Quercus petraea and *Quercus robur*
Recent Genetic Research

Gösta Eriksson

Uppsala - Ljubljana

2015

Quercus petraea and *Quercus robur* Recent Genetic Research

Cover photo:
Over 400 years old pedunculate oaks in The Genetic
Garden in Uppsala, Sweden.

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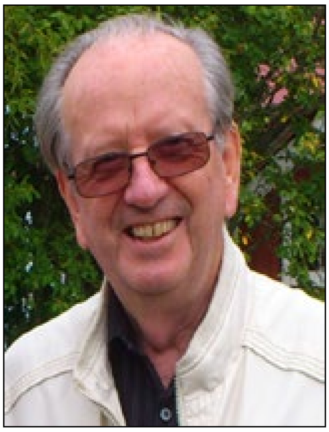
Foreword



Dr. Gösta Eriksson, professor emeritus, has been professor in Forest Genetics and in Progeny Testing at the Swedish University for Agricultural Sciences (SLU), Uppsala, since 1971. His responsibilities included teaching-related tasks in forest genetics and tree breeding as part of the bachelor's, master's and doctoral programmes at the faculty. He has supervised 23 PhDs and served on the selection committee for the appointment of eight professors, two of them in Sweden. Besides domestic teaching duties, he was one of four main lecturers for international courses on forest tree gene conservation organized by EUFORGEN and the Federal Forest Research Centre in Austria, and has been an invited guest lecturer in most western European countries as well as Canada, China, Japan, and the USA. He is still an active member of the Department of Plant Biology, BioCenter, SLU, Uppsala.

His three main research areas were meiotic division of *Larix* and *Pinus* species, *Picea abies* and *Populus tremula*, gene ecology, and forest tree gene conservation. Under his leadership the unique process of meiotic division in pollen mother cells of *Larix* species was detected for the first time, and the dependence on environmental temperature for the progress of meiotic division was elucidated. In the field of gene ecology he carried out thorough investigations of the interaction between progenies and environmental factors, such as temperature, nutrient availability, and water availability under controlled conditions in growth chambers. A technique for early testing for frost tolerance in pine species was developed and is now used in breeding. He initiated pioneering research on genetic variation in the phenology of noble hardwoods. As regards the conservation of forest genetic resources, considerable extension work was carried out for the further development and implementation of the Multiple Population Breeding System for forest tree gene conservation in Europe and elsewhere.

Gösta Eriksson's publication list includes more than 160 research articles, 60 popular science articles, three textbooks in forest genetics and four books on recent genetic research in *Picea abies*, *Pinus sylvestris*, *Betula pendula* & *B. pubescens*, *Quercus petraea* & *Q. robur*.

Dr. Gösta Eriksson is elected member of The Royal Academy for Forestry and Agriculture (KSLA) since 1981. He was a member of the board at KSLA from 1983 to 1986, chairperson of the awards committee at KSLA for prominent achievements in extension work from 1984 to 2005, member of the library committee from 1986 to 1993 and member of the research policy committee from 1986 to 1990.

He was a member of the editorial board of the Scandinavian Journal of Forest Research from 1985 to 1993. He was the first chairperson of the Nordic group of Forest Geneticists and Tree Breeders in the period 1993-1996. He was also the first chairperson of the European group for gene conservation of noble hardwoods within EUFORGEN, 1996-2001, and member of the European group for gene conservation of cork oak within EUFORGEN since its establishment in 1993 until 2001. He was leader of the joint research project NUTRIGEN, funded by the EU, and leader of several Scandinavian forest tree genetics projects as well as a participant in joint projects with scientists in France, Germany, Greece, Italy, Lithuania, Portugal, and Spain. The jubilee medal he received from the Lithuanian Forest Research Institute in January 1997 "For prominent achievements in forest science development and their introduction into practice" expresses well the international appreciation for the pioneering role Prof. Eriksson has played in advancing a future-oriented forest science with a strong connection to field applications.

I had a chance to meet Gösta Eriksson personally in 2003 when he visited Slovenia at the invitation of Prof. Dr. Hojka Kraigher. Prof. Eriksson gave a lecture on quantitative and evolutionary genetics at the Slovenian Forestry Institute to our researchers. Together we visited Triglav National Park, permanent research plots with autochthonous Norway spruce populations on the Pokljuka plateau, and discussed the results of our research and of my PhD thesis on genetic variability of autochthonous Norway spruce, studied by means of isoenzyme genetic markers. His enthusiasm and interest for my work with respect to new ideas and prospects strongly encouraged me to continue studying quantitative and evolutionary genetics as well as to better understand the importance of genetic variation and provenance research interrelated with molecular markers. I have admired Gösta's work since early in my career, for the steady progress facilitated by a supportive professional background.

Dr. Gregor Božič
Slovenian Forestry Institute

Author's preamble

I have tried to summarise recent genetic research related to the two oak species occurring in Sweden, *Quercus petraea* and *Q. robur.* The former has a limited distribution in Sweden and occurs along the southern coastal landscapes and the West Coast. *Q. robur* occurs mainly south of latitude 60°N. Literature from years 1990 - 2014 was searched. However, I am aware that I might have missed papers, which should have been treated in this synthesis. I apologise for this.

Major achievements were presented by graphic illustrations and tables. It should be remarked that none of the illustrations were taken from the original papers. As usual I prepared all illustrations and carried out the editing myself.

The late librarian Lars-Olof Hansson gave me important support in literature searching. Sincere thanks to Dr. Björn Nicander for solving several computer problems.

I am most grateful to my Slovenian colleagues Prof. Dr. Hojka Kraigher, Dr. Gregor Božič, and Dr. Peter Železnik for kind cooperation and for enabling printing of this publication.

Contents

1 Population

1.1 Quantitative traits

In his paper *Intraspecific variation of growth and adaptive traits in European oak species* Jochen Kleinschmit (1993) presented the state of the art of this topic.

A continuous variation of leaf shapes occur in the two species. *Q. robur* has an earlier flushing and later budset than *Q. petraea* but the difference among populations is substantial. Usually populations from localities with short growth periods have an early flushing. Population means for flushing score in six classes of 183 *Q. petraea* and 198 *Q. robur* populations are given in Fig. 1-1. There was limited difference between the two species. One early study reported ecotypic variation for flushing and clinal variation for budset. Budset in southern populations takes place later than in other populations. Lamma shoot formation is more frequent in southern populations.

Stem and trunk form vary considerably among populations. *Q. robur* var. *tardissima* from Slavonia had an excellent stem form under varying site conditions. This population was also characterized by good growth; 40% larger volume at age 69 than the local German populations. As is the case for many other tree species transfer southwards leads to reduced growth. It was noted that some populations perform well independent of the site conditions.

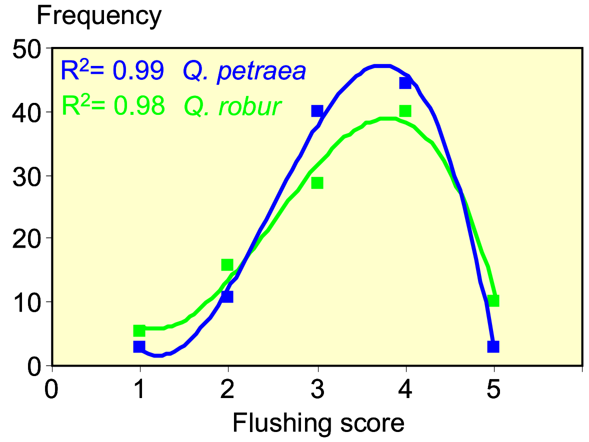


Figure 1-1. The mean flushing stage a certain day in 183 Q. petraea *and 198* Q. robur *populations in a German provenance trial. Kleinschmit 1993.*

Analysis of several wood quality traits in clonal trials showed high broad sense heritabilities. Early wood percentage and basic density showed particularly high heritabilities. Epicorms, which causes poor wood quality, was negatively correlated with flushing date.

It was concluded that *intensive selection and testing of populations and single trees seems worthwhile due to the considerable variation available in growth and quality traits*.

1.1.1 Northern Europe

Two provenance trials in eastern Denmark with 20 and 25 *Q. robur* populations, respectively, and one *Q. petraea* population from Germany were established in Denmark in 1967 (Jensen 1993). They were evaluated at age 25 with respect to growth, stem straightness and occurrence of epicormic shoots. From Fig. 1-2 it is seen that the Dutch *Q. robur* populations showed better growth and stem straightness than the Danish populations. These results were interpreted as a consequence of dysgenic selection in the Danish oak populations. The German *Q. petraea* population showed the best growth but low percentage of quality trunks.

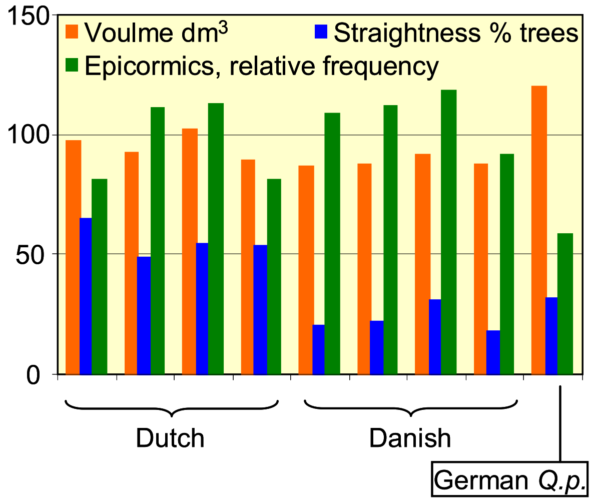


Figure 1-2. The stem volume at age 25 based on two provenance trials with eight Quercus robur *populations and one* Quercus petraea *population. The percentage of trees with acceptable stem straightness and the relative frequency of epicormic shoots are shown. Jensen 1993.*

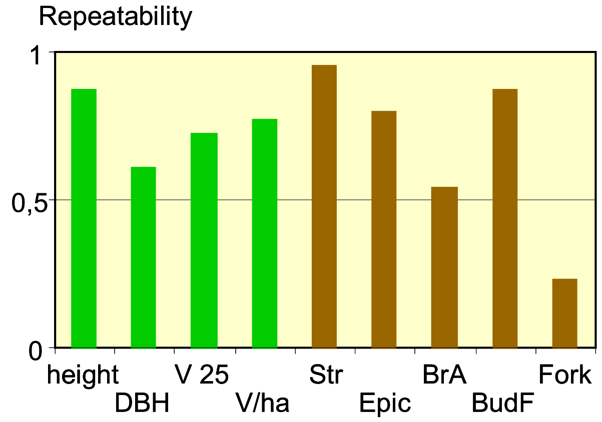


Figure 1-3. The repeatability for four growth traits and five quality traits at age 25 in two provenance trials with 20 or 25 Quercus robur *populations. DBH=breast height diameter, V25=stem volume at age 25, V/ha= volume per hectare, Str=straightness, Epic=epicormic shoots, BrA= branch angle, BudF= bud flushing, Fork=forking. Jensen 1993.*

The repeatability was estimated for the growth traits and some quality traits (Fig. 1-3). Except for forking high and significant estimates were noted for the other traits, which suggests that selection of good performing populations will lead to significant gains. As regards the phenology traits, bud flushing and growth cessation, a clinal variation was noted with early bud flushing and growth cessation of northern populations from Sweden. - There was no correlation between growth in nursery and growth at later stages in field.

