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WITHIN AND BETWEEN POPULATION VARIATION IN ADAPTIVE TRAITS IN *ULMUS LAEVIS*, THE EUROPEAN WHITE ELM

Rachel E. Whiteley[1*], **Sanna Black-Samuelsson**[1] **& Gunnar Jansson**[2]

[1] Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Box 7080,
S-750 07 Uppsala, Sweden.
[2] Skogforsk, Uppsala Science Park, S-751 83 Uppsala, Sweden.
* Corresponding author: Rachel E. Whiteley, Tel: +46 (0)18 673158, Fax: +46 (0)18 673279,
Email: rachel.whiteley@vbsg.slu.se

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ABSTRACT

We investigated within and between population variation for growth and phenological traits in juvenile *Ulmus laevis* Pall. (European white elm). In a field trial near Uppsala, Sweden, we assessed quantitative genetic variation and estimated genetic parameters in 19–23 open-pollinated families from each of five populations from across the western and central region of the species range. Traits studied included height, stem diameter and timing of bud flushing, bud setting and leaf fall. Significant pairwise population differences were found for all traits, and most frequently separated northern populations (Sweden and Russia) from more southerly populations (France and Germany). Heritabilities (h^2) ranged from zero to 0.7, and additive genetic coefficients of variation (CV_A) from zero to 64.6 %. For most traits in most populations, values for both h^2 and CV_A were within the range of previously reported estimates in other broad-leaved trees. Populations differed in terms of their levels of within-population genetic variability, the greatest variability being recorded for the Swedish and the two German populations, and lowest variability in the case of the French population. Height and frost damage were found to be positively correlated with late bud flushing, and significant between-year correlations were recorded for height and leaf fall.

Keywords: conservation, field trial, genetic variation, growth, quantitative genetics, *Ulmus laevis*.

INTRODUCTION

The presence of additive genetic variation for adaptive traits is important if populations are to adapt to a changing environment. Given current predictions for future climate change, it has become an important goal of conservation programs to ensure that adequate additive genetic variation is present in populations to be conserved (ERIKSSON 2001; SOULÉ & MILLS 1998). Studies of existing adaptive genetic variability can provide valuable information for prioritising populations for conservation (LYNCH 1996; STORFER 1996). Most studies of adaptive traits in temperate tree species, both conifers and broad-leaves, report substantial genetic variation within and among the populations studied. However, detailed studies have focused primarily on species and traits of commercial interest.

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. The species tolerates wet soils and periodic flooding, and typically occurs in damp low-lying areas and as a component of riparian forests; and populations tend to be scattered and relatively small. This is particularly true in western Europe, where deforestation and drainage of flood plains for agriculture and industry have left little suitable habitat. Further land-use changes, particularly in some countries in eastern Europe, continue to pose a threat to many riparian forests (COLLIN *et al.* 2000). In Sweden, *U. laevis* occurs only on the Baltic island of Öland and is categorised as vulnerable on the Swedish red data list (GÄRDENFORS 2000). The most significant threat facing the species is probably habitat destruction. Although *U. laevis* is susceptible to Dutch elm disease (DED), the disease is not thought to pose an immediate threat to the species, at least in the western part of its range. Feeding studies have shown that the *Scolytus* beetle species acting as vectors for the fungal pathogen appear to find *U. laevis* unattractive as a food plant, strongly preferring *U. minor* (SACCHETTI *et al.* 1990; WEBBER 2000).

Ulmus laevis is wind pollinated and the seeds are primarily dispersed by wind, although some dispersal of seeds by water is likely to occur where the trees grow along lake-shores or waterways. It does not appear capable of hybridization with other European elm species, and is self-incompatible (MITTEMPERGHER & LA PORTA 1991). A limited amount of information on genetic variability within the species is available. MACHON *et al.* (1997) reported relatively high isozyme variability in *U. laevis* in France. MATTILA & VAKKARI (1997) found high levels of population differentiation using allozymes in Finnish populations (F_{ST} = 0.33). GEHLE & KRABEL (2002) found some allozyme variation both within and between study populations on the Elbe river, Germany. In terms of quantitative trait variation, very little information is available. BLACK-SAMUELSSON *et al.* (In press) studied the effect of drought stress on growth traits and leaf morphology in three of the same populations of *U. laevis* as studied here, and found significant variation between families and populations.

In this study we have focused on growth and growth-rhythm traits which are likely to have adaptive significance. Bud flush and bud set timing, for example, are likely to be important in avoiding frost damage and making the best use of the available growing season. The objectives of this study were: (i) to assess within and between population genetic variation in such adaptive traits; (ii) to estimate genetic parameters for these traits in *Ulmus laevis*, and (iii) to assess the implications of our results for practical conservation efforts and for the long term persistence of the species.

