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Genetic Variation Among and Within Populations of Four Swedish Hardwood Species Assessed in a Nursery Trial

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Abstract

Four broadleaved tree species, *Acer platanoides, Alnus glutinosa, Fagus sylvatica,* and *Fraxinus excelsior,* which vary with respect to pollen vectors or succession stage, were studied in a nursery trial in Uppsala, latitude 59°50', 12 m asl, at ages 2 to 5. Growth rhythm, growth capacity and damage were assessed in 3 to 7 autochthonous Swedish populations. Generally the family variance components were estimated with higher precision than the population components. There was a considerable variation in bud flushing both at the population and within-population level except for *Fagus sylvatica* with no variation at the population level. The family variance components for bud flushing were on average larger for *Acer platanoides* than for the other species. For budset in *Acer platanoides* (age 2 to 3) and *Fagus sylvatica* (age 3) the family variance components were mostly low. For all species the population variance components for plant height were significant. Except for *Alnus glutinosa* there is a trend that the family variance components for height decrease with age. On average the highest family components were obtained for *Fraxinus excelsior.* Mostly there was limited variation in damage among populations and families. The family mean correlations of the same trait studied different years were significant and positive except for budset in *Acer platanoides.* Correlations between pairs of traits and with meteorological variables were in many cases significant but the correlations never explained more than 50% of the variation. The comparatively large family variance components in *Fraxinus excelsior* and *Acer platanoides* were attributed to non-random mating in their populations.

Key words: Acer platanoides, Alnus glutinosa, Fagus sylvatica, Fraxinus excelsior, populations, families, growth rhythm, growth capacity, genetic variation.

FDC: 165.5; 181.525; 232.1; 176.1 *Acer platanoides;* 176.1 *Alnus glutinosa;* 176.1 *Fagus sylvatica;* 176.1 *Fraxinus excelsior;* (485).

Introduction

Broadleaved tree species from the genera *Acer, Alnus, Fagus, Fraxinus, Quercus, Tilia,* and *Ulmus* play a minor role in Swedish forestry. One reason for this is that these species have their northern limit of distribution in southern Sweden south of latitude 60° and in consequence they constitute approximately 1% of the total forest area in Sweden. Some of the species may play a greater role in the future owing to customer resistance to tropical timber for furniture. There is also a desire to utilize domestic seed sources in landscaping (LAGERSTRÖM and ERIKSSON, 1997). Thus there are incentives for

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studies of broadleaved tree species for identification of good seed sources. Another incentive is that Sweden like many other countries has signed the Forestry Convention which means that a gene conservation programme has to be developed also for broadleaved species.

For gene conservation it is important to identify differentiation within a species before sampling. Therefore, adaptation to different site conditions play a major role. At northern latitudes adaptation to climatic conditions probably leads to population differentiation (ERIKSSON, 1997). Since the first report on photoperiodic biotypes by SYLVÈN (1940), a vast literature on this topic has been published for conifers (e.g. CLAPHAM et al., 1998). Under Swedish conditions it is evident that inwintering, building up of frost tolerance and dormancy, is regulated by the night length, e.g. the *Picea abies* studies by DORMLING et al. (1968). Northwards transfers of *Pinus sylvestris* lead to dramatic plant mortality as seen from the classical paper by EICHE (1966). It is highly likely that other tree species growing under Swedish conditions also respond to the night length for their inwintering. This was also proven by HÅBJØRG (1978) for many tree species growing in Norway. Northern populations respond to a shorter night length than southern sources. The time for bud flushing is a frost-sensitive period in *Picea abies* (DORMLING, 1982). Similarly we expect that species belonging to the genera listed above are frost-sensitive during their bud flushing. When the chilling requirement for breaking of dormancy is met the triggering factor for onset of development during the spring is the temperature (for a recent treatment see MENZEL, 1997), with a lower heat demand in northern than southern sources.

Appropriate growth rhythm, i.e. the timing of growth initiation during spring and growth cessation during late summer and early autumn, is probably of high adaptive value (ERIKSSON, 1995) and natural selection has caused populations to differentiate considerably at high latitudes (> 60°, ERIKSSON, 1997). There is probably a trade off as regards the growth rhythm. A too late bud flushing to avoid late spring frosts combined with a too early growth cessation to avoid early autumn frosts will result in a short growth period and trees that have these characteristics may not grow tall enough to compete with trees with somewhat longer growth periods and will in consequence not contribute to the next generation. Studies of genetic variation in growth rhythm is a prime objective in identification of the best seed sources for different site conditions as well as for sampling for gene conservation.

Owing to their minor economic importance little research on broadleaved tree species was carried out either in Sweden or in other countries before the eighties (JONSSON and ERIKSSON, 1989). In recent years several European reports on the above mentioned genera have been published, especially for beech and oak. Reference will be given to the species included in our investigation.