In a combined provenance and progeny trial with 23 Dutch *Q. robur* populations and one *Q. petraea* population Jensen et al. (1997) reported significant effects for tree height and breast height diameter at age 14 as well as straightness at age 17 but no significant difference in formation of epicormics shoots. The strongly significant effect for tree height is somewhat surprising since the populations were selected alongside roads and the true origin of the populations was unknown. The tree height and DBH of the *Q. petraea* population were inferior to all *Q. robur* populations.

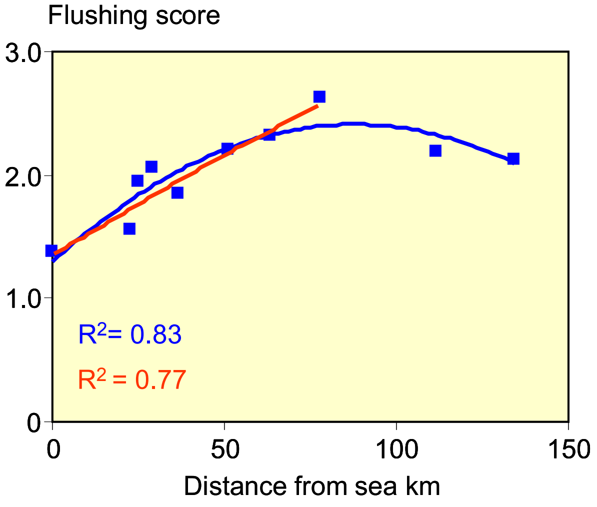


Figure 1-4. The relationship between population distance from sea and flushing score in a Danish provenance trial with ten native Danish populations of Q. robur*. The red line refers to populations originating less than 100 km from the North Sea. Jensen 2000.*

Jensen (2000) summarized information on phenology, growth and damage from a large number of Danish provenance trials of shifting quality.

Phenology. Flushing was found to be under strong genetic control in the three trials, for which genetic parameters were estimated. The repeatability (genotypic variance/phenotypic variance) was estimated at 0.87 and 0.84. The population x test locality effect was non-significant. In one trial flushing was recorded 16 times. In this trial the difference in flushing among populations was strongly significant. There was a strong consistency in timing of flushing among the populations even if the onset date of flushing varied significantly over the years. In general flushing and growth cessation in both species, *Q. petraea* and *Q. robur*, was earliest in northern populations. A striking trend with flushing being dependent on the distance from the sea was noted in one trial with 10 Danish populations of *Q. robur* (the red line in Fig. 1-4). As seen from this figure there was a clear linear trend for populations originating less than 100 km from the coast. In one trial with eight *Q. petraea* and eleven *Q. robur* populations flushing occurred 2-4 days later in *Q. robur* than in *Q. petraea*. Contrary to many other investigations with these oak species, northern populations flushed earlier than the southern populations. However, these Danish results agree with results for other tree species from northern Europe. The probable explanation is that fitness of flushing date in south and north differ.

Damage. Spring frost damage was noted in one trial on a frost prone site. Local populations of *Q. petraea* were unaffected while the local *Q. robur* and a Norwegian *Q. robur* population showed pronounced frost damage. Sudden exposure to strong western winds caused damage in all populations except for the local *Q. petraea* population. Oak decline was observed in two Swedish *Q. robur* populations in two trials while the populations from Denmark and The Netherlands were unaffected.

Growth. Tree height and breast height diameter was measured at age 24 in two Danish provenance trials (Fig. 1-5). The *Q. petraea* population of unknown origin had the tallest stems and widest diameters. The Dutch population had mean values larger than the mean of the 15 or 20 Danish populations in the two trials. The range of performance of the Danish populations was not shown. Since the difference between the single Dutch population and the mean value was small it might be suspected that some Danish populations performed as well as or better than the Dutch population. Such a variation is evident from another trial with eleven Danish and two Dutch

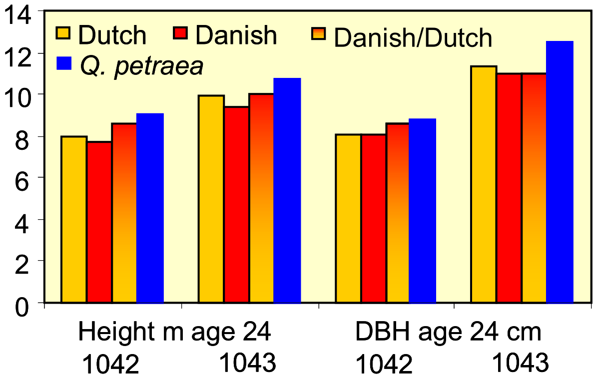


Figure 1-5. Stem height and breast height diameter in two Danish provenance trials, 1042 and 1043, with one Dutch 20 and 15 Danish populations, three Danish populations of Q. robur *but of Dutch origin, and one* Q. petraea *population of unknown origin. Jensen 2000.*

Q. robur populations at ages 7 and 13 from seed (Fig. 1-6). The two Dutch populations did not grow well at this test locality. Generally the Fennoscandia populations grew less than the Danish and western European populations. A Norwegian *Q. petraea* population had the tallest trees in this trial. The mean stem height of the eleven *Q. petraea* populations was also higher than the mean of the *Q. robur* populations, 4.4 versus 3.9 meters.

Stem straightness and branch quality. The stem form in the two trials 1042 and 1043 showed high population repeatability, 0.95. This indicates large population differences in this trait. It was noted from other trials that Norwegian *Q. petraea* populations and Swedish *Q. robur* populations were characterized by good stem form. Preliminary results from Jutland suggested that local land races were formed in this region. The repeatability of branch angle was estimated at 0.54.

Epicormic shoots. Generally, there are higher frequencies of epicormics shoots in *Q. robur* than in *Q. petraea*. In trials 1042 and 1043 a repeatability of 0.80 was obtained for this trait but also a significant population x site interaction.

It was stated that *all Dutch provenances in the present trials reacted as a common geographic group with regard to flushing, budset and lamma shoot formation.* The generally poor stem quality of Danish populations was attributed to dysgenic selection. Besides absence of dysgenic selection in Swedish and Norwegian populations, tolerance against snow pressure might have contributed to their better quality.

Autumn frost tolerance of eleven *Q. robur* and one *Q. petraea* population was reported by Jensen and Deans (2004). The populations originated from England in southwest to Finland in northeast. Twigs were exposed to temperatures in the range -4°C to -32°C at two occasions, November 3-7 and December 1-5. Frost damage was assessed by visual inspection and by electrolyte leakage. Probit analyses were used to estimate the LT_{50} temperature for the different traits.

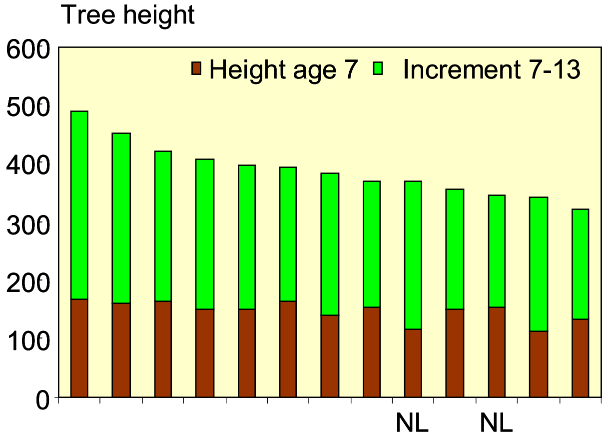


Figure 1-6. The tree height at ages 7 and 13 of eleven Danish and two Dutch (NL) Q. robur *populations studied in a Danish trial. Jensen 2000.*

Especially for bud viability the November freezing, -4°C - -18°C, resulted in an erratic pattern as regards the relationship between temperature and bud viability. Twigs were generally rather frost tolerant and an English and a western Norwegian population showed pronounced damage at the lowest temperature, -18°C at this freezing date. The probit analysis for bud viability revealed significant differences among the populations.

As regards bud viability, a good resolution among the populations was noted for the freeze testing at -20°C in December (Fig. 1-7; unfortunately, the graphic illustrations of the results did not allow identification of all 12 populations. Therefore, only data from eleven of them are shown.). There is a large variation in bud viability among the populations. The highest frost tolerance was noted for the southeastern Norwegian, south-western Finnish, and the *Q. petraea* population from Jutland. The south-western populations were among the most frost sensitive populations.