MATERIALS AND METHODS

During May and June 2000, seeds were collected from 19–23 trees in each of five natural or semi-natural populations from across the central and western region of the species range. The exception was the French population, for which 15 families were sampled in 1999 and a further four families in 2000. The source populations were: Mittlandskogen forest on the Swedish island of Öland; a lowland mixed forest in the Moscow area; the river Elbe close to Torgau and the river Mulde near Eilenburg, both in Germany (these locations are approximately 33 km apart and treated as separate populations for the purposes of the analyses), and a small, possibly relict, population on the Garonne river in southern France. Seed-bearing trees were chosen at random within each population, with at least 20 m between trees in order to avoid clonally related trees, but generally with some of the trees a few tens of meters apart and other trees located at greater distances. Locations of the source populations are given in Table 1. Seeds from all populations were sown in a greenhouse in Uppsala in early July 2000. After six weeks, 30 seedlings per family were selected at random and transferred to pots. The young trees were planted out as single tree plots at Pustnäs experimental field site (59°48′ N; 17°39′ E) four km south of Uppsala, during September 2000, in six fully randomised complete blocks. Each block contained four trees per family, a total of 24 trees per family in the study as a whole. The field site was a west-facing slope with well-drained soil. Watering was carried out as necessary during summer months.

Assessments

Details of the traits assessed, including the timing of assessments and number of classes used for recording class traits are given in Table 2. Measurements of height growth, timing of bud flush, bud set and leaf senescence were made during the growing seasons of 2001 (age two) and 2002 (age three). Height was measured after cessation of growth at ages two and three, and the growth increment during 2002 calculated. Loss of the apical bud in all trees during growth cessation in 2000 (age one) led to a bushy growth habit and all height measurements refer to the height of the longest stem when extended vertically. Stem diameter, at five to ten centimeters above ground level, was measured once, after growth cessation at age three. Branch number was counted during the same period, and was recorded as the number of major stems formed during the third growth period (i.e. during 2002). Autumn frost damage occurring at age two was registered early the following spring. A distinctive flaking or

Table 1. Locations of five *Ulmus laevis* population included in a field trial, and number of open pollinated families sampled from each population. x = data not available.

No.	Location	Latitude (N)	Longitude (E)	Altitude (m)	No. families
1	Mittlandsskogen, Öland, Sweden	56°41′	16°35′	30	19
2	Moscow, Russia	55°45′	37°35′	x	23
3	Mulde valley, Germany	51°32′	12°37′	95	20
4	Elbe valley, Germany	51°30′	13°05′	85	20
5	Garonne valley, France	43°36′	1°26′	110	19

Table 2. Traits assessed in a field trial of *Ulmus laevis*. Times of assessment, measurement units (metric traits) or number of classes (class traits), and any data transformations used. Increment = difference between height age two and height age three; Stem diameter = diameter measured at 5–10 cm from ground level; NS = normal score; LR = logistic regression model.

Trait	Time assessed	Trait type	Assessments made	Units / Description of classes	Transformation
Height	Age 2 (End 2001)	Metric	1	cm	None
	Age 3 (End 2002)	Metric	1	cm	None
Increment	–	Metric	1	cm	None
Stem diameter	Age 3 (End 2002)	Metric	1	mm	None
Branch no.	Age 3 (Late 2002)	Count	1	–	None
Bud flushing	Age 2 (Early 2001)	Class	5	Closed bud (0) to fully opened leaves (6)	NS and LR
Bud set	Age 2 (Late 2001)	Class	5	Still growing (0) to mature bud (3)	NS and LR
Leaf fall	Age 2 (Late 2001)	Class	1	None (0) to all (10) of leaves fallen or coloured	NS
	Age 3 (Late 2002)	Class	1	None (0) to all (5) of leaves fallen or coloured	NS
Frost damage	Age 2 (End 2001)	Class	1	No damage (0) to very severe damage (5)	NS
Bark texture	Age 2 (Late 2001)	Class	1	No blistering (0) to most of bark blistered (4)	NS

blistering of the bark was observed in some trees and the presence and extent of this morphological characteristic in each tree was registered during early 2002. Height and diameter were treated as metric traits, and these data were analysed without prior transformation. All other traits were treated as class variables, with four to six classes, apart from leaf senescence at age two, in which eleven classes (zero to ten) were used. Branch number was a counted trait and was analysed in the same way as the metric traits.

Statistical analysis

Differences between least squares means were calculated to identify pairs of populations which differ significantly, using the LSMEANS function within the PROC MIXED procedure of SAS (SAS Institute Inc. 2001).

For the heritability calculations, height, growth increment and stem were analysed without transformation. Normal score transformation (GIANOLA & NORTON 1981) was used to achieve homogenous variances and normal distribution of residuals for all other traits. Family variance components were calculated using the REML option in the PROC MIXED procedure in SAS (SAS Institute Inc. 2001). The models used for all traits were:

$$y_{ijkl} = \mu + p_i + b_j + f_{k(i)} + e_{ijkl} \qquad [1]$$

for analyses across all populations, and:

$$y_{jkl} = \mu + b_j + f_k + e_{jkl} \qquad [2]$$

for analyses of each population separately.

