Quantitative and molecular genetic variation in *Ulmus laevis* Pall.

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2004

Acta Universitatis Agriculturae Sueciae
Silvestria 313

ISSN 1401-6230
ISBN 91-576-6547-8

Tryck: SLU Service/Repro, Uppsala 2004

Abstract

Whiteley R.E. 2004. *Quantitative and molecular genetic variation in Ulmus laevis* Pall. Doctor's dissertation.
ISSN 1401-6230, ISBN 91-576-6547-8

Genetic diversity is a prerequisite for evolutionary change. The conservation of genetic diversity within species is therefore important in order to ensure the potential for future adaptation in a changing environment. Genetic variation for traits of adaptive importance may be measured by observation of phenotypic variation in common garden experiments. Genetic variation can also be assessed using molecular markers.

Ulmus laevis Pall. is a broadleaved riparian tree with a central and eastern European distribution. *Ulmus laevis* populations tend to be small, and many are thought to be at risk of losing genetic diversity via random drift. The aim of this study was to assess the amount and distribution of variation in *U. laevis* populations, using both quantitative and molecular genetic approaches.

Five *U. laevis* populations from the north and west of the species range were included in a common garden experiment in which quantitative adaptive traits were assessed over two growth periods. Considerable genetic diversity was recorded, both among and within populations, and the populations also varied in the amont of genetic diversity they possessed. Three of the same populations were included in a phytotron-based experiment to test the effect of drought stress on the expression of quantitative genetic variation. Treatment effects were relatively small, but the genetic variation recorded within populations and families again appeared substantial.

For the analysis of molecular genetic variation, two molecular marker systems were used: nuclear microsatellites and chloroplast DNA PCR-RFLPs. Microsatellites were developed for *U. laevis* and tested for utility in other *Ulmus* species. Seven populations were assessed using the two marker systems. Moderate levels of population differentiation were observed for the microsatellite loci. Chloroplast DNA diversity in the study area was very low, with only three haplotypes observed across all populations. A broad concordance was observed between allelic richness at the microsatellite loci, and the level of quantitative genetic variation within populations.

Key words: adaptive, conservation, microsatellite, phenotypic plasticity, quantitative, *Ulmus laevis.*

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Appendix

Papers I – IV

The thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Whiteley R.E., Black-Samuelsson S. and Jansson G. 2003. Within and between population variation in adaptive traits in *Ulmus laevis* Pall., the European white elm. *Forest Genetics* 10(4):313-323.

II. Black-Samuelsson S., Whiteley R.E. and Junzhan G. Growth and leaf morphology response to drought stress in the riparian broad-leaved tree, *Ulmus laevis* Pall. *Silvae Genetica*. In press.

III. Whiteley R.E., Black-Samuelsson S. and Clapham D. 2003. Development of microsatellite markers for the European white elm (*Ulmus laevis* Pall.) and cross-species amplification within the genus *Ulmus*. *Molecular Ecology Notes* 3:598-600.

IV. Whiteley R.E., Black-Samuelsson S. and Demesure-Musch B. Nuclear and chloroplast DNA variation in the European white elm (*Ulmus laevis* Pall.). Manuscript.

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Introduction

Background

Temperate forests provide habitats for many plant and animal species, and protection for soils and watersheds. The destruction of habitat is currently one of the most serious threats to terrestrial ecosystems, reducing both habitat area and the connectivity of the remaining population fragments. Fragmented populations face ecological, demographic and genetic challenges as a result of reduced population size and increased isolation.

The conservation of genetic diversity within species is important in order to assure the potential of populations for future adaptation (Eriksson 2001; Soulé & Mills 1992). In species of economic importance, conservation of genetic variability is also a prerequisite for the success of future breeding programs. Information about genetic variation within and between populations, and about existing adaptedness, can therefore be valuable both in prioritising populations for conservation, and for developing sustainable management practices (Lynch 1996; Storfer 1996; Young *et al.* 2000).

The studies comprising this thesis use a range of approaches to assess the genetic diversity within and among small and fragmented populations of the temperate deciduous tree species *Ulmus laevis* Pall., the European white elm. *Ulmus laevis* often occurs in small populations, and throughout the northern and western parts of the species range, suitable habitat is highly fragmented so that remaining populations may be at risk from genetic drift and inbreeding (Collin *et al.* 2000). This summary of the thesis presents the main findings of these papers and discusses the results and their implications for the conservation of these populations of *U. laevis*.

The study species - *Ulmus laevis* Pall.

Ecology

Ulmus laevis is a broad-leaved deciduous tree characteristic of lowland mixed forests in central and eastern Europe, with a range extending from southern Finland to the Balkans, and from western France to the Volga river valley in Russia (Collin *et al.* 2000) (Figure 1). The species tolerates wet soils and periodic flooding, and typically occurs in damp low-lying areas and as a component of riparian forests. As a consequence, populations are often scattered and relatively small. In western Europe, deforestation and drainage of flood plains for agriculture and industry have left little suitable habitat, and in many countries *U. laevis* is represented only by small population fragments. Towards the centre of the distribution the species is more abundant, but future land-use changes may pose a threat to many riparian forests in this region (Collin *et al*. 2000).

Ulmus laevis is wind-pollinated and the seeds are also dispersed by wind, although some dispersal by water is likely to occur at riparian sites and may be

important in long-distance dispersal. In Sweden, *U. laevis* occurs only on the Baltic island of Öland and is categorised under the World Conservation Union system as 'Vulnerable' (VU) on the Swedish Red Data list (Gärdenfors 2000). The most significant threat facing the species is probably habitat destruction (Collin 2000). Although *U. laevis* is susceptible to Dutch elm disease (DED), it is not thought to be in immediate danger from the disease. Feeding studies suggest that the *Scolytus* beetle species acting as vectors for the fungal pathogen find *U. laevis* unattractive as a food plant, strongly preferring *U. minor* (Webber 2000).

Genetics

Along with the American elm (*U. americana*), *U. laevis* belongs to the Blepharocarpus section of the genus, whereas the other two European elm species, *U. glabra* and *U. minor*, belong to the subgenus *Ulmus*. *Ulmus laevis* does not easily hybridise with the other European elm species, and is self-incompatible (Mittempergher & La Porta 1991). Data from molecular genetic studies using allozymes (Machon *et al.* 1995) and random amplified polymorphic DNA (RAPDs) and inter simple sequence repeat markers (interSSRs) support this classification, distinguishing *U. laevis* genotypes from both *U. minor* and *U. glabra* (Goodall-Copestake, Hollingsworth, Hollingsworth, Jenkins and Collin, unpublished data).

A limited amount of information is available on genetic variability within the species. Machon *et al.* (1997) reported relatively high allozyme variability in *U. laevis* in France, and Mattila & Vakkari (1997), also using allozyme markers, found high levels of differentiation among Finnish populations (F_{ST} = 0.33) but low genetic variability within populations. Gehle & Krabel (2002) found some allozyme variation both within and between populations on the Elbe river in Germany. Machon *et al.* (1995) have suggested that *U. laevis* may be a segmental allotetraploid, since studies with allozymes indicated that some loci show up to four bands at a single locus. However, the interpretation of these banding patterns is not straightforward, and large-scale studies of inheritance patterns would be required to confirm or refute the hypothesis of segmental tetraploidy in the species.

Why study Ulmus laevis?