A clinal variation with respect to bud flushing in *Fagus sylvatica* populations from Romania in east to France in west was reported by VON WUEHLICH et al. (1995a). An elevational cline for Polish population of *Fagus sylvatica* from different elevations was reported by SULKOWSKA (1995). There are several recent reports on population differences in height growth in *Fagus sylvatica* (e.g. KLEINSCHMIT and SVOLBA, 1995; MADSEN, 1995; VON WUEHLICH et al., 1995b) as well as in *Fraxinus excelsior* (SMINTINA, 1993; GIERTYCH, 1995; WEISER, 1995; KLEINSCHMIT et al., 1996).

In *Acer platanoides* WESTERGAARD (1997) reported a clinal variation for growth cessation in the latitudinal range 55° to 60° in Scandinavia. This confirms results referred to above by HÅBJØRG (1978). Based on an isozyme study, RUSANEN et al. (1996) reported an F_{ST}-value, 0.126 for Finnish populations of *Acer platanoides,* which is large compared to corresponding data for conifers from Scandinavia (e.g. GULLBERG et al., 1985).

LEVINS (1963) formulated a theory on the expected optimum population structure with respect to adaptive traits depending on the heterogeneity in space and time. According to his theory, heterogeneity in space would favour monomorphic, specialized, and genetically different populations. Heterogeneity in time as well as space would lead to polymorphy within populations and a clinal variation. This may hold true for random mating populations with strongly limited gene flow among populations. ERIKSSON (1997) presented a graph on the expected ratio of within-/among-population genetic variation for random-mating populations with varying strength of natural selection and among-population gene flow. It is evident that a strong gene flow under random mating will lead to a large within-population variation. With decreased degree of gene flow a larger among-population differentiation would be possible. Based on this discussion it is evident that ecological traits such as distribution, and pollen and seed vectors, as well as stage in the ecosystem, may influence the within-/among-population genetic variation. Allozyme studies reviewed by HAMRICK et al. (1992) support this statement. In non-random mating populations in which genetic drift plays a major role this ratio will become larger than expected in random-mating populations.

To test the above-mentioned hypothesis that ecological traits may influence the within-/among-population variation differently, four tree species having different combinations of the above-mentioned ecological traits were selected for a preliminary study. *Alnus glutinosa* is a pioneer species with wind pollination. *Acer platanoides* and *Fraxinus excelsior* are secondary species with scattered distributions, mostly in mixed stands. The former is mainly insect pollinated while the latter is wind pollinated and dioecious. *Fagus sylvatica* is a climax species with large stands, at least in the southernmost part of Sweden. *Alnus glutinosa* is expected to have the lowest within-population variation while the opposite is expected for *Fagus sylvatica,* the two other species taking intermediate positions. The purpose of this investigation was to get information on the importance of the above-mentioned ecological traits on among- and within-population genetic variation of growth and growth rhythm traits during the juvenile phase.

Materials and Methods

Single-tree progenies from native populations of the four broadleaved tree species *Acer platanoides, Alnus glutinosa, Fagus sylvatica* and *Fraxinus excelsior* were included in the study. The geographic data of the populations and the number of open-pollinated families from each population are given in *table 1.*

For each species, a common-garden test was established in a nursery south of Uppsala (latitude 59°50' elevation 12 m asl) in summer and autumn 1987. The nursery is located on a slight western slope of a river valley at a less frost-prone site. The seedlings were raised in individual pots in spring 1987. The seed of ash, beech and maple were stratified before sowing. The experimental design was a randomized complete block design in 6 or 8 replicates with single-tree plots, spaced 0.65 m x 0.65 m.

Data were recorded during one or more years up to age 5 on the following traits.

Bud flushing was assessed using scores of 6- or 7-classes, with near equal spacing, from class 0 up to class 5 or 6.

Budset: apical bud visible.

Table 1. – For the four species included in the investigation, the number of families studied per population and the geographical characteristics of the population sites are gives.

No.	Population	Number of families	Latitude	Longitude	Altitude m a.s.e.
		Alnus glutinosa			
1	Flytjärn	15	61°36'	17°05'	35-45
2	Andersby	12	60°09'	17°50'	10-20
3	Bergaön	13	59°12'	16°00'	25-30
4	Hyltinge	15	59°05'	16°50'	25-40
5	Vingåker	15	59°03'	15°55'	30-40
6	Trosa	15	58°53'	17°33'	5-10
7	Stenshuvud	17	55°40'	14°16'	35-40
		Fraxinus exelsior			
1	Hamnäs	10	61°13'	16°45'	45-55
2	Broby	10	58°54'	16°29'	20-25
3	Sjöbo	10	55°39'	13°46'	90-100
		Fagus sylvatica			
1	Fiholm	7	59°26'	16°45'	15-20
6	Söderåsen	15	56°04'	13°04'	165-175
7	Stenshuvud	15	55°40'	14°16'	30-85
		Acer platanoides			
5	Gorsingeholm	19	59°21'	17°03'	5-15
6	Bergaön	10	59°12'	16°00'	25-30
7	Hyltinge	15	59°05'	16°50'	25-40
8	Stenshuvud	16	55°40'	14°16'	35-65

Frost damage on *Fraxinus excelsior* assessed in 1990 at age 4: different degrees of apical shoot damage, causing one or two lateral shoots succeeding the apical shoot.

Mildew infection on *Acer platanoides* assessed at age 2 and 3: no infection, light infection, heavy infection.