Where y is the value of an observation, μ is the grand mean, p_i is the population effect (fixed) for the ith population ($i = 1,....5$); b_j is the block effect (fixed) for the jth block ($j = 1,....6$); f_k is the family effect (random) of the kth family ($k = 1,...19–23$, depending on population); $f_{k(i)}$ is the family effect (random) of the kth family within the ith population and e is the error term. Population-by-block effects were not significant and were not included in the model. Heritibilities were calculated from these variance components, treating all progeny within a family as half-sibs, as follows:

$$h^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_e^2} \qquad [3]$$

Where σ_f^2 is the family variance component and σ_e^2 is the error variance component.

To calculate Q_{ST}, population variance components were calculated for each trait using a model similar to model [1] but treating population as a random instead of a fixed effect. Q_{ST} was then calculated as described by SPITZE (1993). Coefficents of additive genetic variation (CV_A) were calculated for metric traits (height, growth increment and stem diameter) for each population using the following formula:

$$CV_A = 100 \times \frac{\sqrt{4\sigma_f^2}}{\bar{x}} \qquad [4]$$

Where σ_f^2 = family variance component, obtained using equation [2], and $\bar{x}$ = the population mean. However, when the phenotypic mean for a trait is very low, CV_As may be significantly overestimated and when classes are used to assess traits, CV_A will vary inversely with the number of classes used. For this reason we have presented CV_A only for metric and count traits. For other characters we present one additive genetic standard deviation change (P_{dev}) from the population mean, presented as a percentage, assuming a mean value for each trait of 50 %. The reference probability (P_{ref}) is the incidence probability for an average entry, and can be arbitrarily chosen between 0 and 100 %. As typical values for comparison of variability for different traits we used P_{ref}= 50 %. A family's normal score value deviating one genetic standard deviation (σ_A, equivalent to $\sqrt{4\sigma_f^2}$) from the mean was converted into percent (probability) breeding value (P) centered around P_{ref} using

$$P = 100\Phi(\Phi^{-1}(P_{ref}) - \sigma_A)\% \qquad [5]$$

where F is the standard normal distribution function (cf. ERICSSON & DANELL 1995). The deviation (P_{dev}) of P from P_{ref} was calculated as: $P_{dev} = P - P_{ref}$.

During bud flush and bud set at age two, five assessments of each trait were made, recording the progress of the trait over time. A logistic linear regression model was used to produce estimates of the proportion of trees within populations and families reaching a chosen stage over the observation period. Calculations were made using the SAS macro Glimmix (SAS Institute Inc. 2001). The models used were:

$$\ln(q_{ijk}/(1-q_{ijk})) = \mu + tc_{k(i)} + p_i + b_j + f_{k(i)} \qquad [6]$$

for the among-population analyses, and:

$$\ln(q_{jk}/(1-q_{jk})) = \mu + tc_k + b_j + f_k \qquad [7]$$

for analyses in each population separately.

Where t is time, q is proportion of trees having reached a given stage and c is the regression coefficient for populations (c_k) or families within populations ($c_{k(i)}$). Other terms are defined as in equation [1].

Additive genetic correlations between different traits, and between assessments of the same trait in different years (height and leaf fall only), were estimated using ASREML (GILMOUR *et al.* 2001). Estimates were made across all populations, using a two-trait model:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{p}_i + \mathbf{Z}_i\mathbf{u}_i + \mathbf{e}_i \qquad [8]$$

i refers to the first and second traits, respectively, $\mathbf{y}_i$ is the vector of individual tree observations for trait i, $\mathbf{p}_i$ is the vector of the fixed block and population effects, $\mathbf{u}_i$ is the vector of the random family effects, $\mathbf{e}_i$ is the vector of random residuals, $\mathbf{X}_i$ is the incidence matrix connecting the observations with the fixed effects, and $\mathbf{Z}_i$ is the incidence matrix connecting the observations with the family effects.

For two traits this can be summarised as: $\mathbf{y}' = (\mathbf{y}_1', \mathbf{y}_2')$, $\mathbf{p}' = (\mathbf{p}_1', \mathbf{p}_2')$, $\mathbf{u}' = (\mathbf{u}_1', \mathbf{u}_2')$, $\mathbf{e}' = (\mathbf{e}_1', \mathbf{e}_2')$, $\mathbf{X} = \mathbf{X}_1 \oplus \mathbf{X}_2$, $\mathbf{Z} = \mathbf{Z}_1 \oplus \mathbf{Z}_2$, where $\oplus$ denotes the direct sum.