The *Ulmus* genus is of interest for a number of reasons. From a commercial point of view, elms are a source of high-quality wood used for furniture and flooring and have been greatly valued as landscape trees. As a result of the two DED pandemics of the last century, in 1919 and 1972, which devastated elm populations throughout Europe and North America, there has been great interest in the possibility of breeding resistant elms to replace lost trees. The taxonomy of European elms has been the subject of much debate, particularly regarding the *U. minor* species complex and the nature and frequency of hybridisation between *U. minor* and *U. glabra* (see Hollingsworth *et al.* 2000 for a discussion of these issues). Elms are also of interest for biodiversity conservation, as they form important habitats for other organisms. For example, elm bark has been found to

support a large number of species of lichens compared with many other temperate European trees (Watson *et al.* 1988).

In the case of *Ulmus laevis*, a major advantage for detailed genetic studies is the absence of interfertile species within the distribution area, removing the complicating factors of hybridisation and introgression. *Ulmus laevis* is one of the few European tree species which thrives in waterlogged soils, and for this reason it is an important component of riparian forests throughout its geographic distribution area. Riparian forests are highly valued for the protection they provide for rivers and surrounding land by stabilising river banks against erosion and reducing the amount of sediment entering the river. Riparian forests also provide important habitats for wildlife, forming forest corridors through cultivated land and providing shaded areas of water for aquatic species (Dosskey *et al.* 1997).

Genetic variation and evolutionary potential

Differential survival of individuals according to the alleles they carry leads to changes in allele frequencies within a population, and gradual adaptation to new conditions. The capacity of a population to evolve in response to environmental change ultimately depends upon the presence of additive genetic variation (Lande & Shannon 1996). In theory, the rate of evolution of a trait's mean phenotype is a function of both the additive genetic variation for the trait and the intensity of selection. This relationship can be expressed as $R=h^2S$, where R is the response to selection, S is the selection differential and h^2 is the narrow-sense heritability, the proportion of the phenotypic variation in a population which is due to additive genetic variation (Falconer & Mackay 1996). It is therefore important that appropriate levels of additive genetic variation are maintained in order for adaptive response to selection to be possible. Over the long term, the rate of evolution is more likely to be limited by ecological opportunity than by the amount of genetic variation present in a population (Lande & Shannon 1996).

Traits likely to be of adaptive importance in trees include height, which may be of significance in competition for light and other resources, and the timing of bud flushing and growth cessation, which determine the length of the growing period. Most adaptive traits appear to behave as quantitative traits, i.e. as characters with complicated inheritance, influenced by many loci, each with a relatively small effect (Lynch 1996), and their phenotypic variation usually contains a strong environmental component. Recent research has also highlighted the importance of single loci with relatively large phenotypic effects (e.g. Bradshaw & Schemske 2003; Orr 1998), and functional studies of individual loci and their adaptive significance are becoming more common (Remington & Purugganan 2003).

Contemporary climate change and natural populations

The capacity to respond to environmental change will be of particular importance if current climate trends continue. In the past thirty years, global mean surface temperature has increased at the unprecedented rate of 0.2°C/decade (Stott *et al.* 2000; Crowley 2000) and it is predicted that this accelerated increase in average global temperature will continue (Houghton *et al.* 2001). Intergovernmental Panel

on Climate Change (IPCC) models suggest that the globally averaged surface air temperature will warm by 1.4°C to 5.8°C by 2100 relative to 1990. An increase in total precipitation and in the frequency of intense precipitation events and consequent flooding are also predicted. Sea levels are expected to rise, leading to flooding of low lying islands and coastal areas. Given these projections, *Ulmus laevis* populations in the northern part of the present distribution area would be likely to experience warmer winters and more rainfall than at present, while southern populations could be exposed to prolonged droughts during summer months. An increase in extreme weather events could lead to a higher frequency of severe flooding, threatening floodplain populations.

Plant responses to climate change are expected to fall into four categories: phenotypic plasticity, adaptive evolution, migration via dispersal to sites where conditions are more appropriate, or extiction (Bawa & Dayanandan 1998). Morphological and phenological changes have been have been observed in wild populations of plants that correlate with climate data, as have range shifts and population density changes (e.g. Root *et al.* 2003). Evidence of adaptive evolution in response to recent global warming has also been reported in some taxa (Rodríguez-Trelles & Rodríguez 1998; Bradshaw & Holzapfel 2001). Within the lifetime of the present generation of trees, such changes could constitute new or increased sources of abiotic stress. Within their own lifetime, plants may adapt to change on an individual level, through phenotypic plasticity (Schmalhausen 1949). In *U. laevis*, climate change could initiate a phenotypic response to increased temperatures, and either increased or decreased water availability, depending on location. In Paper II, we investigated the effect of drought stress on the expression of genetic variation in three *U. laevis* populations.

Life history traits, post-glacial history and population structure

In general, trees maintain higher levels of genetic variation within their populations than do annual plants (Hamrick *et al.* 1992). Austerlitz *et al.* (2000) showed, using simulations, that this observation can be explained in part by the delayed reproductive phase of tree species. Trees take several years to reach maturity and during colonisation events this allows the accumulation of founder individuals prior to reproduction. Hamrick & Godt (1989) found that a range of other life history traits significantly influenced the genetic structure of plant populations. Mating system, dispersal agent and geographic range were the strongest indicators of the amount of within-population genetic diversity of a species. The highest levels were found in long-lived, outcrossing, late-successional species that were wind-pollinated and wind- or animal- dispersed. *Ulmus laevis* is wind-pollinated, wind-dispersed and allogamous, and might be expected to show a relatively high degree of within-population genetic diversity as has been demonstrated in other tree species.

The present distribution of genetic diversity in temperate forest trees has also been influenced by historical factors including the size and number of refugia during the last ice age, post-glacial colonisation routes and the numbers of founder individuals involved in establishing new populations (Hewitt 2000). Petit *et al.* (2003) studied cpDNA variation in a number of European trees and shrubs and

found that differentiation among populations was strongest in refugial areas, while within-population diversity was greater in more recently colonised regions. In *Fagus sylvatica*, allozyme studies have shown higher allelic richness in southern Europe, close to putative refugia, but greater gene diversity (*H*) at higher latitudes (Comps *et al.* 2001). This trend in *H* was unexpected and may be the result of admixture between genotypes originating in separate refugia, by selection favouring heterozygotes, or as a result of meta-population dynamics occurring during the colonisation process. In paper IV, I investigated the distribution of genetic variation in nuclear microsatellite markers and chloroplast DNA in order to gain understanding of the influence of population genetic processes and post-glacial history on the current genetic structure of *U. laevis* populations.

Habitat fragmentation

Habitat fragmentation reduces total habitat area and creates habitat patches separated from one another by a matrix of non-habitat. It can have complex ecological consequences, hindering migration and increasing the vulnerability of local demes to extinction as a result of demographic and environmental fluctuations.

Fragmented populations face genetic threats as a consequence of both their decreased size and increased isolation. Young *et al.* (1996) identified four main ways in which fragmentation can lead to reduction of genetic variation: i) increased losses from genetic drift. Random drift removes a fraction of the genetic variation from a population equivalent to $1/(2N_e)$ per generation, where N_e is the effective population size. Fixation and loss of different alleles at random leads to increasing differentiation among populations. Genetic drift is likely to be the most serious genetic threat to a population that remains small for many generations (Shaffer 1987; Booy *et al.* 2000). ii) increased rates of inbreeding. Inbreeding changes genotype frequencies in a population, reducing heterozygosity and leading to expression of deleterious recessive alleles. Natural selection is less efficient in small than in large populations, so purging of both the existing genetic load and new deleterious mutations may be slower following fragmentation (Lynch 1995). iii) reduced gene-flow between remaining population fragments. iv) increased probability of loss of genetic diversity via local extinction.

