



**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.

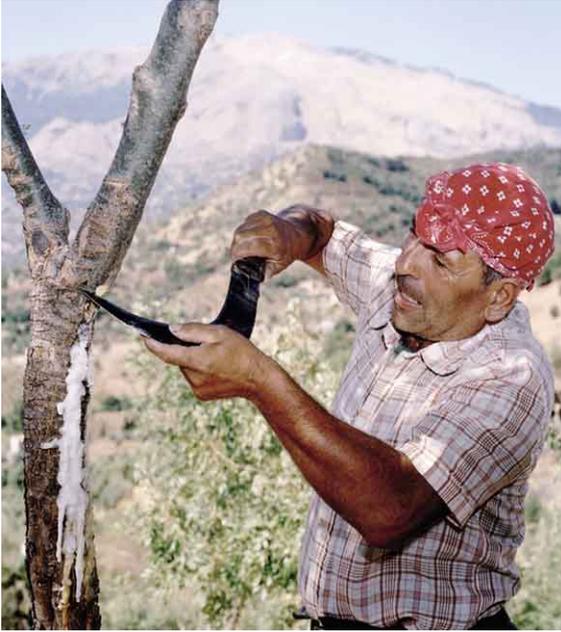
*Fraxinus ornus* is also a very valuable tree species in urban forestry, because it does not reach large dimensions, and in spring is very decorative because of its large white inflorescences.

## Traditional and modern uses

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**F***raxinus 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<sup>3</sup>. 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

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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

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### 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<sup>1</sup>, 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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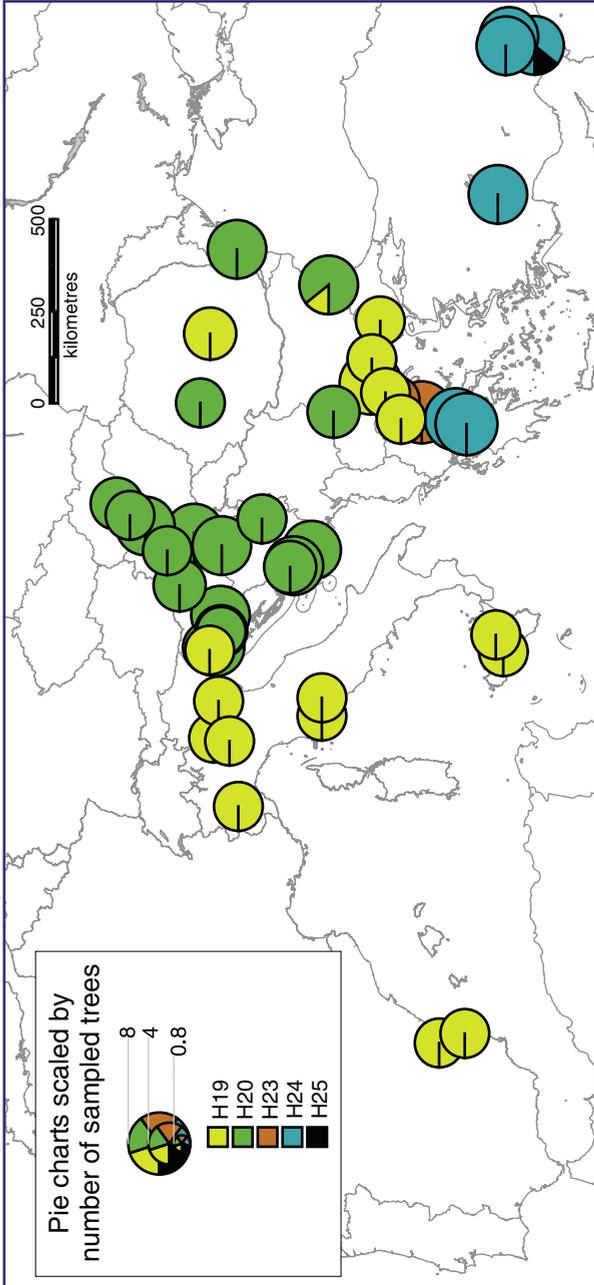


Figure 6.1.- Chloroplast haplotypes in 45 populations of *Fraxinus ornus* from Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Hungary, Italy, Romania, Slovakia, Slovenia, Spain, and Turkey. The size of the pies is scaled to show the number of individuals sampled in each population.

## 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<sup>3</sup>. 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**<sup>25</sup> 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 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.