The random effects are assumed to have a multivariate normal distribution (MND). Means and variances of these effects are:

$$\begin{bmatrix}\mathbf{y}\\ \mathbf{u}\\ \mathbf{e}\end{bmatrix} \sim \mathrm{MND}\left(\begin{bmatrix}\mathbf{Xb}\\ 0\\ 0\end{bmatrix}, \begin{bmatrix}\mathbf{V} & \mathbf{ZG} & \mathbf{R}\\ \mathbf{GZ} & \mathbf{G} & 0\\ \mathbf{R} & 0 & \mathbf{R}\end{bmatrix}\right)$$

where $\mathbf{V} = \mathbf{ZGZ}' + \mathbf{R}$, $\mathbf{R}$ is the residual variance-covariance matrix and $\mathbf{G}$ is the parent variance-covariance matrix. The variances and covariances were estimated from the same data using the average information algorithm (GILMOUR *et al.* 1995).

RESULTS

Genetic variability between populations

Population mean values for each trait are shown in Table 3, and family mean values for height, leaf fall (age two) and frost damage are shown in Figure 1. Pairwise differences between populations, based on the differences between least squares means for each population, are summarised in Table 4. Significant differences between populations were found for all traits studied. Most pairwise differences separated the two most northerly populations (Öland and Moscow) from the three populations originating further south, although differences among the southern or northern populations were apparent for several traits. At age two, the Elbe population was significantly taller than all the others, including the Mulde population, although the difference between the two German populations was not significant at age three. Trees from France and Germany set bud later than trees from Russia and Sweden and were more affected by frost damage. Despite setting bud latest of all, the Garonne trees were significantly less tall than the Elbe population at age two and both the Elbe and Mulde populations at age three.

Figure 2 shows the progress of bud flush and bud set based on data collected over the course of five registrations. The population means at a single assessment for bud flush and bud set are shown in Table 3. Patterns of bud flush and bud set broadly followed the latitude of origin of the populations, with high latitude

Table 3. (a) Population means, additive genetic coefficients of variation (CV_A) and heritabilities (h^2) of five metric traits, and (b) population means, genetic variation expressed as one standard deviation change when the population mean is 50% (P_{dev}), and heritabilities of six class traits, for open pollinated families from five populations of *Ulmus laevis* assessed in a field trial. See equations 5 and 6 in the text for P_{dev} calculation. All population means shown are in the original units, whereas P_{dev} for class traits are calculated assuming a mean value of 50% (see equation five in the text for details). Stem diameter was measured at 5-10 cm from the ground and stem diameter, bark texture and branch number were assessed at age three. Unless otherwise specified, all other assessments were made at age two. The right hand column shows Q_{ST} for each trait.

a	Sweden - Öland			Russia - Moscow			Germany - Mulde			Germany - Elbe			France - Garonne			Q_{ST}
	Mean	CV_A (%)	h^2	Mean	CV_A (%)	h^2	Mean	CV_A (%)	h^2	Mean	CV_A (%)	h^2	Mean	CV_A (%)	h^2	
Height age 2 (cm)	113	12.1	0.26	109	2.9	0.06	126	8.7	0.20	132	10.1	0.33	122	8.6	0.25	0.077
Height age 3 (cm)	256	14.5	0.65	244	2.7	0.03	269	7.5	0.22	273	8.0	0.25	255	0.0	0.00	0.035
Increment (cm)	143	35.8	0.67	135	9.4	0.05	143	19.9	0.21	140	23.2	0.26	133	0.0	0.00	0.010
Stem diameter (mm)	34.9	12.9	0.42	34.3	0.0	0.0	37.2	9.4	0.27	38.1	0.0	0.0	35.6	0.0	0.00	0.025
Branch number	9.5	28.9	0.11	10.5	31.6	0.16	11.9	64.6	0.67	12.9	38.7	0.30	11.3	40.9	0.27	0.041

b	Sweden - Öland			Russia - Moscow			Germany - Mulde			Germany - Elbe			France - Garonne			Q_{ST}
	Mean	*P dev*	h^2	Mean	*P dev*	h^2	Mean	*P dev*	h^2	Mean	*P dev*	h^2	Mean	*P dev*	h^2	
Bud flushing	3.02	19	0.32	2.89	19	0.29	2.81	13	0.18	2.74	20	0.43	2.71	14	0.22	0.017
Bud set	2.15	13	0.21	2.25	0	0.00	1.28	17	0.28	1.3	22	0.44	0.96	0	0.00	0.053
Leaf fall age 2	6.16	18	0.40	7.94	19	0.48	3.50	15	0.26	3.55	24	0.65	2.34	17	0.34	0.302
Leaf fall age 3	3.80	21	0.44	4.51	14	0.15	2.87	16	0.34	2.86	22	0.60	2.60	11	0.19	0.229
Frost damage	0.24	0	0.00	0.27	4	0.02	1.25	20	0.29	1.20	24	0.41	1.25	20	0.29	0.093
Bark texture	0.99	24	0.57	1.21	28	0.69	0.98	29	0.70	1.09	26	0.58	1.50	26	0.49	0.009