The results of empirical studies of the impact of fragmentation on genetic variability have been varied, some showing lower reproductive success of individuals in small population fragments, but also relatively high levels of outcrossed progeny (Nason & Hamrick 1997), and even increased numbers of long-distance pollen-flow events (White *et al*. 2002). However, most studies of the impact of fragmentation so far have been carried out in insect- or animal-pollinated tropical species, so the applicability of their findings for *Ulmus laevis* is uncertain. While the present study does not directly investigate the effects of habitat fragmentation, *Ulmus laevis* frequently occurs in populations of limited size, significantly isolated from one another, and concern over genetic problems related to its fragmented distribution was an important motivation for the study.

Measuring genetic variation

There are several approaches to the study of genetic variation. Quantitative traits with likely adaptive value may be measured directly, or a molecular marker may be employed to estimate variation at the level of DNA or proteins. The variation within a species may be divided into the amount of genetic variation among different populations or fragments, and the genetic diversity within the populations. At the level of the individual, the diversity among chromosomes, or heterozygosity, can also be measured.

Quantitative genetic studies

Environment has a profound effect on plant phenotypes, and studies of genetic variation in natural populations may be confounded by environmental variation. Measurement of phenotypic variation in closely related individuals in common garden experiments allows the additive genetic variation that forms the basis for response to selection to be inferred. Such experiments aim to reduce to a minimum the environmental component of phenotypic variance in the study organisms, to give the most accurate possible estimates of genetic parameters. Heritability estimates provide information on how a population may respond to changes in the environment (Storfer 1996), and is a parameter commonly calculated in studies of quantitative genetic variation. However, the heritability includes an environmental component which makes its value specific to the study population in the environment of the study. Geber & Griffin (2003) reviewed measurements of heritability made in common garden experiments with those surveyed in-situ in natural populations and found that experimental studies overestimated the heritability of a trait 'in the wild' by a factor of two to four. A more appropriate measurement for conservation purposes is likely to be CV_A, the coefficient of additive genetic variation, which is standardised by the mean value of a trait. It is therefore more suitable for comparison between studies, and more likely to provide a better measure of the long term evolvability of a population (Houle 1992). Such experimental studies of quantitative variation require very large numbers of closely related individuals. The use of a phytotron – one or more growth chambers in which environmental variation can be further controlled – allows for increased accuracy of estimates and/or a decreased number of study individuals, but carries the disadvantage of a highly artificial environment, so that extrapolations to the situation in natural habitat must be made with care.

Microsatellite markers

Microsatellites are highly polymorphic, codominant, DNA markers which have become a widespread choice in population genetic studies. They consist of multiple contiguous repeats of short (usually two to five nucleotides long) sequences of DNA, amplified by Polymerase Chain Reaction (PCR) using primers in the 'flanking region' on either side of the repeat sequence. Alleles at a locus differ from one another in length, usually by one or more repeat units. A single locus may show tens of alleles and this high polymorphism is a result of their high mutation rate. Estimates of mutation rates at microsatellite loci range from 10^{-2} to 10^{-6} events per locus per generation, compared with 10^{-9} to 10^{-10} events per locus

per generation for point mutations in non-repetitive DNA (Li *et al*. 2002; Hancock 1999). Changes in the number of repeats (stepwise mutations) are often the result of replication slippage left uncorrected by mismatch repair mechanisms. This mutation mechanism, along with the high mutation rate, raises the significant probability of homoplasy - alleles with identical sequences differing in their recent evolutionary histories, i.e. not being identical by descent. This has led to the development of microsatellite-specific mutation models and measures of population structure, e.g. Slatkin's R_{ST} (1995).

Microsatellites are not randomly distributed within the genome - most are concentrated in non-coding regions (Wang *et al*. 1994), particularly in the centromeric region (Li *et al*. 2002), and in plants there is evidence that they are preferentially associated with non-repetitive DNA (Morgante *et al.* 2002). Microsatellites have often been assumed to be selectively neutral, but there is evidence that they can have functional roles. Some microsatellites appear to play a role in regulating DNA replication and recombination, and even in modifying adaptive mutation rates (Li *et al*. 2002; Chang *et al*. 2001). The possible functional roles of microsatellite loci, their non-random distribution in the genome and the likelihood of homoplasy mean that these data must be interpreted with care. However, their high polymorphism and co-dominance means that these markers remain very useful in studies of population genetic structure, particularly in relatively recently diverged populations.

The possibility of obtaining individual-specific multilocus genotypes also makes these markers useful for parentage-based studies of contemporary gene-flow. A study of pollen-mediated gene-flow in *U. laevis* is planned and this was taken into account in the decision to develop microsatellite markers. Prior to this study no microsatellites had been described for *U. laevis* or any closely related species. Paper III in this thesis describes the development of six microsatellites for use in *U. laevis*, and their potential utility in other *Ulmus* species.

Chloroplast DNA PCR-RFLP

As mentioned above, homoplasies complicate inferences from microsatellite variation over long historical periods. Chloroplast DNA (cpDNA) PCR - restriction fragment length polymorphism (PCR-RFLP) markers lack the power of microsatellites to differentiate between recently separated populations, because they accumulate mutations more slowly, but can provide information on more distant historical events. The chloroplast genome is usually maternally inherited in angiosperms so there is no recombination. Gene-flow is via seed dispersal and therefore likely to be far more limited than for nuclear genes which disperse via both seed and pollen movement. Chloroplast PCR-RFLP employs a combination of PCR primers and restriction enzymes to first amplify a section of cpDNA and then cut the amplification product at one or more restriction sites. The result is a set of fragments that may vary in length among individuals as a result of insertion/deletions or point mutations at restriction sites.

Recently, many studies employing PCR-RFLP to assess cpDNA variation in European forest species have been published, revealing a variety of post-glacial

colonisation routes and identifying possible refugial areas for these species (e.g. Palmé & Vendramin 2002; Rendell & Ennos 2003).

Do molecular markers provide useful information about genetic variation?

Lynch (1996) outlines a number of theoretical reasons why molecular markers should not be expected to predict patterns of quantitative variation: i) variation at single marker loci is introduced via mutation at a slower rate than for polygenic traits, ii) heterozygosity declines linearly with inbreeding coefficient at marker loci, but not for quantitative traits owing to dominance and epistasis variance; iii) the sampling variance of additive variation measured in several populations can be very large iv) as can the variance of heterozygosity measured at molecular marker loci, unless large number of loci and individuals are sampled.

Many empirical studies support the view that molecular markers cannot be relied upon to accurately predict quantitative variation (reviewed by Reed & Frankham 2001). According to Pfrender *et al*. (2000) "measures of within population genetic diversity are essentially unrelated to those at the molecular level." Several authors have cautioned against using only molecular markers in planning for the conservation of genetic resources e.g. Crandall *et al.* (2000). Reed & Frankham (2001) conclude that "When information about a population's short-term evolutionary potential or estimates of local adaptation and population divergence are required, quantitative genetic variation should be measured directly." However, the existence of subdivision at the molecular level may indicate subdivision at the quantitative level, upon which natural selection may have acted or be acting (Pfrender *et al*. 2000). Molecular markers can also provide information about the presence of inbreeding in a population or the extent to which genetic diversity has been affected by random drift.