Crown form in *Acer platanoides* assessed at age 5: strong apical dominance, normal lateral shoots; strong apical dominance, no or weak lateral shoots; apical dominance disappearing, normal lateral shoots.

Final leader length and length at a day close to the growth cessation.

Final height growth.

Meteorological variables included in the correlation calculations were: temperature sum, length of the growing season, start of the growing season (when threshold temperature = 5 °C), global radiation sum. The values were obtained using the geographical data and equations suggested for Sweden (MORÉN and PERTTU, 1994).

Statistical calculations

In addition to ANOVA, variance components and family-mean correlations were estimated. Two types of calculations were made: on individual level and on family mean at block level, the latter being used for traits with discrete values.

If necessary, data were transformed mainly by square root but in some cases logarithmic and arcsine transformations were made to get homoscedasticity for the residuals.

The analysis of variance model used to study the among- and within-population variation of the traits mentioned above was:

$$y_{ijkm} = \mu_o + b_i + p_j + f_{k(j)} + e_{ijkm} \qquad [1.0]$$

where

$$\mu_o \qquad [1.1]$$

[1.1] = overall mean

$$b_i \qquad [1.2]$$

$$N(0, \sigma_b^2) \qquad [1.3]$$

[1.2] ~ [1.3] effect of block, i = 1, ..., n_b (n_b = 6 for *Acer platanoides* and *Fagus sylvatica*, n_b = 8 for *Alnus glutinosa* and *Fraxinus excelsior*)

$$p_j \qquad [1.4]$$

$$N(0, \sigma_p^2) \qquad [1.5]$$

[1.4] ~ [1.5] = effect of population, j = 1, ..., n_p (for n_p, see *Table 1*)

$$f_{k(j)} \qquad [1.6]$$

$$N(0, \sigma_f^2) \qquad [1.7]$$

[1.6] ~ [1.7] = effect of family k within population j, k = 1, ..., $n_{f(j)}$ (for $n_{f(j)}$, see *Table 1*)

$$e_{ijkm} \qquad [1.8]$$

$$N(0, \sigma_e^2) \qquad [1.9]$$

[1.8] ~ [1.9] = the residual error, m = 1, 2, 3, 4.

Since the number of populations is limited, the sample distribution of

$$\hat{\sigma}_p^2 \qquad [2.0]$$

is very skewed. Hence, the standard errors can be misleading. To find confidence intervals for the relative value

$$\theta_p \qquad [2.1]$$

of

$$\sigma_p^2 \qquad [2.2],$$

we used the following transformation:

$$\ln \frac{\hat{\theta}_p + \frac{1}{n_p}\hat{\theta}_f}{1 - \hat{\theta}_p} \qquad [2.3]$$

where

$$\hat{\theta}_p = \hat{\sigma}_p^2 / (\sigma_b^2 + \hat{\sigma}_p^2 + \hat{\sigma}_f^2 + \sigma_e^2) \qquad [2.4]$$

and

$$\hat{\theta}_f = \hat{\sigma}_f^2 / (\sigma_b^2 + \hat{\sigma}_p^2 + \hat{\sigma}_f^2 + \sigma_e^2) \quad [2.5]\,.$$

The transformation for

$$\hat{\theta}_f \quad [3.0]$$

was:

$$\ln \frac{\hat{\theta}_f}{\hat{\theta}_f + n_p \hat{\theta}_p} \quad [3.1]$$

or

$$\ln \frac{\hat{\theta}_f}{1 - \hat{\theta}_f} \quad [3.2]$$

depending on whether

$$\hat{\theta}_p > 0 \quad [3.3]$$

or

$$\hat{\theta}_p = 0 \quad [3.4]\,.$$

These transformations are aimed at stabilizing the variance and improving the approximation to the normal distribution. The third transformation is in fact the logit transformation. Using the standard errors of

$$\hat{\theta}_p \quad [3.5]$$

and

$$\hat{\theta}_f \quad [3.6]\,,$$

the corresponding was found for the transformations by series expansions. Confidence intervals were found by first calculating them in the transformed scale and thereafter using retransformation. The intervals reflect significances, if any, obtained by F-tests in the ANOVA. However the transformations are not perfect as the final intervals may include zero although the tests yield significant results.

All effects studied were considered as random. The numerical computations were done using SAS (SAS Institute, 1988). The analyses of variance were done with PROC GLM, Type III SS. Estimation of variance components was made using PROC VARCOMP, the REML option. The resulting components are presented as percentages of their sums. All correlations calculated were based on family mean values only, since the number

Table 2. – The percentage variance components and 95% confidence intervals (italics below) for bud flushing at population and family levels at various ages. *, **, and *** significant at 5%, 1%, and 0.1% levels, respectively.