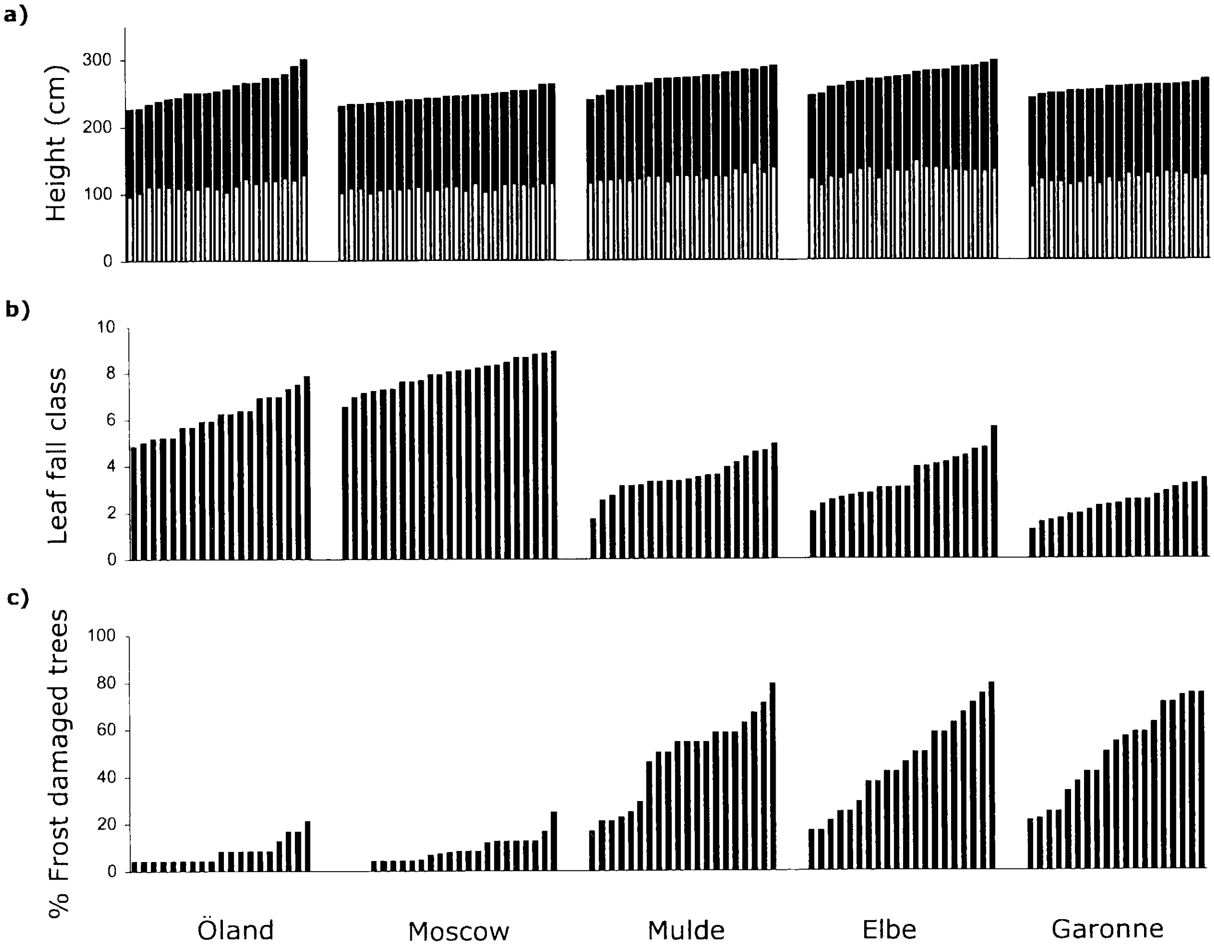

Figure 1. Family mean values for **a**) height at age 2 (white bars) and height age three (black bars), **b**) leaf fall age 2, **c**) autumn frost damage age 2, for five populations of *Ulmus laevis* grown in a field trial.

Table 4. Number of significant comparisons made within and between groups of populations of *Ulmus laevis* grown in a field trial. The Moscow and Öland populations are considered together as 'Northern' populations, Elbe and Mulde as 'Central' populations, and Garonne is considered alone as 'Southern'. Five growth traits: height age two, height age three, growth increment, stem diameter (age three) and branch number (age three), and five phenological traits: bud flush, bud set, leaf fall age two, leaf fall age three and frost damage, are considered. For example, there are 20 possible pairwise comparisons between northern and central populations (four in total) for the five growth traits, of which 17 differences are significant. Among the two northern populations only five comparisons are possible, one for each of the five traits, of which three were significant.

	Growth traits			Phenological traits		
	Northern (Moscow and Öland)	**Central** (Elbe and Mulde)	**Southern** (France)	**Northern** (Moscow and Öland)	**Central** (Elbe and Mulde)	**Southern** (France)
Northern	3/5	17/20	6/10	1/5	19/20	10/10
Central	–	2/5	8/10	–	0/5	4/10

populations flushing and setting bud first. The Moscow population, on average, flushed slightly earlier than the Öland population, but this difference was not significant.