Objectives

The main objective of this thesis was to obtain information about the genetic structure of *U. laevis* populations that would be of use in the conservation of the species. Specifically, my aim was to answer the following questions: i) are the study populations genetically differentiated from one another in terms of adaptive or molecular variation? ii) how much genetic variation exists within the populations? and iii) what are implications of the answers to these two questions for conservation of the *U. laevis* populations studied? A secondary aim was to develop microsatellite markers for use in this project and in future ecological and genetic investigations of *U. laevis*.

Paper I describes an investigation using a field trial to assess levels of quantitative genetic variation within and between five populations of *U. laevis* from the northwest of the species range. Paper II deals with the expression of genetic variation in three of the populations from Paper I under drought stress conditions. Paper III describes the development of a set of microsatellite markers for use in *U. laevis*, and Paper IV describes the use of these microsatellites along with chloroplast DNA markers in an investigation of molecular genetic population structure in seven populations.

Materials and methods

Plant material collection

Seven source populations of *U. laevis* formed the focus of the study. Population locations are shown in Figure 1 and the number of adult individuals or open-pollinated progeny from each population used in each study are shown in Table 1. Seeds and leaves were collected from approximately 20 mature trees in *U. laevis* populations in Russia (Moscow), Sweden (Öland), France (Garonne valley) and Germany (one population from each of the Elbe and Mulde river valleys). Our study populations were chosen to represent both very marginal populations, such as the stands of *U. laevis* on the Garonne river in southern France, and populations closer to the centre of the distribution area (Figure 1). The seed samples were the source of the open-pollinated progeny used in Papers I and II. The leaf samples formed the basis for the molecular studies although these were supplemented both by two additional populations (Hattula and Lolland) and additional individuals from the same populations. As far as possible, both leaf and seed samples were taken from non-adjacent trees in order to minimise the likelihood of clonal relationships among them. Additional samples included in the cpDNA analysis are listed as an appendix in Paper IV.

Field and phytotron trials

To facilitate measurement of adaptive variation for Paper I, a field trial was established in September 2000 at the Pustnäs experimental field site, 4km south of Uppsala (59º48´N; 17º39´E). The field trial was composed of 24 seedlings from each of approximately 20 trees from each of the five populations (Table 1). The trial contained six fully randomised blocks and single tree plots. Trait registrations were made during 2001 and 2002, when the seedlings were aged two and three, respectively. Table 2 shows details of the traits registered.

The study of the expression of genetic variation on drought stress (Paper II) was carried out in a phytotron. The experiment was established using progeny from eight open-pollinated families from the Öland, Elbe and Garonne populations. Each family was represented by 20 seedlings in each of two watering treatments: 'free access' watering and a drought stress treatment, in which watering was withheld until the weight of plant, growth medium and pot combined had fallen to approximately 40% of its saturated weight. Height and bud set stage were registered in seedlings in both treatments over one growth period. At the end of this period the total dry weight and root and shoot dry weights were determined. The morphology of two mature leaves per plant was analysed using two image descriptor systems: moment invariants (Dudani *et al.* 1977) and elliptic Fourier coefficients (Kuhl & Giardina 1982).

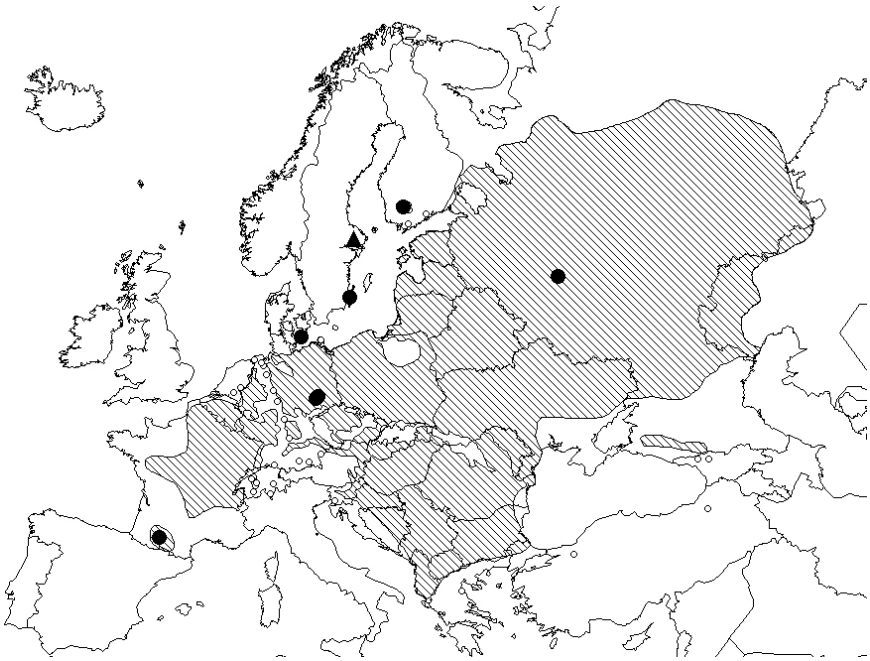

Figure 1. Distribution of *Ulmus laevis* Pall. (shaded area). Small open circles indicate population fragments. Filled circles indicate the populations studied in this thesis. The location of the field trial in Paper I is shown by a triangle. Study populations were located in the north and west of the species range, and included both marginal (e.g. Öland, Garonne), and more central populations (Moscow).

Table 1. *Numbers of individuals or maternal, open-pollinated families used in three studies of genetic variability in Ulmus laevis. For Papers I and II, numbers in brackets indicate the number of progeny per family. For Paper IV the left hand figure refers to the microsatellite analysis and the right hand figure to the cpDNA analysis.*

Population	Country	Latitude	Longitude	Number of families or individuals used		
				Paper I Quantitative traits	Paper II Drought stress	Paper IV Molecular markers
Hattula	Finland	61°03'N	24°22'E	-	-	26 / 20
Öland	Sweden	56°41'N	16°35'E	19 (24)	8 (20)	51 / 20
Moscow	Russia	55°45'N	37°35'E	21 (24)	-	24 / 19
Lolland	Denmark	54°46'N	37°35'E	-	-	32 / 20
Mulde	Germany	51°32'N	12°37'E	20 (24)	-	15 / 20
Elbe	Germany	51°30'N	13°04'E	20 (24)	8 (20)	20 / 20
Garonne	France	43°36'N	01°26'E	19 (24)	8 (20)	18 / 20

Figure 2. *Ulmus laevis* seedlings in a field trial (Paper I) at Pustnäs experimental site, September 2000.

Data analysis

Quantitative traits

Genetic parameters calculated for each trait in Papers I and II are shown in Table 2. In Paper I, narrow-sense heritabilities (h^2) were calculated for all traits, whereas coefficients of additive genetic variation (CV_A) were calculated for metric traits only. For traits registered in classes, the data were transformed to 'normal score' values to achieve homogenous variances and normal distribution of residuals. A logistic linear regression model was used to analyse data collected on several occasions during bud flush and bud set in 2001 (when the trees were age two) to construct curves showing the progress of these phenological events in the families and populations within the field trial (Figure 3). Additive genetic correlations between traits and between registrations of the same trait were also estimated. In Paper II family and population variance components and CV_A values were calculated for the traits assessed, firstly across all treatments then within each treatment separately.