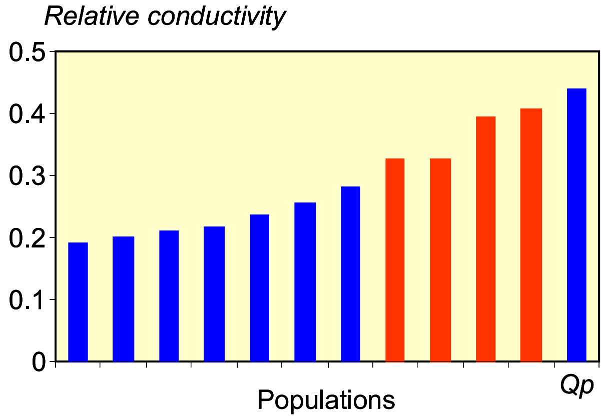


Figure 1-7. Bud viability of ten Q. robur *populations and one* Q. petraea *population after freeze testing at -32°C on December 2. Red columns refer to south-western populations; QP = Q. petraea from Jutland. Score 1 = no damage. Jensen and Deans 2004.*

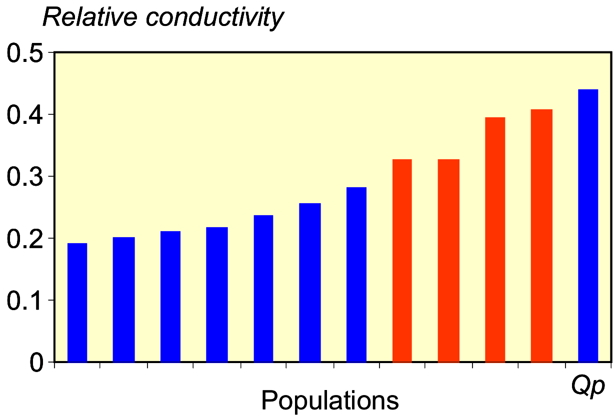


Figure 1-8. Relative conductivity of eleven Q. robur *populations and one* Q. petraea *population after freeze testing at -32°C on December 2. Red columns refer to south-western populations; Qp = Q. petraea from Jutland. Jensen and Deans 2004.*

As regards relative conductivity, the best resolution was noted for the freeze testing at -32°C (Fig 1-8). Both for conductivity and bud viability the south-western populations were among the most hit populations. The *Q. petraea* population that showed high bud viability had the lowest frost tolerance estimated as relative conductivity at -32°C. Also at freezing temperatures -20°C and -25°C it had the highest conductivity. No explanation for this discrepancy was given.

The performance of the two Norwegian populations, one from the south-western tip of Norway and the other from the Oslo Fjord region was discussed. There was no difference in time for budset between the two but a large difference in frost sensitivity. Two Danish populations showed a similar case. It was concluded that local conditions have contributed to the performance. Such adaptation disturbs any simple relationship with climatic variables.

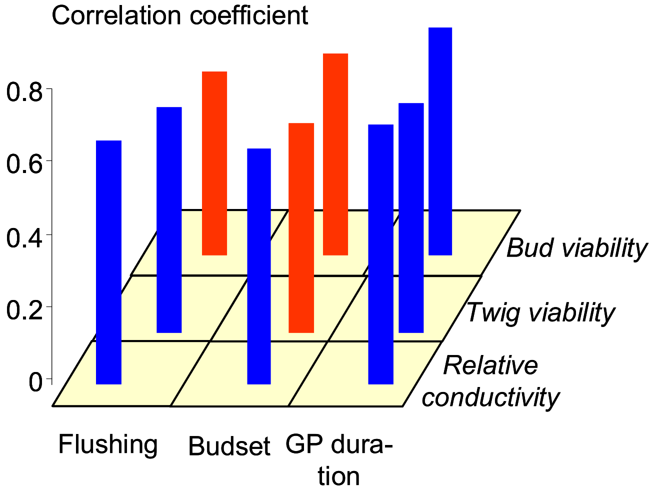


Figure 1-9. The correlation coefficients between phenology traits and frost damage after freeze testing. Frost damage was assessed in three ways, bud viability, twig viability, and relative conductivity. Red columns refer to non-significant correlations. Jensen and Deans 2004.

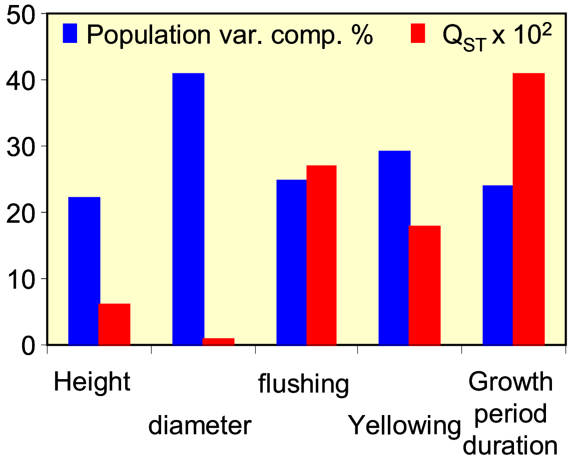


Figure 1-10. The population variance components and Q_{ST}s for growth and phenology traits in 23 Q. petraea *and* Q. robur *populations studied in a greenhouse. Flushing refers to assessments during the second growth period while the other traits were assessed during the first growth period. Jensen and Hansen 2008.*

Correlations between phenological traits and traits related to frost tolerance were presented (Fig. 1-9). This figure reveals that six of the nine correlations were significant. It was unexpected that budset had the fewest significant correlations with frost tolerance traits. However, the size of the non-significant correlation coefficients was not much smaller than the significant ones, 0.50-0.57 versus 0.62-0.71.

In addition to the frost-related information it was shown that growth period duration was strongly correlated with both flushing and budset while the correlation between flushing and budset was non-significant.

Variation in growth and phenology of 23 populations of *Q. robur* and four of *Q. petraea* were tested in a greenhouse trial by Jensen and Hansen (2008). The populations originated from Fennoscandia and central Europe. Seedlings were grown in large containers (16 x 36 x 3 dm) and placed in an unheated greenhouse.

Fig. 1-10 reveals that the population variance component was strong for all traits studied while the Q_{ST}s were unexpectedly small for the two growth traits. The height and root collar diameter were strongly positively correlated while the correlation between leaf yellowing and duration of the growth period was strongly negative, r = -0.85. All other correlations between traits were non-significant. Attempts were made to find out whether any geographic variables could explain the significant differences among populations for most traits. Only for leaf yellowing there was a significant relationship with latitude or temperature conditions. Northern (Fennoscandia) origins had the earliest leaf yellowing. Flushing showed a mixed pattern with several of the Danish populations with extreme late flushing while the Fennoscandia populations were early flushers. The two Dutch populations showed the opposite pattern. Growth was generally larger among southern

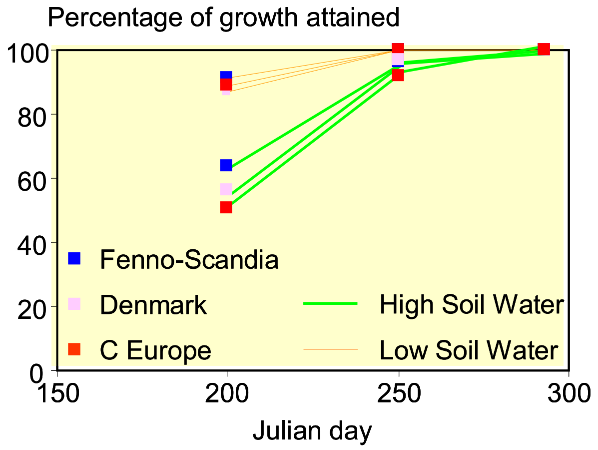


Figure1-11. The percentage of diameter growth in Q. robur *populations from three regions in Europe, Fenno-Scandia, Denmark, and central Europe, in two irrigation treatments in a greenhouse. HSW = 80% of field capacity, LSW = 38% of field capacity. Jensen and Hansen 2010.*

populations. It might be speculated that the absence of geographic trends could be attributed to introduction of oaks during earlier centuries.

Jensen and Hansen (2010) reported on irrigation experiments in a greenhouse with 22 *Q. robur* populations. Data from the first and second growth periods until the start of the irrigation treatment was reported in the above paper. From July 8 (of the second growing season) three irrigation regimes were applied, 38% (LSW), 55% (MSW), and 80% (HSW) of field capacity. Recording of height growth was carried out three times and leaf coloration was recorded on October 8.

The population effect was strongly significant for all traits that were assessed after the start of the treatment, root collar diameter, height, No. of lamma shoots, shoot biomass, root biomass, shoot-root ratio, and growth cessation. This resulted in a high shoot-root ratio in this treatment. The diameter growth in the LSW treatment was almost completed at Julian day 200, *i.e.* shortly after the start of the irrigation treatment on day 189 (Fig. 1-11). In contrast to this, a considerable part of the growth took place between days 200 and 250 in HSW treatment. The growth curves presented suggest that the growth was not completely finished at the end of the experiment. Owing to the favourable growth condition in the HSW treatment the root growth was restricted in this treatment. Except for root biomass and growth cessation significant population x treatment interactions were noted. In spite of the strong interactions one would expect that the ratio *population/population x treatment* for the variance components would be much smaller than revealed in Fig. 1-12. In consequence of the absence of any important ranking changes it was stated that there was no need for many seed zones. However, the strongly significant interactions for the majority of traits suggest a need for many seed zones.

