superscript numbers within the text match the numbers in the references section.

We have tried, throughout the book, to keep the use of scientific language and technical words to a minimum. The glossary explains any technical terms used in the text. These words are highlighted in the text by printing in small capitals in BLUE TEXT.

The FRAXIGEN project

This book is about the three ash (*Fraxinus*) species native to Europe: *Fraxinus excelsior* L. (common ash), *F. angustifolia* Vahl (narrow-leaved ash) and *F. ornus* L. (manna ash). Its aim is to summarise the findings of FRAXIGEN, a research project funded under the Fifth Framework Programme (Biodiversity and Ecosystems) of the European Union from January 2002 to June 2005. It intends to draw out key conclusions and recommendations of relevance to both policy makers and field practitioners, including forest owners and managers, tree nursery operators, and seed collectors.

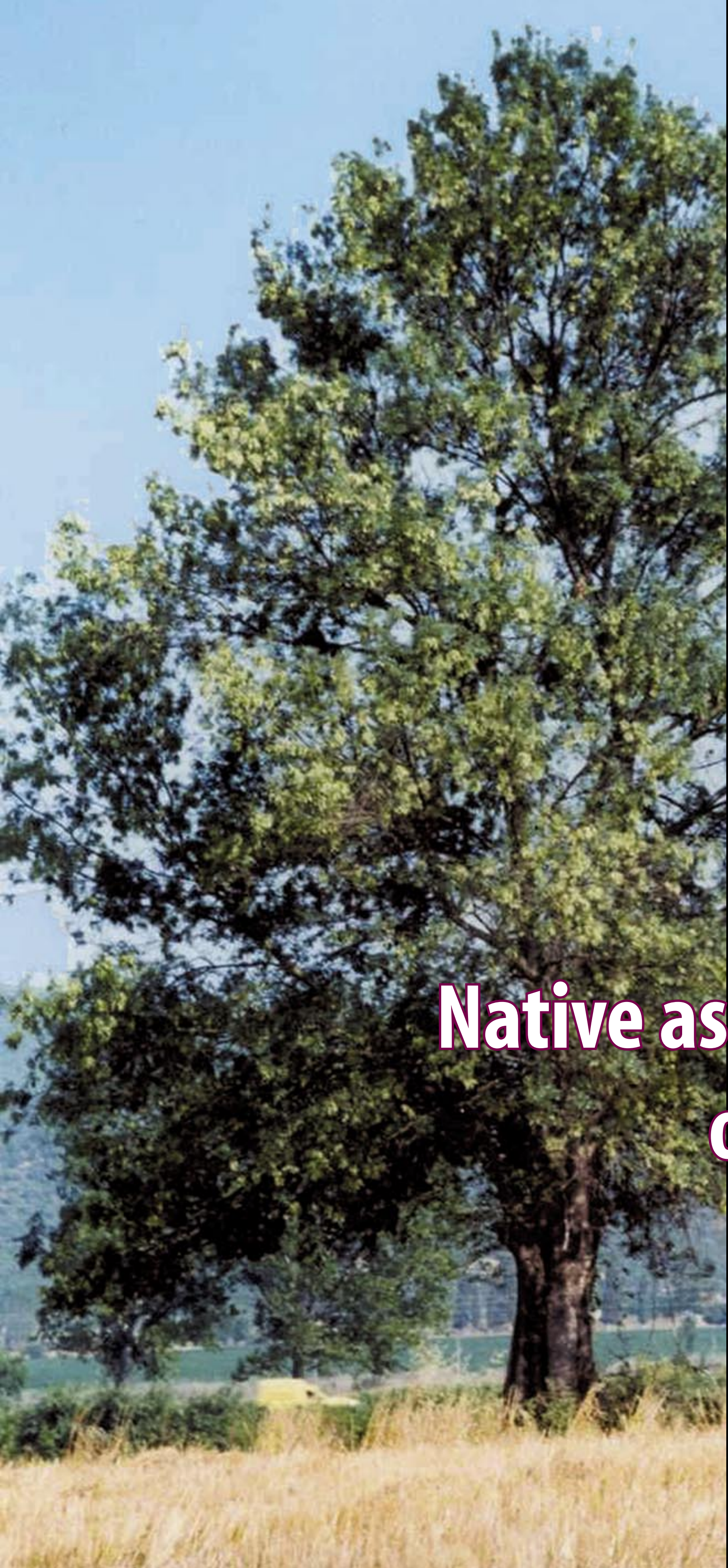
Scientists from ten institutions across Europe worked in close collaboration to address the same research questions relating to the three European ash species in different parts of their distributions. The objectives of FRAXIGEN were:

1. **To study patterns of gene flow and genetic diversity in the three European *Fraxinus* species, and how these are influenced by variation in reproductive biology**. The studies of **GENETIC DIVERSITY** and **GENE FLOW** used **NEUTRAL VARIATION** using **GENETIC MARKERS**, while the work on reproductive biology included both studies of the **MATING SYSTEM** using controlled pollinations, and detailed studies of the species' **PHENOLOGY**.

2. **To study how natural ash populations have adapted to their environment, and how selection by humans for productive characters has affected adaptive variation**. This was addressed through **RECIPROCAL TRANSPLANT EXPERIMENTS**: networks of field trials specifically designed to investigate localised adaptation and the scale over which it operates.

3. **To provide guidance for governmental, private and public interest groups on the collection, exploitation, and conservation of ash genetic resources**. This was done by using findings from all the studies.

The methods used for these studies were standardised as far as possible across the different sites, to make the results comparable. The project's scientific approach is outlined in Chapter 3. The results of the studies are summarised in the species chapters (Chapters 4-6).



Chapter

1

Native ash species of Europe

Native ash species of Europe

The genus *Fraxinus* (ash) is one of 24 genera in the Olive family, Oleaceae[41]. It comprises 43 species[40] distributed in temperate and subtropical areas of the northern hemisphere. The species are mainly trees, most of which are deciduous, but there are also some evergreen shrubs adapted to arid environments. Nineteen species occur in North and Central America (from Canada south to Guatemala) and 24 in Europe, North Africa and Asia. About two thirds of ash species are wind-pollinated and one third insect-pollinated. Most of the insect-pollinated species occur in eastern Asia, but the wind-pollinated ones are more evenly distributed across the temperate forests and arid regions of the northern hemisphere.

Of the three European species of ash[1], the common ash (*F. excelsior*) and the narrow-leaved ash (*F. angustifolia*) are both large wind-pollinated trees. The smaller and often multi-stemmed manna ash (*F. ornus*), with its showy white **INFLORESCENCES** which attract insect pollinators, is more closely related to Asian species of *Fraxinus* than to the other two European species. Common ash and narrow-leaved ash are very closely related, and have such a similar appearance that they are sometimes difficult to distinguish, especially when they are growing in mixed stands. There are reports of **HYBRIDISATION** in areas where they occur together (the Balkans, plus parts of Italy and southern France). For this reason Chapter 5 (on narrow-leaved ash) includes a guide to distinguishing between these two species. Other species of ash are sometimes cultivated in Europe, mainly the American species *F. americana* and *F. pennsylvanica*, but it is doubtful whether they can hybridise with the native European species.

Figures 1.1, 1.2 and 1.3 show the distributions of the three species. Common ash is the most widely distributed, occurring across the whole of northern Europe up to 64°N (in Norway), south to the Mediterranean, and east into continental Russia almost to the River Volga. The distributions of the other two species are more restricted and generally more southern. Narrow-leaved ash occurs naturally throughout most of the Iberian peninsula; on the Mediterranean coasts of France, Italy and the Balkan peninsula as far as northern Greece; south into coastal areas of Morocco, Algeria and Tunisia; and east as far as the Caspian Sea. Manna ash has the most limited range: it is found mainly in Italy, the Balkans, Greece, and western Turkey, with isolated populations in eastern Spain.

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[1] The systematics of other **TAXA** of *Fraxinus* found in south-east Europe, which are sometimes treated as separate species, are discussed in the individual species chapters (Chapters 4-6).

All three species have strong, flexible, resilient wood used historically for weapon handles and agricultural implements. It is still used for tool handles and sports equipment. Common ash, and to a lesser extent narrow-leaved ash, produce good quality timber, used for furniture and flooring. Manna ash is a much smaller tree and usually has multiple stems, so sawn timber is not an important product. This species produces manna, a sweet exudate obtained from the stems by incision. Manna ash is used as a mild laxative, a sweetener and an anti-caking agent. The foliage of all three species has been used historically for animal fodder, and this practice continues today in some countries. The uses of the three species are described in more detail in the individual species chapters. Ash also has great ethnic, cultural and mythological significance in many parts of Europe.

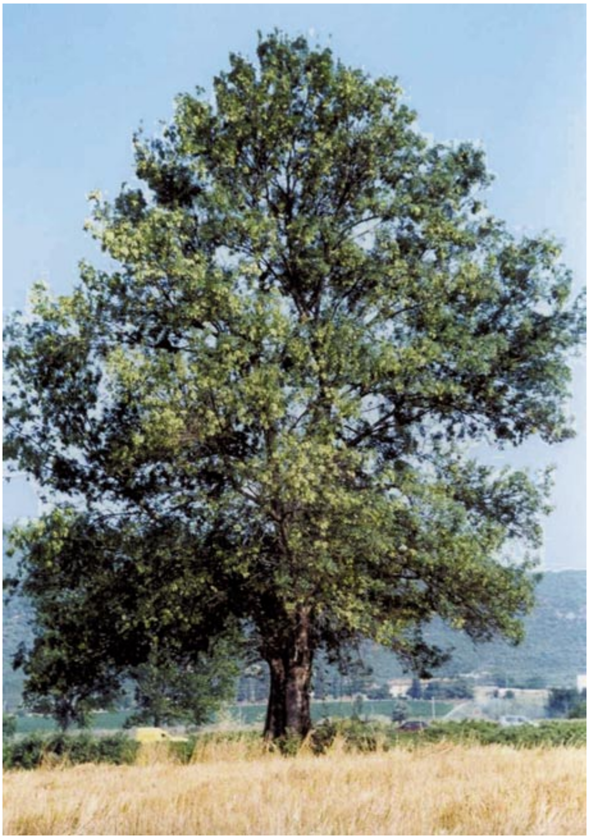


Photo 1.1.- Isolated *F. angustifolia* tree, Greece.

Figure 1.1.- Natural distribution of *Fraxinus excelsior* in Europe

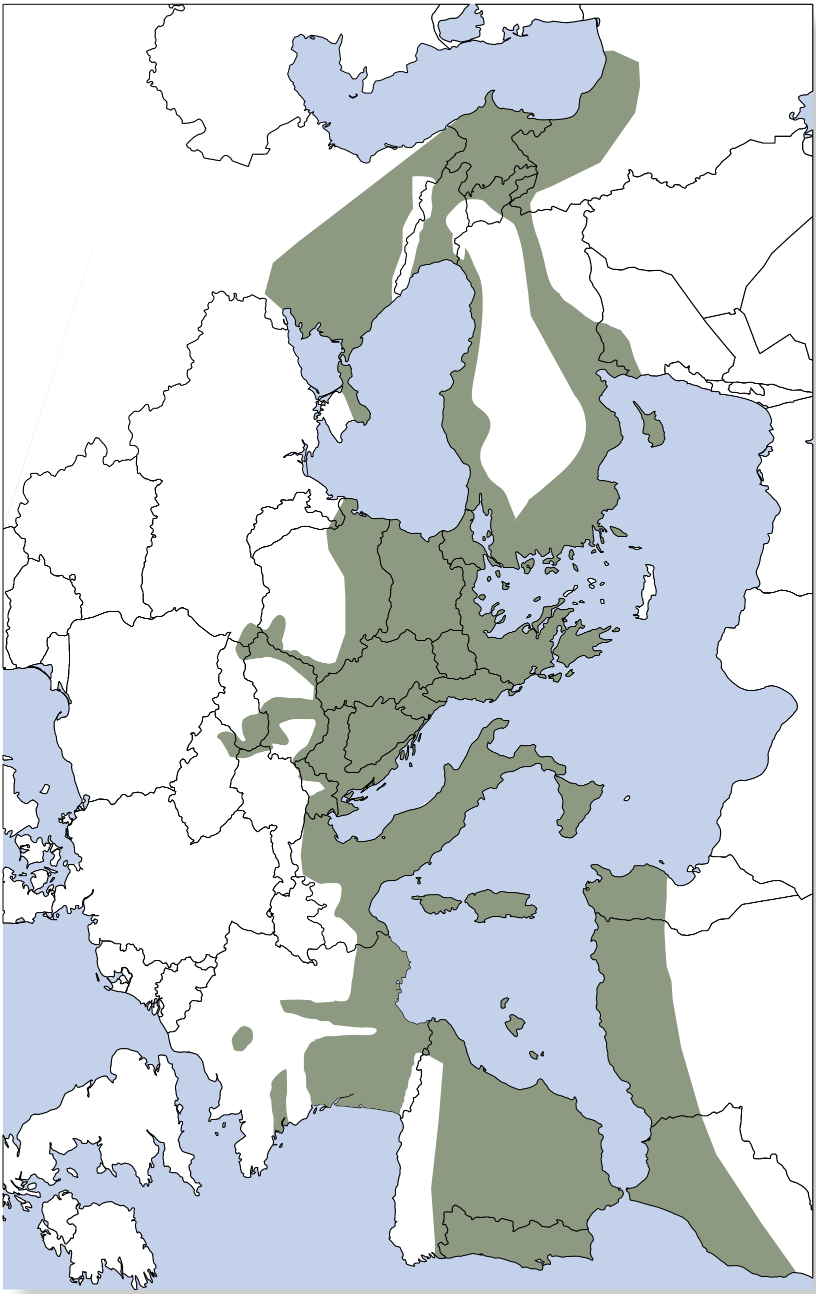


Figure 1.2.- Natural distribution of *Fraxinus angustifolia* in Europe

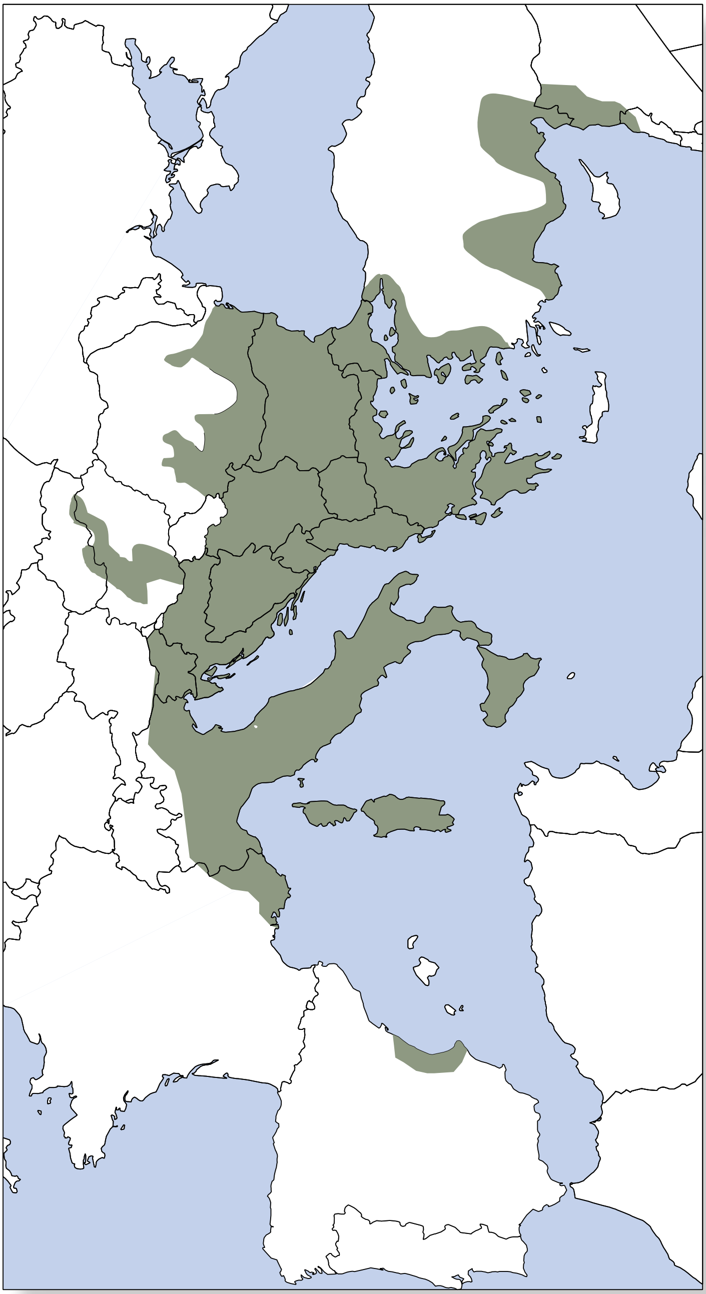
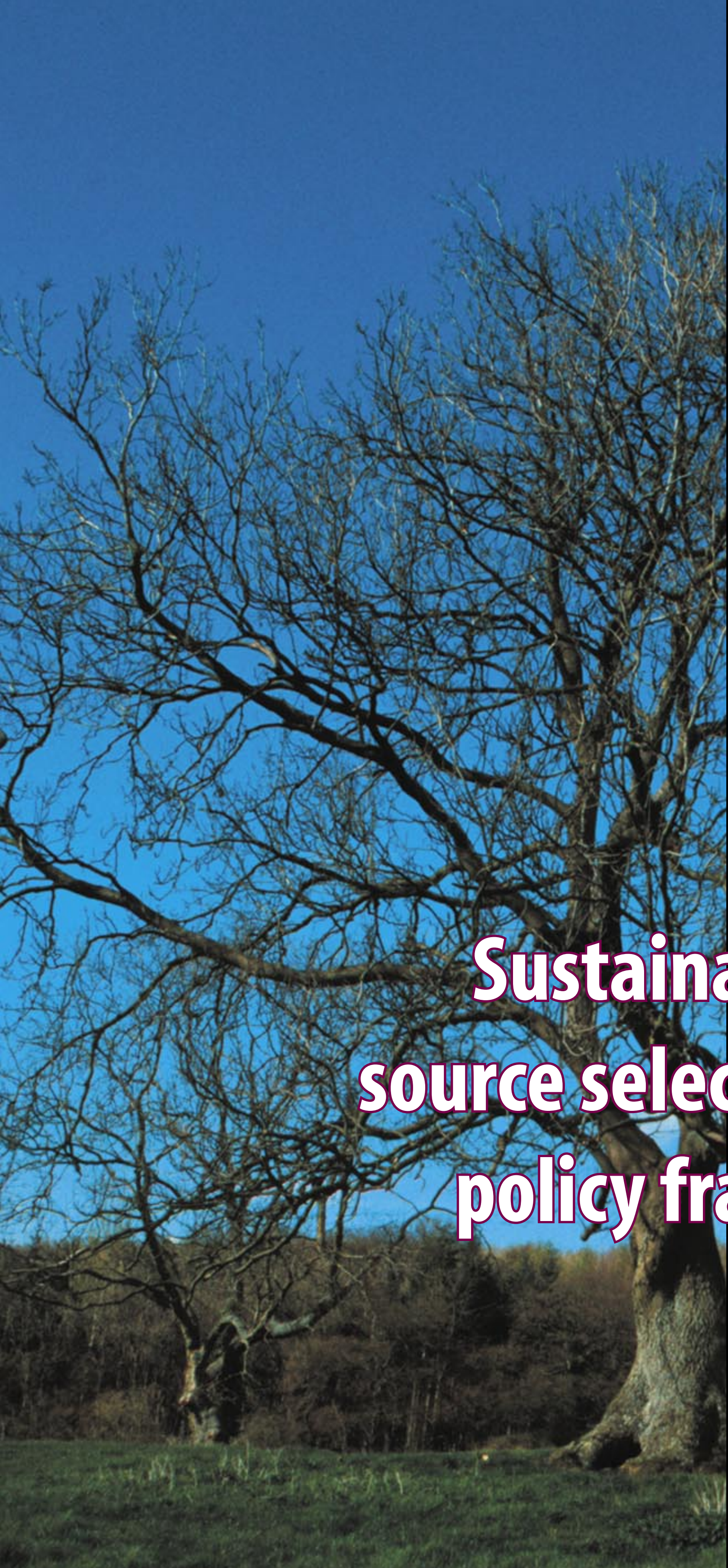


Figure 1.3.- Natural distribution of *Fraxinus ornus* in Europe



Chapter

2

Sustainable seed source selection: the policy framework

Ash for conservation and ecological restoration

Until recently, common ash and narrow-leaved ash have been planted mainly with production objectives. Both species (but particularly common ash) have been widely planted for timber, and there has been some work on genetic improvement for common ash, based on selection for traits considered superior in a timber tree. These include a long, clear, straight bole, a compact crown with little forking, and little or no fluting at the base. Manna ash has historically been exploited mainly for manna production, although to our knowledge there has not been any formal genetic improvement for this.

All three species are important components of European mixed broadleaved woodlands. There has been increased recognition in recent years of the importance of managing forests for a wider range of objectives than simply production. These include long-term sustainability, conservation of biodiversity, ecological restoration, and protection of landscape character and cultural heritage.

Trees planted in the context of ecological restoration, or enrichment of native woodlands, require different qualities for adaptation and competition from those established in plantations specifically for timber production. Selection of genetically superior material for timber production is almost always carried out with plantations in mind. The breeder anticipates that the selected seed will be raised in a highly uniform environment, assisted by careful site preparation, and protected from many of the normal causes of mortality for seedlings in a natural forest setting, such as competition with weeds and surrounding vegetation, and damage by animals such as moose, deer, squirrels, rabbits and rodents. Such favourable treatment minimises mortality in nurseries and plantations at the stages of germination and early growth. In contrast, in a natural setting, mortality is highest at these stages. Therefore, these processes provide the greatest opportunity for natural selection, and hence evolutionary adaptation to local conditions. The adaptive traits that contribute to survival under natural conditions may not, therefore, be selected for in a plantation environment.

The process of conventional genetic improvement in trees starts with the evaluation of seed sources from across a species' native range in provenance trials which may not even be located within that range and are usually on uniform, open field sites. Material with superior productive traits is selected for further improvement (breeding) and multiplication, with the expectation that this material will be moved around both within and outside the species' natural distribution.

Seed source selection for conservation objectives, such as conversion of an exotic plantation back to mixed native woodland, often involves planting within existing woodlands, and therefore requires a different approach. Much more emphasis is given to obtaining seed from local sources, with the implicit intention of capturing localised adaptation and therefore favouring such adaptive traits as competitive ability. This is currently one of the principal considerations driving policy development on seed sourcing in Europe, and is one of the main issues addressed by FRAXIGEN. In this model, the ultimate aim is future management under a continuous cover system, with small clear fellings (less than 0.5 ha) allowing natural regeneration.

European forest policies supporting planting for conservation

Since the early 1990s, a policy framework has developed in Europe to support the concept that "local is best", at least in the context of planting for non-production objectives. The balance in European forest policy regarding seed sourcing has shifted dramatically. The present emphasis is on adaptive rather than productive traits, through a strong focus on local planting material, and showing the more varied roles of forests.

According to the Helsinki Guidelines developed by the second Ministerial Conference on the Protection of Forests in Europe (MCPFE, 1993), "Genetic selection, which is commonly practised in Europe, should not favour performance traits at the expense of adaptive ones except in particular cultures where intensive care may protect them against damage". This wording accommodates both conservation and production objectives, since plantations of selected genetic material for timber production are indeed 'particular cultures' designed to receive 'intensive care'. The same Guidelines also state that "native species and local provenances should be preferred where appropriate". Again, the 'appropriate' context for local material is in planting primarily for conservation and environmental objectives, and this criterion does not necessarily need to be applied in plantations for timber, where the best selected material may not be local. For common ash and narrow-leaved ash, genetic improvement programmes remain important at both national and regional levels to identify superior reproductive material for production planting within a plantation environment. However, there is at the same time a growing interest in seed sourcing for enrichment planting and ecological restoration. The principles in the Helsinki Guidelines have been repeated in the resolutions adopted by the subsequent MCPFE conferences in Lisbon (1998) and Vienna (2003).

The EC Council Directive 1999/105/EC on the marketing of Forest Reproductive Material (FRM) expanded to 46 the list of species subject to regulation: these include both *Fraxinus excelsior* and *F. angustifolia*. The directive is interpreted through legislation in each member state, controlling the marketing of FRM of these species. The certification of FRM falls into one of several categories:

- Source Identified - FRM comes from general or specific locations within a single provenance zone, but with no specific superior qualities recognised.
- Selected - FRM is collected from stands showing superior characteristics, e.g. better form, growth rate, health.
- Qualified - FRM derives from the selection of superior individual trees which have not undergone any form of testing.
- Tested - FRM derives from the selection of individual trees or stands which have undergone evaluation for genetic quality or, in comparison to accepted standards, have been shown to be superior.

How local is local?

The recommendations, guidelines and regulations summarised in the previous section have required the designation, at national level, of provenance zones within which seed should be collected. This enables the seed to be certified as source-identified and so satisfy the criterion that it is 'locally' sourced. It is assumed that, in the long term, seed from a provenance zone will be better adapted to a planting site within that zone than seed brought in from outside. This is a reasonable assumption in areas with strong environmental gradients (rainfall, temperature, topography, altitude, aspect or soil type). In large, environmentally uniform areas, however, it is much more difficult to define provenance zones using environmental criteria. This is because almost nothing has been known until now about the geographic scale over which local adaptation actually occurs in trees. Many herbaceous species do show significant localised adaptation over quite short distances. However, trees are much longer-lived and thus they need to retain much more genetic variation to be able to adapt to changing environments. In addition, the **POLLEN** of trees is also likely to travel much further, carrying genes from one population to another (**GENE FLOW**). These characteristics of trees tend to act against the development of local differences through natural selection. Therefore, the extent to which localised adaptation occurs in a species will depend on the relative strengths of two opposing forces: selection and gene flow. Where there is very strong environmental variation (and hence selection pressure) over short distances it is more likely that there will be adaptive differences, even with gene flow between populations.

Examples of seed sourcing policies in Europe

In the absence of any hard genetic evidence, countries without clear environmental criteria for delimitation of provenance zones have sometimes been forced to define rather arbitrary boundaries. This applies particularly to those with large uniform lowland areas such as England. There is therefore an urgent need for a better understanding of the true extent and significance of genetic variation in trees in these countries. Where strong environmental gradients do exist, it is easier to make generalised recommendations or delimit provenance zones valid for several species.