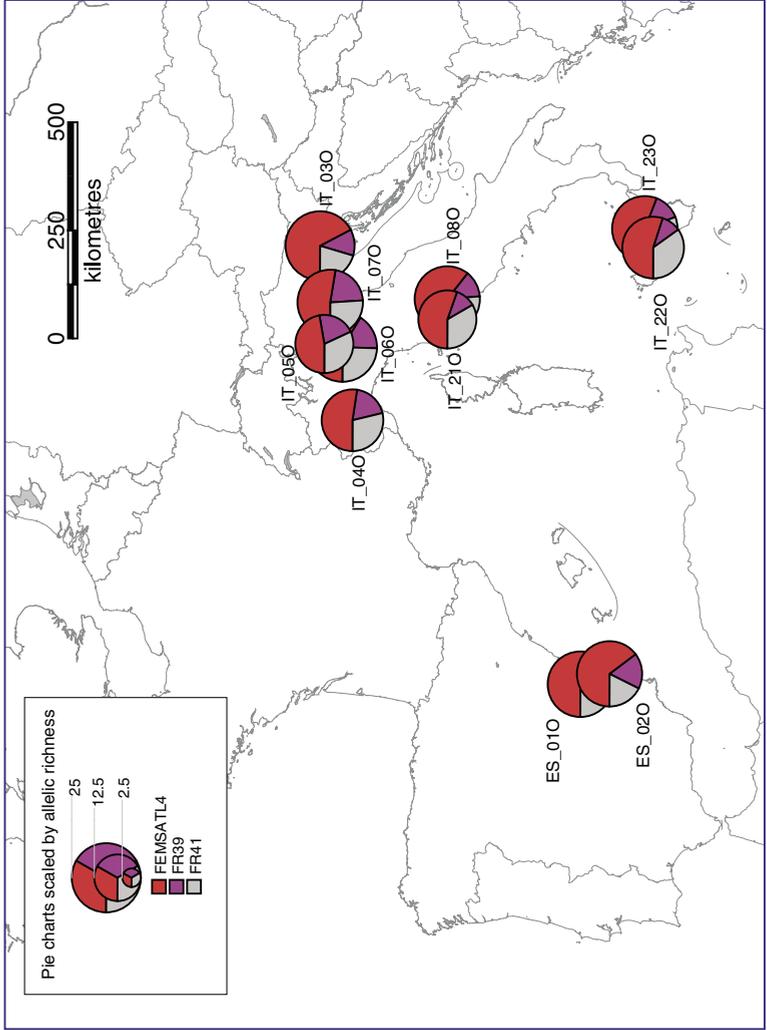


Figure 6.2a.- Allelic richness of *Fraxinus ornus* in populations from Italy and Spain; data from Spanish laboratory.