Strong population differentiation is seen for autumn frost damage, which is significantly more severe in the

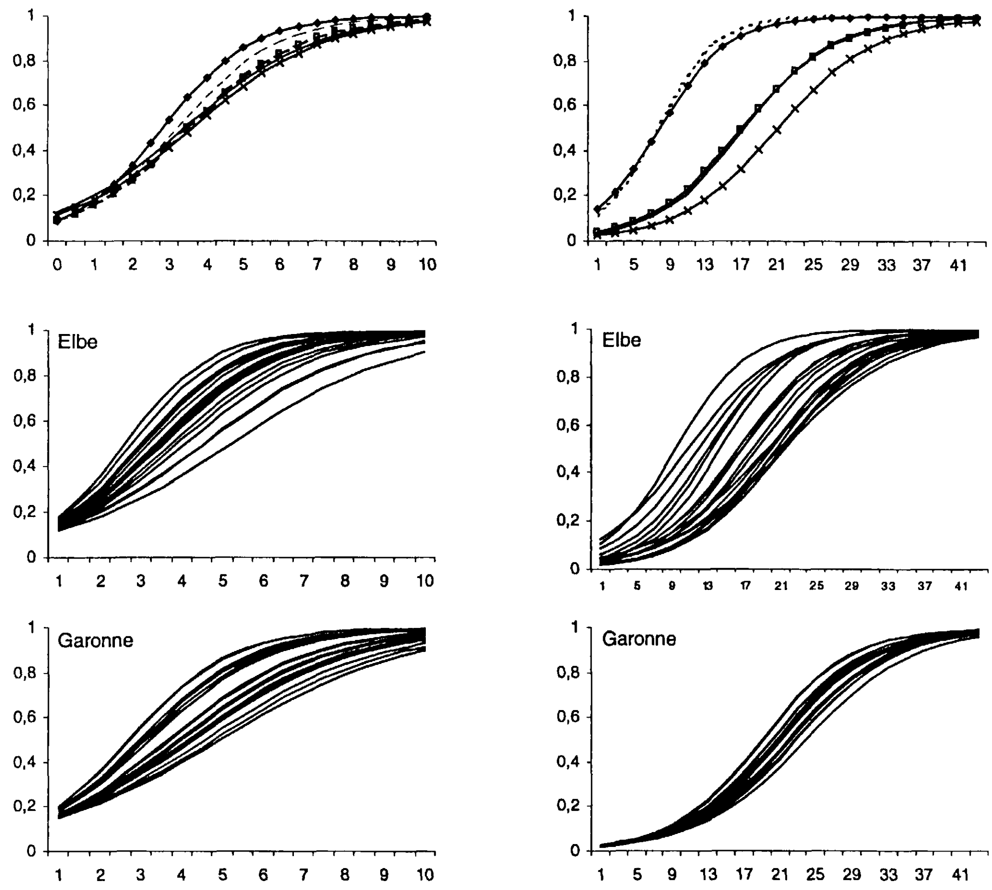

Figure 2. The progress of **a)** bud flushing and **b)** bud setting in five populations of *Ulmus laevis* grown in a field trial. Top: population means (closed squares = Öland, dotted line = Moscow, open squares = Elbe, unbroken line = Mulde, crosses = Garonne); centre: family means for Elbe population; bottom: family means for Garonne population. The graphs show the proportion of trees in a family or population reaching a given stage of bud flush or bud set over time, estimated from data collected at five assessments for each trait using a logistic regression model (see Materials and Methods for details). Of the five populations studied, the Elbe and Garonne populations showed the highest and lowest genetic variability respectively.

Garonne, Elbe and Mulde populations than for Moscow and Öland. Population differentiation for leaf fall was also very strong, and followed a similar pattern to that of bud set, including earlier leaf fall in the Moscow than the Öland population, (significant at the 0.1% level). The French population showed significantly greater scores for the bark texture trait than all other populations except Russia. The final column in Table 3 shows Q_{ST} for each trait. Q_{ST} varied considerably among traits and was highest for the two registrations of leaf fall.

Genetic variability within populations

Table 3 shows CV_A values for metric and count traits, and the distance of one standard deviation (P_{dev}) from an assumed mean of 50 % for class traits. The Öland and Elbe populations showed the highest levels of within population variability in four traits each. The Garonne population showed generally low within-population genetic variability, having the lowest CV_A, or lowest deviation from 50 %, of all the populations for six of the traits assessed. CV_A for height decreased from age two to age three in all populations except for Öland, in which there was a slight increase. In spite of the generally low variability found in the Garonne population, genetic variation for frost damage was high in the Garonne and both German populations, but extremely low for the two more northerly populations. The Moscow population showed relatively low levels of within-population variability for most traits, the exceptions being for bud flushing and for leaf fall at age two. Relatively large additive genetic 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.

Mean heritabilities for metric traits in this study were similar to those reported by CORNELIUS (1994). Patterns of heritability in most cases followed those of CV_A.