Spain has designated specific zones for priority species, for which more information is available, while for the less important species (which include ash) a standard set of provenance zones has been defined. Slovakia, instead, has not defined provenance zones for the less important species, but applies other criteria of environmental similarity. For example, the altitude of the seed collection site must be within 200m of the proposed planting site.

In Germany, the number of provenance regions ranges from two to thirty, depending on the species and based on their ecological requirements: for *F. excelsior*, eight regions are defined, and these are shared with alder (*Alnus glutinosa*) and lime (*Tilia cordata*). One of the most scientifically-based examples of provenance delimitation for an ash species comes from Lithuania, where growth data from 3000 stands has been combined with ecological and bio-geographic information to define five provenance regions for *F. excelsior*. In Italy, a study of patterns of **GENOME** in common ash has made it possible to propose provenance regions which take account of both environmental and genetic variation.

The importance of diversity in adapting to environmental change

Although it is clearly preferable, for ecological restoration, to select planting material that is well-adapted to the competitive pressures of planting in a woodland environment, this may not always be achieved just by using local material. The assumption that local material will always be the best suited to a particular site implies a static situation where the environment does not change throughout the lifetime of the tree. Given the long life-span of trees and the increasing likelihood throughout Europe of accelerating climate change, adaptability to a range of environmental conditions over both space and time could become the most important trait for sustainability. **GENETIC DIVERSITY** offers the potential to adapt to such changes, while a lack of diversity limits this potential. In deforested areas, particularly, an overemphasis on seed collection from the few remaining local trees can result in the establishment of genetically impoverished trees which may be less vigorous and also less able to adapt to future environmental changes. The recommendation to use local seed must therefore be interpreted with caution in areas where ash populations are very small and/or highly **FRAGMENTED**, because there may be a risk of significant **INBREEDING** leading to reduced genetic quality of the seed. Even in genetically healthy populations it is important to collect seed in a way that captures as much of the available genetic diversity as possible.

Chapter

3

Understanding ash genetic resources: research under FRAXIGEN

Photo 3.1.- Reciprocal transplant experiment

Photo 3.2.- Placing pollination bags over closed buds of ash flowers

The concepts and methods described in this chapter apply to the research carried out under FRAXIGEN on all three ash species. The principles underlying the study of genetic variation in trees are outlined, followed by a summary of the experimental methods used in FRAXIGEN.

Concepts in forest genetic resources research

Adaptive and neutral variation

The overall appearance of an individual plant (**PHENOTYPE**) is a result of its **GENES** (**GENOTYPE**) and the environment in which it is found. Variability of both the phenotype and genotype can be measured, although the genotype is more important from the point of view of breeding, conservation and utilisation.

Two broad types of genotypic variation can be measured: variation which is influenced by natural selection (**ADAPTIVE VARIATION**) and variation which is not affected by selection (**NEUTRAL VARIATION**). Adaptive variation is measured in specialised field trials such as **RECIPROCAL TRANSPLANT EXPERIMENTS** (RTEs), where plants are grown in common conditions and their performance measured. Such experiments reveal the capacity of different plants to adapt to their surroundings, and give insights into the scale over which local adaptation occurs. In contrast, neutral variation, which can be measured using **DNA** profiling techniques, does not imply any difference in fitness (survival, vigour or fecundity) between plants with different genotypes. Measuring adaptive variation is difficult and time-consuming in comparison to DNA profiling, so neutral variation is often used as a surrogate. High neutral variation is assumed to reflect high adaptive variation.

In the context of setting policy agendas, information about adaptive variation may intuitively appear to be more valuable than knowledge about levels of neutral variation, since adaptive variation indicates which provenances are likely to perform best at particular sites. However, neutral variation arguably offers a less biased view, since it incorporates information about all possible genotypes which may occur at a particular site, not only those which have been selected for under certain conditions.

NEUTRAL MARKERS can be used to measure **GENETIC DIVERSITY**, **GENE FLOW**, and the parentage of particular seeds. Furthermore, neutral variation can also be used to look at changes in genetic variation over time, for example between seed crops from different years. All of these issues have direct implications for policy, especially for defining populations, designing different sampling strategies for seed collections, and ultimately for designing for provenance zones.

Population identification

There are many ways to define a population. For example, it may refer to a group of individuals that occur at a particular point in time and space. By this definition seed crops from different years would constitute different populations. It is not only temporal factors which may affect the definition of a population. Spatial factors may also be important, since if **POLLEN** or seed dispersal is high then the population may include more individuals. However, the term population is most usually used more loosely to refer to a stand of trees.

Sampling strategies

The distribution of genetic variation (both adaptive and neutral) within and between populations influences how we design strategies for sampling plant material for the conservation and utilisation of genetic resources. Good sampling strategies are important in order to collect a representative sample of the genetic diversity in a population. However there is often insufficient knowledge about diversity and gene flow at the population level for seed collection strategies to be based on scientific evidence. Basic information such as the number of trees to collect from, and the minimum recommended distance between them, are left to guesswork and rules-of-thumb.

Knowing the relative amount of variation found within and between populations allows decisions to be made about how best to distribute available resources in conservation and management plans. For example, if there is high neutral variation within populations but few differences between populations, then sampling should be focused on sampling many individuals from a few populations. In contrast, if populations tend to have low levels of neutral variation and there are large differences between populations, then more populations should be sampled but fewer individuals are needed from each population. These simple rules can be modified in the light of ecological data, the costs associated with material collection and the occurrence of adaptive variation. Patterns of genetic variation are also important when *ex situ* collections (e.g., seed banks) are designed and decisions are made as to which collections to keep and which to discard.

If actual levels of genetic diversity are unknown, population sampling strategies can be developed from general theoretical models[11]. These indicate that the leaves of 30 adults would be sufficient to sample 95% of the **ALLELES** that occur at a frequency of greater than 5% (see Box 7.1 in page 116). Furthermore, standard guidelines for tree sampling indicate that trees should be separated by at least 50 m[11]. However, this may lead to sampling more trees in each population than is necessary (leading to increasing costs of seed storage and maintenance), and the distances between individual samples may be inappropriate. Thus, application of direct knowledge of levels of genetic diversity in a species and in populations can make sampling more efficient and long-term conservation and utilisation more cost effective.

Seed zone designation

Patterns of pollen and seed dispersal in time and space have direct implications for the accurate identification of seed zones. For example, neutral genetic variation may be similar in woodlands which are widely separated geographically, if there are other woodland patches or scattered trees in between which can act like stepping stones, providing a genetic link between distant populations. The term gene flow includes the concept of this indirect genetic link, so the definition is not limited to the direct exchange of seeds or pollen between populations. Thus, seed zones may be large, if gene flow is extensive. Alternatively woodlands which are a only a short distance apart but at different altitudes may be more different genetically than distant woodlands at similar altitudes, because high altitude populations may flower later, preventing genetic mixing between populations.

The neutral genetic variation in adult trees at two sites with different ecology may differ even if there is gene flow between them, if natural selection acts to favour some genotypes over others. Thus, the definition of seed zone must also take into account the influence of adaptive variation and localised adaptation.

In conclusion, delimitation of seed zones must integrate both neutral and adaptive genetic variation, and must try to take into account how genes move across a landscape, rather than relying on arbitrary political or man-made boundaries.

The scientific approach of FRAXIGEN

Estimating genetic diversity

We have studied in FRAXIGEN patterns of neutral genetic variation in all three species, both at the broad scale, comparing diversity within and between populations across Europe, and at the level of individual populations, investigating patterns of genetic diversity and gene flow within woods. It is possible to assess the level and distribution of genetic diversity in trees, at both these scales, using DNA profiling techniques or **GENETIC MARKERS**.

Studies at the broad scale allow us to identify areas of high diversity which might be considered to be of higher intrinsic conservation value at the Europe-wide level. The DNA, or genes, in the cell **NUCLEUS** (the nuclear **GENOME**) are inherited from both of a tree's parents. A tree inherits one copy of each **GENE** from the mother via the egg cell in the **OVULE**, and one copy from the father via the sperm cell in the pollen. Each copy is called an allele. Using genetic markers to measure variation in the nuclear genome gives an indication of the current diversity within populations, and of differences between populations (**GENETIC DIFFERENTIATION**). Genetic diversity can be measured by **ALLELIC RICHNESS**, i.e. the number of different alleles in a population, or by **HETEROZYGOSITY**, which takes into account the evenness of the distribution of different alleles. There is a separate genome in the **CHLOROPLASTS**, and this differs from the nuclear genome in two important characteristics: it is only inherited from the mother, and it evolves much more slowly. Because it remains essentially unchanged from generation to generation, we can use **CHLOROPLAST DNA** markers to look at the history of populations, for example to reveal where populations of temperate trees survived the last Ice Age and how they have since re-colonised Europe. We have looked at diversity in both these genomes in FRAXIGEN, to help us to understand patterns of diversity over both space and time, and at different scales.

Different types of genetic markers are available for different purposes. We used a type called **MICROSATELLITES**, chosen because they have a high level of resolution. They can tell us if the nuclear genotype of a single seed is different from that of its parents or siblings. Microsatellites can also be used to detect small differences in the chloroplast genotype (termed **HAPLOTYPE**) of trees from populations with different colonisation histories.

Using DNA profiling to track pollen movement

We can use DNA profiling to identify individual trees and seeds from a particular population. DNA can be extracted from seeds, leaves, leaf buds or cambium, so the DNA of adult trees and seeds can be directly compared. The DNA in the cell nucleus, inherited from both parents at fertilisation, results in a unique nuclear genotype for each tree or seed. By comparing the genetic profiles of a mother tree and its seed with those of the pollen-producing trees in the population (the potential fathers of the seed), we can deduce whether each sampled seed has been fathered by self pollen, by a tree located within the stand, or by another tree which has not been genotyped. If there is no match between the seed and any of the adult pollen-producing trees, it implies that the pollen for that seed came from outside the sampled population.

By detailed mapping of all the sampled trees in the population, we can not only pinpoint the location of the trees identified as probable fathers, but also see how far the pollen has travelled (the distance between the father tree and the mother tree) for each seed. This type of information is useful in designing seed orchards and planning seed collections from seed orchards, natural woods and registered seed stands.

In FRAXIGEN we have used this approach for common ash in Sweden and Great Britain, and for manna ash in Spain and Greece. The research was replicated in two countries for each species to test how widely applicable the findings are. In each study population we studied the genotype of 20 seeds from each of 20 mother trees, as well as of the mother trees and the potential father trees within the population.

What kind of tree makes the best father?

Reproductive processes and mating patterns determine the genetic structure of each generation. This is why information on reproductive biology, including **MATING SYSTEMS**, **GENDER** of adult trees, and variation in seed and pollen production, are all valuable for planning and managing conservation programmes, including seed collection and planting.

The reproductive biology of each species was studied in detail by FRAXIGEN, including assessing gender and recording **PHENOLOGY** in different years. We also looked at how different mating systems in the three species influence patterns of gene flow and genetic diversity. For example, we used genetic markers to compare the number of seeds produced naturally by fathers of different genders (male or **HERMAPHRODITE**), and also carried out controlled pollinations, using pollen from both male and hermaphrodite trees, to see which produced more seed. We compared the male- and hermaphrodite-fathered seeds in terms of their germination and early growth.

The findings of all these studies are described in more detail for each species in Chapters 4-6, and have contributed to recommendations for genetically sound seed collection strategies. These are described in Chapter 7.

Research on local adaptation

A major objective of FRAXIGEN has been to provide sound evidence to be used as a basis for a more rational definition of 'local' planting material. We have used reciprocal transplant experiments, or RTEs, which are ecological field trials specifically designed to estimate the scale and pattern of local adaptation in plants. In an RTE, seed is collected from several native populations which are thought to have arisen only from natural regeneration (**AUTOCHTHONOUS**). These populations are selected to be from contrasting environments and also at varying distances from one another. Seed or seedlings representing all the populations is then planted at all the seed collection sites, so that at each site the 'home' population (from seed collected at that site) is compared with all the other 'away' populations. This methodology has been used to examine local adaptation in a wide range of herbaceous species, but has rarely been applied to trees.

In FRAXIGEN we have set up RTEs for each of the three species: in UK for common ash and in Greece for narrow-leaved ash and manna ash. The initial results from these trials, and their implications, are described in Chapters 4-6. In all three sets of trials, we have established the experiments within the woodlands where the seed was collected, at close spacing and with minimal site preparation or weeding, to simulate natural regeneration within a semi-natural woodland environment.

Chapter

4

Fraxinus excelsior (common ash)

Photo 4.1.- Marking *Fraxinus excelsior* trees for field studies at Queen´s Wood, UK (see location in Figure 4.11).

Distribution and systematics

Fraxinus excelsior L. (common ash; Photo 4.1) is the most widely distributed ash species in Europe (Figure 1.1, page 10). Its distribution extends across Europe from the Atlantic coast in the west into continental Russia, almost to the River Volga, in the east. Its northern limit (in Norway) is at 64°N, and it extends south to the Mediterranean, through the northern parts of Spain, Italy, and Greece, and as far south as 37°N in Iran.

There are variants of *F. excelsior* with different morphological features, for example *F. excelsior* L. ssp. *coriariifolia* (Scheele) E. Murray. This variant has hairy leaves, branchlets and **SAMARAS**, and occurs around the Black Sea (Crimea, Caucasus, Turkey and Romania), but is not very common.

Fraxinus excelsior is closely related to *F. angustifolia*. The two species are sometimes confused, even though there are characteristics that distinguish them. Of these, the **INFLORESCENCE** type is the most reliable. *Fraxinus excelsior* has a branched inflorescence (compound panicle), *F. angustifolia* an unbranched simple raceme. **HYBRIDS** have been reported in areas where the two species grow together[33] (Figures 1.1 and 1.2; pages 10-11). There is more information in Chapter 5 about hybridisation and how to distinguish the two species (Figure 5.1; pages 66-69).

Ecology and silviculture

The ecology of *F. excelsior* in its natural range is intermediate between a pioneer species and a permanent forest component[30]. It usually occurs in groups within mixed broadleaf woodland; pure stands or scattered trees (Photo 4.2) are less common. *Fraxinus excelsior* is a dominant species in the young and juvenile stages of forests[36].

Climate and altitude

The natural distribution of *F. excelsior* in western Europe appears to be defined by the species' lack of tolerance of winter cold, late spring frosts and dry, hot summers. In particular, the young emerging shoots are sensitive to spring frost[30;35]. Its altitudinal limits vary with both latitude and longitude. In the Pyrenees and the Alps, it is found up to 1600-1800 m above sea level. Further east and south in Asia (Iran), it can be found at much higher elevations, up to 2200 m[30]. In contrast, at more northerly latitudes and further west the species' altitudinal limit is much lower (e.g. 450 m in the British Isles[43]).

Soil

The growth potential of *F. excelsior* is closely correlated with the physical properties of the soil on which it is grown. It requires a fertile soil[30], and usually occurs in woodlands formed on lime-rich soil[26;37], doing best where the pH is greater than 5.5[30]. *Fraxinus excelsior* favours slopes and ravines, where it grows in association with maple (*Acer* spp.), lime (*Tilia* spp.) and elm (*Ulmus* spp.)[30]. It requires moist soil conditions, so is not common on drought-prone sites[35]. Although it tolerates waterlogging[43], it will not survive prolonged flooding, and is also intolerant of compacted soils.

Fraxinus excelsior is not suited to acid heathlands, rarely occurring on sites where the pH of the surface soil is less than 4.2. It should not be planted next to arable fields where severe die back can be caused by damage to the spreading lateral roots. Compared to beech, sycamore and oak, it has a high demand for easily available nutrients, particularly nitrogen and phosphorus[36], and comp etition from weeds for both water and nutrients can be a problem, especially in newly planted areas.

Seedlings of *F. excelsior* may germinate profusely on heavy wet soils because they only need a small depth of well-drained soil on which to become established. However, periodic water shortages during droughts on such sites lead to poor subsequent growth, so they should be avoided because *F. excelsior* never thrives on them in the long term.

Fraxinus excelsior is not frost tolerant, but because it flushes so late, it often escapes spring frost damage. However, late frosts can cause forking, one of the major potential defects in *F. excelsior*[35].

Suitable planting locations

Climate has less influence than soil on the growth of *F. excelsior*. It will grow well under a wide range of climatic conditions provided the soils are suitable[10]. In that sense, *F. excelsior* is a very site-demanding species[17]. It does best on mild, moist sites, and shelter is also important when young[35]. To grow really well, *F. excelsior* requires base-rich, ideally sandy, calcareous loams. (pH 7–8, especially in the lower parts of the soil profile[36]), which are moist, deep, well-drained, and have high contents of available nitrogen and phosphorus. Contrary to popular belief the ideal site is not a damp valley bottom, but a well-drained alkaline soil[13]. This may be rich deep marl, or a shallow soil over limestone.

Silvicultural characteristics

Stands can be established by

- managing natural regeneration, which can be prolific on good sites (Photo 4.3);
- planting;
- a combination of the two, for example by enrichment planting in between patches of natural regeneration.

The species is strongly shade-tolerant for its first seven years or so (up to about 4 m tall), but becomes very light-demanding thereafter. It does not, therefore, grow well with overhead cover or high levels of crown competition, and must be thinned regularly once it reaches 6-7 m in height.

A common mistake in *F. excelsior* management is insufficient thinning. It is important to thin in good time, because it does not respond to delayed thinning, and all thinnings should be heavy and frequent with the aim of keeping crowns entirely free and a live crown on at least one-third of the tree. Stands should be at their final spacing by age 30 to 35. Pruning is also important, to prevent the development of large branches.

Fraxinus excelsior coppices well, and natural regeneration is often so prolific that the species becomes invasive. Regeneration is particularly good in woodlands where canopies are dense. It will also regenerate freely on sites where it will not grow well later, such as compacted clays, and will usually disappear from them within a few years. *Fraxinus excelsior* competes well with *Clematis*, where other trees may become smothered, but not satisfactorily with grasses. It is therefore not suitable for using on previously unplanted ground unless weed control is very good.

Fraxinus excelsior is often regarded as a useful component in mixtures, though in groups, rather than intimate mixtures. It is more rarely successful as a pure plantation species. *Prunus avium* (cherry) is very suitable for mixing with it[13;31], and it also does well in group selection systems with *Acer pseudoplatanus* (sycamore) and *Fagus sylvatica* (beech). In Denmark, scattered *F. excelsior* trees are grown in *F. sylvatica* stands and felled after 70 years, leaving the *Fagus* to grow on for another 30–40 years. In Belgium, it is often grown with sycamore, cherry, oak, elm, aspen and birch.

The two most common stem defects in *F. excelsior* are canker and forking. Bacterial cankers can be caused by the bacterium *Pseudomonas savastanoi* or the fungus *Nectria galligena* and these may damage trees badly if they are grown on unsuitable sites. Forking can be caused by birds and by the ash budmoth (*Prays fraxinella*) in young trees, as well as by late spring frosts[18]. *Fraxinus excelsior* is almost immune from grey squirrel damage (currently a problem mainly confined to Britain, Ireland and Italy), but seedlings are severely browsed by hares, rabbits and deer.

Growth and rotation length

Fraxinus excelsior grows rapidly when young, for a hardwood species, but its growth slows after around 60 years, so it is typically managed on a 60 year rotation. Boles of 6 m in length and 40-60 cm dbh can be achieved on the best sites in 50 years, while on poorer sites this can take up to 80 years. Mean yield classes typically range from 4 to 6 m^3/ha/year, with a maximum of about 10 m^3/ha/year. On the best sites, *F. excelsior* is more productive than oak but less so than beech. Like oa , it should not be felled when in leaf, as this makes the sapwood much more prone to attack by wood-boring insects. The wood is, however, easy to treat with preservatives.

Traditional and modern uses

Fraxinus excelsior is an important timber species, with attractive pale (white or pinkish white) wood which is strong, durable, resilient and easily bent, making it particularly suitable for furniture and house interiors. Although the grain is coarse, *F. excelsior* is easy to work, can be turned, takes a smooth finish, and will stain easily[26]. The strength and elasticity of the wood enables it to carry weight, bear tension and absorb shock better than any other European wood. Before the extensive use of steel, it was used for joists and beams, and many things now made of metal were originally made from ash, such as spears, ploughs, harrows and rakes, as well as carriages, cart axles and the rims of cart wheels, and frames for boats and cars[36]. It is also the wood of choice for tool handles (e.g. hammers) and sports equipment. The best timber for all these purposes comes from rapidly-grown trees on fertile sites. Timber with 4 to 16 rings/25 mm is likely to be suitable for most purposes. Slower-grown timber can be used for veneers. The wood has one of the lowest 'natural' moisture contents of all temperate timbers, and consequently burns well without the need to be dried after felling. It was widely used in the past to produce excellent charcoal[4].

The wood of *F. excelsior* is an excellent material for sports goods, particularly when strength, flexibility and springiness are required. In the past, tennis racquets and skis were made of *F. excelsior*, until man-made materials became available. Nowadays, *F. excelsior* is the time-honoured wood used in Ireland for the manufacture of the *hurley*, the stick used in the sport of hurling. In other countries, ash wood is used for a diversity of sporting goods including oars and rudders, baseball bats, cricket stumps, croquet and polo mallet shafts, lacrosse shafts, hockey sticks, billiard cues, arrow shafts for archery and dog sleds.

The leaves are palatable and nutritious to animals, and were once used as fodder[4]. Pollarded ash trees can still be seen in some parts of Europe (Photo 4.4). The dried leaves were fed to pigs and goats in the winter[26]. The bark was used to tan calf skin, and the white ash from the burnt wood was considered among the best for bleaching linen[26].

Fraxinus excelsior also used to be valued in herbal medicine. For example, before the introduction of quinine a bitter tonic made from the bark was used as a remedy for malaria, which was once common in Europe. An infusion from the leaves has purgative and laxative properties, and this is the main use of sap from the related manna ash, *Fraxinus ornus*. The samaras of *F. excelsior* are edible and used to be pickled. *Fraxinus excelsior* has been widely used in Central Europe as an ornamental tree along roads and city streets[26]. It also has great cultural significance in many parts of Europe. It is central to Norse mythology, Yggdrasil being the hollow ash tree from which the first man, Ask ("Ash") emerged. Ash was also thought to give protection against snakes, and women working in the fields would hang babies' cradles in ash trees to protect them.

Photo 4.2.- Solitary tree of *Fraxinus excelsior* near Göteborg, Sweden.

Photo 4.3.- Prolific natural regeneration of *Fraxinus excelsior* in a forest gap, Sweden.

Photo 4.4.- Ancient pollarded *Fraxinus excelsior* trees in Queen's Wood, Gloucestershire, UK.

Research on *F. excelsior* under FRAXIGEN

The ideas underlying the research done in FRAXIGEN are introduced in Chapter 3. We have carried out detailed research on **GENE FLOW** and reproductive biology in *F. excelsior,* using trees in semi-natural woodlands (i.e. not plantations) in U.K. and Sweden. The **PHENOLOGY** research also included populations of *F. excelsior* in Romania, so that we could make comparisons over a wider geographic area. Most of our findings on these topics are likely to apply also to *F. excelsior* populations in other parts of Europe.

We have also carried out studies of **GENETIC DIVERSITY** in which we included material from Denmark, Norway and Lithuania as well as U.K. and Sweden. These were intended to complement and expand the scope of previous studies in other areas. Because the pattern of genetic variation has been found in these earlier studies to differ in different parts of Europe (see below) readers from areas other than northern Europe should take account of the findings from research outside FRAXIGEN. In UK, the molecular studies of genetic variation have been complemented by a **RECIPROCAL TRANSPLANT EXPERIMENT** (RTE), established at eight sites (Figure 4.11) to investigate local adaptation.

Genetic variation

Background: previous research

Several studies of genetic diversity in *F. excelsior* have been carried out over the last five years. The REGECON project[1] looked at genetic diversity in **NUCLEAR DNA** across most of the range of *F. excelsior* in Europe, whilst the CYTOFOR project[2] surveyed variation in **CHLOROPLAST DNA**(the **HAPLOTYPE**) over a similar scale. microsatellite markers were used to assess nuclear genetic variation. **ALLELIC RICHNESS** and **HETEROZYGOSITY**, which are both measures of genetic diversity, were both found to be high across the range[16], as expected for a long-lived, **OUTCROSSING** tree species. Few genetic differences were detected between populations, as predicted for a species with wind-dispersed

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[1] REGECON (2001-2004): Développement de techniques de conservation ex situ d'essences forestières feuillues: project funded by International Plant Genetic Resources Institute (IPGRI), and Ministère des Finances (Luxembourg), Ministère des Affaires Etrangères, du Commerce Extérieur et de la Coopération (Luxembourg).

[2] CYTOFOR (1998-2001). European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT97-3795, 'CYTOFOR: Measuring molecular differentiation of European deciduous forests for conservation and management.

pollen and seed. Populations in western and central Europe were the most similar to one another, whilst differences between populations were greater in southeastern Europe. These results suggest more genetic connection (gene flow) between populations in western and central Europe, in contrast to southeastern Europe, as a result of the movement of pollen and/or seed. In Sweden the situation was less clear, with populations showing more similarity to those in southeastern Europe than those in western and central Europe.

Comparing information from nuclear DNA with results from chloroplast DNA can help to distinguish whether gene flow between populations is due to the movement of seeds or of pollen, since chloroplast DNA is only inherited from the mother via seed (see Chapter 3). The range-wide survey of chloroplast DNA revealed a clear geographical pattern, consistent with *F. excelsior* surviving the last Ice Age in the Iberian Peninsula, Italy, the eastern Alps and the Balkan Peninsula[15]. Towards the end of the Ice Age, as the climate of central and northern Europe became warmer, *F. excelsior* migrated northwards along with other forest species. However chloroplast DNA from the different lineages of *F. excelsior* can still be distinguished today, indicating that there was little mixing via seed between lineages as they moved north, and that they probably took different migration routes. It therefore seems likely that the uniform genetic pattern observed for the nuclear DNA in western and central Europe has resulted instead from high levels of pollen movement between populations after recolonisation.

FRAXIGEN findings on long-term migration(chloroplast haplotypes)

We identified the same haplotypes within Great Britain and the Scandinavian region (Figure 4.2), as reported in the European-wide study[15] (Figure 4.1). In Great Britain the dominant haplotype is one which spread from the Iberian Peninsula following the last Ice Age. This pattern is found in other woodland species such as oak (*Quercus robur* and *Q. petraea*) and holly (*Ilex aquifolium*), which also recolonised the British Isles from the Iberian Peninsula[29]. We found a different haplotype in a few trees in an autochthonous population on the north-east coast of England (GB_08E). This haplotype is dominant in western Denmark and also occurs in south-western Sweden; it probably originates from the eastern Alps. Following glaciation it spread north and west through Austria, Germany, Denmark and into Sweden. In contrast, in Scandinavia and in the Baltic region, the dominant haplotype originates from south-eastern Europe. This 'Balkan' haplotype spread northwards through eastern Europe into Finland, probably colonising Sweden from the south via the land bridge that existed when the ice was retreating northwards. In three populations in southern Sweden and Denmark, we found mixing of the two lineages, with trees carrying the typical Balkan haplotype occurring in the same stand as trees carrying the haplotype from the eastern Alps.