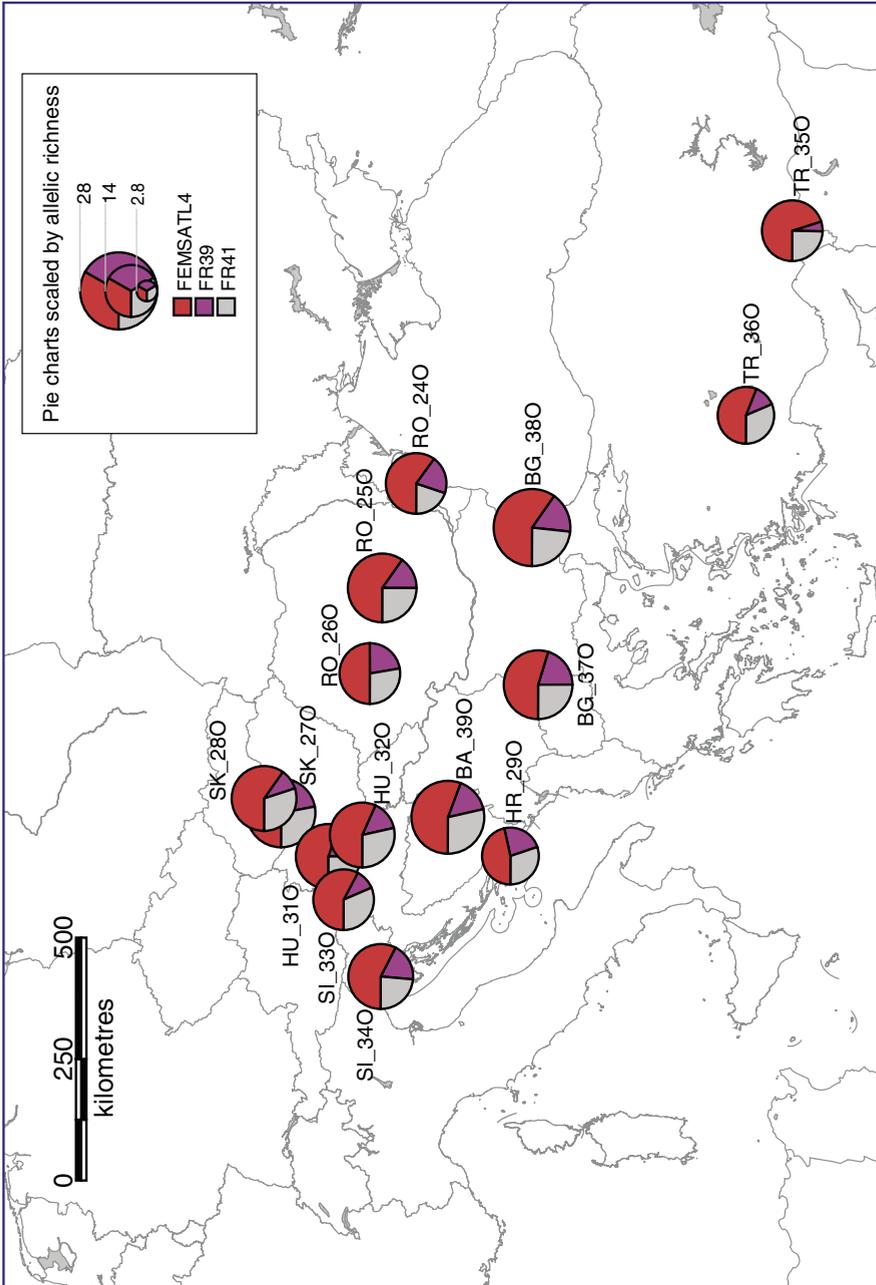


Figure 6.2b.- Allelic richness of *Fraxinus ornus* in populations from Central and Eastern Europe; data from Slovakian laboratory.

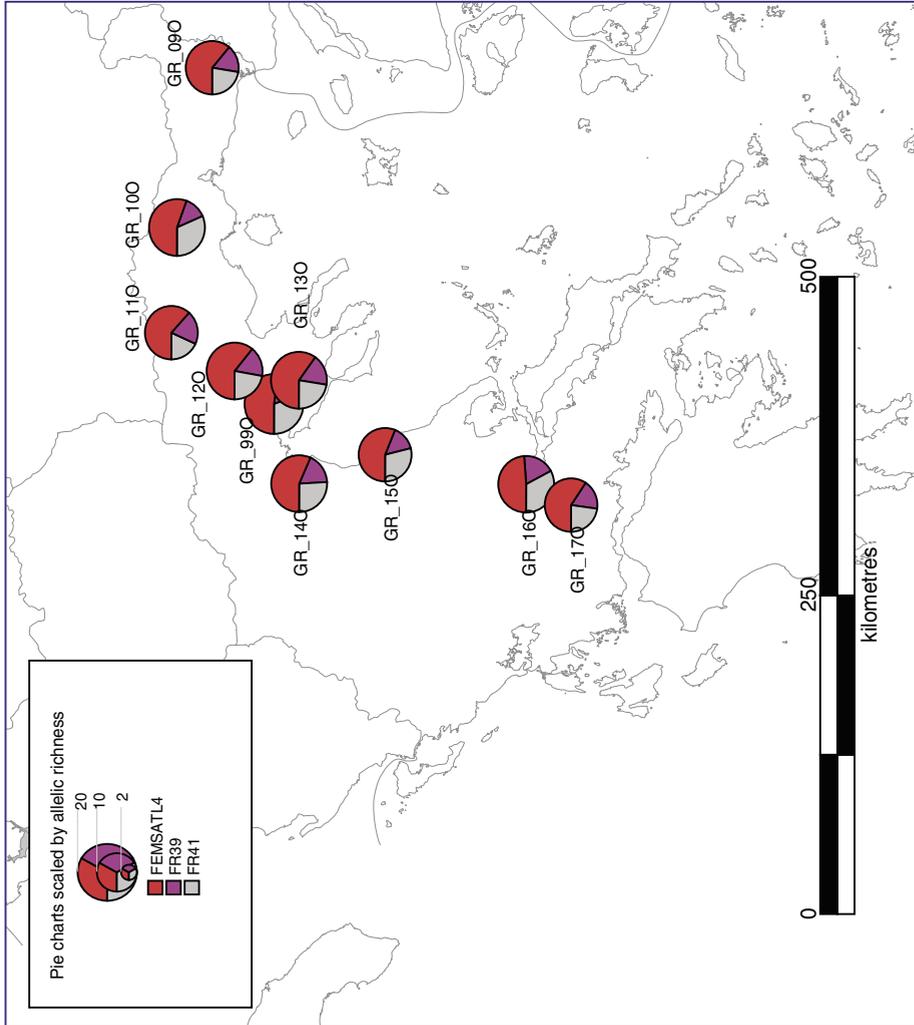


Figure 6.2c.- Allelic richness of *Fraxinus ornus* in populations from Greece; data from Greek laboratory.

**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<sup>9</sup>. 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<sup>27</sup>.

## 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*<sup>39</sup>.

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<sup>39</sup>.

### *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



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

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).



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.