Species	Age 3		Age 4		Age 5	
	Population	Family	Population	Family	Population	Family
Acer platanoides	14.4 *1.0 - 48.1* ***	12.8 *7.2 - 19.2* ***	19.1 *3.0 - 55.8* ***	8.4 *4.3 - 15.0* ***	20.5 *2.7 - 58.1* ***	12.6 *6.8 - 20.6* ***
Alnus glutinosa	11.4 *3.3 - 29.5* ***	8.2 *5.4 - 11.7* ***	12.4 *4.0 - 31.4* ***	3.6 *2.0 - 6.3* ***	–	–
Fagus sylvatica	0	9.0 *4.2 - 18.2* ***	0	8.9 *4.1 - 18.2* ***	–	–
Fraxinus excelsior	15.1 *0.4 - 56.2* ***	8.6 *4.3 - 14.6* ***	16.4 *0.7 - 58.3* ***	8.8 *4.4 - 15.2* ***	16.7 *1.3 - 58.7* ***	6.4 *3.0 - 12.2* ***

Table 3. – The percentage variance components and 95% confidence intervals (italics below) for percentage growth at a day close to growth cessation at population and family levels at various ages in three of the four species included in this investigation. *, **, and *** significant at 5%, 1%, and 0.1% levels, respectively.

Species	Age 3		Age 4		Age 5	
	Population	Family	Population	Family	Population	Family
Acer platanoides	5.0 *-0.1 - 23.0* **	6.3 *3.2 - 9.6* ***	13.3 *2.3 - 45.4* ***	3.1 *0.8 - 11.1* *	5.8 *0.5 - 25.4* ***	3.5 *1.5 - 7.0* ***
Alnus glutinosa	3.4 *0.8 - 11.0* ***	2.7 *1.4 - 4.6* ***	–	–	3.0 *0.5 - 10.2* ***	4.8 *2.9 - 6.8* ***
Fraxinus excelsior	5.6 *-0.2 - 33.4* **	3.6 *1.2 - 8.2* **	–	–	–	–

Table 4. – The percentage variance components and 95% confidence intervals (italics below) for plant height at population and family levels at various ages in four species included in this investigation. *, **, and *** significant at 5%, 1%, and 0.1% levels, respectively.

Species	Age 2		Age 3		Age 4		Age 5	
	Population	Family	Population	Family	Population	Family	Population	Family
Acer	8.6	7.6	9.2	5.4	8.9	5.3	5.5	3.0
platanoides	*0.6 - 33.2*	*4.0 - 12.0*	*1.0 - 35.1*	*2.6 - 9.8*	*1.0 - 34.4*	*2.5 - 9.7*	*0.6 - 24.2*	*1.2 - 6.6*
	***	***	***	***	***	***	***	**
Alnus	9.3	3.9	3.6	3.0	3.6	3.0	3.5	4.3
glutinosa	*2.8 - 25.1*	*2.3 - 6.4*	*0.9 - 11.4*	*1.6 - 5.1*	*0.9 - 11.4*	*1.6 - 5.1*	*0.7 - 11.2*	*2.5 - 6.4*
	***	***	***	***	***	***	***	***
Fagus	6.1	16.8	5.3	5.1	4.9	6.5		
sylvatica	*-3.2 - 36.2*	*9.6 - 18.6*	*-0.6 - 32.5*	*2.0 - 9.3*	*-1.0 - 30.6*	*2.9 – 10.1*	–	–
	*	***	*	**	*	***		
Fraxinus	6.1	8.1	6.7	3.9	11.7	4.6	12.4	2.4
excelsior	*-0.9 - 31.1*	*4.2 - 11.4*	*1.0 - 33.0*	*5.0 – 12.6*	*0.8 - 48.6*	*2.0 - 9.1*	*1.3 - 51.0*	*0.9 - 6.3*
	**	***	*	***	***	***	***	***