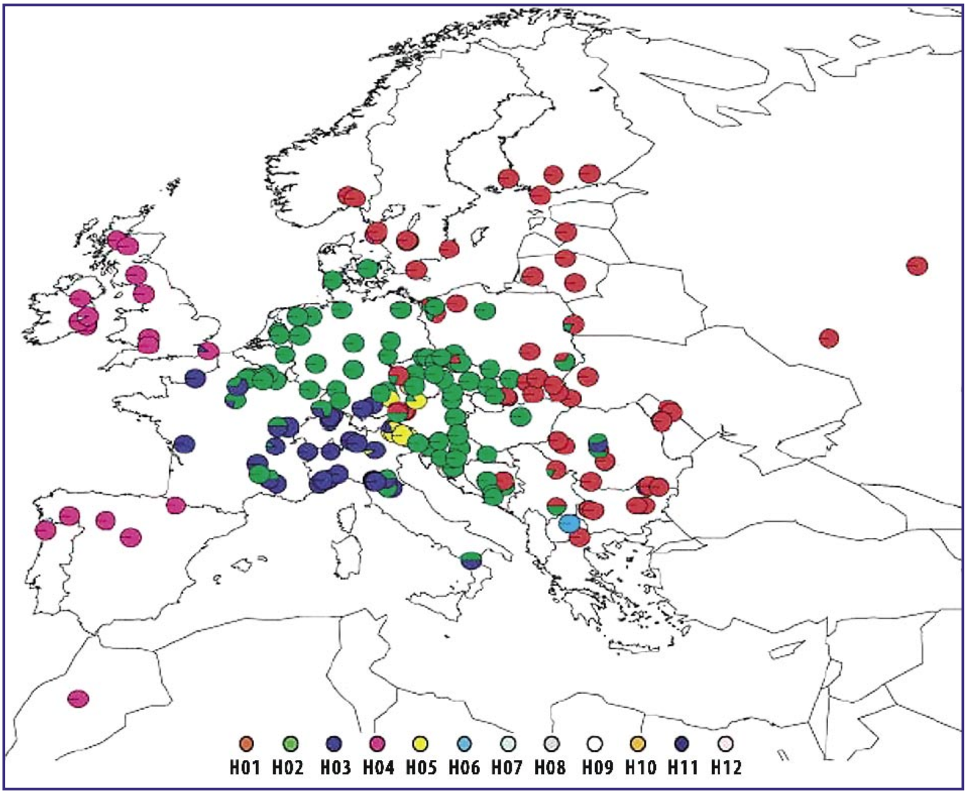


Figure 4.1.- Geographical distribution and frequency of chloroplast microsatellite haplotypes in *Fraxinus excelsior*. Reproduced from Heuertz *et al*. (2004)[15]. [Courtesy of Blackwell Publishing]

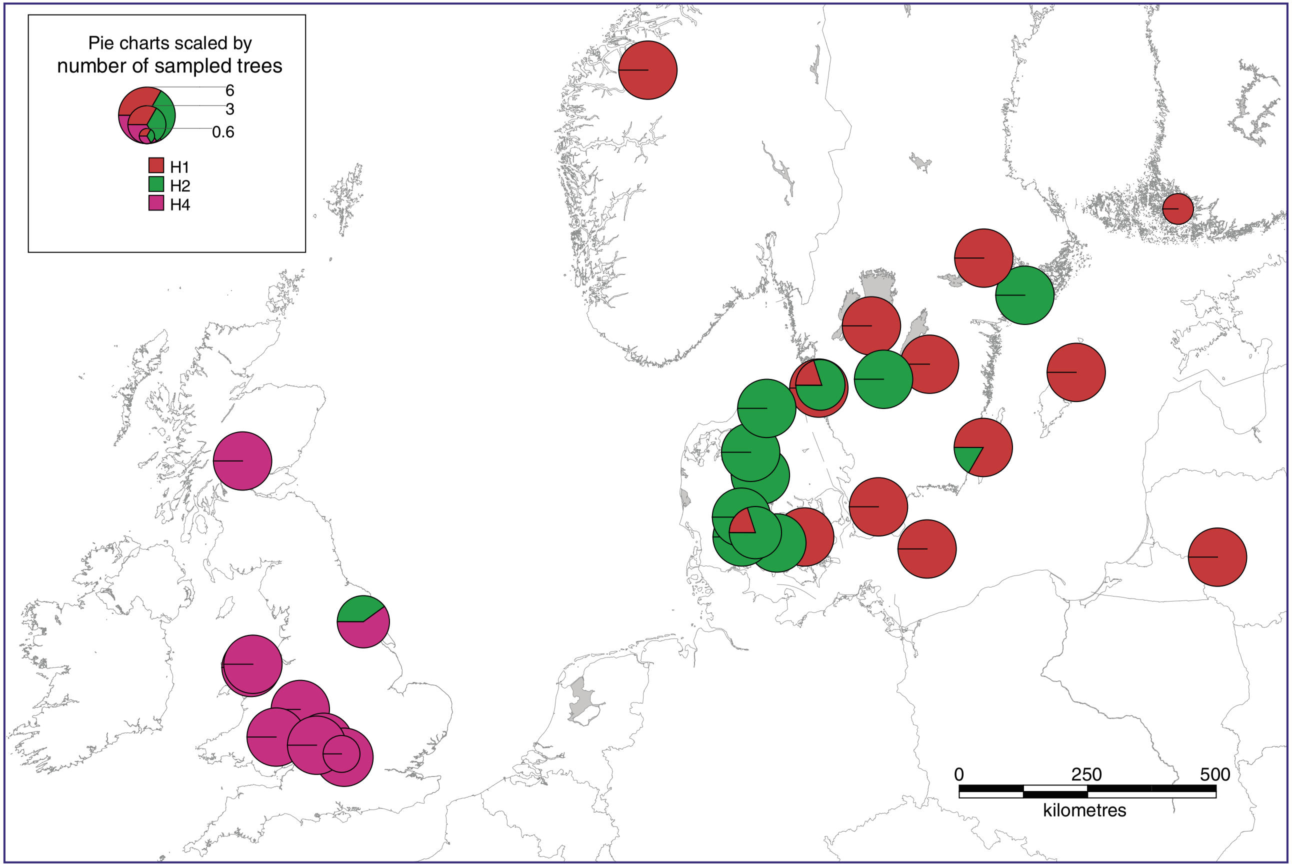


Figure 4.2.- Chloroplast haplotypes in 33 populations of *Fraxinus excelsior* from the UK, Scandinavia and the Baltic region. The size of the circles is scaled to show the number of individuals sampled in each population. Each colour represents a different haplotype, corresponding to those described by Heuertz et al. 2004[16] in Figure 4.1.

FRAXIGEN findings on genetic variation (nuclear diversity)

In the FRAXIGEN project we were interested in investigating nuclear genetic variation in Sweden and Great Britain in more depth, in order to complement results from the European-wide study[16]. We sampled ten populations from Scandinavia and the Baltic region and ten populations from Great Britain, and analysed them using the same five nuclear microsatellite markers which had been used in the earlier European-wide study so that our results would be comparable to theirs. In each population we analysed **DNA** from leaf material from 30 adult trees separated by 30 to 100 m, depending on the size of the woodland. For each marker we recorded the total number of **ALLELES** found in each population (allelic richness; Figure 4.3), and also calculated heterozygosity, which takes into account the evenness of the distribution of different alleles.

In Scandinavia and the Baltic region, populations from Norway (NO_24E) and Gotland (SE_27E), a Swedish island in the Baltic Sea, had the lowest allelic richness. Both are isolated populations: the Norwegian population is at the extreme north-western edge of the range of *F. excelsior* in Europe, and the island of Gotland is 60 km from the mainland. We found the highest allelic richness in populations from Lithuania (LT_26E) and southern Sweden (SE_21E and SE_30E), both located in continuous parts of the range of *F. excelsior* in this region. Within Britain, lower levels of allelic richness were found in populations from the north of Britain (GB_08E and GB_09E) compared to the south. In general, British populations were more similar in their level of allelic richness than were populations from Scandinavia and the Baltic region. These results are consistent with the Nordic populations being at the edge of the range of *F. excelsior* in Europe, where the distribution of the species is more **FRAGMENTED** and populations are more isolated. Thus there is less gene flow between them and they become gradually more differentiated, a process called **GENETIC DRIFT**. Levels of heterozygosity showed the same broad trends as allelic richness, with populations from lower latitudes and/or continuous parts of the range having higher levels of heterozygosity than populations from higher latitudes and/or the extremes of the range.

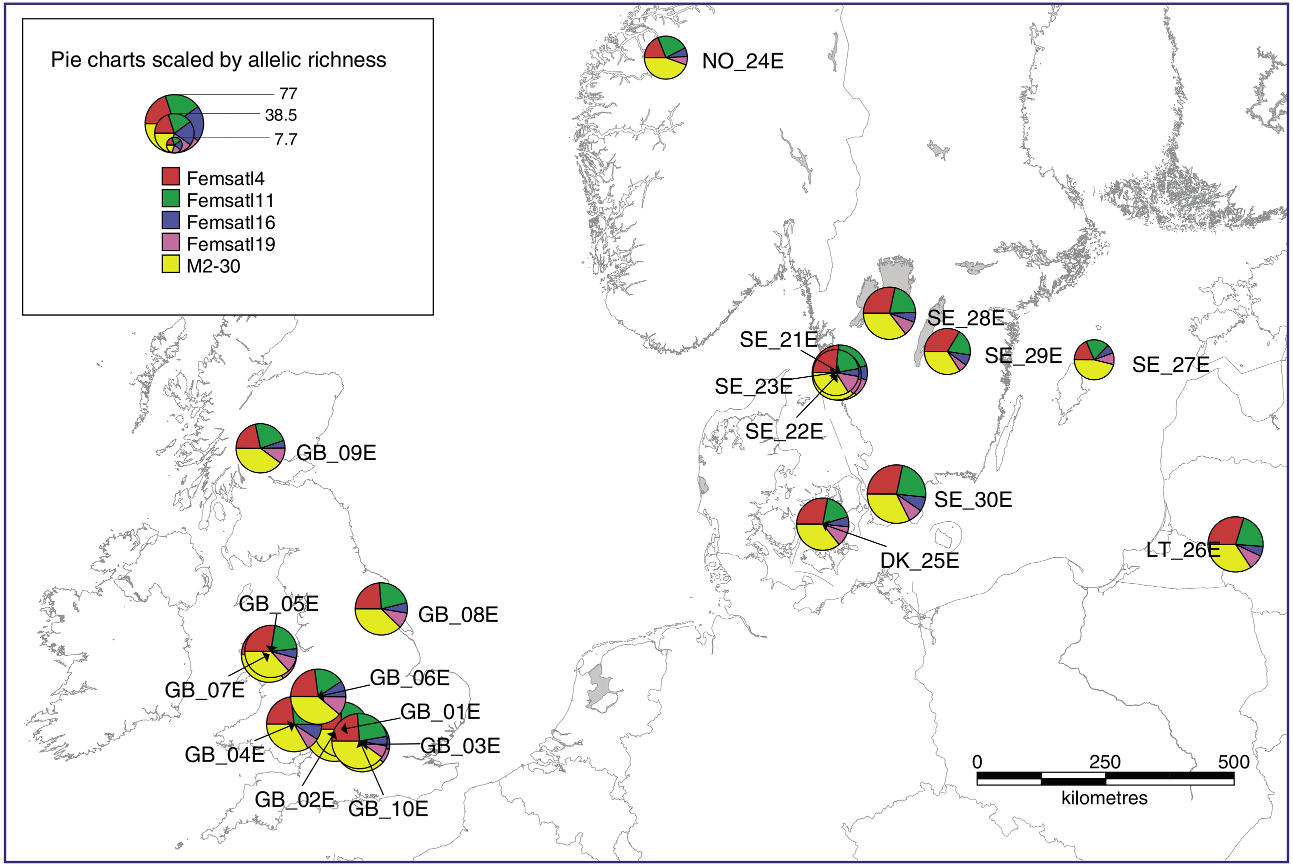


Figure 4.3 .- Allelic richness in 20 populations of *Fraxinus excelsior* from the UK, Scandinavia and the Baltic region. The size of the circles is scaled to show the total number of alleles found at five nuclear microsatellite loci, represented by the different colours. Each circle is divided into slices showing the proportion of alleles found at each locus. The number of alleles in each population is corrected for differences in sample size between populations.

Reproductive biology and gene flow

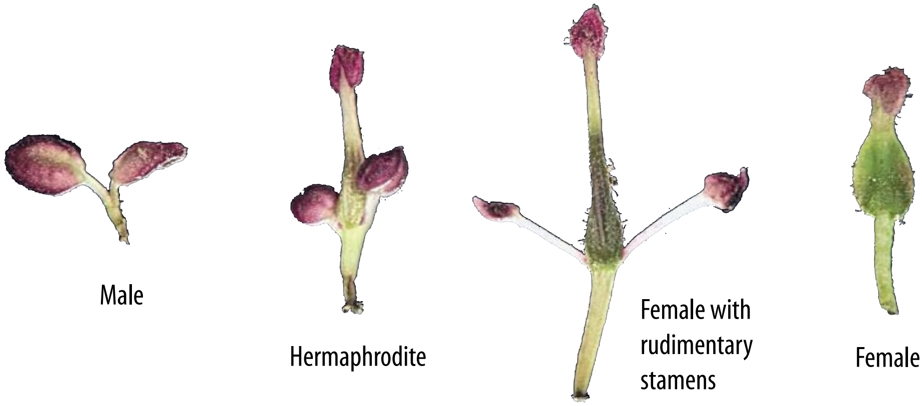
Fraxinus excelsior has been known for a long time to have a complicated reproductive system, and until recently it was poorly understood. In the 1990s, the state of knowledge was summarised in a review[21] which concluded that flowering and fruiting patterns had not been well described, although most evidence suggested that *F. excelsior* set seed irregularly, rather than regularly in alternate years. Different flower types had been noted: female, male, hermphrodite, and intermediates between them, but the function of **HERMAPHRODITE** and intermediate flowers was not understood. In particular it was not known whether **SELF-FERTILISATION** (**SELFING**) occurred, and if so at what level. Trees of different **GENDERS** had been noted but they could not be reliably identified and their relative abundance in different populations was not known. It was also not known whether the proportion of different sexes varied from year to year or in different locations, e.g. at different latitudes or in different climates, and a major unanswered question was whether individual trees changed sex from year to year. Answers to all these questions are important for designing planting schemes and seed orchards, managing woodlands, and planning seed collections. We addressed them by carrying out detailed studies to:

- determine sex ratios and describe patterns of flowering, fruiting and leafing in natural populations over several years;
- investigate seed set and levels of selfing in trees of different genders by means of controlled pollination experiments;
- establish levels of selfing and pollen movement distances by paternity testing, using DNA profiling on seeds collected in natural populations.

Flower types

Fraxinus excelsior is a wind-pollinated tree with small, simple flowers lacking nectar, scent and petals (all of which are used in insect-pollinated plants to attract pollinators). The flowers may be

- male, consisting only of two **STAMENS**,
- female, consisting only of one **PISTIL**, or
- hermaphrodite having both male and female parts and consisting of two stamens and one pistil (Figure 4.4).



Four different types of flowers

Male

Hermaphrodite

Female

Three types of inflorescences

Figure 4.4 .- Flowers and inflorescences of *Fraxinus excelsior*. Male inflorescences are dense clusters with about 200-400 male flowers. Hermaphrodite and female inflorescences both have around 100 flowers.

Female and hermaphrodite flowers can collectively be called **PISTILLATE** flowers because they both have a functional pistil containing an ovary producing the fruit. The ovary contains four **OVULES** (each containing an egg cell) but only one of these is usually fertilised by the **POLLEN**, resulting in a one-seeded fruit called a samara. The samara has a flattened wing facilitating dispersal by wind. Many flowers occur together in clusters called inflorescences (Figure 4.4). Pistillate inflorescences contain around 100 flowers whereas male inflorescences contain at least twice as many flowers, on average 200-400. The inflorescences emerge in the spring (March to April) from lateral buds on the shoot from the previous year. Leafing (or flushing) occurs after flowering has finished, on the shoots that emerge from the terminal buds.

The pistillate flowers frequently have male parts that are reduced in size and function. In particular the size of the **ANTHERS**, which contain the pollen, varies greatly among flowers in an inflorescence and among inflorescences on a tree. There is a continuous range of intermediate pistillate flower types, from hermaphrodite flowers with both male and female function to pure female flowers, with no clear distinction between these two types. Some intermediates resemble hermaphrodite flowers, with anthers that open and shed pollen even though they are reduced in size, whilst other intermediates are more similar to female flowers with much reduced anthers, which do not open. However, pollen production is not always directly related to anther size, because some smaller anthers may release pollen while larger ones may not. Pistillate flowers with functional anthers can potentially father seeds by selfing or outcrossing to other pistillate trees. There are fewer intermediate male flower types, although male flowers with tiny pistils do occur. Different flower types are combined in various proportions in the inflorescences, and different inflorescence types are combined in various proportions on the tree, producing trees with a range of gender expression.

Defining the gender of trees

At the broadest level we can classify trees as male or pistillate. The category of male trees includes all trees in which the majority of flowers are male. This category can be subdivided into purely male trees (with only male flowers) and trees with a mix of male and hermaphrodite flowers. These male-hermaphrodite mix trees can produce a few seeds, although considerably fewer than pistillate trees. The category of pistillate trees includes all trees with mainly hermaphrodite or female flowers, or flower types in between (see Figure 4.4). The main reproductive function of these trees is to produce seed, but because they also produce pollen, in varying quantities, it is possible that they also father some seed. Pistillate trees can be subdivided into hermaphrodite trees and female trees, based on the proportion of hermaphrodite and female flowers.

The most difficult trees to categorise are those with flowers that are intermediate between female and hermaphrodite, with small anthers, which may or may not shed pollen. We carried out experimental pollinations to compare seed set on female and hermaphrodite trees using self (i.e. their own) pollen, cross pollen from males and cross pollen from other hermaphrodites. We also collected seed from female and hermaphrodite trees in natural populations and used DNA profiling on the collected seeds to find out how common selfing is in nature and what proportion of seeds are fathered by other pistillate trees. These concepts are explained further in Chapter 3.

Seed set, selfing and seed germination in controlled pollination experiments

The aims of our pollination experiments were:

- to assess the ability of pistillate trees to self-fertilise;
- to compare seed set in different types of pistillate trees (females and hermaphrodites);
- to compare the fertility of pollen from males and hermaphrodites;
- to compare the viability and vigour of seeds produced by male and hermaphrodite pollen.

In two countries (UK and Sweden) we selected a total of 48 pistillate 'mother' trees and enclosed inflorescences on the trees in bags before the buds opened in the spring. The mother trees were chosen to represent the full range of pistillate tree types, and we gave each mother tree a score on a scale of increasing maleness, from females (low score) through intermediate types to hermaphrodites (high score). We collected and mixed pollen from five 'father' trees, either males or hermaphrodites. We used the two pollen mixtures to hand-pollinate inflorescences in some bags, whilst in other bags we left the inflorescences to self-pollinate. We replicated each treatment five times on each mother tree and also marked some inflorescences on the same mother trees as open-pollinated controls. These were bagged later so that the seed could be collected. In the autumn we counted and weighed the samaras from all the bags. To estimate seed set we calculated the seed:flower ratio for each bag as the total number of samaras with fully formed seeds divided by the number of flowers in the bag.

For each mother, we compared germination of 100 seeds produced by the three different pollination treatments: pollination by male pollen, hermaphrodite pollen and self-pollen. *Fraxinus excelsior* seeds have a two year dormancy so they were first pre-treated with warm and cold stratification to break this dormancy[12]. Once they started to germinate we recorded the number of germinated seeds every second or third day until germination ceased. We used the germination percentage after 15 days and 60 days as measures of the vigour and viability respectively of each seed lot. The following paragraphs summarise the main findings from these experiments.

Fraxinus excelsior is self-fertile

Out of 48 mother trees (18 in Sweden and 30 in the UK) only one tree in the UK produced no selfed seed at all, although two other UK trees and three in Sweden produced only a few selfed seed. The weight of selfed seeds (measured as mean samara weight) did not differ from the weight of seeds produced by male pollen or hermaphrodite pollen. This shows that almost all pistillate trees of *F. excelsior* are able to produce selfed seed in the absence of competing pollen from other trees.

Selfed seeds may not survive because of inbreeding depression

When counting the fruits from the experimental pollination treatments, we found many aborted fruits, ranging in size from expanded ovaries to full-sized samaras containing no seeds (empty fruits). Empty fruits were more common in the case of self pollination, resulting in a lower seed:fruit ratio, i.e. the number of samaras with fully formed seeds divided by the number total number of samaras (Figure 4.5). This suggests that selfed seeds, which have lower genetic variability, may not survive because of **INBREEDING DEPRESSION**.

Selfing rate and seed set do not differ between females and hermaphrodites

There was no relationship between field observations of gender (expressed as low and high maleness scores) and levels of seed set and selfing. We found no tendency for female trees to set more seed than hermaphrodites, nor for hermaphrodites to set more selfed seed than females. This suggests that female function does not differ between female and hermaphrodite trees.

Male pollen, hermaphrodite pollen and self pollen differ in fertility

We compared the seed:flower ratio (seed set) for the different pollination treatments. All experimental pollination treatments set fewer seeds than the open pollinated controls. This was probably an effect of the pollination bags, which increased temperature and humidity around the inflorescences. Of the experimental treatments, self pollination gave the lowest seed set, male pollen the highest, whilst hermaphrodite pollen gave intermediate levels (Figure 4.6). Hermaphrodites have fewer flowers per inflorescence than males, and the anthers are often smaller, which would decrease their pollen fertility relative to males. In addition, a previous study has shown that hermaphrodite anthers contain fewer pollen grains and have lower pollen viability[40].

Seeds produced by hermaphrodite and self pollen have similar vigour and viability to seeds produced by male pollen.

Seeds produced by male pollen, hermaphrodite pollen and self pollen had similar germination profiles (Figure 4.7), with no significant differences in vigour (defined as germination percentage after 15 days), nor in final germination percentage (viability). To continue this work, we potted up 10 germinants per mother from each pollination treatment and these seedlings will be harvested at the end of the 2005 growing season. We will measure final seedling height and root:shoot biomass to see if there are any differences in mortality and growth among seedlings produced by the different pollination treatments.

Paternity testing and pollen flow

We carried out detailed studies of paternity and pollen flow in a 12 ha mixed deciduous woodland in southern England and a 10 ha woodland in southern Sweden during a **MAST YEAR** (2003). In the UK the study was also carried out in a non-mast year (2002) to compare pollen flow during these two contrasting flowering events. The aims of our study were to:

- estimate levels of outcrossing and selfing in natural populations of *F. excelsior*;
- calculate levels of pollen flow in ash woodlands;
- compare mating success of hermaphrodite and male trees;
- identify factors influencing fathering success, such as size or age;
- compare results between sites (UK and Sweden) and years (mast and non-mast).

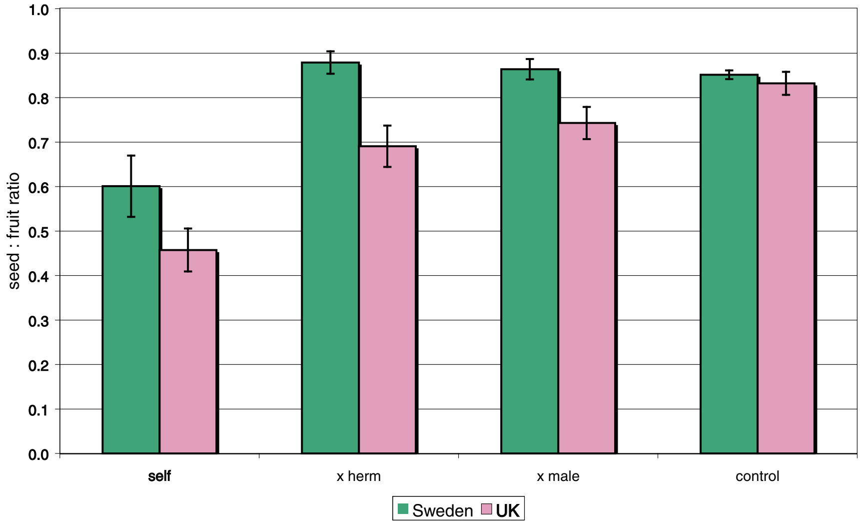


Figure 4.5 .- Mean seed:fruit ratio and standard error of the mean for pollination experiments with *Fraxinus excelsior* using self pollen, pollen from hermaphrodites, pollen from males and open pollinated controls. Data from 24 trees (UK) and 11 trees (Sweden).

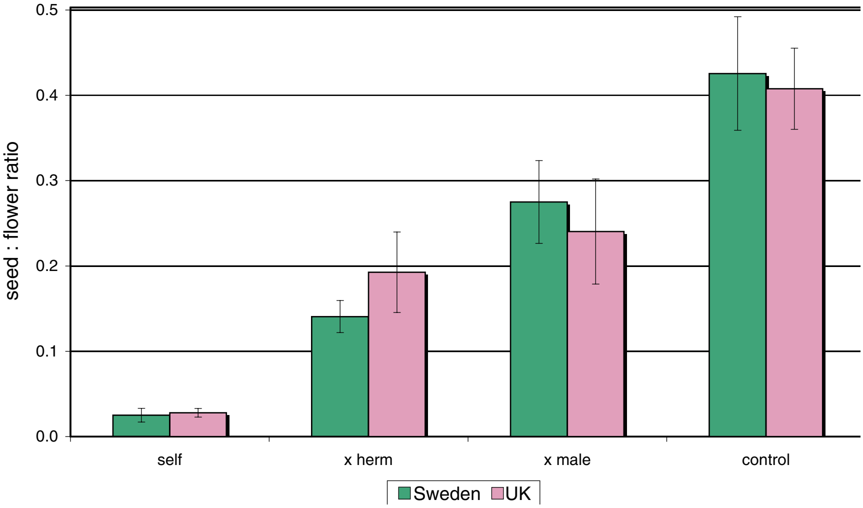


Figure 4.6 .- Mean seed:flower ratio and standard error of the mean for pollination experiments with *Fraxinus excelsior* using self pollen, pollen from hermaphrodites, pollen from males and open pollinated controls. Data from 24 trees in the UK and 10 trees in Sweden.