Genetic correlations

Genetic correlations are shown in Table 5. 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 shorter 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. Frost damage sustained in autumn 2001 does not appear to have affected height gain in the following year, since there is no correlation between frost damage and either growth increment or height at age three. Leaf fall was also negatively correlated with frost damage, but not as strongly as bud set. No significant correlations were found between bud flushing or bark texture and any other trait measured.

DISCUSSION

The trees in this study were very young, assessments being made in only the second or third growth period. Predictions for the expression of genetic variation in fully grown trees must therefore be made with caution. The northern location of the field trial, at a higher latitude than all the source populations, is likely to have affected the results, particularly for phenological traits, and for the trees from the most southerly populations. The field site was also drier than typical *U. laevis* habitat, but this may not have strongly influenced the results. BLACK-SAMUELSSON *et al.* (In press) found little effect of drought stress on the expression of genetic varibility in three of the same populations as studied here.

Between population variation

Significant pairwise differencess between populations were seen for all traits. Most differences involved phenological traits or frost damage, and tended to separate the Öland and Moscow populations from the Garonne, Elbe, and Mulde populations. Differences among southern or among northern populations did occur, and primarily involved metric traits.

Estimates of Q_{ST} provide a measure of quantitative genetic differentiation analogous to Wright's F_{ST} (1951), a measure of population subdivision commonly used with molecular markers. Values for Q_{ST} were highest by far for the two registrations of leaf fall, which might suggest that divergent selection has contributed to among-population differences for this trait. We are currently investigating genetic structure in the same populations using microsatellite markers, and comparison of these two data sets will allow us to make inferences about the contribution of natural selection to population differentiation.

The greater frost damage in the Garonne, Elbe and Mulde trees is likely to be a result of their failure to set bud before the earliest severe autumn frosts. Bud set is an aspect of growth cessation, which is thought to be an

Table 5. Between-trait and between-year additive genetic correlations calculated across five populations of open-pollinated *Ulmus laevis* families grown in a field trial. Abbreviations: Inc = Growth increment; Diam = Stem diameter; Leaf = Leaf fall. Bud flush and bud set refer to data collected at age two (2001). For height and leaf fall, '2' indicates data collected at age two, '3' data collected at age three.

	Height 3	Inc	Diam	Frost	Flush	Bud set	Leaf 2	Leaf 3	Branch
Height 2	0.76***	0.36*	0.61***	0.31*	-0.26	-0.56***	-0.15	-0.19	0.32*
Height 3	-	0.86***	0.43**	0.06	-0.11	-0.43**	-0.16	-0.03	-0.14
Increment		-	0.20	-0.14	-0.01	-0.20	-0.12	0.10	-0.27
Diameter			-	0.20	-0.03	-0.44*	0.00	-0.30	0.27
Frost				-	-0.26	-0.71***	-0.34**	-0.37**	-0.03
Bud flush					-	0.21	-0.07	-0.06	-0.05
Bud set						-	0.34**	0.43**	-0.14
Leaf 2							-	0.80***	-0.15
Leaf 3								-	-0.23

important adaptive trait and recent studies in poplar have shown it to be under strong genetic control (FREWEN *et al.* 2000). Timing of growth cessation appears to be strongly linked to increasing night length (ERIKSSON & EKBERG 2001; ANDERSON *et al.* 2001 and refs. therein), and also influenced by temperature (CHEN *et al.* 2002). At higher latitudes the response is likely to be stronger in order to avoid frost damage, so growth cessation begins earlier in these populations. This response also shortens the time available for growth and total height is expected to be greatest in populations from lower latitudes. A general separation of southern and northern populations, in terms of budset, height and frost damage, is seen in our results.

Despite setting bud later, the Garonne trees did not grow as tall as either of the German populations. The shorter stature of the French trees might be an indication of inbreeding, or may simply be a consequence of ecological conditions at the source location placing less selection pressure on height growth. Relatively shorter stature, and later budset, in the Garonne compared to the Elbe population, was also recorded in our study of drought stress response, carried out under phytotron conditions, using a subset of the same populations (BLACK-SAMUELSSON *et al.* In press). Given the shorter growth of the Garonne trees, it would be premature to attribute the height difference between the Moscow and Öland populations to the difference in timing of bud set.

The Elbe and Mulde populations were significantly different from one another with respect to height at age two, branch number, and budset at age three (data not shown). The source populations are separated by approximately two minutes latitude, and lie at very similar altitudes. The involvement of budset timing as well as height may point to an adaptive explantion such as differing temperature microclimates experienced by the source populations.

Timing of bud flushing is thought to be controlled primarily by temperature. It is advantageous for northern populations to respond quickly to rising temperatures in the spring so as to make the best use of the available growing time. Hence, northern populations are expected to begin bud flushing first. This is the trend observed in the present study, but few pairwise population differences are significant. Weather conditions during spring 2001 at the field site may have played a role in reducing phenotypic differences between the populations.