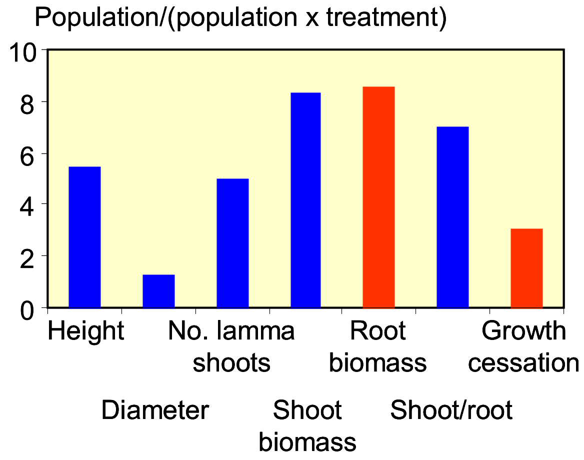


Figure 1-12. The ratio population/population x treatment *interaction variance components for traits studied in a greenhouse trial with 22 central and northern European populations of* Q. robur. *Red columns indicate the two traits with non-significant interaction. Jensen and Hansen 2010.*

1.1.2 Eastern Europe

Gračan (1993) reported on the performance of 12 *Q. robur* populations and four commercial seed lots in two Croatian field trials at age five. The mean survival after two years in nursery was 85.6%. At this age the tallest populations had a height of 35.6 cm. One of the trials was severely attacked by mice with poor survival and growth. This made it less meaningful to carry out a joint analysis of the two trials. Fig. 1-13 with data from the other trial reveals that there was a considerable difference in height growth that was significant. The survival varied between 91 and 98.5% in this trial and obviously there was no significant difference for this trait.

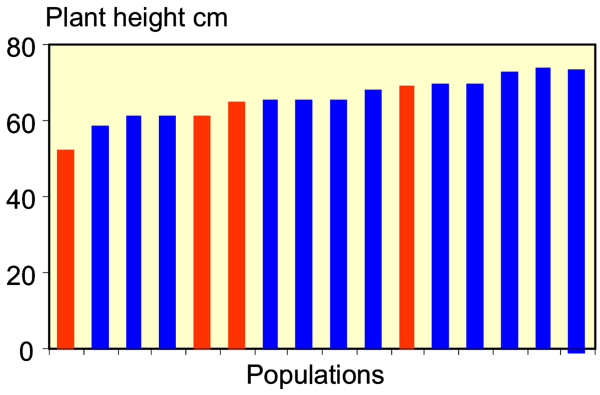


Figure 1-13. Tree height of 12 Q. robur *populations and four commercial lots (red columns) in two Croatian field trials at age 5. Gračan 1993.*

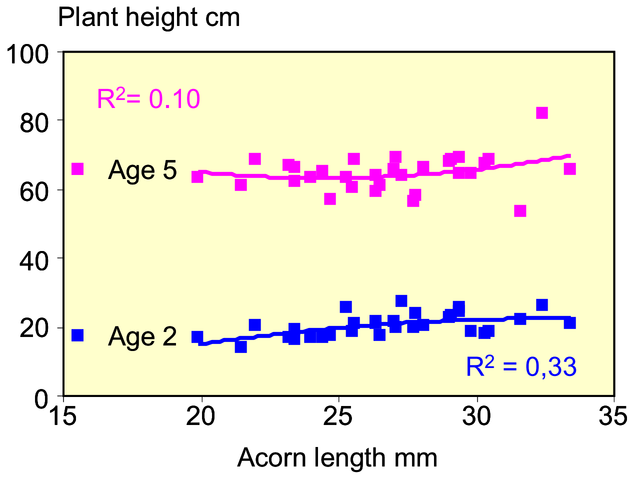


Figure 1-14. The relationship between acorn length of Polish oak populations and height at age 2, nursery, and age 5 in field. Barzdjan 1993.

Preliminary results from a provenance trial in Poland containing 31 populations with assessments of acorn lengths, acorn diameter, height at age 2 and 5, and diameter at age 2 in nursery were presented by Barzdajn (1993). Mainly *Q. robur* populations were included, but hybrid and *Q. petraea* populations were also present but no separate analysis for them was presented. One of the advantages with this study was the assessments of the acorns that allowed estimating the impact of acorn size on growth at ages 2 in nursery and 5 in field.
As seen from Fig. 1-14 there was only a weak relationship between acorn length and plant heights at both ages. The relationships between height and acorn diameter were still weaker. It is remarkable that maternal effects largely are absent in a species with such large acorns. The ANOVAs disclosed that there were significant population differences for all growth traits studied. There was no geographic trend for any of the growth traits. It was pointed out that the transfer of acorns occurred frequently during the 19th century. If some or several of the populations originated from such transfers, geographic trends may be hard to disclose. Two other explanations for the results were given; existence of ecotypes and poor outcrossing rates. I find both of them as very unlikely considering the wind-pollination and high outcrossing rates reported elsewhere.

Q. robur in the Middle Volga region (between Nizhnii Novgorod and Kazan) in Russia occurs at its north-eastern boundary of distribution (Yakovlev 2000). Superior trees were selectively cut for ship building, which may have resulted in dysgenic selection. The variation in bud flushing was reported to be limited in these populations, 5-10 days, while defoliation was said to vary between 55 and 75 days. Three types of defoliation were identified, early, intermediate, and late. Not surprisingly the late defoliation type showed the best growth at age 28 (Fig. 1-15), which must be attributed to its longer growth period. The late flushing type showed superior growth from the first to the final assessment. It should be noted that there was no detailed information on the progeny trial with these three types of progeny.

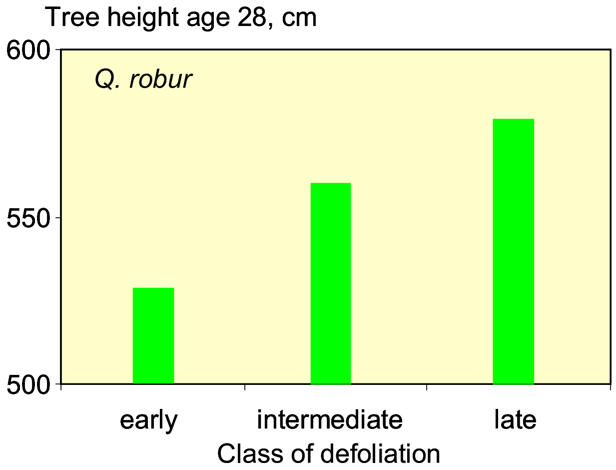


Figure 1-15. Tree height at age 28 of different phenological classes of Q. robur *from the middle Volga region in Russia. Yakovlev 2000.*

Baliuckas and Pliura (2003) reported on growth, bud flushing, and autumn colouring in three Lithuanian field trials and one nursery trial with six *Q. robur* populations represented by 6-10 OP-families.
Bud flushing was the only trait that showed a strongly significant population and population x site interaction effect (Fig. 1-16). Based on this graphic illustration it is easy to understand that the population effect for bud flushing was strongly significant but hard to understand that population x site interaction was that too. There was no clinal trend for the population difference in bud flushing.

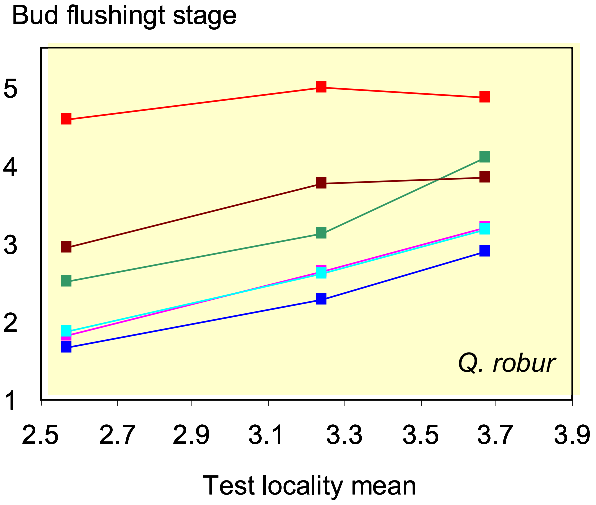


Figure 1-16. The bud flushing stage in six Lithuanian Q. robur *populations at age four at three Lithuanian combined provenance and progeny trials plotted against the test locality means. Six classes of budburst were used. Baliuckas and Pliura 2003.*

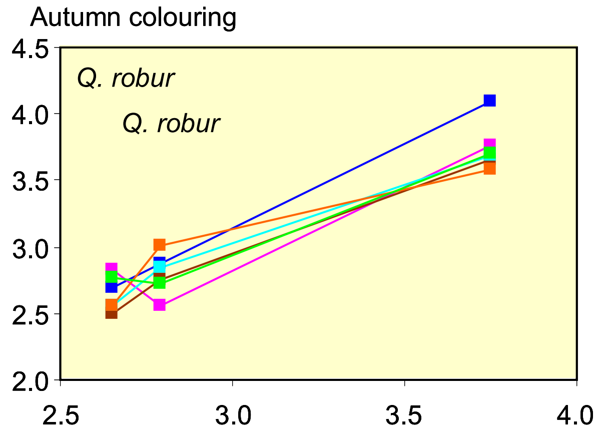


Figure 1-17. The autumn colouring in six Lithuanian Quercus robur *populations plotted against trial mean for autumn colouring at age 4 in three Lithuanian combined provenance and progeny trials. Seven classes of autumn colouring were used. Baliuckas and Pliura 2003.*

It was noted that the polycyclic growth of young oak plants may disturb the process of growth cessation and it might be one reason for absence of any population effect. The largest difference in the seven-degree scale used was 0.5 units in one of the trials.

The population x site interaction was significant for autumn colouring (Fig. 1-17). This figure reveals that there were several substantial rank changes between two of the test sites that probably explained the significant population x site interaction for this trait. In spite of the substantial rank changes of tree height between test sites this interaction was non-significant. Similarly, the population effect was non-significant for tree height (Fig. 1-18).

1.1.3 Central Europe

Bud flushing of 34 *Q. petraea* populations, mainly from Central Europe, was followed in a northern Germany nursery (Liepe 1993). In addition, a growth chamber study of variation in freezing tolerance of ten of these populations was reported. Plants at three different flushing stages were exposed to temperatures in the range -1.5 - -13ºC for four hours following gradual lowering of the temperature to the treatment level. Frost damage was recorded separately for terminal buds, lateral buds, and lamma shoots.