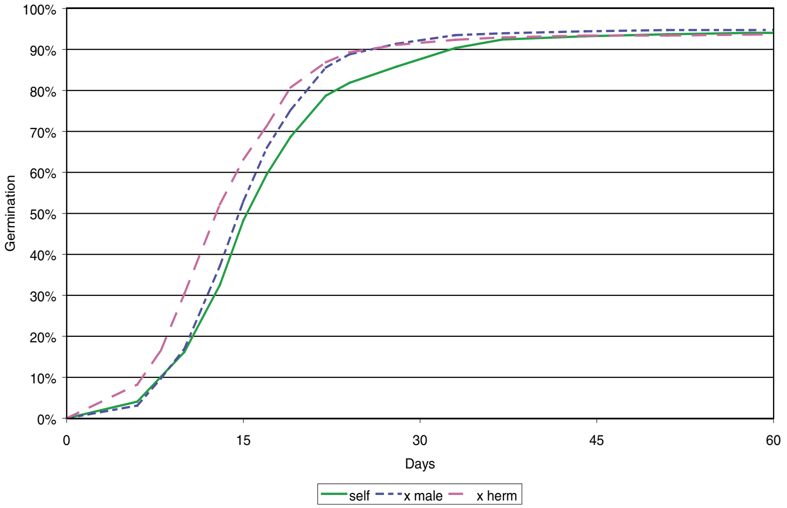
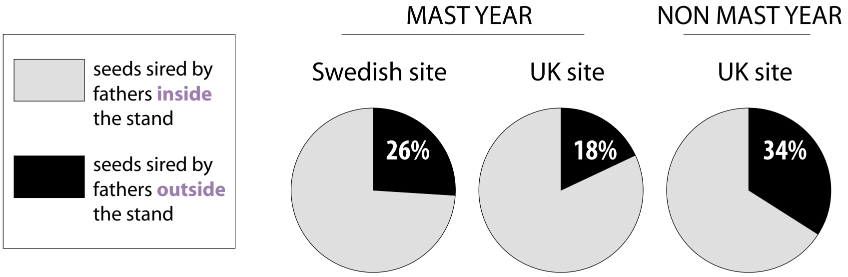


Figure 4.7 .- Germination curves for samaras of *Fraxinus excelsior* produced under three different pollination treatments: pollination by self-pollen (green line), male pollen (blue line), hermaphrodite pollen (pink line). Data from 9 trees in the UK.



Mean pollen dispersal distance:	49m	41m	72m
Mean distance to all flowering males:	177m	173m	180m

Figure 4.8 .- Pollen flow and movement: for *Fraxinus excelsior*: comparisons between sites and years

Fraxinus excelsior is outcrossed

We **GENOTYPED** seeds collected from about 20 trees at each site to see if they were produced by self pollen or outcrossed pollen. We detected very high levels of outcrossing at both sites, in both mast and non-mast years. The mother trees were classified as hermaphrodite or female based on field observations of flower types and anther size. In the UK there was some indication of a small amount of selfing in hermaphrodites in both years, although the level of outcrossing was not significantly different from females (in the Swedish populations there were too few hermaphrodite trees to test this). We pooled data from all mothers to compare levels of selfing between sites and between years. Similar levels of selfing were observed in the UK and Sweden in the mast year (0.3% and 0.7% respectively), whilst selfing was higher in the UK in the non-mast year (1.7%).

Pollen flow is higher in non-mast years compared to mast years

In the non-mast year 53% of the 650 *F. excelsior* trees in the UK woodland flowered, whilst in the mast year this increased to 91%. This difference was mainly due to smaller (younger) trees flowering in the mast year but not in the non-mast year. The number of seeds fathered by trees outside the investigated stands varied between sites and years (Figure 4.8). The highest level of incoming pollen was detected in the UK in the non-mast year, when 34% of seeds were fathered by trees from outside the stand. In the mast year this decreased to 18%. Effective pollen flow (i.e. pollen that fertilises) from outside the stand was thus higher in the non-mast year. In the non-mast year effective pollen flow was also higher to trees on the edge than to trees in the centre of the stand.

The average distance between mates, a measure of pollen movement, was similar in the UK and Sweden in the mast year (41 and 50 m respectively), reflecting the fact that the average distance between a mother and all males that flowered was also similar in both sites. However, in the non-mast year the average distance between mates increased greatly to 72 m, although the average distance between a mother and all males that flowered only increased slightly. Pollen flow occurs over greater distances in non-mast years relative to mast years, because fewer trees are flowering and the distance between successful mates is greater. By comparison, in a different study in the highly exposed landscape of the southern uplands of Scotland, average pollen movement was estimated to be 328 m among isolated patches of *F. excelsior*[1]. Wind dispersal of pollen and subsequent siring of seed in *F. excelsior* is therefore highly dependent on the density of the stand of trees, the proportion of trees flowering, and on the local environment and landscape.

Fathering success

Gender was the main factor determining fathering success. Most seed is produced by pollen from males but pistillate trees ('females' and hermaphrodites) do father some seed. In the mast year in the UK the fathering success of male trees was 6.6 times that of pistillate trees, whilst in the non-mast year the ratio decreased to 4.0. Pistillate trees thus fathered proportionately more seed in the non-mast year when fewer potential mates were available. This increase also reflects the slight increase in selfing in non-mast years. Distance from the mother tree and size (dbh) of the father also had significant, but small, effects on fathering success, with closer and larger males fathering more seeds than distant and smaller ones. We found no evidence of an effect of flowering intensity (proportion of crown in flower) or age on fathering success.

Sex ratios and phenology of flowering, fruiting and leafing

The relative timing of pollen release and stigma receptivity within individuals and populations of *F. excelsior* is likely to influence the mating system (outcrossing or selfing) and the extent of pollen flow within and between populations. It will also influence the number of individuals mating with each other (effective population size) and the levels of genetic diversity in natural regeneration and seed collections. It was already known that *F. excelsior* is **PROTOGYNOUS**[40], i.e. the stigmas of the flowers are receptive at bud burst whereas pollen is not released until several days later. However, the extent to which the protogynous phase varies between trees, populations and years, and its influence on mating patterns, was unknown. Similarly, although the breeding system of *F. excelsior* has been characterised in general as **SUBDIOECIOUS**[40], i.e. populations predominantly consisting of trees with either male or female function, it was uncertain whether individual trees changed sexual function from year to year, and whether the proportion of different genders varied across the species range. It was not clear either whether sex had an effect on resource allocation between vegetative and reproductive growth, at least in masting years.

Phenological observations were carried out every two to three days (over flowering, leafing, and leaf fall periods) on at least 60 trees in two populations, over two years, in Romania (2003, 2004), Sweden (2002, 2003) and U.K. (2002, 2003). This allowed us to study the following traits within trees, populations and across the species distribution:

- flowering phenology (timing of start and finish of flowering, including male and female phases);
- flowering and fruiting intensity;
- vegetative phenology (timing of leaf flush and leaf fall);
- sex expression at flower, inflorescence and tree level and its variation over years and at different sites (estimates of relative numbers of female, male and hermaphrodite trees in the populations);
- between-year differences in flowering and fruiting within individuals and how this correlates with vegetative growth (relative increase in basal area between years);
- the influence of temperature on the various phenological phases.

The research followed a standardised protocol to ensure consistency of observation and interpretation of flower types across sites. The results are summarised as answers to questions of importance in the management, utilisation and conservation of the genetic resources of *F. excelsior*.

Does the proportion of sexes vary from site to site and year to year?

There were marked differences between sites (Figure 4.9), with a very low occurrence of hermaphrodites in Sweden. However, the proportion of sexes at any one site did not change much over time, contrary to the popular belief that *F. excelsior* can change its gender from year to year. We did observe that a small proportion of hermaphrodite trees appeared to vary in their sex, becoming more female or male in the mast year.

Does the start of stigma receptivity vary across sites and years?

Yes. Stigmas in *F. excelsior* are receptive to pollination as soon as the flower buds open. British sites were the earliest for flower buds to open, being three weeks to a month earlier than those in Sweden (depending on the year), with the Romanian sites being intermediate. The start of stigma receptivity shows a similar response to temperatures as leaf flushing, with flowers opening much earlier when preceded by warm winters.

Does the start and length of pollen release vary across sites and years?

Yes. The timing of pollen release shows marked differences between sites and between years at the same site. Within the same site, the start of pollen release varied by 1–2 weeks over a two year period. It also varied by 3–4 weeks between the earliest sites in Britain and the latest in Sweden; again, Romania was intermediate. Where sites were geographically close they showed similar timing, except where there were large altitudinal differences, where lower temperatures delayed pollen release. The length of time over which an individual tree released pollen varied between countries from 5–10 days in Sweden, and 4–13 days in Romania, to 15–18 days in Britain.

The timing of floral bud opening is influenced by the response of bud development to winter and spring temperatures. However, anther maturation is further influenced by temperatures immediately after flower buds open. High daytime temperatures and dry conditions cause the anthers to open and pollen to be released into the atmosphere. Wet and cold conditions lead to delays in pollen release and to an increase in the overall period of pollen dispersal within a stand, as seen in the British sites. Delays in local pollen release may increase the likelihood of pollination by airborne pollen from outside a stand.

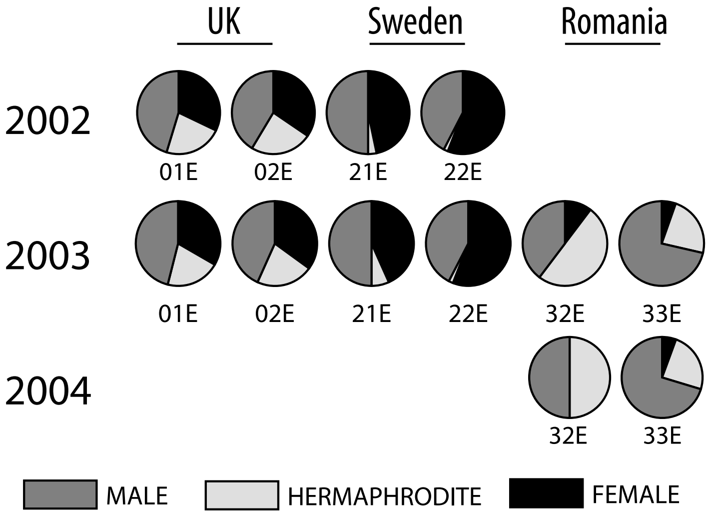


Figure 4.9.- Proportion of sexes of *Fraxinus excelsior* in 6 sites across 3 years.

Does the extent of overlap between the male and the female phase within sites vary?

Yes. The female and male phases showed considerable overlap at the Swedish sites, whereas at the British and Romanian sites it was much less (Figure 4.10). In particular much of the peak of stigma receptivity at the British sites was before large-scale pollen release at the same site.

Within a stand of trees, the extent of overlap between pollen dispersal (male phase) and the period of female receptivity (female phase) may influence the proportion of pollination by trees within the same stand. Thus the degree of synchrony in male and female phases can influence the degree of pollen contamination in a seed orchard, or the connectivity (pollen flow) between different patches of forest (see section on paternity testing and pollen flow). The much greater overlap between pollen release at site GB_02E and stigma receptivity at GB_01E, compared to the overlap between these within GB_01E (Figure 4.10), shows the potential for gene flow between two sites which are only 15km apart. Similarly, the lower synchrony between countries reduces the possibilities of gene flow through long distance pollen dispersal, maintaining **GENETIC DIFFERENTIATION** between regions (see section on genetic variation).

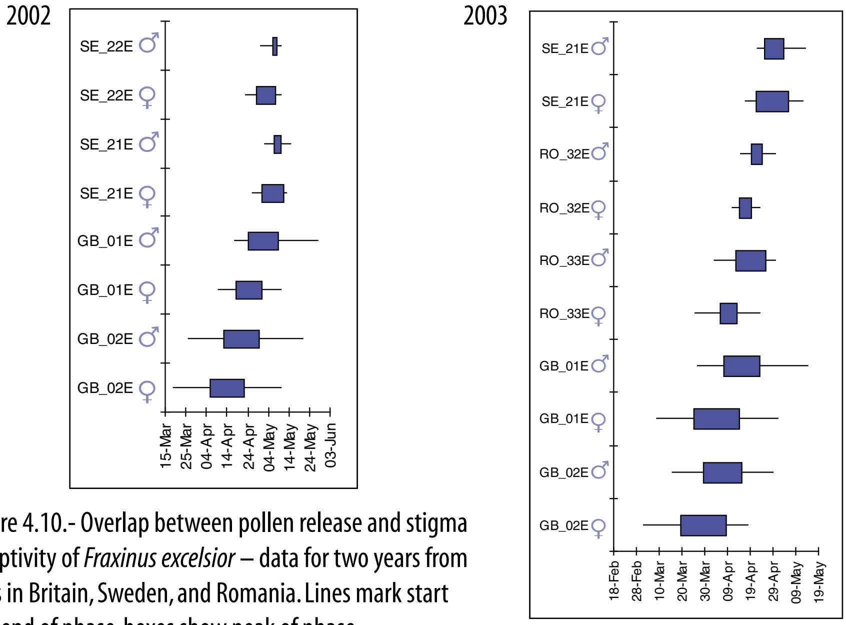


Figure 4.10.- Overlap between pollen release and stigma receptivity of *Fraxinus excelsior* – data for two years from sites in Britain, Sweden, and Romania. Lines mark start and end of phase, boxes show peak of phase.

Are the male and female phases synchronised in hermaphrodite trees?

Generally pollen release and stigma receptivity do not occur at the same time within hermaphrodite trees. Only a few trees showed high synchrony (5% of trees showing more than 80% within-tree overlap of male/female phases, 50% showing less than 15% overlap). The difference in timing between female and male phases reduces the likelihood of selfing in individual trees and supports the mating system study results, which showed high levels of outcrossing in hermaphrodite trees.

Does flowering and fruiting (seeding) intensity vary from year to year?

Yes. Ash is well known for having mast years and the data reflects this. In 2002, no seed was produced at either Swedish site, despite 40–60% of the trees flowering to some degree, whereas in 2003 there was heavy flowering and seeding at both sites. 2003 similarly saw mass flowering and seeding at both British sites, with low seed production in 2002. In Romania, flowering and seeding was less consistent across sites. One site showed low seed production in both years, despite substantial flowering, whilst at the other site there was little flowering and seed production in just one year.

More long-term data from a similar site in Sweden shows large variation from year to year, but there is no particular pattern in the occurrence of mast years[40]. Flowering intensity is mainly determined by summer temperatures two years preceding flowering (when flower buds are initiated in leaf axils of next year's shoots)[8]. Seed production is affected by pollination success, so weather that is unsuitable for effective wind pollination (*e.g.* rain) decreases pollination. Thus, even a good flowering year may lead to a low seed crop if pollination fails. A late frost may also destroy the flowers, preventing seed production.

Does the start of leaf flushing vary from site to site and year to year?

Yes. Such variation is well known, and unsurprisingly there were differences between countries and years in the start of leaf flushing. The British sites were the earliest to flush, being 1-4 weeks earlier than those in Sweden (depending on the year), with the Romanian sites being intermediate. The British sites are 15 km apart, and only differ by 150 m in altitude. However even the small difference in altitude influenced the phenology and flushing at the colder site (300 masl) was approximately 1 week later than the other. Variation over two years at the same site was anything from one week (Romania and Sweden) to three weeks at both British sites.

Variation in flushing date is not simply a factor of latitude, but a more complex relationship of the accumulation of **GROWING DEGREE HOURS** (GDH - sum of mean hourly temperatures above 5°C), with milder winters leading to earlier flowering and flushing[8].

Previous research shows that timing of leaf flushing can be an important factor in adaptation and production. For instance, material of tree species introduced into Britain from parts of continental Europe flushes early under the milder winter conditions, but often suffers from late spring frosts that are typical of the British climate. This has been reported for Romanian *F. excelsior* growing in Britain[7].

Does duration of leaf activity vary from site to site, year to year, with sex, flowering or non-flowering?

Similarly to leaf flushing, the length of leaf activity (from new leaves becoming fully expanded to leaf fall starting) also showed variation between sites and years, with differences of 10-40 days, depending on the year, between Britain/Romania and Sweden. Variation over two years at the same site was as much as 40 days in Romania and as little as one week at the British and Swedish sites.

The length of leaf activity is influenced by previous winter temperatures (through leaf flushing), differences in day length, and the occurrence of frosts during leaf senescence. In Sweden the period of leaf fall was much shorter (5–6 days) than in Romania (19–29 days) and Britain (18–20 days). In 2002, early frosts at the Swedish sites caused sudden leaf fall. There was no difference between males, females and hermaphrodites in the length of leaf activity, neither did flowering trees show any difference from non-flowerers, nor pistillate trees from male trees.

The resources needed for sexual reproduction (flowering and fruiting) may vary between sexes, resulting in differing allocation of resources between vegetative and reproductive growth. There was, however, no evidence of differences in terms of the duration of flowering or leafing. In any site, both sexes flower and flush leaves at the same time, with the growing period being similar across sexes, such that none has longer to photosynthesize and thus accumulate more resources. However, we detected a tendency for all trees to show decreased vegetative growth following mast seeding. At the British sites, seed-bearing trees in particular responded negatively. This suggests that the real costs of reproduction may differ across sexes and be compensated for by reduced vegetative growth in pistillate trees, an observation which deserves further investigation in a long term study using a larger sample of trees.

SUMMARY OF RESULTS FROM STUDIES OF REPRODUCTIVE BIOLOGY AND GENE FLOW

Levels of selfing are very low

Our results showed that *F. excelsior* is highly outcrossed in both good and poor seed years. Although self-fertilisation is possible, selfing in natural populations is very low, probably due to inferior pollen quantity and quality as well as lack of synchrony between stigma receptivity and pollen release in hermaphrodite trees.

Hermaphrodites have both male and female function

Levels of selfing and seed set are similar in hermaphrodite and female trees. This means that seed collections from hermaphrodite trees, or their inclusion in seed orchards, is unlikely to result in the use of selfed seed or reduced levels of genetic diversity. Although experimental pollinations demonstrated that hermaphrodites are able to father similar quantities and quality of seed to males, at least in the absence of competition from male pollen, the actual male fertility of hermaphrodites in natural populations appears to be much lower than that of male trees, where pistillate trees father at least four times fewer seeds. The breeding system of *F. excelsior* is here called subdioecy, referring to the predominantly male or female nature of individuals in a population.

Sex ratios are generally stable between sites and years

The proportion of trees of different genders was fairly stable: 40-50% male and 50-60% pistillate trees were found in all sites in all years (except for one site in Romania, with about 70% male trees). The relative proportions of hermaphrodites and females varied from year to year and site to site, but for the purposes of planning seed orchards, seed collections and planting schemes all pistillate trees can be considered as a single group.

Phenology varies with climate, altitude and temperature

Flowering, leafing and fruiting phenology varied between sites and years depending on factors such as latitude, altitude and temperature. This should be taken into account if transferring planting material between sites, for example to avoid frost damage in particular during leaf flushing, which leads to increased forking in trees.

Pollen flow into a stand is higher in non-mast years compared to mast years

In poor flowering years, pistillate trees receive pollen from further away than in good flowering years. Hence, pollen flow into a stand from the outside is higher in non-mast years than in mast years. Seeds collected from edge trees more often have fathers from outside the stand compared to seed from trees in the centre. As explained on p.54, gene flow through pollen movement across the landscape is also influenced by the degree of synchrony between stigma receptivity and pollen release between stands.

Local adaptation

One of the main factors currently shaping European policies on forest reproductive material is the concept that local seed is preferable. The underlying assumption is that this is more likely to be well adapted to the planting site than seed from further away. The FRAXIGEN project has used reciprocal transplant experiments (RTEs) to examine the extent to which local adaptation is apparent in European ash species, and the scale over which it operates. The concepts of localised adaptation, and the RTEs, are discussed in Chapter 3.

For *F. excelsior*, we have conducted an RTE comprising trials at eight sites across the southern part of Britain (England and Wales). Each trial includes the seed from that wood (the *home* population) and seed from the seven other sites (*away* populations). The same eight seed sources are planted at all eight sites, in openings under the woodland canopy (Photo 4.5), and evaluated in terms of germination, survival and growth. At each site one trial measures germination and growth of direct-sown seed (Photo 4.6), while a second measures survival and growth of seedlings raised in a nursery and then planted out in the wood (Photo 4.7). All the sites are semi-natural mixed deciduous woods (woodland composed mainly of locally native trees and shrubs including *F. excelsior*, which derive from natural seedfall or coppice rather than from planting). This means that the seed was collected from trees which have had the opportunity to adapt to that site. The sites cover a range of distances from one another, to enable us study the geographic scale over which localised adaptation operates. The closest pair of sites is 7 km apart; others are separated by 50-100 km, and some by up to 280 km (Figure 4.11). There are also differences between the woods in their soils, altitude, and aspect, such that sites closest together are ecologically distinct.

The trials are currently at an early stage, and so far only data from the nursery stage and the first year in the field is available. The seedlings were raised in the nursery in the soil of the site into which they would eventually be planted. Top soil was collected from each site, and transported to the nursery. In conventional provenance trials, the use of highly fertile nursery soil makes it difficult to say anything about the extent of local adaptation until several years after planting out in the field. In the RTE, in contrast, the raising of seedlings in soil from their eventual planting site should reduce the extent and length of nursery effects, so that adaptive differences are visible at a much earlier age. It also allows assessment of the influence of soil at the nursery stage.

Photo 4.5 .- Seed (left) and seedling (right) trials in an RTE of *Fraxinus excelsior* in an opening under the woodland canopy at Sally Beds, UK (see location in Figure 4.11).

No fertiliser treatment was applied, but the plants were irrigated during their time in the nursery. The seedlings were assessed at the end of one growing season for height and root collar diameter before planting in the field. The nursery data show significant differences between provenances for diameter growth in each of the soil types, for volume in five of the eight soils, but not for height in any soil[2]. At the end of one year's growth in the field (two years since germination) there were significant differences between provenances for height at six of the eight sites, while germination and survival in the seed trial showed differences between provenances at only two of the sites.

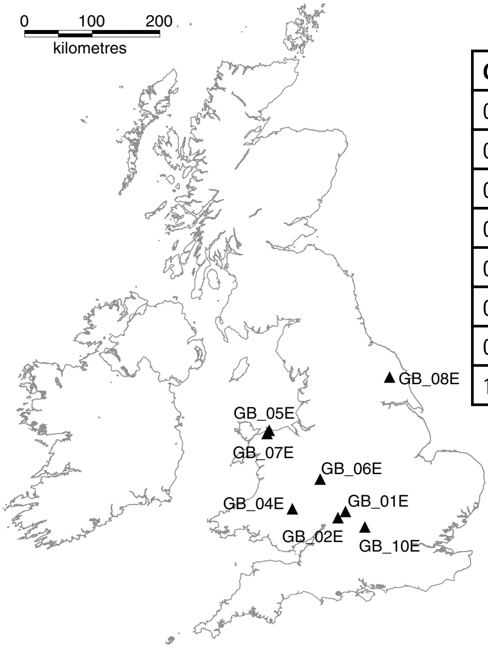
In both the nursery and field, there was no geographic pattern as to which provenances showed the best growth. The consistently second best performer (01E) comes from only 15 km away from the worst (02E), while growth of provenances from highly calcareous soils (05E, 08E, 10E) was not any better on the highly calcareous sites. Whilst the 01E and 08E provenances performed best on their own soil, this was no more than a reflection of the fact that they were the best performers over all the sites. Across-site analysis showed significant differences both between provenances and between sites. However, the interaction term between site and provenance was non-significant, so there is no evidence of genotype-environment interaction, and hence of home site advantage at this age.

Although there is currently no evidence for home site advantage in initial seedling growth, it is too early to draw broader conclusions related to the scale of adaptation in *F. excelsior*. Results from traditional provenance trials of *F. excelsior* in Europe suggest that while genotype by environment interaction may occur, this may not be due to a home site advantage[7;20]. In other words performance of provenances may be unstable across sites, but not because local seed sources perform better. Thus, geographical proximity may be a poor indicator of adaptive fitness.

The extent of local adaptation in *F. excelsior* within Britain, and therefore its practical importance to woodland restoration, remains in doubt. While trials such as this RTE mature and provide more definitive results, planting of native broadleaves within Britain, for a wide variety of purposes, continues to increase, and so does demand for certified sourced seed. A study of the few remnant *F. excelsior* and *Sorbus aucuparia* (rowan) trees in the denuded Carrifran Valley in southern Scotland has shown that large amounts of genetic diversity are maintained, making them suitable for local use in restoration despite their highly fragmented nature[1]. In contrast some of the locally sourced rowan planted as part of the Carrifran wildwood restoration project has been shown to be low in genetic diversity[19], presumably due to poor collection practices, and this will impose limitations on the future potential of the population. Thus, whether or not local adaptation turns out to be a major determinant of performance in British ash, it is beyond doubt that poor adherence to basic good seed collection practice will lead to the establishment of trees with restricted genetic diversity, with poor future adaptive potential, whatever the provenance of the seed.

Photo 4.6.- Seed trial in a Reciprocal Transplant Experiment (RTE) of *Fraxinus excelsior*.

Photo 4.7.- Close up of a seedling trial in a RTE of *Fraxinus excelsior*.



GB_	01E	02E	04E	05E	06E	07E	08E	10E
01E	0							
02E	15	0						
04E	91	79	0					
05E	198	194	143	0				
06E	76	75	69	122	0			
07E	196	191	139	7	121	0		
08E	237	246	269	217	200	223	0	
10E	41	51	129	238	115	236	251	0

Site #	Site name	Latitude N	Altitude m.a.s.l.	Mean annual rainfall (mm)	Soil type	pH	Continentality+	FC seed zone*
GB_01E	Sally Beds	52°01′	310	622	clay loam	4.3	8	403
GB_02E	Queen's Wood	51°55′	110-200	622	calcareous clay	5.7	9	403
GB_04E	Cilcenni Dingle	52°04′	130-160	846	seasonally wet, coarse loam	6.8	8	304
GB_05E	Marl Hall	53°18′	60-90	788	shallow calcareous loam	7.7	6	304
GB_06E	Meadowley	52°32′	100-170	656	red-brown, silt loam	5.8	10	403
GB_07E	Coed Merchlyn	53°14′	50	788	seasonally wet, alluvial, silt loam	5.5	6	304
GB_08E	Settrington	54°07′	80-130	729	shallow calcareous loam	8.0	9	401
GB_10E	Wytham	51°46′	120-150	642	shallow calcareous loam	7.7	11	404

Figure 4.11.- *Fraxinus excelsior* reciprocal transplant experiment site locations, details, and matrix of distances between sites (km). + Pyatt *et al.* 2001 * Herbert *et al.* 1999 in Literature cited list.