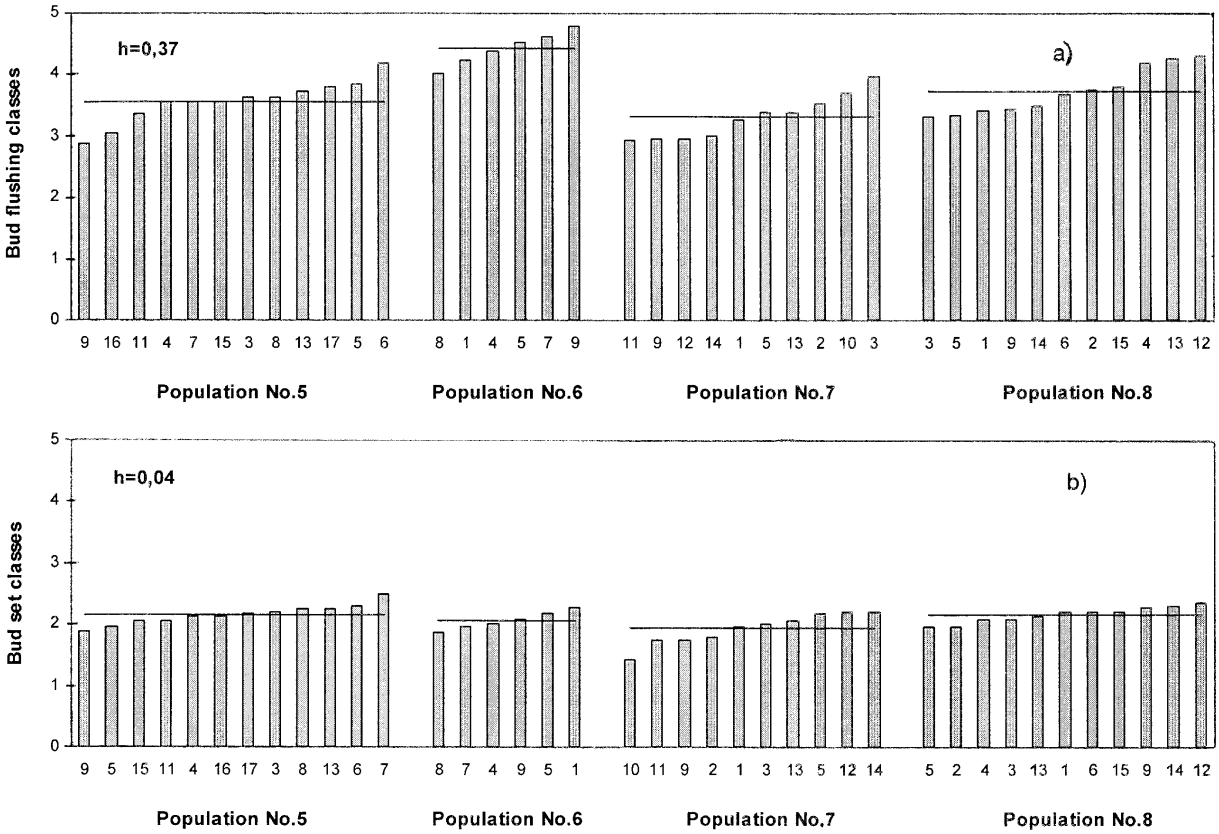

Figure 1. – Bud flushing (a) and bud set (b) mean values at age 5 for families of *Acer platanoides* populations.

of entries was too low to allow any precise estimates of genetic correlations.

Results

Figure 1 shows that there was considerable variation in *Acer platanoides* with respect to bud flushing whereas the variation was extremely low with respect to budset at the same age. The variance components of individual populations as well as the separation of the variance components among populations *(Figure 2)* revealed that there was considerable variation in bud flushing both at the population and within-population level. The precision in the estimates at the population level was low owing to the low number of populations included *(Table 2)*. The low precision of the population variance components estimates was shared with the other species. In spite of this, both the population and family effects were in most cases significantly different *(Tables 2* to *4)*. The family variance components for bud flushing were on average larger for *Acer platanoides* than for the other species *(Table 2)*. For *Acer platanoides* (age 2 to 3) and *Fagus sylvatica* (age 3) the family variance components for budset were low except for age 3 in *Acer platanoides,* where this component was estimated at 10.7. Also for the other approach to studying the variation in inwintering, percentage growth at a day close to growth cessation, only one variance component exceeded 10% at the population level, though estimated with low precision *(Table 3)*. Generally the family variance components were estimated with higher precision.

There was a considerable difference in plant mean height in *Fagus sylvatica* on the one hand and *Alnus glutinosa* and *Acer platanoides* on the other hand *(Fig. 3)*. For all species the population variance components for plant height were significant but estimated with low precision *(Table 4)*. Except for