Table 2. *Details of quantitative traits assessed and parameters calculated in Papers I and II.* CV_A *= Coefficient of additive genetic variation;* h^2 *= narrow-sense heritability;* Q_{ST} *= population differentiation in terms of quantitative traits; % Var = variance components expressed as a percentage of total variance.*

Paper no.	Traits assessed	Parameters calculated			
I	Height	CV_A	h^2	Q_{ST}	-
	Height increment	CV_A	h^2	Q_{ST}	-
	Stem diameter	CV_A	h^2	Q_{ST}	-
	Branch number	-	h^2	Q_{ST}	-
	Bud flush timing	-	h^2	Q_{ST}	-
	Bud set timing	-	h^2	Q_{ST}	-
	Leaf fall timing	-	h^2	Q_{ST}	-
	Frost damage	-	h^2	Q_{ST}	-
	Bark texture	-	h^2	Q_{ST}	-
II	Leaf size	CV_A	-	-	% Var
	Height	CV_A	-	-	% Var
	Bud set timing	CV_A	-	-	% Var
	Shoot dry mass	CV_A	-	-	% Var
	Root dry mass	CV_A	-	-	% Var

Molecular markers

Individuals shown in Table 1 were genotyped using three of the microsatellite loci described in Paper III. Estimates of the amount of differentiation between population (F_{ST} and R_{ST}) were calculated. F_{ST} is a measure of the proportion of total inbreeding in a population which occurs as a result of differentiation among sub-populations. R_{ST} is an equivalent measurement of differentiation developed for use with microsatellite markers. Allelic richness and heterozygosity within each population were also calculated. Allelic richness calculations used the rarefaction method (Petit *et al*. 1998) to compensate for the varied number of individuals per population. Chloroplast DNA PCR-RFLP was used to assess variation in the chloroplast genome. Fragments of chloroplast DNA were amplified using two PCR primer pairs, and the PCR products were then cut using three different restriction enzymes. Of the resulting fragments, five were found to be polymorphic when separated by electrophoresis and these were found in three combinations or 'haplotypes' in the study populations.

In order to compare levels of population differentiation measured with molecular markers and quantitative traits, I calculated Q_{ST} (Spitze 1993) for the 10 quantitative traits assessed in Paper I. Q_{ST} is a measure of differentiation analogous to F_{ST}, allowing direct comparison between molecular and quantitative genetic information.

Main results

Adaptive trait variation in a field trial - Paper I

Genetic differentiation among populations

Significant pair-wise differences between populations were found for all traits studied and for the most part separated the two most northerly populations (Öland and Moscow) from the three populations originating further south. Patterns of bud flush and bud set broadly followed the latitude of origin of the populations, with high latitude populations flushing and setting bud first. Despite setting bud latest of all, the Garonne trees were significantly less tall than both the Elbe and Mulde populations at age three. There was strong population differentiation for autumn frost damage, which was significantly more severe in the southern populations. Differentiation for leaf fall was also very strong, and followed a similar pattern to that of bud set, including significantly earlier flushing in the Moscow population than the Öland population.

Genetic variability within populations

The Öland and Elbe populations showed the highest levels of within population variability. The Garonne population showed generally low within-population genetic variability, having the lowest CV_A or σ^2_f of all the populations for six of the traits assessed. CV_As for height decreased from age two to age three in most populations. Genetic variation for frost damage was high in the Garonne and in both German populations, but extremely low for the two more northerly populations, which also showed much lower mean values for frost damage.

The Moscow population showed relatively low levels of within-population variability for most traits. Relatively large additive variance components for leaf fall, compared to other traits, were found in both years in the Öland, Moscow and Elbe populations at age two and for all populations at age three.

Additive genetic correlations

The correlation between height age two and the growth increment during the following year was weakly significant; the between-year genetic correlation for leaf-fall was strongly significant. Height was strongly negatively correlated with bud set i.e. early bud set was associated with short stature. A strong negative correlation was obtained between bud set and frost damage, i.e. those trees that set bud earlier were less affected by frost. Leaf fall was also negatively correlated with frost damage, but not as strongly as with bud set. Frost damage was not correlated with either growth increment the following year or height at age three.

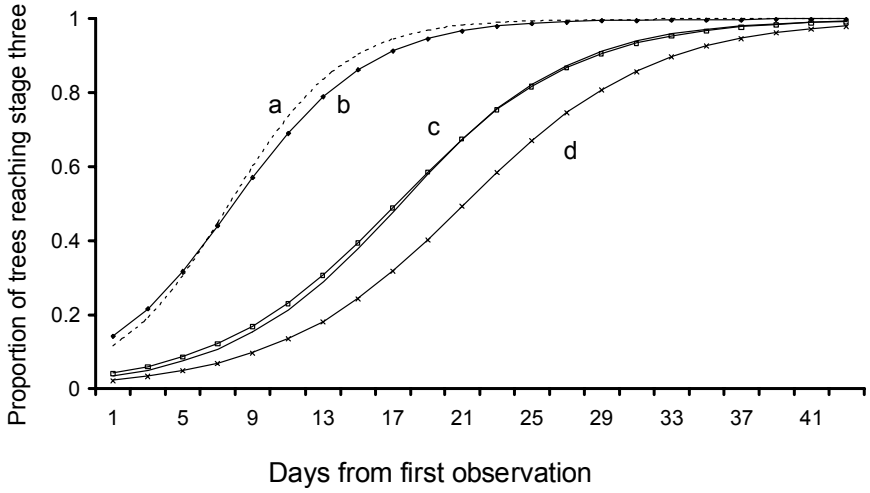

Figure 3. Bud set in five populations of *Ulmus laevis* Pall. grown in a field trial. The Y axis shows the proportion of trees reaching stage three (fully mature bud) of the bud set scale (0-3). The populations are: a) Moscow, b) Öland, c) Elbe (line with points) and Mulde, d) Garonne. The two most northerly populations (Moscow and Öland) started and finished setting bud first, and the most southerly (Garonne) started and finished last. There was a difference of more than 16 days between 80% of the Moscow trees reaching stage three, and 80% of the Garonne population reaching the same stage.

Drought stress response – Paper II

Genetic variation and phenotypic plasticity in Ulmus laevis

Results from Paper II are summarised in Figure 4. Families and populations possessed large genetic variation for a number of the growth, bud set and leaf shape characters. However, the effect of the water regimes was significant for only a few of the traits. The Öland population showed the lowest mean growth increment and leaf number and the earliest bud set. Genotype x treatment interaction was moderate for growth and bud set, and low for the leaf shape components. Family variances were higher than the treatment x family variances.

The effect of the water regimes on the expression of genetic variation

Similar amounts of genetic variation for growth and bud set were expressed within both water regimes: families varied for a majority of the traits irrespective of treatment, while population differences were slightly more common in the drought stress than in the well-watered treatment. The family variances were in general lower for growth and bud set in the drought than in the well-watered treatment.

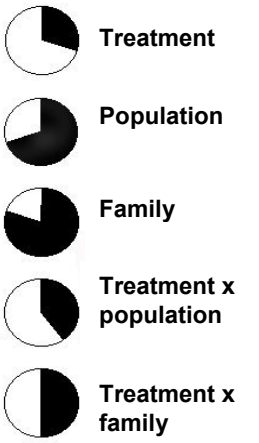

Figure 4. Sources of variation in a study of the effect of drought stress on adaptive and morphological traits in *Ulmus laevis*. Each pie corresponds to one source of variation and the shaded section represents the number of traits, out of ten traits assessed, for which a significant effect was observed. Eight families from each of three populations were used in the study; each family represented by 15 individuals in both a 'free access' water treatment and a drought stress treatment. Treatment effects were seen for only three traits: bud set stage and two leaf-shape components. Population and family effects were observed for seven and eight traits respectively. Treatment by population and treatment by family interactions were observed for several traits incuding height, biomass traits and leaf size.