Chapter

5

Fraxinus angustifolia (narrow-leaved ash)

Photo 5.1.- Dense *F. angustifolia* forest at Ploiesti, Romania.

Distribution and systematics

Fraxinus angustifolia Vahl (narrow-leaved ash) is found throughout southern and eastern Europe. Its distribution extends from Spain and Portugal in the west, northwards to Slovakia and southern Moravia, and eastwards to Turkey (Mediterranean and Black Sea region), Syria, Caucasus, Iran and southern Russia (see distribution map on page 11: Figure 1.2). In Central Europe, the Pannonian Basin and the Balkans it occurs mainly in riparian and floodplain forests (Photo 5.1). These forests are found along the large rivers and their tributaries (such as the Drava, Sava, Danube, Váh, Morava, Tisa, Nester, Maritza, Tundja and Iantra), or associated with lakes and wetlands (e.g. Lake Balaton, Burgas and Varna). In the Mediterranean region (Spain, France, Italy, Greece, southern Turkey), *F. angustifolia* can be found on drier sites at higher altitudes (500m-2000m), as well as on riverine and wetland sites. Mixed stands of *F. angustifolia* and *F. excelsior* are also found (*e.g.* in Romania and Moldova) on the banks of large rivers, where *F. excelsior* seeds have been transported downstream by the water.

Fraxinus angustifolia is closely related to *F. excelsior*. Its morphology is quite variable, and several **TAXA** have been described based on differences in the **SAMARA** and leaf morphology. *Fraxinus angustifolia* has three subspecies, restricted by geographical region:

- ssp. *angustifolia* (western Mediterranean);
- ssp. *oxycarpa* (Bieb. ex Willd.) Franco & Rocha Afonso (east Central Europe and southern Europe from NE Spain eastwards);
- ssp. *syriaca* (Boiss.) Yalt. (Turkey and eastwards to Iran).

Apart from these geographical criteria, the three subspecies differ from one another in the number of leaflets and the shape of the samara, but there are many intermediate forms that make subspecies determination difficult. There are also different phenotypic forms of *F. angustifolia* (form *parviflora*, form *rotundifolia*), which are not considered as separate taxa. The form *rotundifolia*, with wide leaves like those of *F. excelsior*, is common in Moldova and the eastern part of Romania.

Distinguishing *F. excelsior* and *F. angustifolia*

Fraxinus angustifolia is sometimes confused with *F. excelsior*, although there are several characters that distinguish them (see Figure 5.1):

The clearest distinction is in the **INFLORESCENCE** type. *F. excelsior* has a compound inflorescence (panicle), whereas *F. angustifolia* has a simple, unbranched raceme. There are many more flowers in the large inflorescences of *F. excelsior* than in the smaller inflorescences of *F. angustifolia*.

This can also easily be seen on trees bearing fruits or fruit remnants: there are many branches in a *F. excelsior* fruit cluster, but only one branch in *F. angustifolia*.

Although bud colour is variable, *F. excelsior* has black terminal buds whereas *F. angustifolia* buds are usually brown.

The leaflets of *F. angustifolia* are usually narrower than in *F. excelsior*.

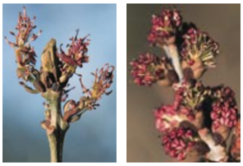
N.B. One should not rely on leaf and bud characters alone to distinguish the two species, since these vegetative characters are quite variable in both species. Seedlings of the two species, as well as **HYBRIDS**, may therefore be indistinguishable.

Fraxinus pallisiae, a hairy variant common in the Danube Delta

Synonyms

The name *F. pallisiae* Willmott, and its synonym *F. holotricha* Koehne, is also treated in this book as a synonym of *F. angustifolia* (although some practitioners, in its natural range in southeastern Europe, consider it as a distinct species in afforestation programmes). This variant is similar to "normal" *F. angustifolia* ssp. *oxycarpa* in most characters, but is distinguished by its pubescent (hairy) or partly pubescent leaves and branchlets, especially when young. The samaras may be pubescent too. It is usually found on wet sites (such as the Danube Delta), flood plains and streams, but more or less pubescent individuals occur elsewhere too, often mixed with intermediate forms and "pure" *F. angustifolia*. The variant is found in various proportions in the eastern part of Romania (from 10-15% in Ploiesti district and Oltenia region, to more than 80% in the Danube Delta), and also in Moldova.

Figure 5.1.- Practical guide for the differentiation of *Fraxinus excelsior* and *Fraxinus angustifolia*

Species / Criteria	*Fraxinus excelsior*	*Fraxinus angustifolia*	Validity of criteria
Buds	 Colour: black to dark brown. Terminal buds with flattened node, forming a "snake-head"	 Colour: brown (variable). Flattened node does not form a "snake-head"	 Weak: because of the variation of bud colour in *F. angustifolia*
Shoots / Stem	 Non-hairy[1]	 Mostly non-hairy[1]	Indifferent, but see comments[1]
Leaflets	 Oval-shaped to spear-shaped	 Narrower, spear-shaped	Slightly discriminative: because of a large variation in leaflet shape and atypical forms (see comments[2])
Gender	 Pure male trees and hermaphrodite or female trees	 Hermaphrodite trees only[3]	 Powerful only in the case of pure male *F. excelsior* trees
Inflorescences and fructifications	 Branched (Panicle with 50-150 flowers/fruits)	 Unbranched (Raceme with 15-20 flowers/fruits)	Very powerful with the exception of male *F. excelsior* trees that may form some fruits (see comments[4])
Fruits (samaras)	 Flattened, distally winged	 Flattened, distally winged	Indifferent[5]

(see Comments overleaf...)

Figure 5.1.- (...continued)

Comments

1. The subspecies *F. excelsior* ssp. *coriariifolia* (occurring in eastern Romania) is densely hairy on shoot, buds, leaves and samaras but the more widespread *F. excelsior* ssp. *excelsior* is always non-hairy.

The hairy variant of *F. angustifolia* (sometimes distinguished as the separate species *F. pallisiae* and *F. holotricha*) is hairy on shoots, buds, leaves and samaras. Intermediate forms which are more or less hairy, or partly hairy, exist in some populations mixed with non-hairy individuals. The hairy ash is mainly found in eastern Europe, and is especially common in the Danube Delta where some *F. angustifolia* populations consist of only hairy individuals.

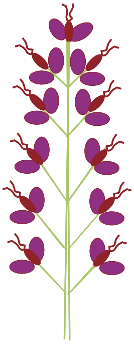
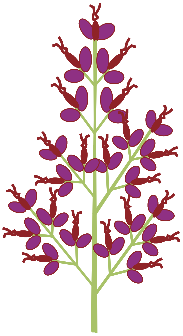
2. The narrow-leaved *F. excelsior* (f. *acuminata*), with leaves like those of *F. angustifolia* is found in large areas in the eastern part of Romania (forest district Iasi) and also in Moldova Republic. The wide-leaved *F. angustifolia* (f. *rotundifolia*) is present in 10-50 % of the individuals of the population in almost all *F. angustifolia* forests in Romania, more frequent in the eastern part of country.

Narrow-leaved *F. excelsior*

Leaflet variation in *F. angustifolia*

3. Female trees of *F. excelsior* can have flowers with reduced **STAMENS**. The hermaphrodite trees of *F. angustifolia* can have hermaphrodite inflorescences (usually with some pure male flowers at the base) and some pure male inflorescences.

4. The mixed inflorescences of some *F. excelsior* trees, with hermaphrodite flowers only on the main axis and male flowers on the secondary branches, can be mistaken in the fruit stage because of the appearance of a raceme after the fall of male flowers.

F. angustifolia	*F. excelsior*	*F. excelsior*
Unbranched inflorescence with hermaphrodite flowers	**Branched** inflorescence with hermaphrodite flowers	**Branched** inflorescence with hermaphrodite flowers on the main axis and **branches with male flowers that have fallen off**
		

5. Another dangerous mistake: common ash versus American ash

Species / Criteria	*Fraxinus excelsior*	*Fraxinus americana*	Validity of criteria
Fruits (samaras): seed cavity	Flattened 	Cylindrical 	Very powerful
Other	 Hermaphrodite or unisexual flowers in a panicle	 Unisexual flowers only (male or female) in dense panicles on separate male and female trees	

Ecology and silviculture

Fraxinus angustifolia is commonly found in riparian forests, sometimes in pure stands but more commonly mixed with other broadleaves including *Quercus*, *Carpinus*, *Ulmus*, *Acer*, *Alnus*, *Populus*, *Salix*, *Platanus*, *Juglans*, *Tilia*, *Sorbus* and *Prunus* species, as well as various small trees and shrubs including *Pyrus*, *Malus*, *Corylus*, *Tamarix*, *Cornus*, *Crataegus*, *Ligustrum*, *Ramnus*, *Viburnum*, *Sambucus* and *Rosa* species[6; 22; 24; 37] In contrast to *F. excelsior*, *F. angustifolia* tolerates wet and flood-prone sites.

Soil and climate

Fraxinus angustifolia grows well on moist, rich clays in bottomlands and moist, well-drained soils in uplands. It does best on rich soils at lower altitudes and will withstand temporary flooding. It prefers aerated or only moderately compacted sandy clays. It also grows well on loams, sandy loams and sandy clay loams with pH in the range 5.0-8.0 (but usually 6.0-8.0). The optimum soil depth is between 40 and 100 cm, with a soil volume of 0.45 to 0.9 m^3/m^2. Sub-soils are usually alluvial, with various textures.

Fraxinus angustifolia requires a mild climate and precipitation between 400 and 800 mm. Such conditions ensure a growing season of 6 to 7 months. *Fraxinus angustifolia* is a light-demanding species, requiring 60-100 % of total direct light.

Silvicultural characteristics

Riverine mixed forests (Photo 5.2), including those containing *F. angustifolia,* represent very stable ecosystems, rich in species diversity. In these forests, most of the hardwoods forming the canopy layer have valuable timber. Silvicultural management of these forests can be achieved using continuous cover methods (shelterwood or group systems) in which natural regeneration of *F. angustifolia* and *Quercus robur* is achieved by regeneration cuttings in small areas (strips or groups) in years with abundant seed crops. Secondary fellings are repeated several times in the same stand, allowing a regeneration period of 15 to 25 years. Another option is to encourage stands of one or two species by selective cutting at long intervals (as practised in Austria, Croatia and Romania). In stands dominated by *F. angustifolia* where the soil is temporarily flooded in spring and autumn, natural regeneration from seed is limited. In such cases, coppicing is carried out on a cycle of 25 to 35 years.

The proportion of *F. angustifolia* to plant in mixed forests depends on the site and on the target mixture. On typical oak sites, the proportion of other hardwoods (e.g. ash, cherry, black alder) should be around 30-40 %, whereas on wetter sites (typical riverine forests, or those subject to temporary flooding) the proportion of *F. angustifolia* should increase to 70-80 %. The remainder is typically a mixture of black alder, willows and poplars. In southern Europe and the Mediterranean, the most productive natural stands are usually found in valley forests. However, due to the patchy distribution of *F. angustifolia* in the lowlands and to human pressures such as deforestation, farming and water extraction in these areas, many stands are left simply for conservation purposes. Silvicultural regimes for *F. angustifolia* on drier sites in southern Europe (Photo 5.3) are usually not so intensive and are linked with coppicing and/or mixed coppicing and planting.

For a hardwood, *F. angustifolia* grows rapidly when young, but its growth slows after 50–60 years. In general, it is considered a fast-growing species: the mean annual increment can reach 12–15 m^3 ha^{-1} in natural stands and 20–25 m^3 ha^{-1} on good plantation sites. The suggested rotation is 40-60 years to achieve 40-60 cm DBH, though site and wood quality objectives must also be taken into account. The rotation time for a larger target size varies in different parts of the natural range. In Central Europe it is around 60–80 years, and in Croatia and Serbia about 60 years, though in the riverine forests of northern Turkey, where growth rates are particularly high, it may be as short as 40 years. Continuous cover forestry can be applied in mixed stands of *F. angustifolia* and *Quercus robur*, using 60-80 year rotations for *F. angustifolia* and 120-140 years for *Q. robur*. As with most broadleaved trees, harvesting of *F. angustifolia* should be in winter.

Drainage to remove excess soil water is important when establishing new plantations in riparian forests on gleyed soils. On alluvial soils, it is important to thin the seedlings and control competing vegetation, as *F. angustifolia* does not tolerate heavy competition from weeds. In plantations, the number of plants established per hectare is usually about 5000 (i.e. 1m x 2m spacing) if the seedlings are small. Intensive plantations now tend to use fewer, larger (2–3 year old) plants at spacings of 2m×2m to 3m×3m. Although more expensive, this gives higher survival by reducing the effect of weed competition.

As with all tree species, a major issue in *F. angustifolia* plantations for wood production is the planting spacing. Wide spacing adversely affects both stem and wood quality. Narrow spacing (e.g. 2m x 2m, 2.5m x 2.5m, 2m x 3m, 3m x 3m) is recommended for high quality wood production. Ash plantations are generally established from nursery-grown seedlings and rarely from rooted cuttings. Regeneration by direct seeding is not recommended for *F. angustifolia* because its slow initial growth results in high weeding and tending costs. Intimate mixtures of oaks (*Quercus*) with *F. angustifolia* and other fast-growing species (e.g. *Acer, Tilia, Alnus, Salix, Populus*) should be avoided since the fast-growing species out-compete the oaks.

Traditional and modern uses

Fraxinus angustifolia is an important timber species with similar wood properties to *F. excelsior* (see p. 32). There have been many studies to compare the wood quality of the two species. *Fraxinus excelsior* has a higher proportion of heartwood (52-74%) than *F. angustifolia* (30-56%)[23]. In trees grown on comparable sites, the width of annual growth rings of *F. angustifolia* is 1-3 mm, while in *F. excelsior* the rings are 15-30% narrower. The wood of *F. excelsior* is denser (665–728 kg.m–3) than that of *F. angustifolia* (645–684 kg.m–3)[23], but its volumetric shrinkage is higher. The mechanical properties of *F. angustifolia* are slightly inferior, in terms of compression strength and tensile strength along the grain, bending strength, impact bending strength, and the bending modulus of elasticity.

The wood properties of *F. angustifolia* vary according to the growing site. In southern and south-western Europe (Photo 5.4) the wood quality tends to be higher on the relatively dry sites preferred by the species. In other areas, *F. angustifolia* tends to grow faster, and its yield on optimum sites in northern Turkey is comparable to that of poplars[5]. The leaves, both fresh and dried, are palatable and nutritious to animals, and were used as fodder in southern Europe, where pollarded ash trees can still be seen. The fodder is also favoured by red and roe deer, which frequently damage young trees in plantations and natural regeneration, initially by browsing and later (in the case of red deer) by bark stripping. *Fraxinus angustifolia* has also been widely used in central and southern Europe as an ornamental tree along roads and city streets.

Photo 5.2.- Riverine *F. angustifolia* population along the Morava river at the Austrian/ Slovakian border (near Hohenau, Austria).

Photo 5.3.- *F. angustifolia* planted around Castelbuono, Sicily, Italy.

Photo 5.4.- Remnant tree of *Fraxinus angustifolia* in an open field at Melia, Greece.

Research under FRAXIGEN

In FRAXIGEN, we have studied genetic variation in *F. angustifolia* in laboratories in three countries (Greece, Romania and Spain), looking at populations from Bulgaria, Croatia, Greece, Hungary, Italy, Moldova, Romania, Slovakia, Slovenia, Spain, and Turkey. The level of genetic variation was studied using nuclear and **CHLOROPLAST** microsatellite markers. The methods used in all these studies are described in Chapter 3. Each laboratory used a different method for the visualisation of microsatellite markers (because different laboratory equipment was available in each country), so standardisation of the data between laboratories is necessary and was still in progress when this book was printed. The results on nuclear **MICROSATELLITES** (from Spain and Greece) are therefore presented separately here, whereas the chloroplast data (from all three laboratories) has been standardised. Data on nuclear genetic variation from Romania was not available at the time of writing.

The molecular studies of genetic variation have been complemented, in Greece, by a **RECIPROCAL TRANSPLANT EXPERIMENT** (RTE) established at the same sites which were sampled for the molecular studies (Figure 5.7). This trial network was designed to examine the extent of local adaptation and the scale over which it operates, as described in Chapter 3.

The flowering and leafing **PHENOLOGY** of *F. angustifolia* was studied in Spain, Greece, Slovakia and Romania, giving a broad east-west geographic coverage. The studies of pollination biology and **GENE FLOW** described for *F. excelsior* and *F. ornus* in Chapters 4 and 6 were also replicated for *F. angustifolia* in Spain and Greece, but did not give any results because the developing seed was killed by frost in two consecutive years. The implications of the frost sensitivity of *F. angustifolia* are discussed further in the section on reproductive biology.

Genetic variation

Background

No molecular genetic data has previously been published on *F. angustifolia*. An Italian research group[1] has been working on chloroplast microsatellites, and kindly provided unpublished data on the distribution of *F. angustifolia* **HAPLOTYPES** across most of Europe. We have assigned numbers to our haplotypes which correspond to this larger study.

FRAXIGEN findings on long term migration (chloroplast haplotypes)

Thirty-four populations of *F. angustifolia* were studied in FRAXIGEN and eight different haplotypes were identified (Figure 5.2). The haplotypes matched those reported in an earlier European-wide study of chloroplast variation in *F. excelsior*[15], which has the same haplotypes as *F. angustifolia*.

One dominant haplotype (H4) was identified within Spain, which was also present in single populations from Croatia and Moldova. The results from Italy showed the existence of two haplotypes (H3 and H5). The earlier European-wide study[15] reported that H5 was the dominant haplotype in *F. excelsior* in southern and central Italy, whilst haplotype H3 was common in northern Italy and southern France.

Haplotype H1 was the most dominant in Greece and the Balkan Peninsula. The second most dominant haplotype in Greece (H3) seems to have migrated northwest to the Mediterranean area (Italian Peninsula and southern France). Haplotype H5 was also found in one population.

Haplotype H2 was found to be dominant in the populations studied in Slovakia, Hungary and Slovenia, whilst haplotypes H6, H7 and H9 were found in Romania, Moldova and Turkey.

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[1] Dr GG Vendramin, Instituto di Genetica Vegetale, Consiglio Nazionale degli Ricerche, Via Atto Vannucci 13, 50134 Firenze, Italy.

The majority of haplotypes were found in populations from the Balkans, suggesting that this was a glacial refugium, i.e. an area where *F. angustifolia* survived the last Ice Age. However, both the Iberian and Italian peninsulas may have been additional refugia, as they contain haplotypes poorly represented in the Balkans.

FRAXIGEN findings on genetic variation (nuclear diversity)

The thirty-four populations of *F. angustifolia* sampled in FRAXIGEN were analysed using the same microsatellite markers used previously in a European-wide study of *F. excelsior*[16] so that our results would be comparable. In each population, **DNA** from leaf material was extracted from 30 adult trees separated by 50 to 100m, depending on the size of the woodland. Mixed stands of *F. angustifolia* and *F. ornus* were found in Italy (IT_17A, IT_18A, IT_19A), Greece (GR_12A) and Spain (ES_04A).

We can only present in this chapter the results from 19 populations (Greece, Italy and Spain), since the analysis of the remaining populations was still in progress at the time of writing. For each marker we recorded the total number of **ALLELES** found in each population (**ALLELIC RICHNESS**; Figure 5.3), and also calculated **HETEROZYGOSITY**, which takes into account the evenness of the distribution of different alleles. Both indicated overall high, and similar, levels of **GENETIC DIVERSITY** across all the populations. In Greece, however, the most southern populations tended to have lower diversity. This may be explained by increased relatedness (crossing between relatives) in these populations, caused by various factors (e.g. isolation, southern distribution limits, human influence). The overall levels of diversity did not differ among pure and mixed stands.

The differences between populations (**GENETIC DIFFERENTIATION**) were small. The Spanish and Italian populations formed two separate groups. Exceptions were population IT_18A, from southern Italy, which appears to be more similar to Spanish populations. In contrast, population ES_06 from eastern Spain is more similar to the Italian populations. The Greek populations showed little differentiation, suggesting that there may be high gene flow between them.

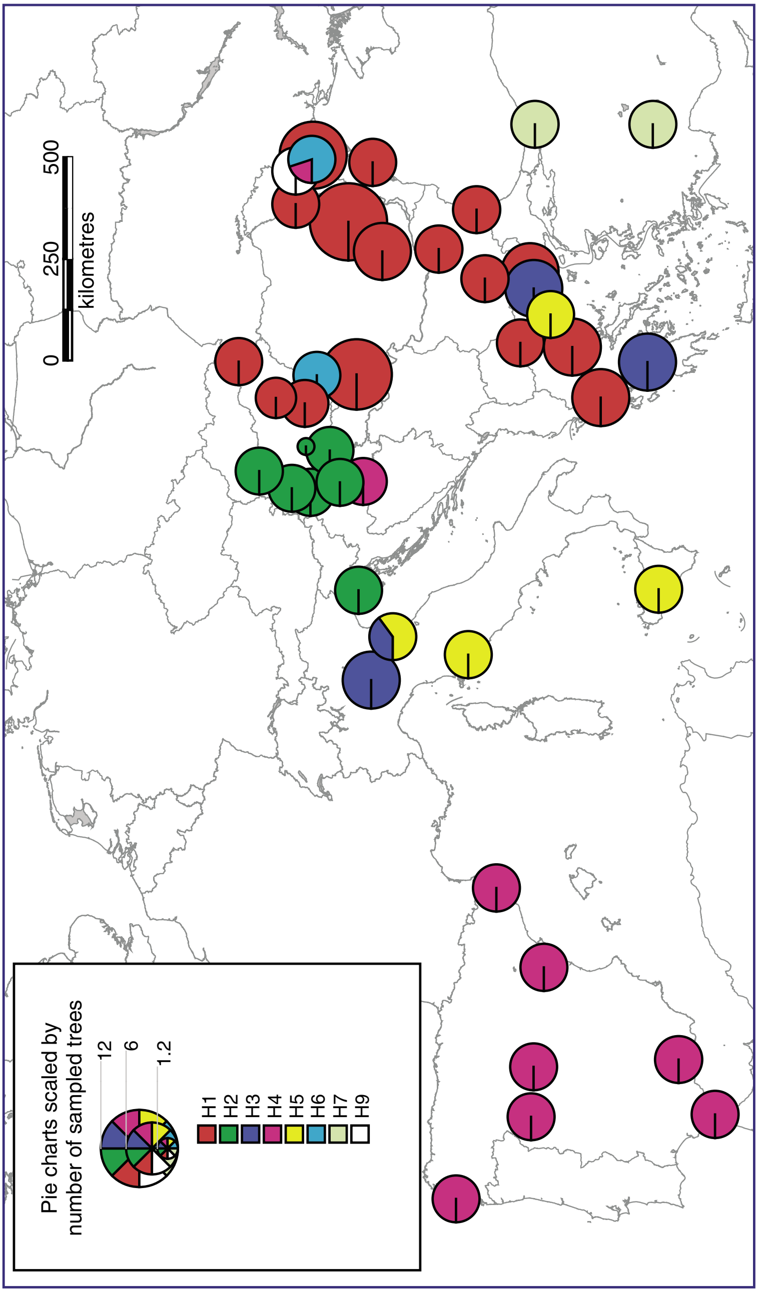


Figure 5.2.- Chloroplast haplotypes in 34 populations of *Fraxinus angustifolia* from Bulgaria, Croatia, Greece, Hungary, Italy, Moldova, Romania, Slovakia, Slovenia, Spain and Turkey. The size of the pies is scaled to show the number of individuals sampled in each population.

Figure 5.3.- Allelic richness in 19 populations of *Fraxinus angustifolia* from Greece, Italy and Spain. The size of the circles is scaled to show the total number of alleles found at five nuclear microsatellite loci. Each pie is divided into slices showing the proportion of alleles found at each locus. The number of alleles in each population is corrected for differences in sample size between populations.

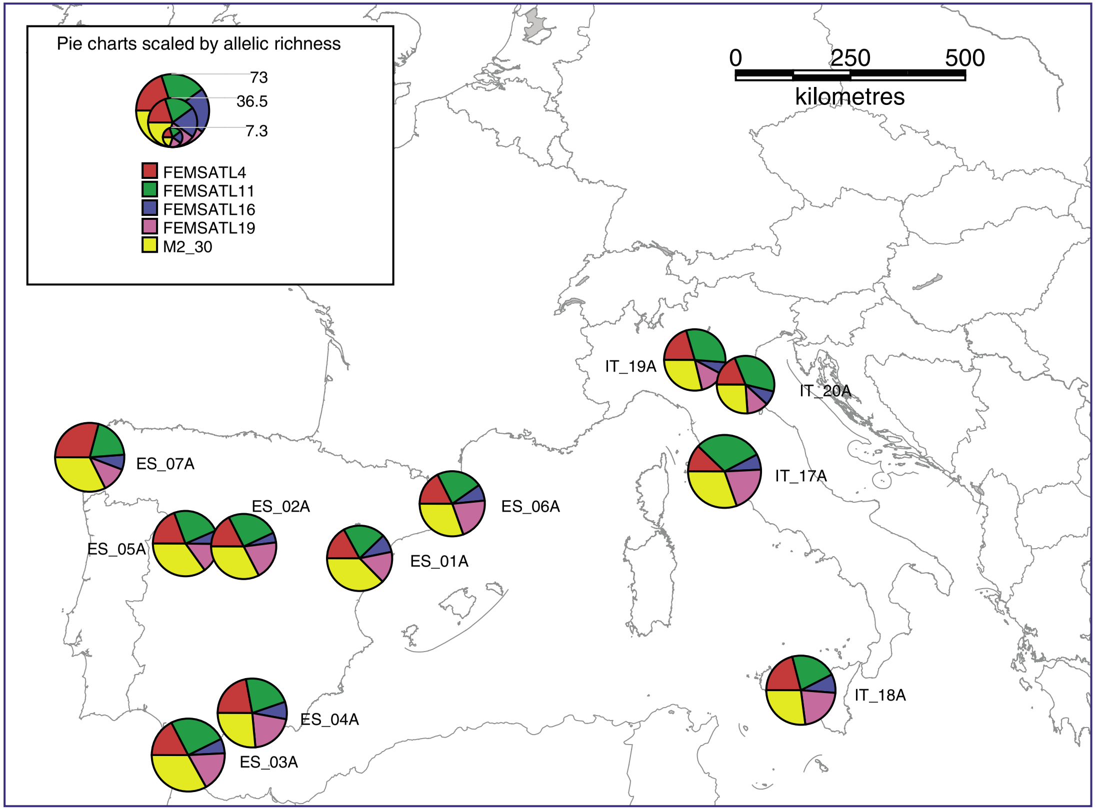


Figure 5.3a.- Allelic richness of *Fraxinus angustifolia* in populations from Italy and Spain; data from Spanish laboratory.