Within population genetic variation

HOULE (1992) recommended that CV_A be used wherever possible in reporting the results of studies of adaptive variation, since CV_A is a better guide than heritability to the long-term evolvability of a trait. In estimating genetic parameters, we have assumed all progeny within a family to be half-sibs. This may have lead to overestimation of these parameters, since most of the populations in this study are relatively small and some progeny may be full sibs. The CV_A values for metric traits are similar to the averages found for eucalyptus and other broad-leaves in studies reviewed by CORNELIUS (1994). However, it is possible that some of these studies underestimated genetic variances, since they involved populations which had undergone selection for the traits assessed. Our estimates of CV_A for metric traits are rather low compared with those from some recent studies of European broad-leaves. BALIUCKAS *et al.* (2000) reported CV_A values for height ranging from 7.9 % to 28.3 % in *Prunus avium*, and up to 41.8 % in *Fraxinus excelsior* at age three, although in later registrations (at age five or six), CV_A was generally lower. Our estimates of h^2 and CV_A for height may have been kept rather low by the trees' bushy growth habit. During the first winter after planting, almost all trees in the study lost their apical bud and growth the following spring continued from axillary buds...

BALIUCKAS (2002) found high family variance components for bud flushing compared to other traits in a number of broad-leaved species; for example, in Swedish populations of *Quercus robur* and *Fagus sylvatica*, estimates of family variance components and CV_A were highest for bud flushing, compared with height, leaf senescence, and frost damage. However, we found that bud flushing in *U. laevis* showed among the lowest variabilities of all the traits studied. Low variability for this trait was seen both within and between populations, and may be a consequence of the late spring at the field study site, compared to the source locations.

The Elbe, Mulde and Öland populations showed, on average, higher within population variability than the Garonne and Moscow populations. The high genetic variability for the very marginal Swedish population is perhaps more surprising than for the German populations, which are more central to the distribution area. One explanation for the high variability on Öland may be non-random mating within the population. The area on Öland over which *U. laevis* mother trees were sampled is considerably larger than for the other populations in the study. Many *U. laevis* on Öland exist in small stands separated by agricultural land, with up to one kilometer between them. In this situaton it might be expected that some population sub-structuring exists, which in turn could lead to inflated values for within-population variability.

High values for both h^2 and genetic variation were seen for the bark texture trait. We have no evidence that this trait is of adaptive significance. High heritabilities for traits of low adaptive sigificance are predicted but such traits are also expected to have lower long-term evolvabilities than those of adaptive importance (MOUSSEAU & ROFF 1987; HOULE 1992).

Genetic correlations

The trees in this study were very young and the data should thus be interpreted with caution. Early events influencing the traits we have assessed may lose their importance later; for example, any effect of the loss of the apical bud in the first winter may decline with time, as dominance is re-established in other leader shoots. However, other studies have reported strong between-year correlations for many adaptive traits in a variety of species (e.g. BALIUCKAS 2002; EKBERG *et al.* 1994), and the between-year correlations for height and leaf fall suggest that our data show trends which may continue in the later life of the trees.

Implications for conservation

In fragmented habitats, opportunities for migration are limited and the conservation of evolutionary potential as a basis for response to environmental change is likely to become especially important (LANDE & SHANNON 1996). Lack of suitable *U. laevis* habitat may mean that the possibility of migration to areas of suitable climate is limited for most populations in western Europe. Our results suggest that there is variation among populations for important adaptive characters. Within-population variation is also present, but its extent differs between populations. The Elbe and Mulde populations both possess considerable genetic variability which could form a strong basis for adaptation in the future. Within-population variability is also fairly high in the Öland population. However, because of the highly fragmented nature of *U. laevis* habitat on Öland, this result should be interpreted with caution. In the context of a study of local-scale population structure and pollen-mediated gene-flow on Öland we are currently using newly developed microsatellite markers (WHITELEY *et al.* 2003) to investigate the possibility that the Öland population is genetically substructured, which could lead to inflated values for within-population variability. Further research using molecular markers would also be valuable in testing hypotheses of inbreeding or genetic drift in the very isolated *U. laevis* populations in southern France. Finally, molecular studies might also shed light on the reason for the rather low variation in the Russian trees, which was not expected given the size of the source population and its central location within the distribution area. This may be especially important if DED is a greater threat in the eastern part of the species' range.

The European Forest Genetics Resources Program (EUFORGEN) has as its goal, the creation of good conditions for the future adaptation of forest species in a changing environment. It has been suggested that, for *U. laevis*, this could be achieved through *in situ* conservation in conjunction with other riparian tree species such as *Alnus glutinosa* L. Gaertn. and *Populus nigra* L., as well as the formation of *ex-situ* collections, in the form of clonal hedges (ERIKSSON 2001).

Work to conserve *U. laevis* both *in-* and *ex-situ* is underway in a number of countries within the species' range (COLLIN *et al.* unpublished). Along with complementary molecular genetic studies, the results of this study provide new information about *U. laevis* population genetics that may inform future conservation decisions.

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