Microsatellite and cpDNA variability – Papers III and IV

Microsatellite marker development

Six microsatellite markers were developed for use in *Ulmus laevis*, and were tested for cross-amplification in *U. americana*, *U. glabra*, *U. minor* and *U. pumila*. All but one of the primer pairs showed clear, polymorphic amplification in at least one of the four non-target species tested. A seventh locus was identified which did not amplify clearly in *U. laevis*, but showed potential utility in three other species. These are the first microsatellite markers developed in *Ulmus* species.

Microsatellite marker variation

Three of the microsatellite loci developed in Paper III showed consistent, polymorphic amplification patterns and an absence of null alleles (no null homozygotes observed) when used to genotype the larger data set analysed in Paper IV. Allele frequency distributions in the Hattula and Garonne populations are shown in Figure 5. Weir and Cockerham's (1984) θ, an estimator of F_{ST}, was 0.09 when calculated across all populations and ρ, the estimator of R_{ST}, was 0.126. Pairwise θ values were also calculated for all pairs of populations. All those involving the Hattula and Garonne populations were significant, while all other comparisons were non-significant. F_{IS} estimates (f) showed a slight deficit of heterozygotes in most populations when averaged across loci. The exceptions were the Lolland and Mulde populations for which a slight excess of heterozygotes was observed.

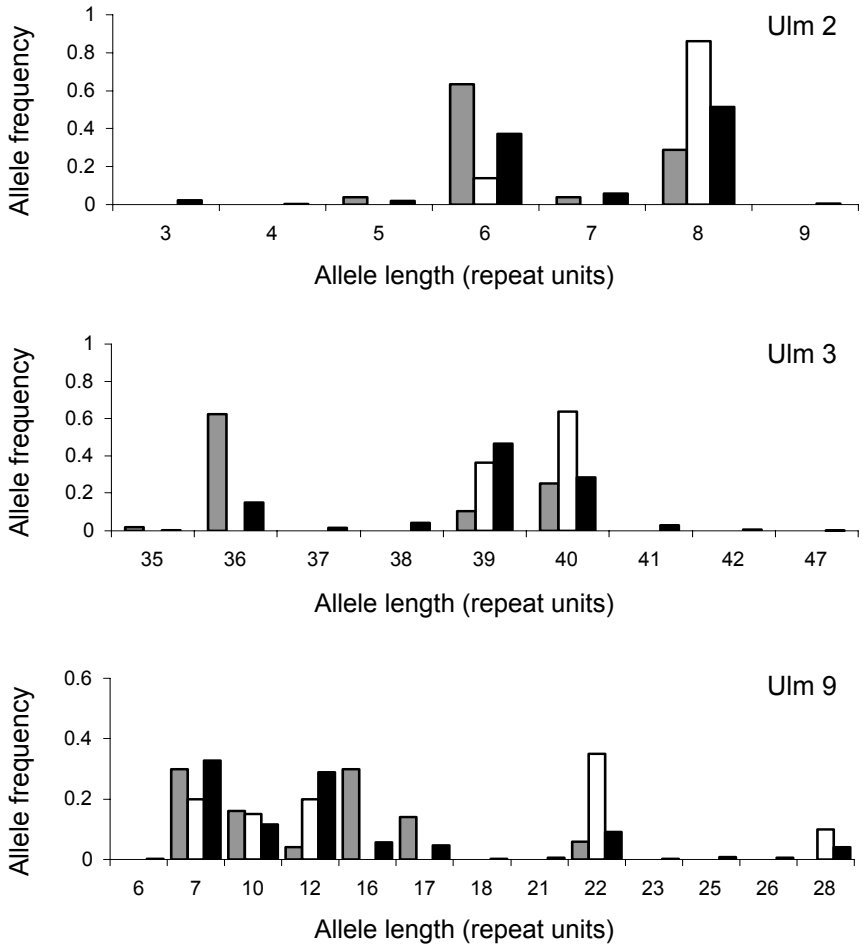

Figure 5. Allele frequencies for three microsatellite loci in *Ulmus laevis*. Black bars show overall allele frequencies across seven populations. Allele frequencies for two single populations that appear relatively genetically divergent (pairwise F_{ST} = 0.38) are also shown: the grey bars show data for the Hattula population and the white bars the Garonne population. The X axis of each graph shows allele length measured as the number of microsatellite repeat units. For example, allele six at locus Ulm2 (repeat unit = CAG) has the sequence: CAGCAGCAGCAGCAGCAG. Ulm2 showed the lowest polymorphism and Ulm9 the greatest, with a total of 7 and 13 alleles observed respectively. Clear differences in allele frequencies can be seen between the populations. For example, at locus Ulm3, allele 36 is more common in the Hattula population than in either the Garonne population or the study as a whole. Note that the scales for Ulm3 and Ulm9 are not completely continuous, owing to 'empty' size classes. For example, in the case of Ulm9, no alleles with a length of 11 repeat units were observed.

Table 3. *Relative amounts of within-population genetic variation in seven populations of Ulmus laevis. This table summarises the CV_A values for growth traits and 'P_{DEV}' values for other quantitative traits, as reported in Paper I, and the allelic richness (corrected for unequal sample sizes) presented in Paper IV. Within each trait, populations are assigned a low, medium or high level of within population genetic diversity based on the relative value of CV_A, P_{DEV} or R_S compared with the other populations. The Garonne population consistently showed a low level of variation compared with other populations. Öland and Elbe showed relatively high levels, and Moscow and Mulde were intermediate. The Lolland and Hattula populations were not included in the quantitative trait analysis.*

	Hattula	**Öland**	**Moscow**	**Lolland**	**Mulde**	**Elbe**	**Garonne**
Quantitative traits							
Growth traits	-	med	low	-	low	high	low
Bud flush	-	-	high	-	low	high	low
Bud set	-	med	low	-	med	high	low
Leaf fall	-	high	low	-	med	med	low
Molecular markers							
Allelic richness (R_S)	med	high	med	med	med	high	low

Chloroplast DNA PCR-RFLP marker variation

Three cpDNA haplotypes were observed. Total haplotype diversity (h_T) was therefore low (0.155). Haplotype A was extremely common and widespread, occurring across the entire study area from Russia and Finland to southern France, while haplotypes B and C were uncommon and geographically localised. Haplotype A and B occurred together in the Garonne population while all other populations were fixed for a single haplotype, resulting in a very low value for within-population diversity (h_S = 0.091). Haplotype C occurred only in two populations in the southeast of the study area.

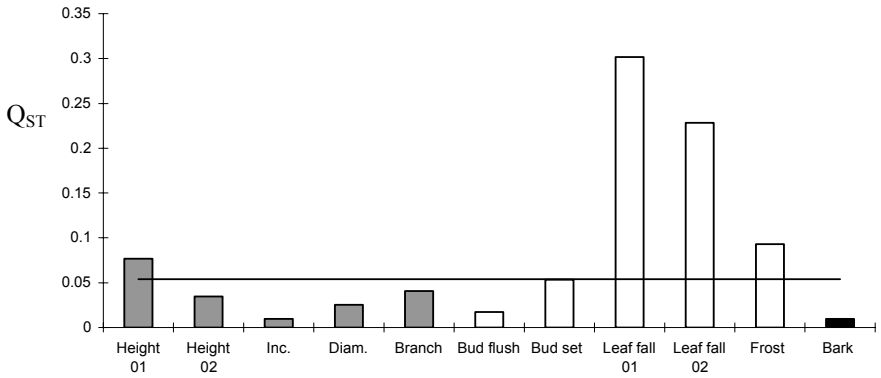

Figure 6. Q_{ST} values for quantitative traits for the five *Ulmus laevis* populations studied in Paper I. The horizontal line shows the value of F_{ST} calculated for the same five populations based on microsatellite data (Paper IV). Shaded bars refer to growth-related traits, white bars refer to phenology traits. Leaf fall showed the highest levels of population differentiation. Inc. = growth increment from age two to age three, Diam = stem diameter at age three, Branch = number of major stems produced during the third growth period, Frost = frost damage sustained during autumn at age two, Bark = a bark morphology trait registered at age three.