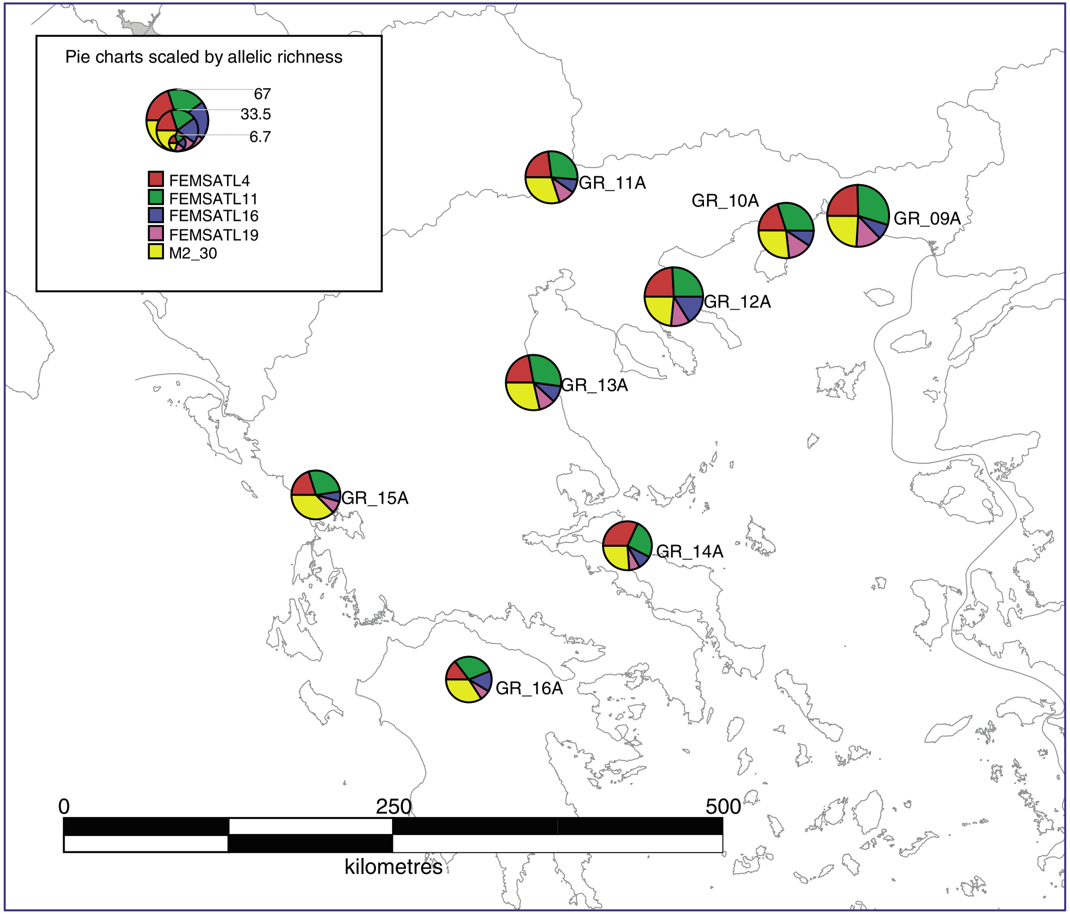


Figure 5.3b.- Allellic richness of *Fraxinus angustifolia* in populations from Greece; data from Greek laboratory.

Reproductive biology

Fraxinus angustifolia is a wind-pollinated tree up to 30 m tall. It is closely related to *F. excelsior,* to the extent that they are able to hybridise[33;34]. Inflorescences can be male, **HERMAPHRODITE** or mixed male and hermaphrodite. No female inflorescences (with pure female flowers or female with rudimentary stamens) have been found in the study populations (or in any reports to date). The male and hermaphrodite flowers of *F. angustifolia* occur on all individuals, i.e. all trees are functionally hermaphrodite.

The start of flowering of *F. angustifolia* varies with latitude, but it always starts before *F. excelsior* in the same area. The inflorescences emerge from lateral buds in the spring, 2–11 weeks before the leaves expand from the terminal buds. The difference between flowering and leaf flushing is much more apparent in the Mediterranean than in central Europe (Figure 5.4).

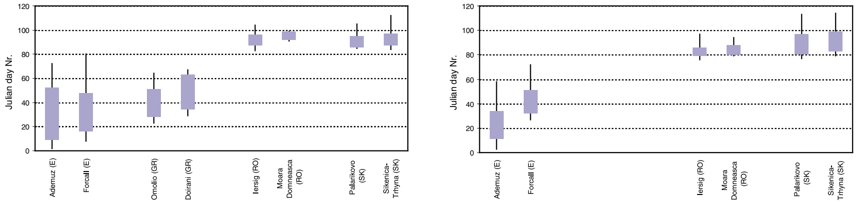
Hermaphrodite inflorescences have 15 flowers on average. Although the mean size of mixed (hermaphrodite + male) inflorescences is slightly larger (17.3 flowers on average), the total number of flowers in the racemes of *F. angustifolia* is always much lower than in the panicles of *F. excelsior*. Therefore, the type and size of inflorescence is considered the best morphological **TRAIT** for the separation of these two closely related species.

In Mediterranean countries (Spain, Greece), the phenological observations in 2003 and 2004 revealed a flowering period between January/early February and February/early March. At higher latitudes (Romania, Slovakia), flowering usually starts in the second half of March and may last till the second half of April. Over two years of investigations, the time of flowering varied by up to 18 days. Although *F. angustifolia* is **PROTOGYNOUS**, i.e. the start of the stigma receptivity precedes the start of the opening of **ANTHERS** and the release of **POLLEN**, there is a marked overlap of the male and female phases within individuals, making self-pollination possible (Figure 5.4).

Occasionally, depending on the autumn and winter temperatures, flowering starts in autumn (mostly in late November, after leaf fall). This happens not only in the Mediterranean area but also in central Europe. In such cases, the inflorescences are extremely vulnerable to winter frosts. Another consequence is that the flowering and fruiting intensity are not correlated, owing to the frequent damage to developing seeds by spring frosts. The overall result of this vulnerability is that seed production is often

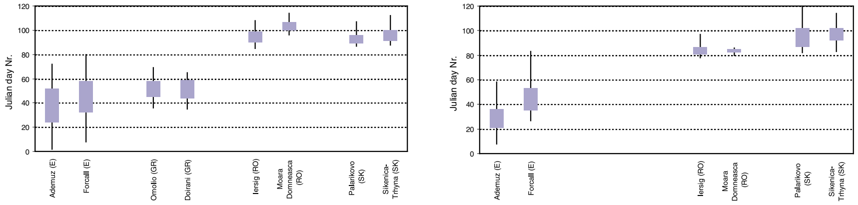
a) FAN female (stigma) receptivity in 2003

b) FAN female (stigma) receptivity in 2004



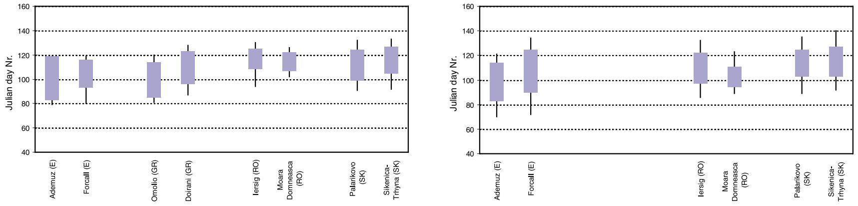
c) FAN pollen release in 2003

d) FAN pollen release in 2004



e) FAN leafing in 2003

f) FAN leafing in 2004



g) FAN leaf fall in 2003

h) FAN leaf fall in 2004

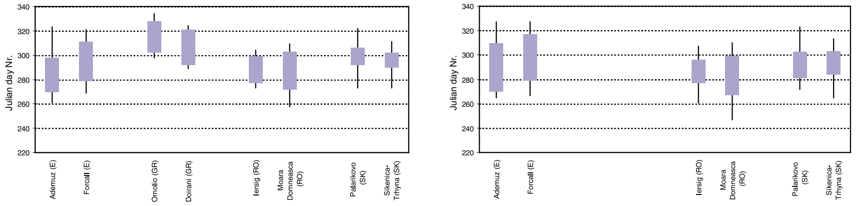


Figure 5.4.- Phenological observations of *F. angustifolia* (FAN) in populations in the Mediterranean and Central Europe in 2003 and 2004.

low or non-existent in *F. angustifolia*, and frost is likely to be a major factor limiting the species' distribution. It is therefore a species which might be expected to increase its range if the European climate becomes warmer.

Leaf flushing shows similar trends to flowering, although there is less difference between regions. Leafing starts earlier in the Mediterranean countries (mid March to early May) compared to the countries in central Europe (late March to mid May). The opposite occurs in autumn, where the most frequent date for complete defoliation falls on the turn of October and November in Romania and Slovakia, in early November in Spain, and in the second half of November in Greece (Figure 5.4). As a result, the vegetative period of *F. angustifolia* is longer in the Mediterranean region and shorter in Central Europe.

One of the most important aspects of phenological studies is to assess the chances of natural hybridisation between *F. excelsior* and *F. angustifolia*. *Fraxinus excelsior* always starts flowering after *F. angustifolia*, but in some years the end of *F. angustifolia* flowering may overlap with the beginning of *F. excelsior* flowering. In a young mixed seed orchard in southern Slovakia (Figure 5.5), and in natural mixed stands of the two species in Romania (Figure 5.6), we found a marked overlap in flowering duration which would make natural hybridisation possible. It is important to note that hybrid seed would be more likely to be found on *F. excelsior* than on *F. angustifolia* trees, because there is more overlap between *F. excelsior* female receptivity and *F. angustifolia* pollen release than between *F. angustifolia* female receptivity and *F. excelsior* pollen release.

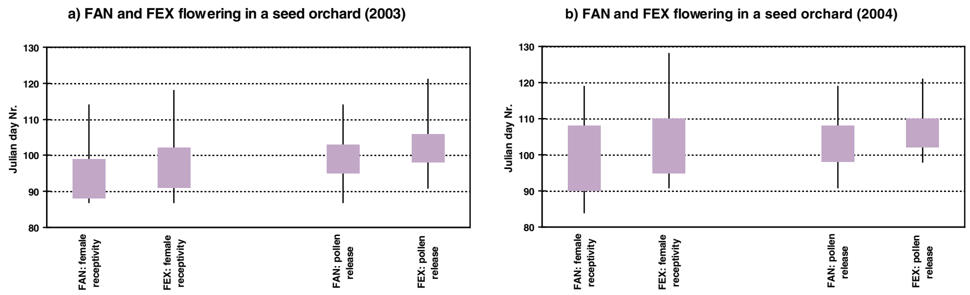
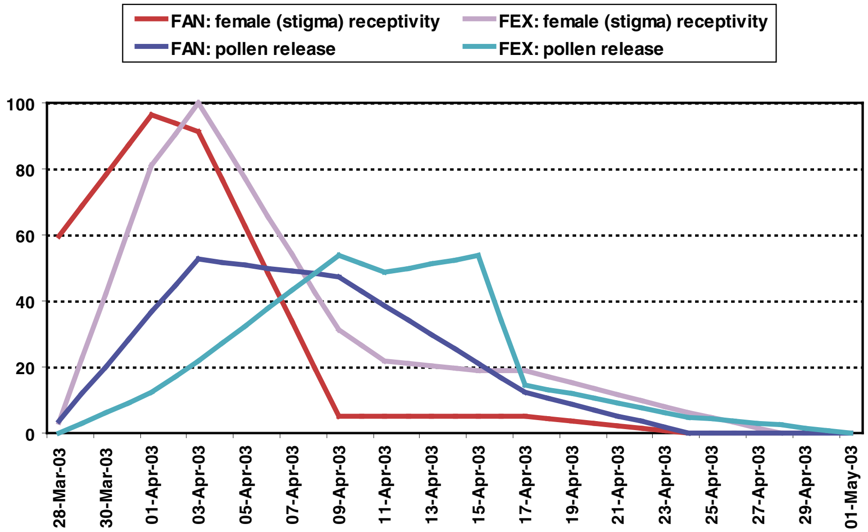


Figure 5.5.- Phenological observations of *F. angustifolia* (FAN) and *F. excelsior* (FEX) in a seed orchard in Southern Slovakia in 2003 and 2004.



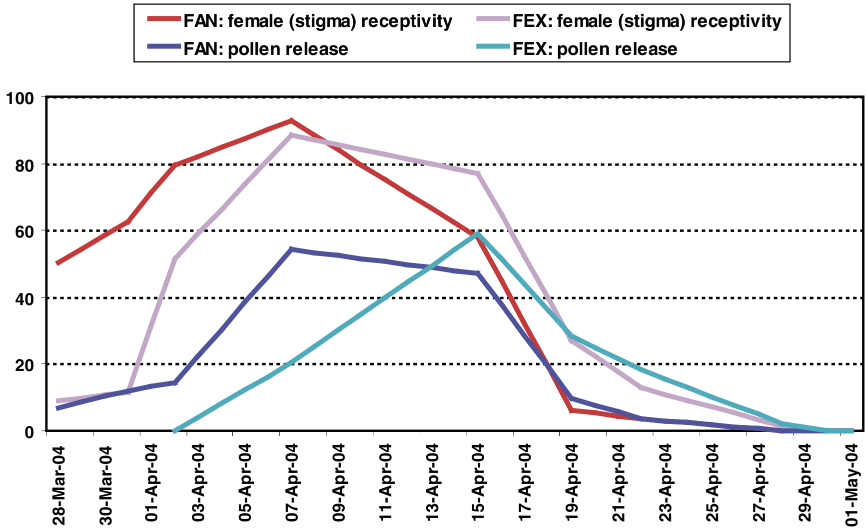


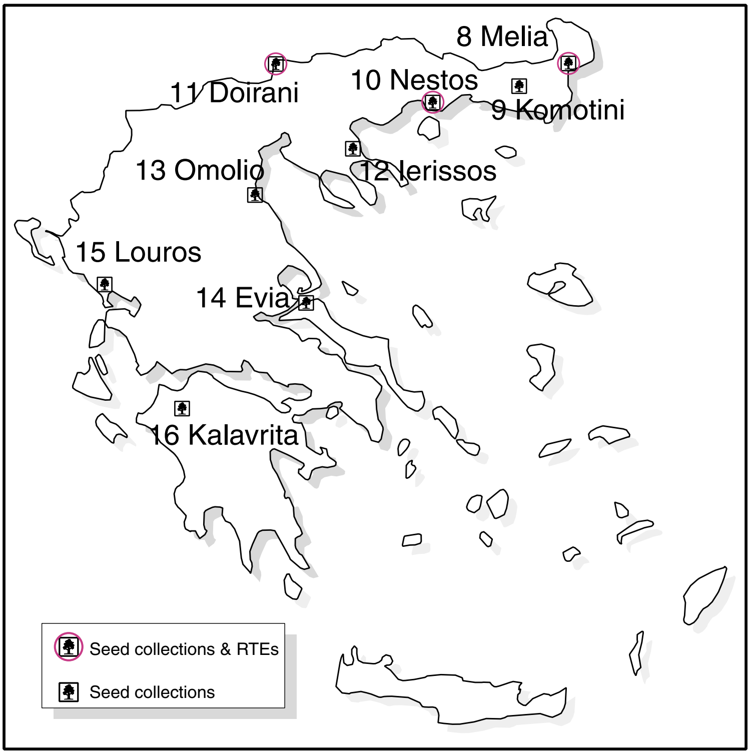
Figure 5.6.- Phenological observations of *Fraxinus angustifolia* and *F. excelsior* in a natural mixed stand in Romania in 2003 and 2004.

Local adaptation

The assumption that locally sourced seed is preferable, because it is more likely to be well adapted to the planting site, is central to current European policies on forest reproductive material (see Chapter 2). The concepts of localised adaptation, and of reciprocal transplant experiments (RTEs) to examine the extent and scale of local adaptation, are discussed in Chapter 3.

In FRAXIGEN we established an RTE for *F. angustifolia* at nine sites in Greece. Poor survival at six of the sites has meant that the experiment is not fully reciprocal, but we have been able to compare the nine seed sources at three of the sites (Figure 5.7). Survival and growth of two year old seedlings were assessed in the field. The results show that survival rates were not significantly different between provenances, but were between planted sites. There were significant differences between seed sources for height increment (at all three sites). Height increments were also significantly different among the sites (probably due to different site conditions - climate and soil). The interaction between seed sources and planted sites (often called **GENOTYPE** by environment interaction) was significant for height growth, showing that the relative performance of the seed sources was different at different sites. There was however, little indication of home site advantage. At the Melia site the local seed source and the closest other site (57km away) are performing best, but there is no overall correlation between height growth and geographic distance. In contrast, at Doirani, a high correlation was found between height and geographic distance, but poorer performance was only apparent in material from sites more than 200 km away. There was no apparent relationship between seed source location and performance at the third site, Nestos.

After only two years growth it is hard to draw firm conclusions about the performance and extent of any local adaptation in the seed sources evaluated. The results so far suggest that if there is any local adaptation at this life stage it is at a very broad scale (more than 100 km) and not restricted to within a few km. Genetic analysis using neutral molecular markers[28;38] showed low differentiation among tested populations, which may equate with the low level of **ADAPTIVE VARIATION** found. Longer term evaluation may be required for the genotypes' expression to show in the different environments. When seed is needed for regeneration and ecological restoration purposes it should be collected from relatively local populations and from as many trees as possible (more than 20; see box 7.1 Chapter 7). This would ensure seed collections maintain the high levels of genetic variation found within *F. angustifolia* populations[28;38].



Site number	Site name	Latitude (°)	Longitude (°)	Altitude (m.a.s.l)
8	Melia *	40.58.3 N	26.07.4 E	155-170
9	Komotini	40.59.5 N	25.23.3 E	10-15
10	Nestos *	40.53.7 N	24.46.5 E	18-20
11	Doirani *	41.14.7 N	22.46.4 E	50
12	Ierissos	40.27.6 N	23.49.0 E	10-20
13	Omolio	39.53.6 N	22.37.2 E	16-20
14	Evia	38.49.1 N	23.25.3 E	120
15	Louros	39.09.2 N	20.45.8 E	50
16	Kalavrita	37.56.3 N	22.04.1 E	870

Figure 5.7.- Location of seed collection sites and *reciprocal transplant experiments for *F. angustifolia* in Greece.

Chapter

6

Fraxinus ornus (manna ash)

Photo 6.1.- *Fraxinus ornus* tree with seeds

Distribution and systematics

The manna ash, *Fraxinus ornus* L. (Photo 6.1) has the most limited distribution of the three European ash species. It occurs mainly in southern Europe and its main distribution range is in Italy (mostly in the south and Mediterranean islands), Greece, and in karst regions of the Balkan Peninsula and western Turkey. There are also isolated populations in eastern Spain (see map in Figure 1.3 in Chapter 1, page 12).

Fraxinus ornus is easily distinguished by the appearance of its flowers from the other two native European ash species. In fact, the 15 species most closely related to *F. ornus* are all native to eastern Asia rather than Europe. They are all small deciduous trees, usually not more than 15 m tall (rarely up to 20 m in humid and rich soils). The small flowers have four white petals and occur in large showy **INFLORESCENCES** (see Photo 6.2) which are mainly pollinated by insects. The reproductive biology of *F. ornus* is discussed in more detail on pages 97 to 104.

Like other ash species, *F. ornus* shows great variation in vegetative characters (leaves, shoots, bark etc.), and several subspecies and varieties, as well as cultivars, have been described. The taxonomy of *F. ornus* is not as complicated as that of *F. angustifolia*, for which many more variants have been described. The only well-known subspecies is *F. ornus* L. ssp. *cilicica* (Lingelsh.) Yaltirik, occurring in Turkey. It is distinguished from the main ssp. *ornus* in having longer petiolules (leaflet stalks) and leaflets which are glabrous (hairless) on both sides, and more acuminate (pointed). These characters are not constant and there are intermediate forms. The names *F. kotschyi* Schneider and *F. petiolulata* Boiss. are synonyms of *F. ornus* ssp. *cilicica,* whereas *F. cappadocica* Juss. ex Bosc., *F. millelacuum* Koch and *F. rotundifolia* Lam. are synonyms of *F. ornus* ssp. *ornus*.

Ecology and silviculture

Fraxinus ornus occurs naturally on warm south-facing slopes in broadleaved and mixed forest (see Photos 6.3 to 6.5), typically forming groups and small stands in association with Mediterranean oaks (*Quercus* spp.) and chestnut (*Castanea* sativa) as well as hornbeams (*Carpinus* spp.), hop hornbeam (*Ostrya carpinifolia*) and maples (*Acer* spp.). It is seldom found in mixtures with conifers, apart from some species of juniper (*Juniperus*), and in Greece and Turkey as an understorey species with firs (*Abies* spp.) and beech (*Fagus sylvatica*).

Photo 6.2.- Inflorescences of *F. ornus*

Photo 6.3.- Native *F. ornus* stand in Greece

Photo 6.4.- *F. ornus* habitat in a population from the western extreme of the distribution area (eastern Spain)

Photo 6.5.- *F. ornus* habitat in a population from the eastern extreme of the distribution (Danube river population)

The high density of trees in natural populations, and the availability of efficient pollinators, combined with rapid seed germination, enable *F. ornus* to colonise new habitats, making it a valuable species for ecological restoration. Another important **TRAIT** allowing *F. ornus* to persist in disturbed populations is its ability to resprout after cutting. Such disturbance is very frequent in its area of distribution because of wildfires, browsing by animals and logging. Together, these qualities enable this tree to be a dominant or codominant species in most of its habitats. For example, after coppicing of mixed oak-manna ash forest, *F. ornus* can quickly out-compete oak because of its greater sprouting capacity and faster growth, and so can become the dominant species in these forests.

Climate and altitude

Fraxinus ornus is found from sea level up to 1,500 m altitude. It requires high air temperatures, and in the northern part of its natural range grows mainly on warm, south-facing slopes. The optimum rainfall is from 500 to 650 mm

Soil

In central and eastern Europe, *F. ornus* grows mainly on chalk and dolomite substrata, and occasionally on andesite, basalt and loess. In contrast, in southern Europe it also grows on silicate substrata. The root system is extensive and the roots require well-drained soils with sufficient stones to allow aeration.

Limitations

Low temperatures limit the distribution of *F. ornus* in central Europe. The northern limit of its natural range is in southern Slovakia and it has been used very successfully for afforestation of degraded and eroded sites in the karst regions of southern Slovakia and Hungary. In the Mediterranean region, in contrast, the limitations are high humidity and rich soils, on which *Quercus* and other broadleaved tree species can out-compete *F. ornus*.

Silvicultural characteristics

Depending on the silvicultural management, *F. ornus* forest takes three different forms: high forests (of seed origin), coppices and mixed shrubs. The trees are frequently multi-stemmed or shrubby owing to coppicing, grazing or re-sprouting after wildfires.

Fraxinus ornus forests on carbonate soils possess rich species diversity. At present the main silvicultural significance of *F. ornus* in southern and central Europe is in the reforestation or afforestation of eroded and degraded soils in karst regions and other degraded ecosystems around the Mediterranean Basin. On such sites it covers the soil very quickly, and ameliorates poor soils with its annual litter-fall. It also provides good microclimatic conditions for the growth and regeneration of other tree species. It grows rapidly when young (in contrast to the oaks which grow with it), but only to a height of 8-15 m.

Fraxinus ornus is also a very valuable tree species in urban forestry, because it does not reach large dimensions, and in spring is very decorative because of its large white inflorescences.

Traditional and modern uses

Fraxinus ornus is not an important timber species. Its wood never reaches large dimensions. Stem discs are characterised by narrow annual rings and small differences in yellowish colour between the heartwood and the sapwood. The wood is lighter than that of the other two European ash species, with a density of about 650 kg/m^3. Although *F. ornus* wood has good properties, it has few industrial uses because of the bad trunk form and quality. However, it is used for tool handles, components of cart wheels, and other household uses. It is a multipurpose tree species which still plays a significant role in many parts of the Mediterranean as a source of fodder for cattle, goats and sheep (managed by pollarding) and also as a source of fuel wood, for which it is usually managed by coppicing.

Fraxinus ornus is the source of manna, an oxidised dried exudate from the bark of young trees which was formerly used as a laxative. Originally it was only collected from trees with damaged bark, but later in southern Italy and northern Sicily plantations were established for manna production, in which the bark is intentionally damaged for exudation and collection of manna. Manna is still produced in Sicily, mainly in the Castelbuono and Pollina areas (see Photos 6.6 and 6.7). The trees are grown in plantations at about 2 m spacing. Manna collection can start when the trees are 8-10 years old, and the trunk is at least 8 cm in diameter. In July-August a vertical series of oblique incisions are made in the bark on alternate sides of the trunk. A glutinous liquid exudes from this cut, hardens as it oxidises in the air into a yellowish crystalline mass

Photostudio Mazzola

Photo 6.6.- Manna being collected in Castelbuono, Sicily. In July-August a vertical series of oblique incisions are made in the bark on alternate sides of the trunk.

Photostudio Mazzola

Photo 6.7.- Manna being collected in Castelbuono, Sicily. A glutinous liquid exudes from this cut, hardens as it oxidises in the air into a yellowish crystalline mass with a bittersweet taste, and is then harvested.

with a bittersweet taste, and is then harvested. Harvesting can continue for up to nine years, after which the tree is cut and a single stem allowed to regenerate. Dry, warm weather is essential for a good crop of manna. The larger pieces, which are collected in September-October when the heat has begun to moderate, are known as flake manna: this is the highest quality product.

The quality and yield of manna of *F. ornus* depend on the cultivar used as well as on the climatic conditions. The main component of manna is the alkaloid mannitol; it also contains glucose, laevulose and resin. Many beneficial medicinal properties have been attributed to it, particularly for digestive problems.