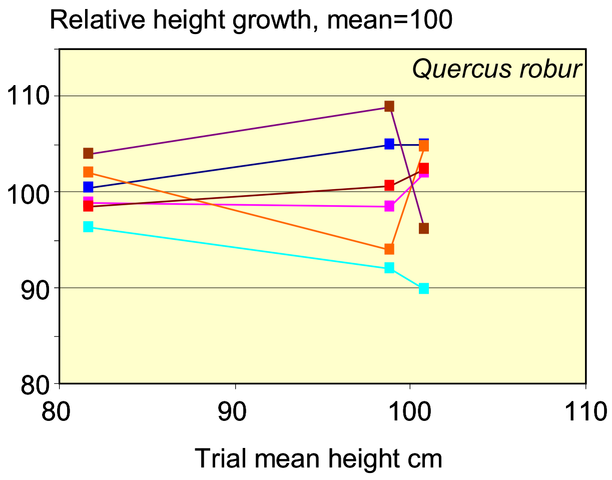


Figure 1-18. The relative height growth of six Lithuanian Quercus robur *populations plotted against trial mean for plant height at age 6 in three Lithuanian combined provenance and progeny trials. Baliuckas and Pliura 2003.*

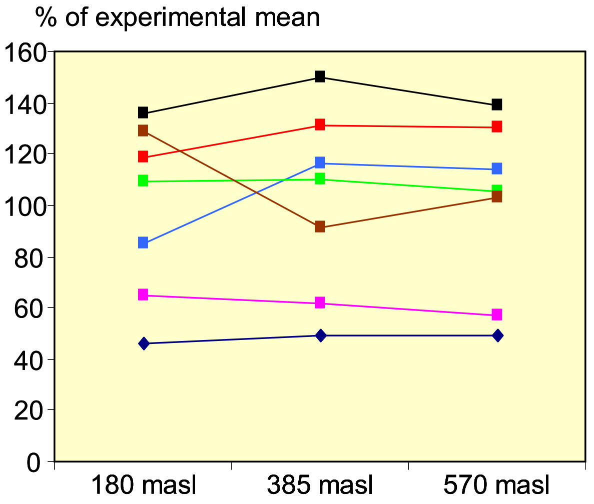


Figure 1-19. The percentage deviation from trial mean heights at age 6 for seven Q. robur *populations tested in three German provenance trials located at three elevations. These seven populations were selected to illustrate the span of response to the trial conditions among the 30 populations included in the trials. Maurer et al. 2000*

Earliest flushing was noted for Austrian, Hungarian, Turkish, and French populations. The early flushing populations were hardy by a natural frost down to -4ºC occurring during advanced stages of flushing.

Unfortunately, no quantitative data on variation in frost damage after artificial freeze testing were given. The author stated that *While interprovenance variations of frost hardiness were found, the hardiness ranking was not consistent over the range of temperatures applied.* Two French and the only studied Turkish populations seemed to be the least frost tolerant.

Preliminary results from two provenance trial series with *Q. petraea* and *Q. robur* were presented by Maurer et al. (2000). The *Q. robur* series contained 26 German and four Dutch populations. Some of the populations were mixtures and one of them being a seed orchard progeny. No estimates of genetic parameters were given. Fig. 1-19 shows that there was a large variation in tree height at age six in the three trials included in this series. The mean values for tree height at age six did not vary much among the trials, 120-134 cm. I have selected a representative sample of populations to illustrate possible population x locality interactions. This figure reveals that no dramatic ranking changes occurred in this material. The most pronounced rank change took place in a population from upper Rhine valley (brown squares). The mortality

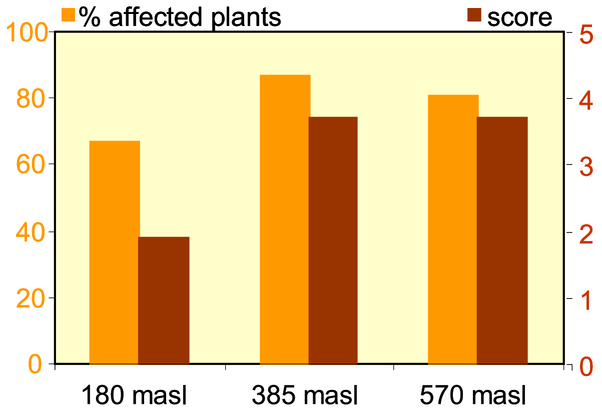


Figure 1-20. The percentage of Q. robur *plants affected by frost damage and mean frost damage score in three German provenance trials at different elevations. 0 = no damage 5 = all leaves and shoots are damaged. Maurer et al. 2000.*

was lowest in the trial at 385 masl, 6%, and highest in the low-elevation trial, 18%. At age four assessments of frost damage was carried out with six classes, 0-5, with class 5 = all leaves and shoots damaged. An extremely large percentage of plants was damaged by frost and the trials at 385 and 570 masl had high scores of frost damage (Fig. 1-20). This high degree of frost damage was not discussed. With such high percentage of damaged trees it was interesting to test the relationship between frost damage and growth (Fig. 1-21). I selected data from the 385 masl-trial but the relationship explained only a minor part of the variation. The relationship with percentage of damage and height had a still lower R^2 value. Exclusion of the population with a tree height percentage of 116 and frost damage score 2.1 improved the strength of the relationship just a little.

Data from one German provenance trial with 38 *Q. petraea* populations were also presented. It belongs to an international series of provenance trials with more German populations than in the international series. At age eight the population mean values varied between 138 and 188 cm (Fig. 1-22). The mortality was low, 4.1-19.0%. Fig. 1-22 reveals that there was no relationship between tree height and mortality.

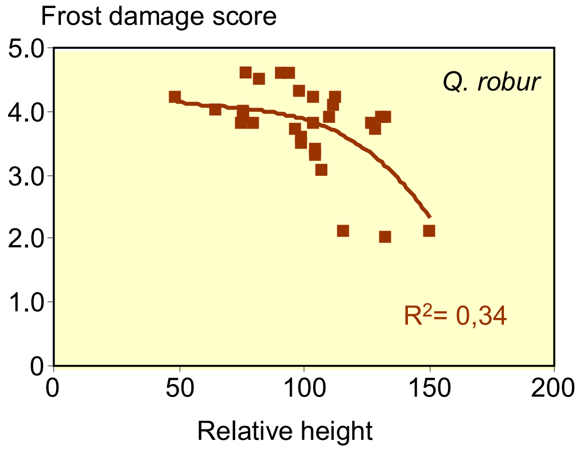


Figure 1-21. The relationship between relative tree height and frost damage score in 30 Q. robur *populations in a German provenance trial at 385 masl. Frost damage was classified from 0, no damage, to 5, severe damage. Maurer et al. 2000.*

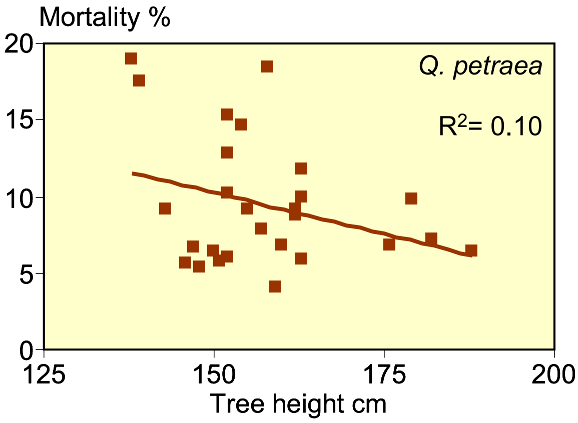


Figure 1-22. The relationship between tree height at age 8 and mortality of Q. petraea *populations tested in one south-western German provenance trial. Maurer et al. 2000.*

Multilocus gametic diversity was calculated based on 18 and 20 isozyme loci in *Q. petraea* and *Q. robur,* respectively. In both species a few populations with high estimates deviated much from the other populations. This was particularly the case for *Q. petraea*. One of the *Q. petraea* populations was one of the least tall populations while the other population showed average growth. Contrary to this, one of the tallest *Q. robur* populations had the highest gametic diversity while another with high diversity was a little above average as regards tree height.

The authors concluded that large variation occurred for growth and frost damage but it was premature to draw any far-reaching conclusions on such young oak material.

A detailed study of offspring from two Dutch *Q. robur* populations growing adjacent to each other close to Wageningen, The Netherlands, was carried out in a field trial in England (Harmer 2000). One stand was characterized by good phenotypes while the other contained many crooked trees and many epicormics shoots.

In greenhouse the poor quality stand offspring had slightly higher number of flushes than the other stand offspring. There was a focus on growth units defined as the elongation taking place during one flush of growth. The first and the fourth growth units differed significantly with larger growth units of the high quality population. Final height did not differ between the two populations. Number of branches and percentage of buds forming branches was highest in the poor stand population. The poor quality of the parent population was at least partly found in the filial generation.

In the field study tree height at age 8 was significantly higher in offspring from the good quality stand while

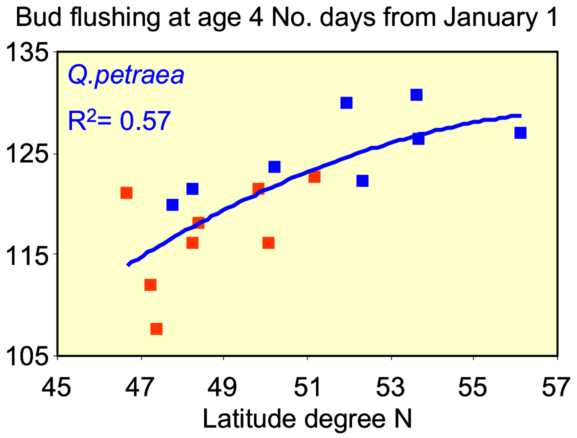


Figure 1-23. The relationship between latitudinal origin and bud flushing in 16 mainly western European Q. petraea *populations studied in a nursery in Scotland at latitude 55.87°N. Red squares indicate populations originating above 200 masl. Deans and Harvey 1995.*

stem diameter did not differ significantly. Nor was there any difference in length and diameter in major branches. For the individual trees strong relationships between diameter and length of major branches or stems were noted for both populations.