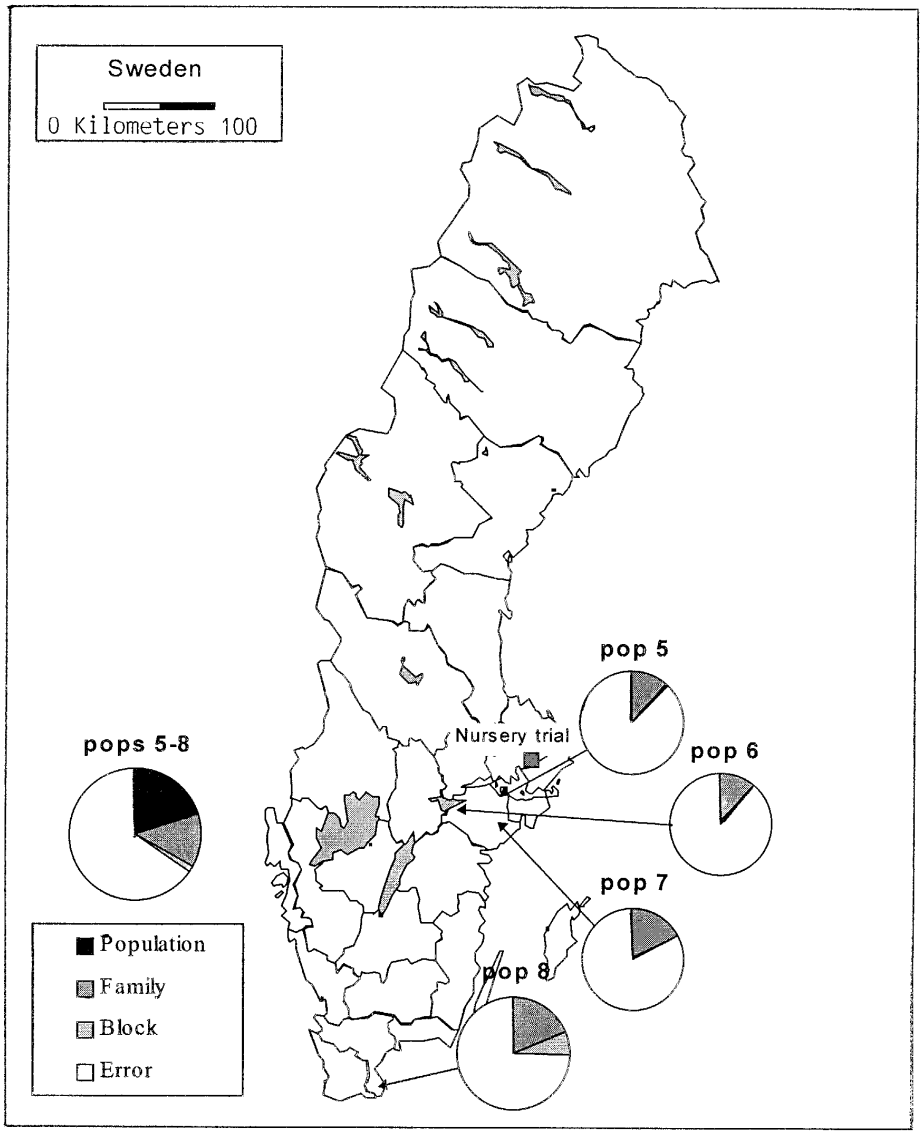

Figure 2. – Variance components for bud flushing at age 5 in *Acer platanoides.* The origins of the four populations are indicated.

Alnus glutinosa there is a trend for the family variance components to decrease with age. On average the highest family components were obtained for *Fraxinus excelsior.* The young shoots in all plants of this species were severely hit by a frost on May 10, 1990, at age 4, which probably affected the height growth. After the frost damage 1 to 3 new leaders developed later on.

Damage caused by mildew was recorded for *Acer platanoides* with a population variance component of approximately 6% at age 3. The family component amounted to 19.3% the same year. The crown form of *Acer platanoides* varied in a similar way with a family variance component of 20.6% at age 5. Details on damage and crown form in *Acer platanoides* were given by LAGERSTRÖM and ERIKSSON (1996). The shoot damage did not reveal much differentiation in any of the four species either at the population or at the family level.

The family mean correlations of the same trait studied different years were significant and positive except for budset in *Acer platanoides (Table 5)* with an estimated correlation of ± 0.24 only. In *Acer platanoides* there were several negative and significant relationships between late bud flushing and plant height. The strongest were obtained with bud flushing at age 3 as one variable explaining up to 40% of the variation.

For *Alnus glutinosa* there were many significant family mean correlations between meteorological variables and bud flushing as well as with plant height but most of them did not explain more than 15% of the variation.

In *Fagus sylvatica* there were several significant correlations between meteorological variables and plant height, which explained approximately 25% of the variation. Bud flushing did not show any significant correlations with other variables.

In *Fraxinus excelsior* there was a positive and significant relationship between early bud flushing and frost damage at age 4, explaining up to 50% of the variation. Tree height was significantly and negatively correlated with latitude and longitude meaning that trees from northern latitudes and eastern longitudes had the smallest trees *(Table 5).*

Discussion

Based on the data presented by HÅBJØRG (1978) our hypothesis was that the population differentiation would be large enough in southern Sweden to be able to disclose population differences with 3 to 4 populations per species. As seen from *tables 2* to *4* the population effect was in most cases significant.