Research under FRAXIGEN

In FRAXIGEN, we have studied both the reproductive biology and the genetics of *F. ornus*, and in particular the implications of its unusual breeding system. Detailed studies of **GENE FLOW** and pollination biology have been carried out in both Greece and Spain, complemented by detailed phenological observations which were also replicated in Slovakia to give a broader geographic perspective. At the same time we have studied genetic variation in *F. ornus* in laboratories in all three countries (Greece, Slovakia and Spain). The methods used in all these studies are described in Chapter 3. In Greece, we have also investigated the extent of local adaptation using **RECIPROCAL TRANSPLANT EXPERIMENTS** (RTEs) as described in Chapter 3.

In the **GENETIC DIVERSITY** studies each laboratory had a different geographical focus, although populations were sampled across a much wider geographic area, including Bosnia-Herzegovina, Bulgaria, Croatia, Greece, Hungary, Italy, Romania, Slovakia, Slovenia, Spain, and Turkey. They each used a different method (depending on the laboratory equipment available) for the visualisation of nuclear microsatellite markers, making it difficult to compare directly **NUCLEAR DNA** microsatellite results between laboratories. For this reason the nuclear DNA microsatellite results from each laboratory are presented separately, whilst the **CHLOROPLAST DNA** data have been standardised. The three regions covered by the three laboratories are shown in Figures 6.2a, 6.2b and 6.2c.

Genetic variation

Background: previous research

Although no genetic data had been published for *F. ornus* before FRAXIGEN, unpublished data on the distribution of chloroplast DNA **HAPLOTYPES** across most of Europe was kindly supplied by Dr G.G. Vendramin[1], so we have assigned numbers to our haplotypes which correspond to this larger study.

FRAXIGEN findings on long term migration (chloroplast haplotypes)

In FRAXIGEN we assessed variation in the **CHLOROPLAST GENOME** in 45 *F. ornus* populations (Figure 6.1). We identified five different haplotypes across the species' range, two of which (H24 and H25) had not previously been identified.

Haplotype H19 is found over most of the species' range, whilst H20 is predominant in the central part of the range and H24 is common in the south-eastern part (Turkey and Greece). Haplotype H23 is present in two populations from the Balkan Peninsula (GR_150 and GR_990), whilst H25 is restricted to a single Turkish population (TR_350). Most populations were not variable, containing only a single haplotype, but we found two mixed populations, one in Bulgaria and one in Turkey. Four of the five haplotypes were found in Greece and Bulgaria.

During the last glaciation the species' range would have been reduced to restricted areas called refugia. The high haplotype diversity found in Greece and Bulgaria suggests that *F. ornus* may have survived the last Ice Age in this region. In addition, the different haplotypes found in central and south-eastern parts of the range indicate that populations of *F. ornus* found in these areas represent lineages descended from different refugia.

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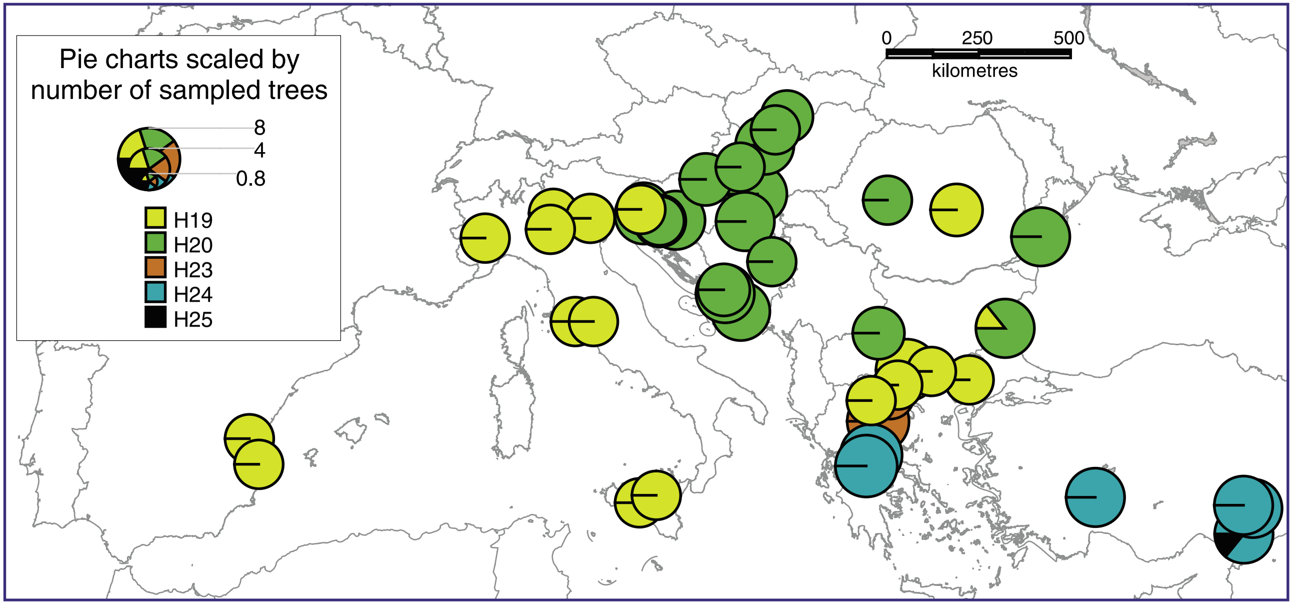


Figure 6.1.- Chloroplast haplotypes in 45 populations of *Fraxinus ornus* from Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Hungary, Italy, Romania, Slovakia, Slovenia, Spain, and Turkey. The size of the pies is scaled to show the number of individuals sampled in each population.

FRAXIGEN findings on genetic variation (nuclear diversity)

We investigated nuclear genetic diversity in 36 populations (Figure 6.2) across the species' range using microsatellite markers[3]. In each population, we analysed **DNA** in leaves from 30 adult trees separated by approximately 30 m. Three of the Italian populations (IT_060, IT_210, IT_220) and one Greek (GR_12A) population contained both *F. ornus* and *F. angustifolia*. Similarly high levels of allelic richness were found in all three regions (Figures 6.2a,b,c). Levels of **HETEROZYGOSITY**[25] in all three regions were also high, and similar, among the studied populations. There was no consistent latitudinal or longitudinal pattern of variation, and levels of genetic diversity did not differ between pure and mixed stands. The levels of heterozygosity shown by the Spanish populations are within the range of values for the species as a whole, even though they are located at the edges of the species' range (Figure 6.2a). In contrast, the two Turkish populations had rather low allelic richness and heterozygosity (Figure 6.2b), which is more typical of populations at the edge of a species' range, where fragmentation and isolation of populations may lead to **GENETIC DRIFT**.

The differences between populations (**GENETIC DIFFERENTIATION**) were small. The Spanish and Italian populations formed two separate groups. One Italian population (IT_230, close to Ficuzza) which appears more similar to the Spanish populations, may be contaminated with **GENES** from cultivated *F. ornus* trees. Central European populations were clearly distinct from the Turkish ones, and the small Slovakian population SK_280, on the northern edge of the species' range, also appears to be different from the other Central European ones. The grouping of the Greek samples follows no clear pattern, probably because genetic differentiation between populations is low (Figure 6.2c).

Reproductive biology and gene flow

An unusual breeding system

Fraxinus ornus is a small deciduous tree with small white flowers grouped in conspicuous inflorescences. The flowers are scented and attract a variety of insects (mainly bees and beetles), although they do not produce any nectar; however wind pollination may also occur. Flowering occurs in spring at the same time that the leaves flush. Large variations in fruit production can be observed between years, a common characteristic of ash species. **MAST YEARS** tend to alternate with non-productive years. The fruits are

Figure 6.2.- Allelic richness in 36 populations of *Fraxinus ornus* from Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Hungary, Italy, Romania, Slovakia, Slovenia, Spain, and Turkey. The size of the pies is scaled to show the total number of **ALLELE**s found at five nuclear microsatellite loci. Each pie is divided into slices showing the proportion of alleles found at each locus. The number of alleles in each population is corrected for differences in sample size between populations.

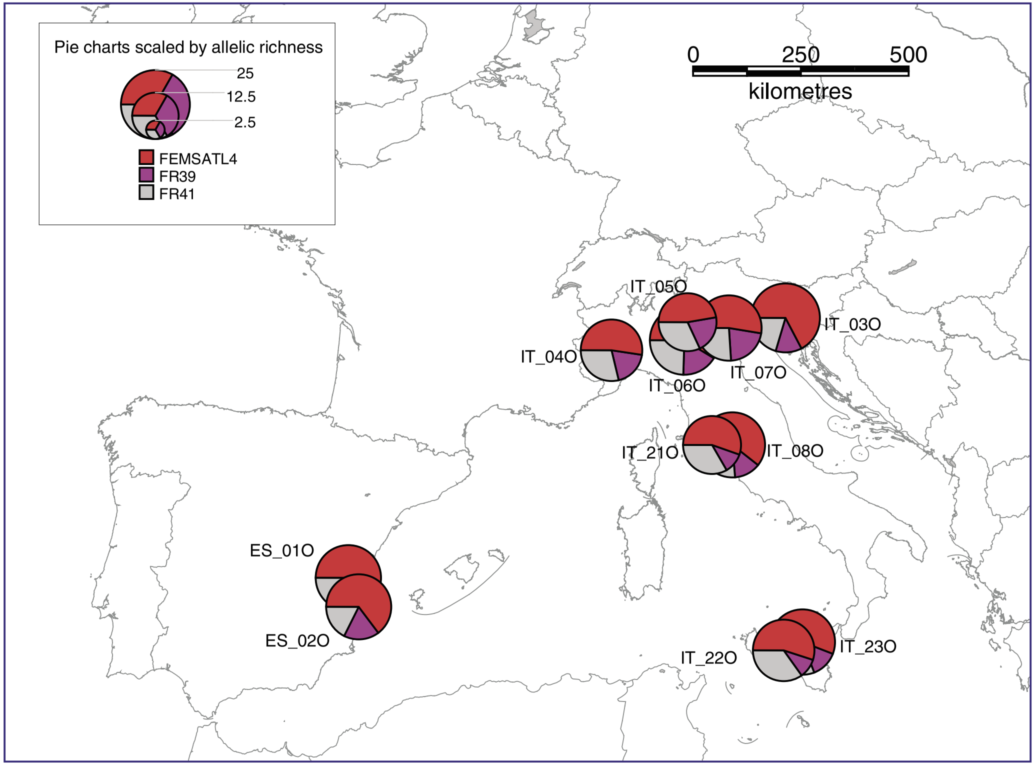


Figure 6.2a.- Allelic richness of *Fraxinus ornus* in populations from Italy and Spain; data from Spanish laboratory.

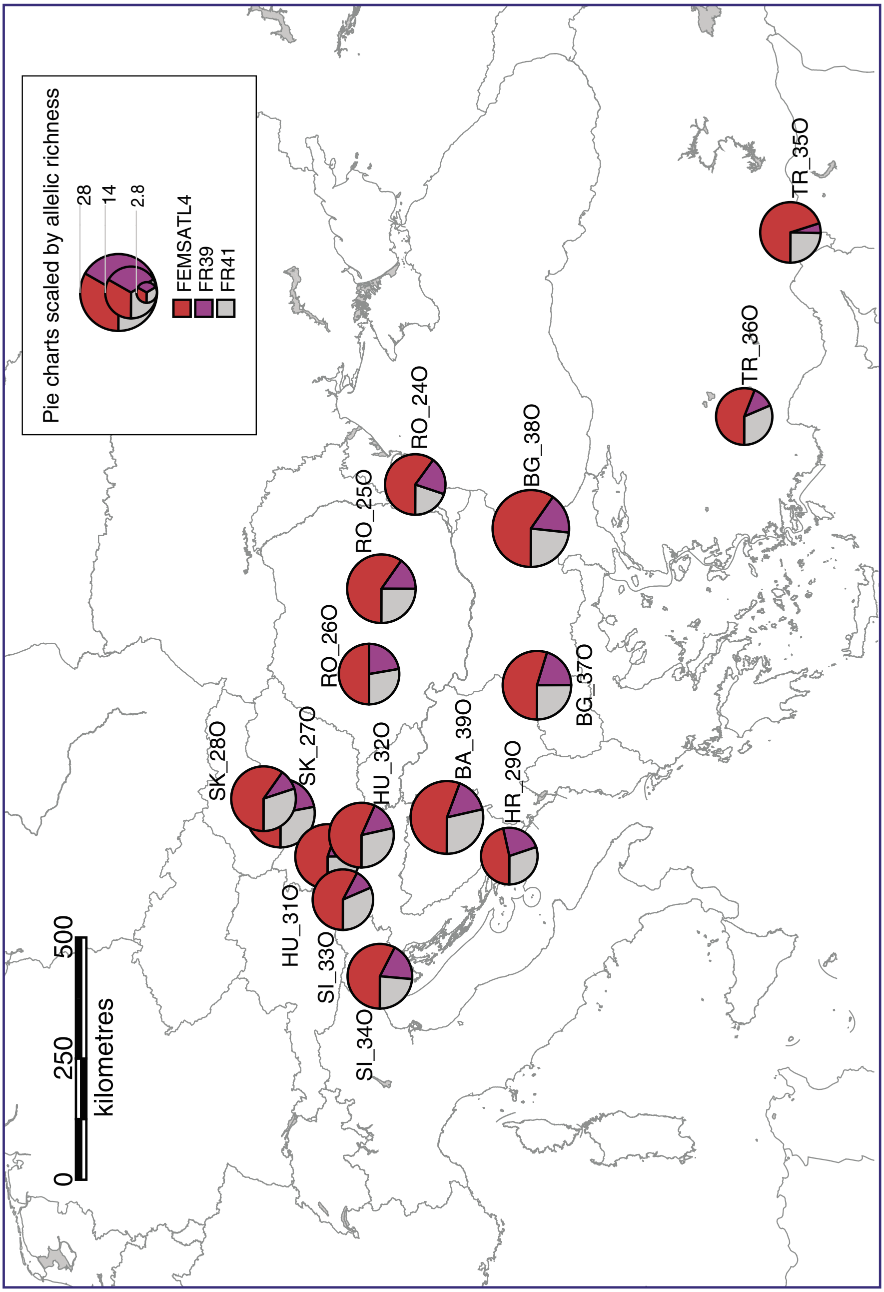


Figure 6.2b.- Allelic richness of *Fraxinus ornus* in populations from Central and Eastern Europe; data from Slovakian laboratory.

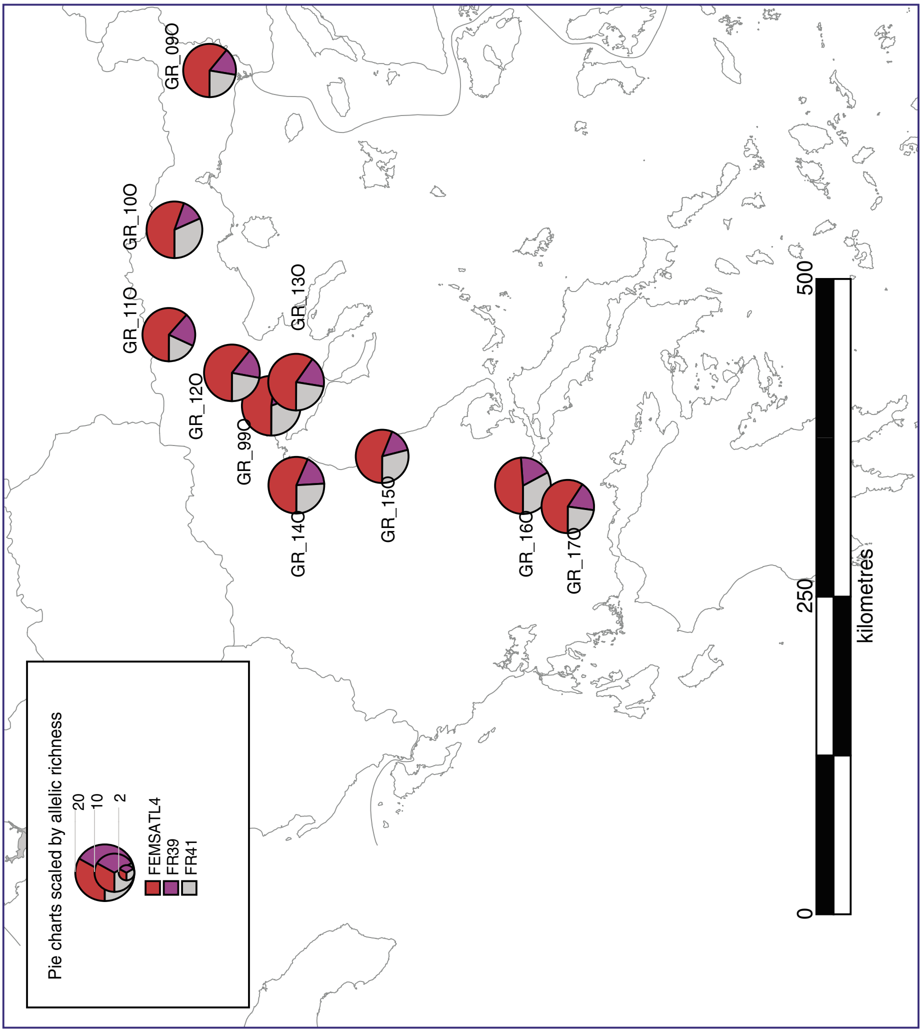


Figure 6.2c.- Allelic richness of *Fraxinus ornus* in populations from Greece; data from Greek laboratory.

SAMARAS which ripen in autumn and are dispersed by wind and also by water along rivers. Seed germination occurs the following spring (from the end of March to May), once the cold winter has broken the physiological dormancy.

The reproductive biology of *F. ornus* remained unknown until 1999, when the first scientific report revealed the unusual breeding system of this species[9]. Until that date, the botanical literature described it as **HERMAPHRODITE**: containing perfect flowers with both male and female reproductive organs. However, close inspection in the field revealed that half of the trees in the population had flowers with only rudimentary female parts, and those trees therefore behaved as males. The co-occurrence of males and hermaphrodites, in the absence of any females, is known as androdioecy and is an extremely rare breeding system[27].

DNA profiling and pollen flow

Pollination and seed dispersal: implications for genetic diversity in seeds

Our DNA profiling studies showed that many of the seeds (around 34%) from a particular mother have the same father. This suggests that insect pollinators usually deposit most of their pollen load on flowers of the same mother. For the same reason, seeds from different mothers do not often share the same father. Exact travelling distances of pollinators are unknown, but paternity analysis using molecular markers showed that more than 90% of the pollen travels more than 20 m.

Seed dispersal is more limited than pollen dispersal, so neighbouring trees are more closely related than more distant ones. The relatedness between two trees decreases drastically beyond 30 m because seeds are seldom dispersed further than this from their mother.

It is therefore important to collect approximately equal quantities of seed from as many mother trees as possible, to minimise the relatedness of the seeds. The trees from which the seed is collected should be at least 30 m apart, so that the mothers are less likely to be related.

Male mating success

Males can only reproduce by acting as fathers, whereas hermaphrodites can act both as fathers and as mothers, and can also sometimes fertilise themselves (**SELFING**). In this asymmetrical situation, we would expect that males would have to be more successful than hermaphrodites as fathers, to compensate for their overall lower probability of reproduction (because they are unable to function as mothers, i.e. to set seed).

To be a better father would have a cost in terms of investment in male reproductive function, because trees would need to produce more and/or better quality pollen. To see whether this is true, we first needed to know what resources each **GENDER** allocates to reproduction, before evaluating males as being better fathers (i.e. producing more progeny from pollen) than hermaphrodites. The results from FRAXIGEN research showed that male trees are indeed better fathers than hermaphrodite trees in *F. ornus*[39].

Investment in male function can be evaluated by estimating how much pollen the individuals of each gender produce and its relative quality. Results from populations studied over several years indicate that, on average, males produce 1.8 times more flowers (and therefore more pollen) than hermaphrodites. This is despite the similar size of **ANTHERS** in both sexes. Furthermore, although both genders have similarly sized pollen, its quality from males is higher than from hermaphrodites. We found this out by doing controlled pollinations, comparing pollen from male and hermaphrodite trees. On average, inflorescences pollinated with pollen from males produced twice (2.1 times) as much seed as those pollinated with pollen from hermaphrodites, indicating that males father more progeny than hermaphrodites. This is consistent with the findings of our study of gene flow in a wild population, using molecular markers (nuclear **MICROSATELLITES**): we found that in the wild, males also fathered twice as many seeds as hermaphrodites. Chapter 3 explains how this type of molecular study is carried out.

When the seeds from the experimental pollinations were planted in pots in a greenhouse experiment, differences in growth between seedlings fathered by males and those fathered by hermaphrodites quickly became apparent. Males were shown to be better fathers not only because their pollen produces more seeds, but also because their offspring grow more vigorously: on average, the height growth of seedlings with male fathers was 12% greater than that of seedlings fathered by hermaphrodites[39].

Fraxinus ornus is outcrossed

An important consequence of the difference in the effectiveness of male and hermaphrodite pollen is that the seedlings produced by self pollination in a hermaphrodite tree will be out-competed by seedlings produced by male pollen, as shown by our greenhouse experiment. Hence, populations of *F. ornus* will not have problems of **INBREEDING DEPRESSION** in spite of the ability of hermaphrodites to self-fertilise. Indeed, the molecular markers confirm that the **INBREEDING** rates for different life stages (seed, seedling and adult trees) are all very low. If inbreeding depression was causing selfed trees to have higher mortality than **OUTCROSSED** ones, the inbreeding rate would be lower at later life stages, as relatively fewer inbred (selfed) trees would still be alive in the population.

Males grow faster because pollen is cheap

In general, trees with male function (pollen) only might be expected to use less resources than trees with a female function (seed), because the former only requires the tree to produce and release pollen, whereas the latter requires the tree not only to produce **OVULES** but also to feed the embryos during development until seed maturity. This difference in the cost of reproduction is the reason why males might also be expected to grow more than hermaphrodites; that is, males invest less in reproduction and more in growth than hermaphrodites. We tested this in *F. ornus*, by comparing the growth rate of the two genders. We found differences between males and hermaphrodites in shoot and annual ring growth and diameter increment, although there was high geographical variation (Figure 6.3). These results support our initial assumption that the costs of reproduction do indeed differ considerably between the two genders.

	Shoot growth	Ring growth or DBH increment	Length of vegetative period
 Spain	♂ = ⚥		♂ = ⚥
 Slovakia	♂ > ⚥	♂ ≧ ⚥	♂ = ⚥
 Greece		♂ > ⚥	♂ = ⚥

Figure 6.3.- Differences in vegetative characters between males (♂) and hermaphrodites (⚥) found in 3 countries.

Sex ratios and phenology of flowering, fruiting and leafing

Leafing phenology

It is important to consider the time at which the investment of resources is made, when estimating the real costs of reproduction. For example, the real costs of flowering may be lower if flowers are produced once leaves are fully expanded and functional, rather than before leafing, at the expense of stored nutrients. Differences in the **PHENOLOGY** of the two genders could be important in this case. However, when we studied the phenological behaviour of males and hermaphrodites, we found no differences in the timing of either flowering or leafing. On average, both sexes flower and flush their leaves at the same time, and the leaves also fall in autumn at about the same time for both genders. This indicates that the length of the growing period is the same for both sexes, and neither has extra time to photosynthesise and so to obtain further resources (Figure 6.3).

Another mechanism that could balance the costs of reproduction between males and hermaphrodites is the photosynthetic ability of the samaras. These are green during development (until they ripen in August/September), and during that stage they contribute to their own maintenance by photosynthesis.

Flowering phenology

Males and hermaphrodites of *F. ornus* flower at the same time. Synchronisation of flowering between genders is an essential feature of a species in which different plants have different genders, because otherwise cross-pollination could not occur. Flowering synchronisation is a requirement for males to out-compete hermaphrodite pollen, as explained above: if males did not flower at the same time as hermaphrodites, they would waste their pollen. Thus, a strong synchronisation of flowering between genders is expected, and we observed this in our study populations. This allows hermaphrodites to be successfully pollinated by males.

Local adaptation

Genetic variation in native populations provides a basis for restoring and expanding natural ecosystems. In *F. ornus*, an understanding of patterns of **ADAPTIVE VARIATION** will help in the selection of appropriate seed sources for genetic conservation and restoration. Genetic improvement for production objectives is unlikely to become important for this species (though in Greece there has been some selection of superior **PHENOTYPES** for stem form and wood quality).

Under FRAXIGEN, local adaptation and growth in *F. ornus* were studied in a reciprocal transplant experiment (RTE) established at six sites in Greece (Figure 6.4), using one-year-old seedlings (Photo 6.8). The methods were the same as for the parallel experiments with *F. angustifolia* in Greece and *F. excelsior* in U.K., and are described in Chapter 3. Survival and growth of 2 year old seedlings were assessed in the field. The differences among the six seed sources were not significant for survival, and only marginally significant for height. In contrast, there were highly significant differences between the sites, for both survival and height. There was little interaction between seed source and site: in other words, the ranking of the seed sources did not differ significantly from site to site, as would be expected if local seed sources were out-performing distant ones. So far, there is no evidence of home site advantage in terms of either survival or height growth. However it is important to recognise that these results are only after two years of growth, and longer-term observations are needed before we can draw firm conclusions about the performance and local adaptation of the different seed sources. Until we know for certain whether or not local adaptation affects the survival and growth of *F. ornus*, we recommend applying the precautionary principle: that is, to use locally sourced material where possible. However an even more important principle is to ensure adequate genetic diversity in the seed by collecting it from as many trees as possible, and ensuring that these trees are at least 30 m apart (see Box 7.1 in p. 116).

Photo 6.8.- RTE of *F. ornus* at Poligiros, Greece

Site number	Site name	Latitude (°)	Longitude (°)	Altitude (m.a.s.l)
10	Stavroupolis (10)	41.14.4 N	24.39.9 E	332
11	Nevrokopi (11)	41.16.6 N	23.44.3 E	817
12	Nigrita (12)	40.51.8 N	23.24.0 E	542
13	Poligiros (13)	40.26.5 N	23.19.1 E	577
14	Kastania (14)	40.26.4 N	22.24.4 E	300
15	Stomio (15)	39.52.6 N	22.39.8 E	380

Figure 6.4.- Location of seed collection sites and reciprocal transplant experiment for *F. ornus* in Greece.

Chapter

7

Conclusions and recommendations

Photo 7.1.- Native ash woodland of *Fraxinus excelsior* at Delsjön, Sweden

Seed source selection - how local is local?