1.1.4 Western Europe

In two papers by Deans and Harvey (1995 and 1996) phenology and results of freezing tests were presented for 16 mainly western European *Q. petraea* populations. Eleven populations were planted in 1990 while the remaining five populations were planted in 1991. Flushing was assessed during two and three years, respectively while growth cessation was assessed at ages 2 or 3 (11 populations).

The dates for flushing differed between years and the population effect was significant for bud flushing and the ranking was kept during the three years of study. Bud flushing took place later in high-latitude populations than in low-latitude populations and the relationship between flushing and latitudinal origin was moderately strong (Fig. 1-23). Similarly, there was a relationship between altitude and flushing of the same strength (Fig. 1-24). Both relationships suffer from the variation in altitude and latitude, respectively. A true latitudinal effect can only be obtained if all populations originate from the same altitude. Analogous to that, a true altitudinal effect can only be obtained in material from the same latitude. A multiple regression analysis would have overcome these shortcomings. Late spring frost and early autumn frost damage were recorded during the second growing season. Data on terminal budset and leaf coloring were presented for an assessment on September 28. There was only a weak relationship between budset and population latitude (Fig. 1-25). A still poorer relationship was noted for budset and population altitude (not shown). It is evident that assessments from earlier dates might be instructive for these two relationships.

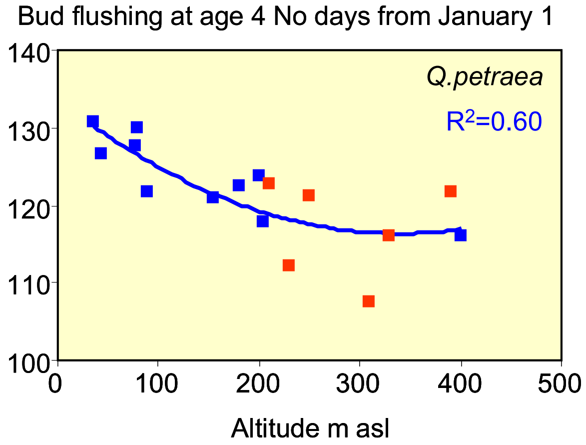


Figure 1-24. The relationship between altitudinal origin and bud flushing in 16 mainly western European Q. petraea *populations studied in a nursery in Scotland at latitude 55.87°N. Read squares refer to populations originating south of latitude 50°N. Deans and Harvey 1995.*

Most spring frost damage was noted in the northernmost and late flushing population from Denmark, 40.2%. Eight of the eleven populations studied this year did not pass 10% damage. There was no relationship between percentage damage and any of the geographic variables. It might be speculated that most of the early flushing populations had passed the most sensitive stage for frost damage when the frost appeared. Leaf autumn frost damage varied in the range 15-48%. The percentage of autumn stem frost damage was much lower, 0-11% and there was a correlation between growth cessation and frost damage according to the authors, R^2=0.60. Populations with late growth cessation were most severely damaged.

Limited information of height growth was given but height growth varied among populations. However, using initial plant size at plantation as covariate in the analysis resulted in non-significant differences.

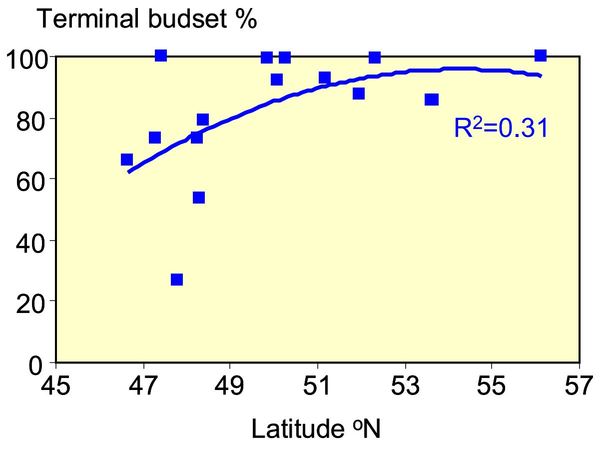


Figure 1-25. The relationship between population latitude and percentage of terminal buds in mainly western European Q. petraea *populations growing in Scotland at latitude 55.87°N. The assessments were carried out on September 28. Deans and Harvey 1995.*

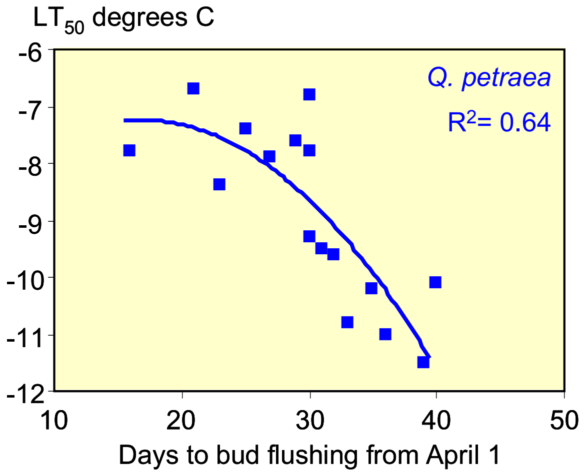


Figure 1-26. The relationship between 50% shoot lethal temperature and bud flushing in 16 mainly western European Q. petraea *populations after artificial freeze testing. Deans and Harvey 1996.*

Detached twigs were taken from plants of the 16 *Q. petraea* populations reported on above for freeze testing (Deans and Harvey 1996). The tests were carried out at intervals of 2-3 weeks from mid-September to mid-April. The temperatures for each testing occasion were selected such that the entire range of 0-100% damage should be accomplished. Frost damage was assessed by conductivity measurements.

There were population differences during the three phases, autumn, winter, and spring, with least variation during winter. The hardiest populations could stand -24°C while the least hardy only -20°C. The difference among populations was non-significant during winter. As seen from Fig. 1-26 there is a moderately strong relationship between days to flushing and LT_{50} (lethal to 50% of the individuals). Late flushers had the lowest LT_{50}s and were thus hardiest. Analogous to this, populations with early growth cessation had lowest LT_{50}s (Fig. 1-27). The authors concluded that it is of importance to select late flushers and early growth cessation populations for reforestation at localities with risks for late spring frosts and early autumn frosts, respectively.

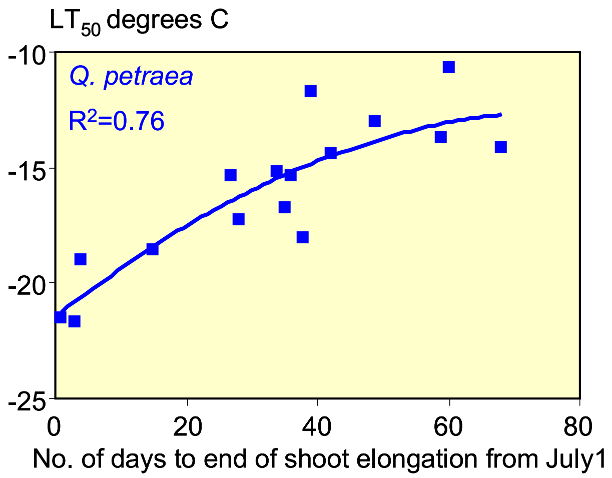


Figure 1-27. The relationship between lethal temperature for 50% of shoots and the final day for shoot elongation in 16 mainly western European Q. petraea *populations after artificial freeze testing. Deans and Harvey 1996.*

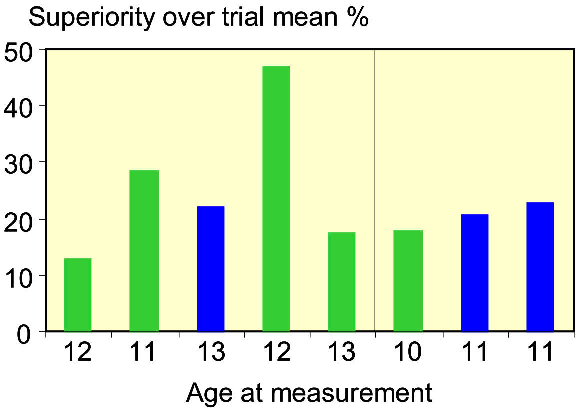


Figure 1-28. The percentage superiority in tree height of the best population in individual trials of two series of provenance trials with oak populations, mainly from western Europe and mainly Q.petraea. *Data from five trials in the 1990 series are shown to the left. To the right data from the three trials in the 1992 series. Hubert 2005.*

Two series of provenance trials, mainly with *Q. petraea*, were established in Great Britain in 1990 and 1992 (Hubert 2005). Heights were assessed at age 6 and again at ages 10-13 in the series with six and three trials. The majority of populations originated from Western Europe.

The percentage superiority of the best population over trial means is presented in Fig. 1-28, which reveals that a considerable superiority exists in all trials. A Dutch *Q. robur* population was the tallest in three of the five trials in the 1990 series. It was noted that populations originating from selected stands in Britain had better growth than populations from non-selected stands. It was recommended to select reforestation material from phenotypically good stands and not base the selection on proximity to the reforestation site. The two Danish populations performed poorly in this series and so did British populations transferred from their northern origins. The latter was attributed to reduced growing season after the southwards transfer. It might be mentioned that no figures were given for tree height at the Scottish trial, at which eight of the 16 populations studied had a survival of less than 20%. The survival was good or fairly good at all other test localities. There was no clear trend that *Q. petraea* should be better than *Q. robur* in these trials.

Hubert and Cundall (2006) in their paper on selection of best populations of oaks for reforestation in Britain largely based their recommendation on the Hubert (2005) paper presented in the previous paragraph.