Discussion

Methodological issues

The design of the studies in this thesis required a compromise between the extent of the geographic area that could be covered, and the quality of the information obtained. This is especially true in the case of the quantitative studies, which require large numbers of individuals to be sampled in each study population. The geographic area studied in this thesis is, however, relatively large for a survey of genetic variation in natural plant populations, with 3000km separating the two most distant populations. This is probably an advantage in the studies of chloroplast DNA and quantitative trait variation, as large-scale patterns and processes can be revealed, such as the length of the growing season along a latitudinal cline, and long-distance post-glacial migrations. On the other hand, problems are associated with the use of microsatellite markers at large spatial scales as their high mutation rate and the occurrence of stepwise mutations can lead to underestimation of the differentiation between more distantly related populations (Slatkin 1995). The large spatial scale should also be taken into account when comparing the global estimates of differentiation reported here (e.g. F_{ST} and Q_{ST}) with those published in other studies.

Although the study area is large it still represents only the north-west region of the species distribution range. Since the largest and most continuous populations are located further south and east, and are not covered by this thesis, important reservoirs of genetic diversity have almost certainly been missed. Another limitation associated with the sampling scheme is that the geographic areas covered by each of the source populations varied from one population to another. This was to a great extent an unavoidable consequence of the fragmented nature of many *U. laevis* populations. Most of the groups of trees designated 'populations' in this study show some geographical fragmentation (e.g. interruption by agricultural land), and the extent of this fragmentation is likely to influence the degree to which genetic variation within the population is structured.

The location of the field trial in paper I was at a higher latitude than all the source populations. This is likely to have affected the results, particularly for phenological traits, and particularly for trees from the most southerly populations. The field site was also arguably drier than typical *U. laevis* habitat. Ideally, the field trial would have been replicated at other sites within the species distribution area, allowing measurement of genotype by environment interaction. The trees studied in papers I and II were very young, assessments being made in the first, second or third growth period. Predictions for the expression of genetic variation in fully grown trees must therefore be made with caution. However, the early years of growth are likely to be very important with regard to natural selection, and in many cases, the expression of adaptive traits during this period will be highly relevant to individual survival.

Genetic variation in *Ulmus laevis*

Differentiation between populations

Populations were found to be differentiated for quantitative traits in both Papers I and II. Differentiation in terms of nuclear microsatellite loci was also moderately high. In papers I and II, as expected, population differentiation for height and bud set broadly followed the latitude of origin of the populations: northern populations in both studies set bud earlier than southern populations and generally did not grow as tall. The three populations common to Papers I and II follow the same pattern in both studies: the Öland trees set bud first, followed by the Elbe and then the Garonne trees. A marked differentiation between populations of the timing of growth cessation has been reported in several species and has been interpreted as an adaptation for avoidance of autumn frost damage mediated primarily by a response to photoperiod (Anderson *et al.* 2001). Growth cessation and height are expected to be negatively genetically correlated. The correlation between these traits was found to be negative and highly significant in Paper I.

An interesting observation in both Papers I and II, is that the Garonne trees grew less tall than the Elbe trees, in spite of the longer growing season afforded by later bud set in the French population. In Paper I this difference in height is significant at the 0.1% level. In Paper II, significant population effects were observed for leaf morphology; the Garonne population had small leaves compared with the other two populations. Evidence for a selective advantage of smaller leaf size in wetter compared to drier habitats has been found in *Cakile edentula* (Brassicaceae) (Dudley 1996). The southerly location of the Garonne population suggests that small leaf size may represent an adaptation for increased water-use efficiency.

The value of 0.090 for θ (ρ = 0.126) among these populations of *U. laevis* is higher than has been reported for some other anemophilous temperate tree species, for example, F_{ST} values of 0.059 in *Fagus sylvatica* (Comps *et al*. 2001) and 0.032 in *Betula pendula* (Rusanen *et al*. 2003). However, both the latter estimates are based on allozyme data and comparisons of data from different markers should be made with caution. Measurements of F_{ST} may underestimate population differentiation when calculated using microsatellite data, in part because the estimation of F_{ST} assumes a negligible mutation rate, which is unlikely to be the case at microsatellite loci (Scribner *et al.* 1994; Freville *et al.* 2001). However, since the markers described here are not 'hypervariable' as is the case with many microsatellite loci (the highest number of alleles observed per locus was 13), the above F_{ST} estimate may be less affected by such bias. The differentiation measure R_{ST} was developed specifically for microsatellite markers, and assumes a higher mutation rate and a stepwise mutation model (SMM), in which new alleles arise from existing ones by the addition or loss of a repeat unit. However, R_{ST} is unlikely to provide reliable estimates of population differentiation for loci that do not follow a fairly strict SMM. As can be seen in Figure 4, the distribution of allele frequencies shows a number of missing size classes. This might indicate that an SMM cannot necessarily be assumed for these loci.

Measurements of F_{ST} using microsatellites at large spatial scales in European broadleaved trees are scarce in the literature. Recent studies of mahogany

(*Swietenia macrophylla*) in Central and South America are among the most similar to that described in Paper IV, in terms of the number of populations studied, the spatial scale (2-3000 km between the most distant populations) and the use of microsatellite markers. For example, Lemes *et al*. (2003) reported values of θ = 0.09 and ρ = 0.147 for seven populations of mahogany in the Brazilian Amazon. These estimates are very similar to those found for *U. laevis* in Paper IV, in spite of the species' very different life-history characteristics and ecology.

Within-population genetic diversity

The amount of within-population genetic diversity varied among populations (Table 3) but in general, the levels of CV_A and h^2 across most traits and populations were within the range of those that have been reported for other broadleaved tree species (e.g. Cornelius 1994; Baliuckas 2002). The summary of within-population variation given in Table 3 shows a general concordance between the within-population diversity measured in quantitative traits and the allelic richness in terms of microsatellites. The exception is Moscow which shows relatively low quantitative diversity, but moderate levels of allelic richness, compared with the other populations. Although the number of populations assessed is low and the trend is not universal, this result might provide some support for the assertion made by Petit *et al.* (1998), that allelic richness is a suitable molecular measure of within-population diversity for conservation purposes. The Garonne trees showed considerably lower levels of within-population diversity than the other three populations over a range of traits, and this was accompanied by a low allelic richness at the three microsatellite loci. There was not, however, a strong deficit of heterozygotes (Paper IV, Table 2), which suggests that inbreeding is not a significant problem among the adult population. The relatively low CV_A values for the Moscow population are unexpected, since this population lies close to the centre of the distribution range. In the case of the French population, a low level of genetic diversity might be expected, since the population is extremely geographically marginal, highly isolated from other populations and very small.