European and national guidelines currently encourage the planting of local tree seed rather than seed from non-native sources. But what does local mean in this context? One objective of the research carried out in FRAXIGEN was to provide scientific evidence to answer the question: "How local is local?" In other words, do stands of ash show local adaptation, and, if so, over what geographic scale does this occur? How much similarity is there between different woods, and are neighbouring stands of ash more similar than distant ones? The field and laboratory studies described in this book aimed to address these questions for all three European ash species (*Fraxinus excelsior*, *F. angustifolia*, *F. ornus*), and in different countries (U.K., Sweden, Spain, Greece, Romania and Slovakia). Our main findings are summarised here and the recommendations arising from them are shown in bold blue text.

Observations of phenology

We studied flowering, fruiting and leaf flushing to find out how these vary between stands. We found that the time of flushing is influenced both by latitude and longitude (e.g. Mediterranean or continental climate), and by elevation. For example, Romanian material planted in provenance trials of *F. excelsior* in southern England flushes earlier than local material and can be damaged by late frosts, causing forking. Late frosts can also kill flowers and reduce fruit set, even in local material. *Fraxinus angustifolia* is particularly at risk of this because it flowers early in the year, between January and March. In Spanish and Romanian study sites there was no seed set in *F. angustifolia* in two successive years following heavy frosts in early April. The distribution of *F. angustifolia* is probably limited by this frost susceptibility caused by its very early flowering, so it may become more widespread if the climate becomes warmer in Europe.

⇨ **Avoid transferring material over large latitudinal or longitudinal ranges since it is likely to be poorly adapted to local climate, causing forking and poor seed set.**

Genetic variation

Reciprocal transplant experiments

We established field trials in U.K. (for *F. excelsior*) and Greece (for *F. angustifolia* and *F. ornus*) to assess the geographic scale of **ADAPTIVE VARIATION**, by testing whether local material had a "home advantage". Local material was tested against material collected at sites increasing distances away, and the trial was replicated at all the sites where the seed was collected. We measured germination percentage of direct sown seed, and survival and growth of two year old seedlings.

The results so far show little (*F. angustifolia*) or no correlation (*F. excelsior*, *F. ornus*) between height growth and patterns of ecological or geographic variation. No local adaptation was observed; instead some provenances grew well on all sites whereas others were uniformly poor by comparison even on the site they originated from. At the scale of the experiments, covering distances up to 250-450 km (depending on the species), no differences were observed between provenances in terms of germination or survival. At this early stage, there is therefore little evidence for local adaptation in ash, suggesting that geographical proximity is a poor indicator of adaptive fitness.

⇨ **Provenance delimitation for ash can operate at the scale of at least 200-300 km without compromising local adaptation, i.e. good seed sources can be moved to other sites within this range without compromising their performance.**

⇨ **These conclusions may also apply to other wind pollinated, light demanding broadleaved species, since high levels of adaptability would be an evolutionary advantage for such widely dispersed species.**

Molecular markers

We assessed levels of neutral genetic variation in stands of ash across Europe using both **CHLOROPLAST** and **NUCLEAR MARKERS**.

Chloroplast markers

Chloroplast markers, being maternally inherited, can be used to investigate the movement of species via seed dispersal over long time periods. They also provide a good picture of broad scale population structures which exist over large distances, and which have been conserved for millennia, as long as human interference has been minimized. **DNA** "fingerprints" from chloroplast markers are called **HAPLOTYPES**.

The southern European peninsulas (Iberia, Italy, Greece and the Balkans) provided temperate refuges for ash during the Ice Ages. Following the last Ice Age, ash colonized northern areas of Europe that were exposed as the ice retreated. The retreat of the ice also opened up new pathways for east-west migration.

Fraxinus angustifolia and *F. ornus* both have southerly distributions in Europe, with postglacial migration predominantly from east to west, originating from glacial refugia in south-eastern Europe and western Asia (although the Iberian and Italian peninsulas may have been additional refugia for *F. angustifolia*). *Fraxinus excelsior* is today widespread across central and northern Europe, and there were probably four glacial refugia for this species, in Iberia, Italy, the eastern Alps and the Balkan Peninsula. Migration was predominantly northerly from these refugia, after the retreat of the ice. A similar pattern has been found in oak.

⇨ **Where conservation of genetic diversity is the highest priority, it can be guided at the largest scale by the distribution of haplotypes.**

⇨ **In countries where only a single ash haplotype is present, chloroplast markers can be used to detect some non-native material.**

⇨ **Most haplotypes have widespread distributions, giving little support to a strategy of subdividing large provenance zones into small areas for seed collection.**

Nuclear markers

We used nuclear microsatellite markers to assess overall levels of genetic variation in ash species, the differences within stands and between stands, and how this variation is distributed across Europe. Nuclear markers reveal recent levels of **POLLEN** and seed movement between stands, termed **GENE FLOW**. If stands are genetically similar, there must be high levels of gene flow between them, mainly due to pollen movement. Conversely, stands with very different genetic compositions must have low levels of gene flow resulting in little genetic mixing between them.

GENETIC DIVERSITY within stands is high in all European ashes: the same is true for most other tree species. *Fraxinus excelsior* and *F. angustifolia* show few genetic differences between stands, suggesting high levels of gene flow via pollen movement, as expected for species with wind-dispersed pollen. For example, at the northern edge of the range of *F. excelsior*, stands are more isolated and have lower levels of diversity. They are more differentiated because there is less pollen flow between them.

Fraxinus ornus pollen is dispersed mainly by insects. As in the other species, there are few genetic differences between stands, but the differences increase with geographic distance. The foraging behaviour of the insect pollinators tends to move pollen relatively short distances. Stands close together are therefore more likely to be closely related than they would be in species pollinated by wind-borne pollen coming from many different trees located further away.

⇨ **High levels of gene flow between stands provide evidence against the strategy of subdividing large provenance zones into small areas for seed collection.**

⇨ **Geographically distant stands show more genetic differences in insect-pollinated** *F. ornus* **than in wind-pollinated** *F. excelsior* **and** *F. angustifolia***.**

Good seed collection practice - collecting genetically diverse seed

Poor seed collection practice leads to establishment of trees with restricted genetic diversity and poor future adaptive potential, whatever the provenance of the seed. Collected seed needs to have a broad genetic base to avoid **INBREEDING DEPRESSION**, to ensure that planted stands remain viable and produce seed in the short term, and to maintain the ability to adapt to changing environments in the future. To maximise the genetic diversity in seed collections it is necessary to collect from as many seed trees as possible; the minimum number of trees depends on the species and the purpose of the collection (see Box 7.1). However decisions often need to be made about which stands to collect from; which trees within the stands; whether to collect from isolated trees; and whether to collect in poor seed years. Studying the reproductive biology of a species (see Chapter 3, page 25) can tell us about the amount and pattern of distribution of genetic variation and also about the factors which determine this distribution.

To maximise the genetic diversity in seed collections the most important considerations are the number of trees collected from, the distance between trees and collecting equal amounts of seed from each tree

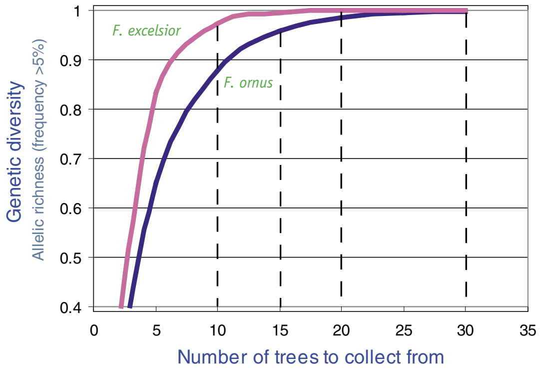
Box 7.1.- How many trees to collect from?

This question is often raised by seed collectors, policy makers and others. There is no single correct answer since it depends both on the aim of the collection and the way in which genetic diversity is distributed within the stand. It is not possible to sample all the diversity present in a population unless the whole population is collected, and this is clearly impractical. So while ideally one might look for 'conservation of as much of a population's genetic diversity as possible', in terms of sample size there are diminishing returns. As the number of seed trees sampled increases, the amount of extra diversity collected is reduced. There is therefore a need to define a criterion for an adequate sample size. We can consider two different scenarios:

- Collection of clonal material,
  e.g. cuttings from selected trees to establish a clonal seed orchard
- Seed collection

In the first case, genetics theory tells us that sampling 30 trees in a species with random **OUTCROSSING**, and 60 trees in a completely **SELFING** species, allows us to conserve an arbitrary 95% of **ALLELES** with a frequency >5%. In outcrossing *Fraxinus* species, cuttings taken from 30 trees per population to establish a clonal seed orchard would therefore meet this arbitrary level.

In the second case, however, when fruits/seeds are collected, adequate sampling also depends on the number of seed collected per tree and the mating/pollination system of the tree species. As FRAXIGEN research has shown, wind pollination (*F. excelsior* and *F. angustifolia*) is likely to result in trees producing seed from many fathers, whereas insect pollination is more likely to produce seed of fewer fathers per tree (*F. ornus*). To make a seed collection with the same levels of



genetic diversity, it is necessary to collect from approximately 50% more trees in *F. ornus* than in *F. excelsior* or *F. angustifolia*. In *F. ornus*, seed from15 trees will contain 95% of alleles with frequency >5%, while a collection from 30 trees will include additional rare alleles (95% of alleles with frequency >2.5%): only a slight increase in the genetic diversity. For *F. excelsior* and *F. angustifolia*, the corresponding numbers are 10 and 20 trees. **These calculations assume collection of at least 500 viable seeds per tree**.

Spacing between seed trees is also important in ensuring a diverse collection. Collecting at distances greater than seed dispersal will ensure that sampled seed trees are unrelated, although the pollen pools siring seed may still be related. Thus, in *F. excelsior* collection of seed from trees which are 100m apart is likely to ensure the seed trees are unrelated, but they are still likely to be mating with a similar selection of fathers. In *F. excelsior* seed trees need to be at least 150m apart to ensure collection of seed from both unrelated females and different pollen pools. With *F. ornus* most seed trees are mating with different fathers, so it is sufficient to collect from trees that are separated by the seed dispersal distance (30m) to ensure a diverse collection.

Suggested guidelines for seed collection

To obtain an efficient sample of the genetic diversity of the species	⇨	Collect >500 seeds from each tree, ensuring that trees are separated by at least	⇨	This will conserve 95% of alleles with frequency	
		(m)		>5%	>2.5%
				when collecting from at least	
F. ornus		30		15 trees	30 trees
F. excelsior		150		10 trees	20 trees
*F. angustifolia**		150		10 trees	20 trees

* *The optimal strategy for F. angustifolia is likely to be the same as for F. excelsior.*

For more details see: Brown, A.H.D., Hardner, C.M. 2000. Sampling the gene pools of forest trees for *ex situ* conservation. In: Young, A., Boshier, D.H. Boyle, T.J. eds. *Forest conservation genetics: principles and practice.* CSIRO, Melbourne, Australia / CABI Publishing, Wallingford, UK. 185-196pp.

Reproductive biology

Phenology and gene flow

Ash has years with high seed production (**MAST YEARS**) every 2-3 years. The intensity of flowering also varies from year to year but not every mast flowering event will result in a mast seeding event. For example, late frosts can kill flowers and decrease seed production.

Geographically close stands tend to flower at the same time unless separated by large altitudinal differences, maximizing the chance of gene flow between stands. Although many more trees flower in a mast year, gene flow between stands is higher in non-mast years, when there are fewer mates available and pollen has to travel further between mates. We found that more pollen from outside the stand contributed to seed production in non-mast years compared to mast years, particularly on the trees along the edge of the stand.

In *F. excelsior* and *F. angustifolia*, female flower parts open before pollen is shed within a stand, maintaining high levels of gene flow between stands. *F. angustifolia* flowers before *F. excelsior* but pollen production in *F. angustifolia* may overlap with the opening of female flowers in *F. excelsior*. This means that there is potential for **HYBRIDISATION** in mixed stands, and the hybrid seed is most likely to occur on *F. excelsior* trees.

⇨ **Seed for conservation, native woodland restoration and breeding programmes can be collected in mast and non-mast years, since genetic variation is high in both**

⇨ **Clonal seed orchards of** *F. excelsior* **should be designed with a predominance of male trees around the edge and female trees in the centre to minimize contamination of seed by external pollen**

⇨ **Avoid collecting in seed orchards and registered seed stands in non-mast years to minimize contamination**

⇨ **Avoid registration of, or collecting in, mixed** *F. excelsior* **and** *F. angustifolia* **seed stands since the seed from** *F. excelsior* **trees may be hybrid.**

Gender effects

Each European ash species has a different breeding system. *Fraxinus excelsior* has male, female and hermaphrodite-type trees, but there are no clear divisions between the different **GENDERS**. *F. ornus* has only males and hermaphrodites, whilst only in *F. angustifolia* are all trees hermaphrodite. Sex ratios are generally stable from year to year, and contrary to popular belief that *F. excelsior* trees change sex from year to year, only a small proportion of hermaphrodite trees varied, becoming more female or male in the mast year.

Males always father more seed

- than hermaphrodites
- on close females than distant females
- if they are large trees

Seeds of *F. excelsior* and *F. ornus* produced by male and hermaphrodite pollen showed similar percentages and rates of germination. However, the seedlings of *F. ornus* fathered by males grew significantly taller than seedlings produced by hermaphrodite pollen. In *F. excelsior* hermaphrodites and females produce similar amounts of seed.

⇨ **Seed can be collected from all seed-bearing trees in *F. excelsior*. Thus in a clonal seed orchard of *F. excelsior*, female and hermaphrodite trees can be included without affecting seed production.**

Selfing and mating patterns

Selfing is possible on **HERMAPHRODITE** trees in experimental pollinations, but in nature *Fraxinus* is highly outcrossing with negligible selfing in both mast and non-mast years. This is because female parts of hermaphrodite flowers open before pollen is released by the same flowers, so selfing is minimized. Mating between closely related individuals rarely occurs, because most pollen is regularly moved over tens of meters by insects (*F. ornus*) or hundreds of metres by wind (*F. angustifolia*, *F. excelsior*). Therefore there is little risk of **INBREEDING** in natural populations.

The insect pollinators of *F. ornus* tend to forage within the crown of a tree before moving on to another tree. This means that about a third of the seeds on one tree share the same father. In contrast, seeds from different trees rarely share the same father. In the wind pollinated species, *F. excelsior* and *F. angustifolia*, pollen comes from many trees located further away, such that seeds on the same tree have many different fathers.

- ⇨ **Seed can be collected from hermaphrodites, isolated trees (as part of a larger collection), and in non-mast years, without risk of inbreeding. Thus it is better to spread a collection over a wider area (e.g. combine between seed stands) keeping the minimum distance between seed trees (see Box 7.1), rather than restrict the number of trees or reduce the distance between collected trees.**

- ⇨ **In** *F. ornus* **collect few seeds from many mothers to increase the genetic diversity of collected seed; avoid collecting in isolated, small stands.**

Lessons for natural regeneration

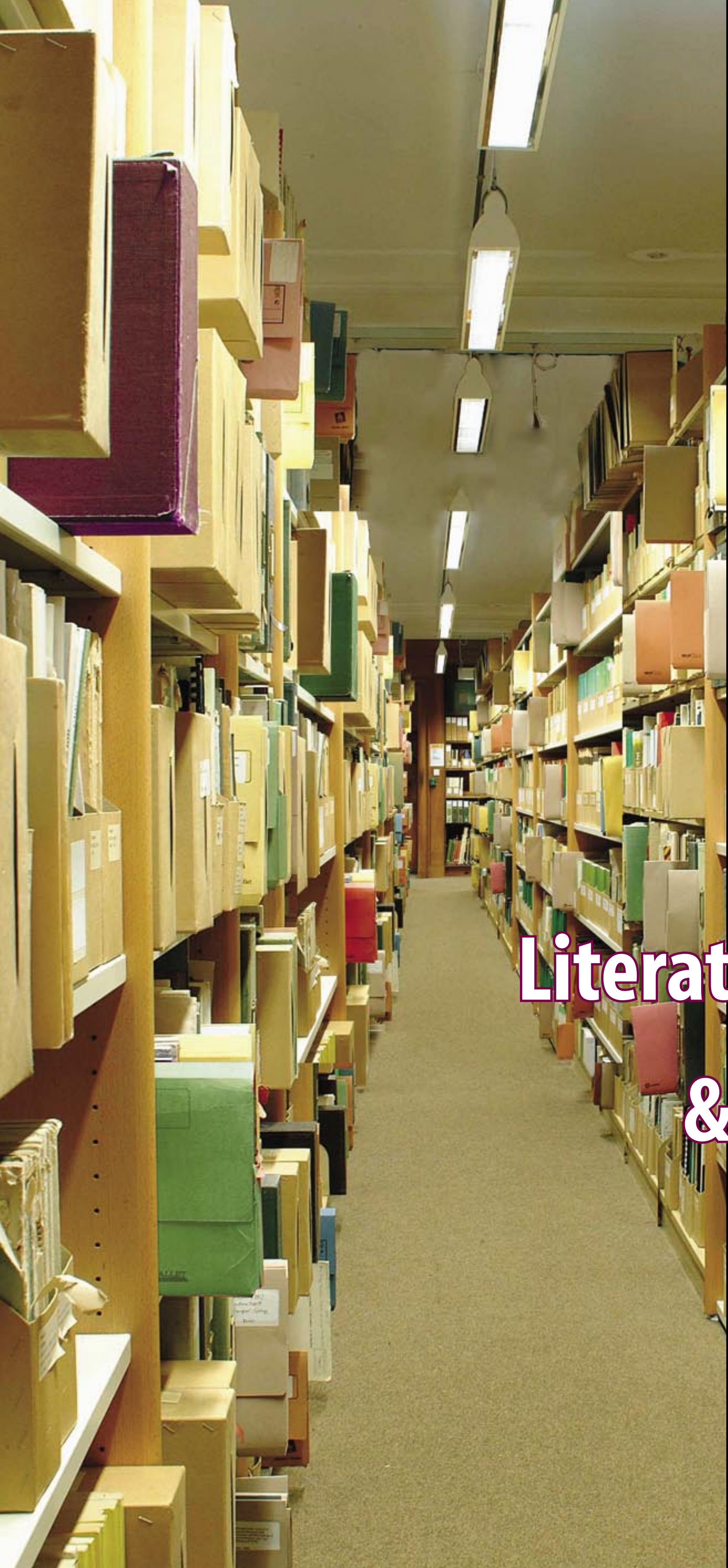
The sustainable management of native woods or their restoration through natural regeneration requires genetic diversity in the seed that fuels the regeneration. In *F. angustifolia* and *F. excelsior* the high levels of pollen flow mean that natural regeneration in woods will maintain high levels of genetic diversity, even in small **FRAGMENTED** woodland remnants. Even where isolated trees form the basis for natural regeneration, the diverse pollen pool that fathers the seed means that regeneration is likely to be genetically diverse. Conversely, the high level of gene flow means that native woodlands are not isolated and do not represent an uncontaminated local gene pool. Thus the natural regeneration in a reserve will also contain 'foreign' alleles from external pollen sources.

In *F. ornus* small (fewer than 15 seed bearing trees) isolated remnants should be avoided as the only source of natural regeneration, since the pattern of insect pollination means that the regeneration is likely to be lower in genetic diversity than in larger remnants.

Summary

⇨ **Native populations of all three European *Fraxinus* species maintain high levels of genetic variation. None of the species are under threat, nor are their genetic resources seriously threatened and in need of specific conservation programmes. Nevertheless activities such as seed collection, planting and restoration through natural regeneration need to take note of FRAXIGEN's recommendations if genetic diversity is to be maintained locally and future productivity and adaptability are not to be affected.**

⇨ **High levels of gene flow and broad adaptability suggest that the definition of a 'local seed source' needs to be broad (up to 250 km scale), and that collection should stress increased distances between seed trees to ensure genetically diverse collections. Too narrow a vision of what is 'local' (*e.g.* only one stand) is likely to result in the use of genetically less diverse material, with poorer adaptive potential.**



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Glossary

Adaptive variation: Differences in genes which improve the fitness of an individual or species, and are influenced by selection. Compare neutral variation.

Allele: One of the different forms of a gene or DNA sequence that can exist at a single locus. Most cells, except the ovule and pollen, have two alleles at each locus. If both alleles are the same (or indistinguishable), the locus is homozygous, if the alleles differ, it is heterozygous.

Allelic richness: A measure of genetic diversity, specifically the number of alleles observed across all markers in a population, corrected for differences in sample size across populations.

Androdioecy: A rare sexual system of plants where male and hermaphrodite flowers occur on separate individuals, thus making the trees either purely male or hermaphrodite. Only the hermaphrodite has a female function but its male function is questioned. Compare andromonoecy (*F. angustifolia*) and subdioecy (*F. excelsior*).

Andromonoecy: A sexual system of plants where male and hermaphrodite flowers occur on the same individual (making all individuals functionally both male and female). Compare androdioecy (*F. ornus*) and subdioecy (*F. excelsior*).

Anther: The part of a stamen that contains the pollen grains. In ash it consists of two pollen sacs that split and open up (dehisce) to release the pollen grains.

Autochthonous: Native or indigenous; not introduced. Often used to refer to a population or stand of trees.

Chloroplast: Part of a cell containing most of the genetic information necessary for photosynthesis, and the site of photosynthesis in the cell.

Chloroplast DNA: The genetic information (DNA) located in the chloroplast. Compare nuclear DNA.

Chloroplast marker: See genetic marker.

DNA: Deoxyribonucleic acid (DNA) molecules carry the genetic information necessary for the organization and functioning of most living cells and control the inheritance of characteristics.

Fragmented: Isolated or detached. For example a "fragmented population" is a stand of trees which is physically isolated from other stands of the same species.

Gender: Sexual function, either male or female. Also used as meaning 'sex', which may be male, female or hermaphrodite.

Genes: Units of hereditary information.

Gene flow: The movement of genes from one population to another via the movement of pollen or seed.

Genetic differentiation: Variation among populations or species that is attributable to differences in genes. See also genetic diversity.

Genetic diversity: Variation within a population or species that is attributable to differences in genes. See also genetic differentiation.

Genetic drift: A force that reduces heterozygosity by the random loss of alleles. The effect of drift is greater in small populations.

Genetic marker: Alleles or genes or DNA variants whose pattern of inheritance can be monitored to keep track of an individual, trace inheritance in families, or reveal genetic diversity. There can be genetic markers in the nuclear or chloroplast DNA.

Genome: The total genetic information (DNA) of an individual.

Genotype: The genetic characteristics of an individual. Compare phenotype.

Growing Degree Hours (GDH): Sum of mean hourly temperatures above 5°C. Hourly mean temperatures below 5°C add nothing to GDH. Temperatures above 5°C add to GDH by their numerical value. Summed across days or months GDH is a strong indicator of biological activity and used in calculations for predictions of start of flowering and leafing.

Haplotype: A group of genes inherited together as one unit. Usually used to refer to the chloroplast genotype of an individual.

Hermaphrodite: A flower having both male and female parts or function, or an individual with both sexual functions.

Heterozygosity: A measure of genetic diversity, specifically the mean probability, across markers, that two randomly chosen alleles in a population are different.

Hybridisation: Mating between individuals of distinct taxa. Usually refers to crosses between two different species.

Inbreeding: Mating between individuals which are more closely related than pairs chosen at random from within the population. Includes **Selfing.** Compare **outcrossing**.

Inbreeding depression: Reduction in fitness due to increase in number of homozygous loci (see **allele**) in offspring from closely related adults.

Inflorescence: The structure bearing the flowers. In ash, two types of inflorescences occur: panicles (*F. excelsior* and *F. ornus*) and racemes (*F. angustifolia*). Panicles have many branches from the main axis whereas racemes consist of a single axis with flowers.

Locus (plural: loci): The position in the **genome** of a **gene** or other **genetic marker**. For example, a '**microsatellite** locus' is a stretch of **DNA** being analyzed for variability.

Marker: See **genetic marker**.

Masting: Synchronized mass production of flowers or fruits/seeds.

Mast year: Years in which flowers or fruits/seeds are produced in large quantities.

Mating system: Description of the patterns of mating in a population or species, for example which individuals mate with each other and under what circumstances. Often used in plants to refer to the degree to which a species is **self fertilising** or **outcrossing**.

Microsatellites: Repetitive stretches of short **DNA** sequences used as **genetic markers** to trace inheritance in families.

Neutral markers: DNA variants or **genetic markers** which do not affect fitness. See also **neutral variation.**

Neutral variation: Differences in **genes** which have little or no effect on the fitness of an individual or species, and are influenced by gene flow. Compare **adaptive variation**.

Nuclear DNA: The genetic information (**DNA**) located in the **nucleus**. Compare **chloroplast DNA**.

Nuclear marker: See **genetic marker.**

Nucleus: Part of the cell containing the genetic information (**DNA**).

Outcrossing: Mating to a random sample of the population. Compare **inbreeding.**

Ovule: An ovule contains the egg cell where fertilization with the sperm cell from the **pollen** grain takes place.

Phenology: Study of the timing of recurring biological phases, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species

Phenotype: The observable characteristics of an individual, the result of interaction between genotype and environment. Compare **genotype**.

Pistil: The female part of a flower. It consists of an ovary, where the **ovules** are borne, and a style the top of which is called the stigma. **Pollen** grains landing on the stigma when it is receptive may germinate and grow down the style to fertilize the egg in the ovule.

Pistillate: A collective term for female and **hermaphrodite** flowers, since both types contain functional **pistils.** May also be used to describe **inflorescences** containing female and hermaphrodite flowers or trees bearing such flowers.

Pollen: Grains produced by the **anthers**, which contain the sperm cell.

Protogynous: A characteristic of **hermaphrodites**, when the female phase occurs before the male phase.

Reciprocal transplant experiment: Network of field trials designed to investigate localised adaptation and the scale over which it operates. See detailed explanation on page 26.

Samara: A type of fruit with a wing that facilitates wind-dispersal. The samaras on ash trees usually contain only one seed each (even though there are four **ovules**) and therefore the fruit is sometimes referred to as a seed.

Self-fertilization: See **Selfing.**

Selfing: Fertilization of an **ovule** with **pollen** from the same plant or clone. Compare **outcrossing**.

Stamen: The male part of a flower, consisting of a filament bearing the **anther**.

Subdioecy: A **mating system** of plants where male and female flowers may occur on the same or different individuals. Most individuals are either male or female but there are **hermaphrodites** or intermediates that specialize either as males or females. Compare **androdioecy** (*F. ornus*) and **andromonoecy** (*F. angustifolia*).

Taxon (pl. taxa): A name of any rank used in plant taxonomy, e.g. species, subspecies, variety, form, etc.

Trait: Inherited characteristics.