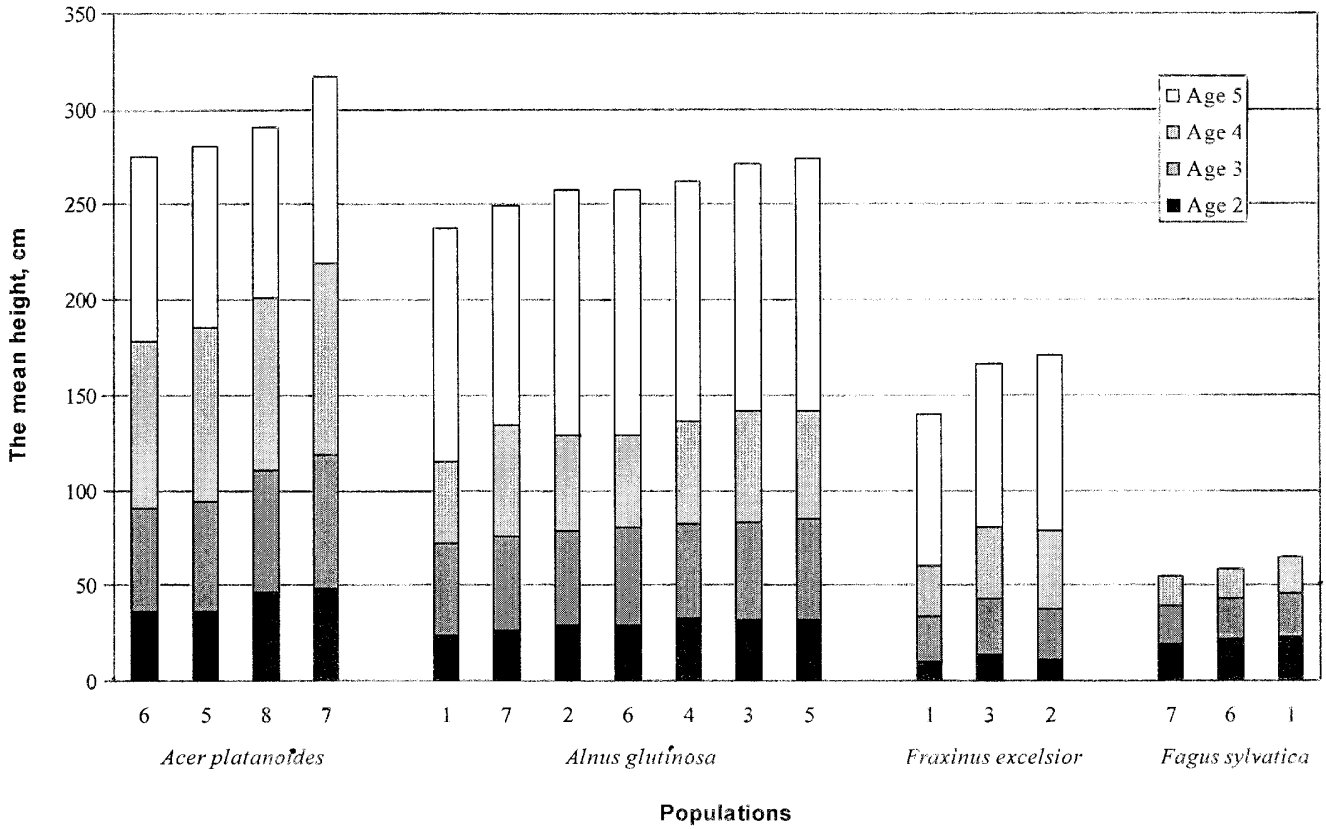

Figure 3. – The performance of plant heights over years by species and populations.

Table 5. – Summary of family mean correlations among traits and geographical (see *Table 1*) or meteorological variables (see Material and Methods) in the four species included in this investigation.

		Bud flushing	Budset	Geographic variables	Meteorological variables
Acer platanoides	Bud flushing	significant, positive; 64 -75 %	see reciprocal cell	NS	NS
	Budset	with one exception NS	NS	NS	NS
	Height	negative, significant; 12 - 41 %	mostly negative and significant; 20 - 30 %	NS	NS
Alnus glutinosa	Bud flushing	significant, positive; 56 %	–	significant, positive; 4 - 42 %	significant, negative; 10 - 43 %
	Height	NS	–	with one exception NS	3 of 8 significant, negative; 5 - 10 %
Fagus sylvatica	Bud flushing	significant, positive 55 %	–	NS	NS
	Height	NS	–	latitude: significant, positive, 20 - 28 %	significant negative; 22 - 33 %
Fraxinus excelsior	Bud flushing	significant, positive; 68 - 73 %	–	with one exception NS	4 of 6 NS
	Height	significant, negative; 25 - 37 %	–	significant, negative; 16 - 40 %	significant, positive; 29 - 52

The low precision in population variance component estimates, with the possible exception of *Alnus glutinosa,* must be attributed to the low number of populations, which causes a skewed distribution of the [2.0] estimate. Evidently, the population differentiation in the latitudinal range of 56° to 60° is not as pronounced as at the higher latitudes that were included in HÅBJØRG's study.

The variance components for bud flushing in *table 2* lend some support to our hypothesis that *Alnus glutinosa* should have a larger population differentiation than *Fagus sylvatica.* The northernmost population of *Fagus sylvatica* originates from latitude 59° while the other two originate from latitude 55° to 56° which rules out a direct gene flow between the two origins. Gene flow can therefore hardly explain the lack of population differentiation. Another possibility is that the northern stand is not autochthonous but has its origin at latitude 55° to 56°. However, before collection of beechnuts we tried to verify that the stands selected were autochthonous. Therefore, we regard the second explanation for the absence of population differentiation in *Fagus sylvatica* as less likely. Our data differ from recent reports on bud flushing in *Fagus sylvatica* (WUEHLICH et al, 1995a; SULKOWSKA, 1995). However, their populations originated from a broader span of site conditions than our three populations which probably favours a population differentiation (cf. ERIKSSON, 1997). The large number of German populations of *Fraxinus excelsior* studied by KLEINSCHMIT et al. (1996) did not show any clinal pattern of variation. This suggests that random genetic drift might have played a role for the traits studied, bud burst, bud set, and height. When populations from Romania and Switzerland were included, a clinal variation was traced for bud burst.