The roles of random drift and natural selection

As mentioned above, it is clear that the study populations are genetically differentiated from one another. It is apparent from the estimates of pairwise F_{ST} reported in Paper IV that the Garonne and Hattula populations contribute strongly to the overall population differentiation in terms of molecular markers. Low values for allelic richness were also found for both these populations. This may indicate that these populations have been subject to either severe founder effects or a more prolonged period of genetic drift. Vakkari *et al*. (In prep) found low genetic diversity within Finnish populations of *U. laevis*, and a very high degree of differentiation between them, which might suggest that the trees originally recolonising Finland were more genetically diverse than the Hattula population, and that drift has occurred subsequently.

Comparison of F_{ST} and Q_{ST} can provide insight into the relative importance of natural selection and random genetic drift in population differentiation. In theory, the value of Q_{ST} for a selectively neutral trait should be the same as that of F_{ST}. That is, if Q_{ST} is significantly different from F_{ST}, the null hypothesis of the trait arising by genetic drift alone can be rejected (Spitze 1993; McKay & Latta 2002). For the five populations common to Papers I and IV, Q_{ST} was greater than F_{ST} for leaf fall in both years (Figure 6), suggesting that natural selection has favoured different phenotypes in different populations. Although other traits show values of Q_{ST} which deviate from that of F_{ST} it is unlikely that many of these differences are significant. Merilä and Crnokrak (2001) reviewed results from a number of taxa for which estimates of both F_{ST} and Q_{ST} were available, and found that F_{ST} only rarely exceeded Q_{ST}, suggesting that natural selection is a widespread cause of differentiation between populations. From the results represented here it appears likely that natural selection and drift have both played a role in the differentiation of the *U. laevis* populations studied.

Chloroplast DNA diversity and postglacial history

The cpDNA data presented in Paper IV show a very low level of haplotype diversity in the study populations, with a total of only three haplotypes observed. Such low levels of differentiation are relatively unusual in temperate tree species that have recolonised Europe since the last glaciation (Petit *et al*. 2001), but are not unheard of. *Carpinus betulus* (Grivet & Petit 2002), *Corylus avellana* (Palmé & Vendramin 2002) and *Fagus sylvatica* (Demesure *et al*. 1996) also show low cpDNA diversity and the dominance of a single haplotype across a large part of their present distributions. The extremely low level of cpDNA diversity was not anticipated and while this may reflect a lack of genetic diversity in the chloroplast genome of *U. laevis*, it may also reflect the relative insensitivity of the cpDNA PCR-RFLP technique to some sequence information. Chloroplast microsatellites or DNA sequencing might reveal more diversity than PCR-RFLP, and this in turn might affect the conclusions that can be drawn regarding refugia and migration routes.

One difficulty in the study of *Ulmus* post-glacial history is the lack of a species-specific pollen record as fossil pollen from *U. laevis* cannot be easily distinguished from that of the other European elms (Huntley & Birks 1983). Given the current distributions of the three European elm species, Huntley & Birks (1983) suggest that *U. laevis* is the most likely of the three to have had a refugium in Russia. The absence of samples from the eastern region of the species range is a particular limitation in terms of the cpDNA analysis. If, as suggested by some palynological evidence, *U. laevis* had an important refugium in Russia, a much more detailed sampling of this area would have be extremely valuable. However, our results appear compatible with the hypothesis of an eastern location for *U. laevis* from which most of Europe, excluding the Balkan region, was recolonised.

Conservation implications and suggestions for future research

In-situ conservation is generally considered the ideal method for conserving wild populations (Graudal *et al.* 1995) and it would seem natural for *U. laevis* populations to be conserved as part of riparian ecosystems, perhaps in conjunction with the conservation of species such as *Alnus glutinosa* (Eriksson 2001). Ex-situ collections can be used as a complement to in-situ conservation, allowing genotypes to be sampled in populations where in-situ management would be more difficult (Collin 2003). Both in-situ and ex-situ conservation require careful consideration of which populations should be prioritised. Eriksson (1998) states that when genetic drift is assumed to have taken place, comparatively more populations must be sampled in order to capture the existing genetic variation. Our results suggest that both natural selection and genetic drift have played a role in bringing about the present population genetic structure. It is clear that the populations included in Paper I are substantially differentiated in terms of adaptive traits. Furthermore, the results obtained for the Moscow trees suggest that central populations should not be assumed to maintain higher levels of within-population variation than marginal populations. In order for a conservation programme to encompass the existing range of adaptedness in *U. laevis* populations, it would be necessary to sample a number of populations.

The CV_A values for growth traits found in Papers I and II are similar to those found in other broadleaved trees for most of the populations studied, indicating that these populations have at least as good a possibility for responding to long term evolutionary change as have other species. In these populations, the threat of habitat destruction is likely to be of more immediate concern. However, the results strongly suggest that diversity in the Garonne population has been eroded by genetic drift. This should be taken into account in future conservation efforts in order to minimise further loss of genetic variation which may threaten the long term survival of this population. The microsatellite data for the Garonne population did not suggest a high level of inbreeding in the adult population. However, inbreeding may become a concern in future, given the population's generally low genetic variation and small size.

If patterns of genetic variation in *U. laevis* are to be understood at a species-wide scale, more information from populations in the eastern half of the distribution is required. In particular, two questions could be addressed: i) is the Moscow population representative of other central populations in terms of adaptive trait variation? ii) is greater cpDNA diversity observed in southern Russia, or in other putative refugia?

On Öland, it may be most urgent to obtain information on demographic processes and the success of natural regeneration. Mittlandsskogen is unlikely to be under immediate threat from deforestation, and the amount of forest on the island may actually be increasing. However forest grazing is currently increasing and this may compromise natural regeneration (Karlsson *et al.* 2001). Data on flowering phenology and on gene-flow via pollen in this population could shed light on the extent of population subdivision within Mittlandsskogen. Patterns of

pollen-mediated gene-flow on Öland are presently being investigated using the markers described in Paper III.

While growth rhythm traits are clearly important for survival for temperate trees, some authors have argued for a greater range of traits to be considered in studies of adaptive variation (e.g. Geber & Griffin 2003). These include physiological characters such as secondary metabolite production which influences the ability of a plant to repel attack from herbivores, or biochemical traits associated with photosynthesis. The latter may be of adaptive significance if current climate change trends continue. It is possible that the rate of climate change in coming decades will be more rapid than plausible plant migration rates (Davis & Shaw 2001), increasing the importance of individual response to the environment. As such, studies of phenotypic plasticity similar to that described in Paper II would be useful in assessing the capacity of populations to survive future change.

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Acknowledgements

To my supervisors, for introducing me to the world of elms. Gösta for sharing your great knowledge of quantitative genetics, David for sharing your scientific philosophy, Gunnar for generously sharing your statistical expertise, and especially to Sanna, for lots of things, including great supervision, patience and organisational skills – I hope I have not put you off being a PhD supervisor for ever!

To Hartmut, without whom I would still be stapling together the ground-cover stuff at Pustnäs.

To Eric for your seemingly endless enthusiam for all things elm-related.

To Yvonne Tillman and Ingrid Schenning for running sequencing and GeneScan gels, and kindly returning my ice-box whenever I forgot.

To the staff of the phytotron, for your help and good company in my phytotron days.

To Justin and Åsa, for top-quality help and fun in the greenhouse and the lab.

To Martin Lascoux and others at the Conservation Biology Department at Uppsala University for interesting and inspiring discussions, and the Plant Ecology Department at Stockholm University for organising some great courses that taught me most of the ecology I know.

To Xavi Domingo, for first showing me what a microsatellite looks like.

And most of all,

To all my friends in Sweden and elsewhere. To Chris for checking my English, and especially to Shoo, for letters and inspiration.

To Paul, and to Mum, Dad, Jane and Jude XXXXX

THANK YOU! :o)