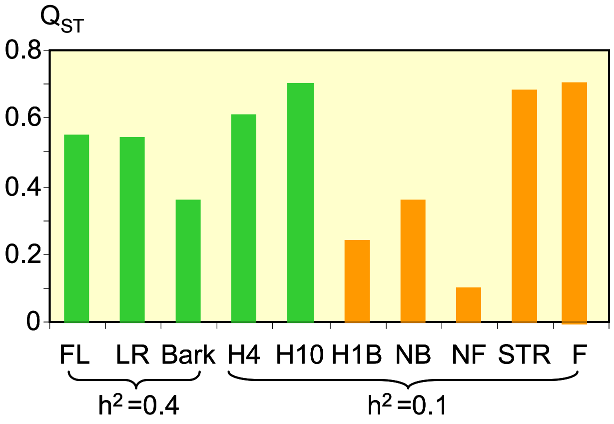


Figure 1-29. Q_{ST} estimates for phenology, growth, and quality traits from a series of four provenance trials in France with 107 Q. petraea *and 17* Q. robur *populations from the entire area of distribution. FL = flushing, LR = leaf retention, Bark = roughness, H4 = Height at age 4, H10 = height at age 10, H1B = height to 1st branch, NB = number of branches, NF = number of forks, STR = straightness, F = tree form bush-tree. Ducousso et al. 1995.*

1.1.5 Southern Europe

Data from a series of provenance trials with 107 *Q. petraea* and 17 *Q. robur* populations were reported by Ducousso et al. (1995). The populations originated from Azerbaijan in southeast to Ireland in west and from southern France to Denmark in north. Four trials in France were included in this series with two types of soil, three on brown soil and one on podzol. Phenology, growth, and quality traits were assessed at ages 4 and 10.

The differences between the populations were strongly significant for all traits. Q_{ST} was estimated for all ten traits (Fig. 1-29). Since heritability is a component of the nominator in estimates of Q_{ST}, the heritabilities used for calculation of Q_{ST} are given in this figure. The estimates were high for all phenology and growth traits. The use of such a low heritability as 0.10 for the quality traits might be questioned. If a higher heritability were used the difference between this type of trait and the two others would have been more pronounced. Still these Q_{ST}s are much larger than F_{ST}s for markers (see Marker section).

The survival was good in two of the trials in the others mortality was attributed to drought during the growth period in one trial and to lack of maintenance in the other. In the latter case there was a significant and positive relationship with the distance of population transfer and mortality, r = 0.66. In the former trial there was a significant and negative relationship between precipitation and mortality, r = -0.54.

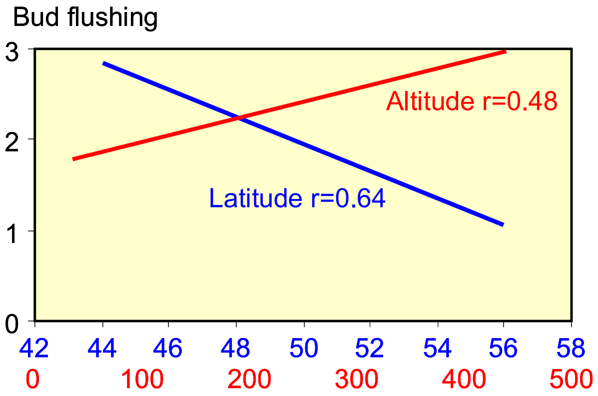


Figure 1-30. The relationships between mean bud flushing stage from four trials and latitudinal (blue) and altitudinal (red) origins respectively. Six classes of flushing were used, 0-5. Ducousso et al 1996.

A detailed report on flushing in a subset of 50 western European *Q. petraea* populations was given by Ducousso et al. (1996). The population effect was strongly significant in all four field trials. The correlation between the flushing among the trials was strong in all pairwise comparisons. The correlation coefficients, r, varied in the range 0.86-0.95. Contrary to many other relationships between flushing and geographic variables of tree species, flushing was most advanced in southern populations with a gradual decrease of flushing stage towards higher latitudes (Fig. 1-30, blue line). In the larger material analyzed in Ducousso et al (1995) there was a positive relationship between latitudinal origin and flushing date, r = 058. The relationship between flushing and altitudinal origin followed the traditional pattern for temperate tree species with earliest flushing of the high-altitude populations (Fig. 1-30, red line). In this case there was the same type of relationship for the entire material, r = 0.34. It should be remarked that all relationships explained a minor part of the variation in flushing. It was speculated that the southern populations might benefit from early flushing that would enhance the competition power against other plant species. It is assumed that competition increases with decreasing latitude. In analogy with this explanation, the flushing should be most advanced in the low-altitude populations, which it was not. Since there were relationships between flushing on one hand and latitudinal or altitudinal origin on the other hand it means that the relationships with one geographic variable at a time will include "noise" from the other relationship. With such a large number of populations it would have been advantageous to carry out a multiple regression analysis. In one of the trials there was a spring frost that damaged several plants. There was a strong and positive relationship between damage frequency and flushing stage. This indicates that adaptation for flushing is important in this oak species.

In a series of papers important information on gene ecology in two French altitudinal transects in The Pyrenees; valleys Gave (Luz, latitude 43.75°N longitude 0.10°E) and Ossau (latitude 42.78°N longitude 0.74°W), respectively, were presented in a series of papers (Vitasse et al. 2009a, 2009b, 2009c, Alberto et al. 2010, 2011 and 2013). The papers will be presented together, even if some of the results belong to other sections of this chapter.

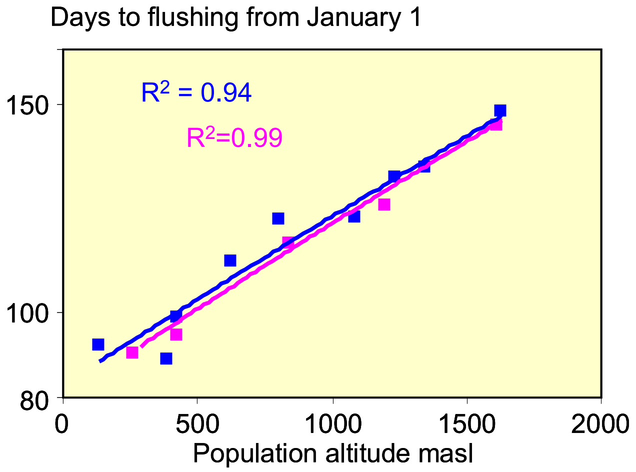


Figure 1-31. The relationship between population altitude and flushing in adult Q. petraea *populations growing in two valleys in The French Pyrenees. The* in situ *assessments were carried out in 2005. Vitasse et al. 2009c.*

Bud flushing and growth cessation was followed *in situ* in the two valleys every ten days during March – June and September – December during years 2005 – 2007 (Vitasse et al. 2009c). Temperature was recorded by data loggers in each of the 14 populations included.

I have selected to illustrate the relationships between altitude and flushing or growth cessation for year 2005 separately for the two valleys (Figs. 1-31 and 1-32). Fig. 1-31 reveals that there was an extremely strong relationship between altitudinal origin and flushing in both valleys. As regards growth cessation the relationships differed between the two valleys but the relationships were also this case extremely strong. This results in a strong reduction of the growth period with altitude. The corresponding relationships were noted when the phenology traits were related to temperature at the test localities. These results are expected since spring arrives later at high altitudes than at low altitudes, and similarly autumn arrives earlier at high altitudes than at lower ones. Based on the mean values for the relationships [flushing-temperature March-June (1)], [growth cessation –temperature August-November (2)], and [growth period duration-temperature January-December (3)] the following slopes were reported:

1. -6.5 days
2. +5.1 days
3. +13.0 days

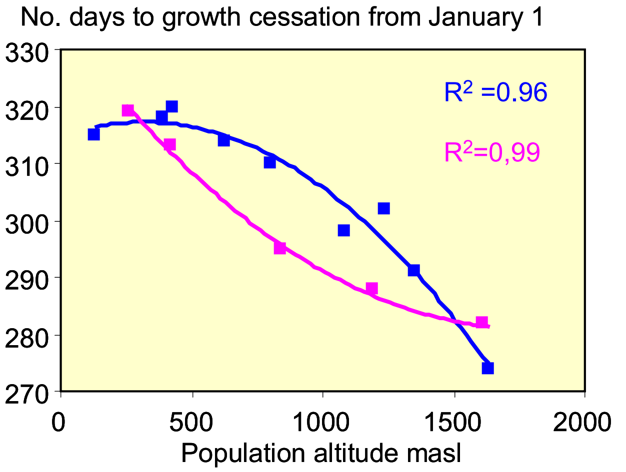


Figure 1-32. The relationship between population altitude and growth cessation in adult Q. petraea *populations growing in two valleys in The French Pyrenees. The* in situ *assessments were carried out in 2005. Vitasse et al. 2009c.*

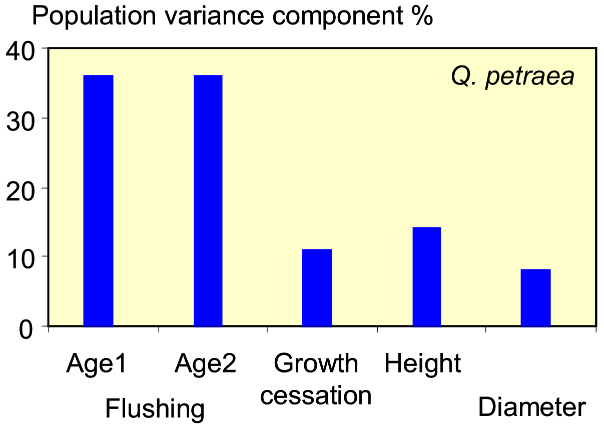


Figure 1-33. The population percentage variance components for growth and phenology traits in a provenance trial with 13 Q. petraea *populations from two Pyrenean valleys in France. The populations originated from two altitudinal transects. The population effect was significant for all five traits. Vitasse et al. 2009a.*

It was noted that *temperature better explained phenological variations rather than altitude whatever the variable….* However, no individual R^{2}s were presented and as seen from Figs. 1-31 and 1-32 it is hard to beat the R^{2}s obtained and this statement seems unjustified. As a matter of fact temperature is strongly correlated with altitude. Since the difference in day length did not vary much among the populations the authors ruled out day length differences as responsible for the variation in growth cessation among the populations. Thus, temperature should be the triggering factor for onset of growth cessation. However, the night length triggering growth cessation may equally well vary from low altitude to high altitude in the same way as temperature does. Therefore, the sole impact of temperature may be illusive.