There is a real within-population variation for bud flushing in all species (cf. *Table 2*). If it is assumed that the *Acer platanoides* open-pollinated families are half-sibs the heritability for bud flushing would vary between 0.3 to 0.5. The data on F_{ST} published by RUSANEN et al. (1996) for Finnish populations close to the margin of the distribution suggest that genetic drift has been in operation. It is likely that genetic drift has occurred in the Swedish stands as well and that the above heritabilities are largely exaggerated. In agreement with the hypothesis put forward above it seems that the within-population variation is lowest in the pioneer species *Alnus glutinosa.*

Our data on inwintering showed low resolution both at the population and within-population levels. Our data at the population level are different from what has been presented before for *Acer platanoides* by HÅBJØRG (1978) and WESTERGAARD (1997) as well as for many other tree species (cf. Introduction). However, the discrepancy with WESTERGAARD (1997) who studied populations from the same latitudinal range as we did is not so pronounced since he found a clear clinal variation but the difference in critical night length for growth cessation did not vary more than 37 minutes between the populations from latitudes 55° and 60°, respectively. Noteworthy is that the estimates for *Alnus glutinosa* have the highest precision but are generally low. Mostly phenological traits show high heritabilities and are consistent over years (e.g. EKBERG et al., 1994). This was not the case for budset in *Acer platanoides (Table 5).*

The variance components for plant/tree height follow the general pattern of limited precision in the estimates of population differentiation with a higher precision in the within-population estimates *(Table 4).* Moreover, the population variance components for *Alnus glutinosa* have a higher precision than in the other species. We have no beechnut weights of individual female trees but the development of the family components over time in *Fagus sylvatica* suggests that the large family component at age 2, 16.8 ± 5.4, might be attributed to differences in beechnut weights. In provenance studies significant variations were found for height growth in contrast with our observations (references Introduction). However, in these provenance studies the material originated from a broader range of site conditions than our populations. Our data on height growth in *Fraxinus excelsior* differ from the data presented by KLEINSCHMIT et al. (1996) who reported that populations and families within populations contributed equally much to the variation in height at age 6 to 7. For *Fraxinus excelsior* the total genetic variance components remained fairly constant over the years, 11% to 16%, but there was a shift such that the population effect from age 4 is the most important *(Table 4).* This shift occurred after the severe early frost in May 1990 when all young shoots died and were replaced by 1 to 3 lateral shoots. It might be a larger population than family-within-population effect in the ability to recover from a severe frost. However, this is not supported by the data on damaged shoots.

Damage caused by mildew in *Acer platanoides* varied considerably between ages 2 and 3, which might have been caused by difference in weather conditions between the two years. The probability of revealing differences in such damage varies, probably much dependent on the weather conditions.

There is a tendency for the variance components both at the population and family levels to be highest for *Acer platanoides* and *Fraxinus excelsior.* This might be attributed to a limited gene flow among populations and between cohorts within populations in these two species which always grow in mixed stands. This would be in agreement with the observations on high population differentiation in Finnish marginal populations of *Acer platanoides* (RUSANEN et al., 1996) and *Ulmus laevis* (MATTILA and VAKKARI, 1997).

Owing to our assumption that non-random-mating prevailed in *Acer platanoides* and *Fraxinus excelsior* we could not obtain a definite answer to our hypothesis that ecological traits should influence the within/among-population variation for adaptive traits. An estimation of the mating pattern (= the genetic structure of the zygotes formed in a filial population) in populations of the different species would be most useful as a complement to our study.

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The Development of a Protocol for the Encapsulation-desiccation and *In Vitro* Culture of Embryonic Axes of *Quercus suber* L. and *Q. ilex* L.

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Summary

Quercus species have seeds recalcitrant against long-term storage. Cryopreservation of embryonic axes could be a feasible way of preserving their genetic diversity. Several cryopreservation protocols are based on desiccation, among them the so-called encapsulation-dehydration. However, it is previously necessary to establish an adequate *in vitro* culture development of desiccated axes. Embryonic axes of *Q. suber* and *Q. ilex* were aseptically excised, encapsulated in alginate beads, cultured in a sucrose-rich liquid medium, desiccated for different periods in a flow bench and cultured on basal WPM medium. Moisture content of encapsulated axes dropped from 74% to 71% (fresh weigh basis) to 24.5% to 21% after 6 h desiccation, respectively for the two species. Germination decreased to 20% in both species. Germination and shoot elongation of encapsulated embryos (non-desiccated or desiccated for 4 h) was studied for both species after culture on WPM medium supplemented with different concentrations of BAP and IBA. Medium with 0.1 mgl^{-1} BAP resulted in a high percentage of germination and development of shoots in both species.

Key words: aliginate bead, dehydration, *in vitro* culture, oak, *Quercus,* recalcitrant seed.

FDC: 165.442; 163; 176.1 *Quercus ilex;* 176.1 *Quercus suber.*

Introduction

In temperate developed areas, forests have suffered a reduction in their distribution due to the destruction of their habitat by human activities which led in many cases to habitat fragmentation (MCNEELY *et al.,* 1995). These events could provoke the genetic impoverishment of forest species. The genus *Quercus* is one of the most important ones of temperate regions of the northern hemisphere. Many species are autochthonous to the Iberian Peninsula and have a protective role in the ecosystems, besides their social and economic importance.

Seed or embryo cryopreservation is an adequate alternative for the storage of species with recalcitrant seeds (GROUT, 1986). However, the number of species where appropriate cryopreservation protocols have been developed is still limited (PENCE,

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