Growth rhythm and phenology in 13 populations from the two Pyrenean valleys were studied in a French provenance trial at latitude 44.57°N at sea level (Vitasse et al. 2009a). Except for the low-altitude population and the population located at 841 masl all other populations were south-facing.

As seen from Fig. 1-33 the population variance components were large for flushing while they were a few times smaller for growth cessation and the two growth traits. Time for flushing was strongly correlated between the two years. The authors pooled the data from the two valleys

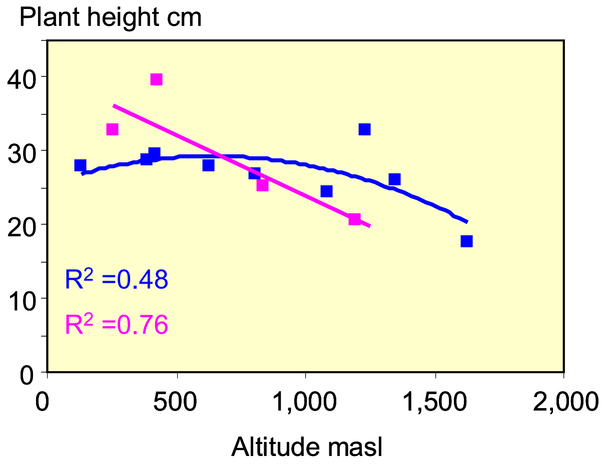


Figure 1-34. The relationship between population altitude and plant height at age 1 in Q. petraea *populations from two valleys in French Pyrenees. Vitasse et al. 2009a.*

and found a significant relationship between plant height and altitudinal origin. Since selection might not have acted similarly in the two valleys I preferred to test the relationships separately for the two valleys (Fig. 1-34). The relationships differed as may be seen from this figure and both R^2s were larger than the R^2 for the pooled data. There was a significant relationship between temperature conditions of the population origin and growth cessation but the degree of explanation was low, $R^2 = 0.34$. For bud flushing and duration of the growing season the corresponding relationships were non-significant. However there was a tendency that high-altitude populations had a later flushing. The duration of the growth period did not differ much among populations.

Except for the relationship *duration of the growing season – growth cessation*, the other relationships between phenology traits and growth were weak.

The late flushing of the high-altitude populations was explained as selection for late flushing genotypes for avoidance of frost exposure. Populations growing under summer drought might benefit from early flushing to enable much growth before the summer drought appears.

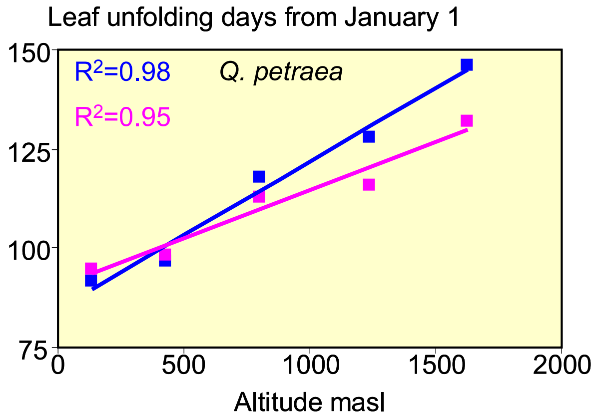


Figure 1-35. The relationship between population altitudinal origin and leaf unforlding during two consecutive years of five Q. petraea *populations studied in a French trial at latitude 48.42°N and 120 masl. Vitasse et al. 2009b.*

I am not aware of any extended summer drought at the localities of the low altitude populations. The variation in growth cessation among populations was attributed to adaptation to different temperature conditions. The differences in night length at sites of origin is marginal and can be ruled out as an explanation for variation in growth cessation.

Ten of these 13 *Q. petraea* populations were also studied in a trial at latitude 48.42ºN, longitude 2.67ºE, and 120 masl in France by Vitasse et al (2009b). The year of establishment of the trial was not given. Based on the information given I assume that data were pooled two and two since only five points were shown graphically in the paper. I have tried to read the figures shown in Fig. 2 of the paper to enable an estimate of the relationship between altitude origin and leaf unfolding during two years, 2005 and 2006. As seen from Fig. 1-35 these relationships are extremely strong and with fairly similar slopes. The temperature decreased linearly with 0.51 and 0.44 degrees in 2005 and 2006, respectively. This difference explains the difference in slope of the relationships between years.

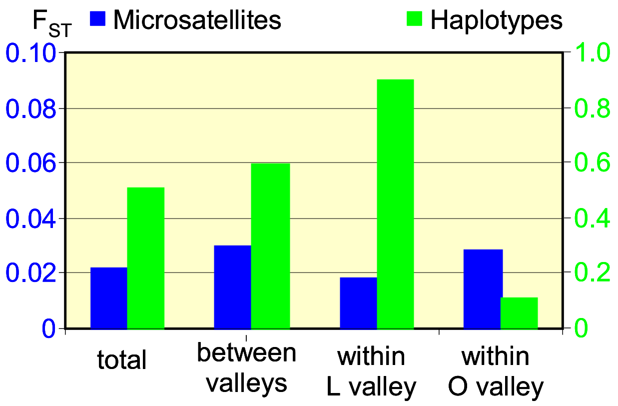


Figure 1-36. F_{ST} estimates for 12 Q. petraea *populations in two altitudinal transects in The Pyrenees estimated separately in the valleys, between valleys and for all 12 populations;. L = Luz, O = Ossau. All estimates being significant. Note the different scales for the two markers. Alberto et al. 2010.*

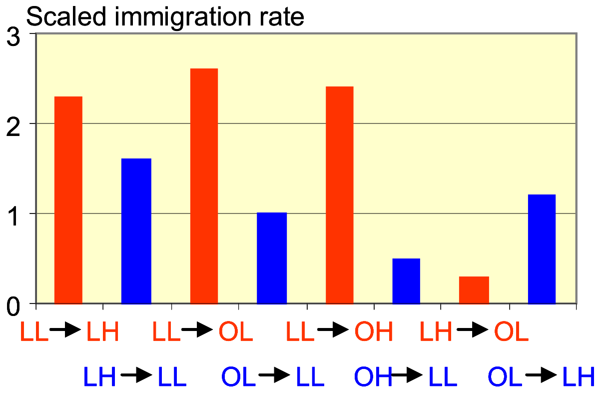


Figure 1-38. The gene flow between low and high elevation populations (> 1,100 masl) in two valleys with Q. petraea *populations in The Pyrenees. L = Luz valley, O = Ossau valley. The first letter refers to the valley and the second to low (L) or high (H) elevation. For three of the twelve possible combinations no migration was detected. Alberto et al. 2010.*

Microsatellite and haplotype variation were studied in these transects by Alberto et al. (2010). Five and seven populations from each transect were included; the altitudinal ranges being 131-1630 and 259-1614 masl, respectively.

Differentiation among populations was estimated within valleys, between the two valleys and for the total material (Fig. 1-36). The F_{ST}s for microsatellites did not exceed 0.03 in any case while the F_{ST}s for haplotypes were several times higher. Six of the seven populations from the L valley were monomorphic while one of the O valley populations was monomorphic. This explains the high F_{ST} for the L valley populations, 0.90. The haplotype F_{ST} for the O valley must be regarded as extremely low (0.106) for this type of marker. In the L valley only haplotype 12 occurred while both western haplotypes 10 and 12 were found in the O valley. It was suggested that the founder population of the O valley had both haplotypes 10 and 12. The slightly higher microsatellite differentiation in the O valley was attributed to the population from 1194 masl. In both valleys there was a clear difference between high and low altitude populations. This was attributed to demographic events. Gene flow between low and high elevation populations might be caused by physical barriers like mountain chains or to difference in flowering times. Two of the microsatellite alleles showed a clear clinal pattern, 109 in the QrZAG39 locus and 113 in the QpZAG15 locus (Fig. 1-37). These two alleles showed the same pattern in the two valleys but the absolute frequencies were at slightly different levels. One explanation for the clinal variation of these two microsatellites might be attributed to hitchhiking with closely linked alleles regulating flushing.

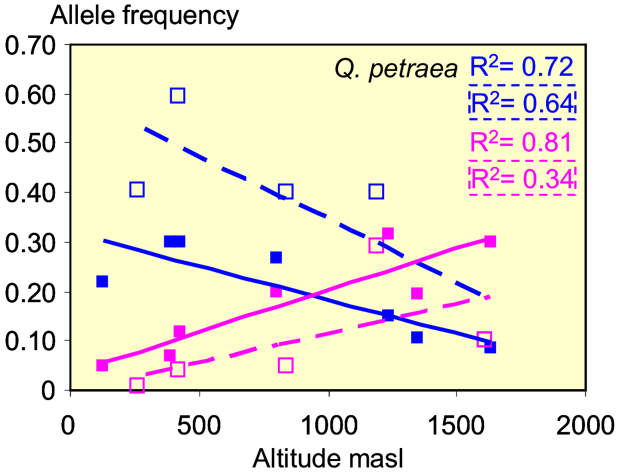


Figure 1-37. The relationship between altitude and allele frequencies of two microsatellite alleles – 109 in QrZAG39 locus (blue) and 113 in QpZAG15 locus (purple) - of Q. petraea *in Luz (full line) and Ossau (hatched line) valleys in The Pyrenees, France. Alberto et al. 2010.*

Fig. 1-38 reveals that the gene flow is larger from low elevation to high elevation than the opposite direction. Moreover, the Luz valley low elevation populations had the highest effective population size. There was a larger variation in flushing dates in the low altitude populations, which was suggested as one explanation for the dominating gene flow from low altitude to high altitude. The lower effective population size at high altitudes may also contribute to this asymmetry in pollen migration.

The frequency of species hybrids increased with increasing altitude and the relationships in the two valleys were almost identical (Fig. 1-39). Contrary to the situation at high latitudes, the pollen migration at high altitudes was mainly from *Q.robur* to *Q. petraea.* The observation of similar diversities in low and high altitude populations was also attributed to increased species hybridization at high altitudes. The species hybridization may also be beneficial in case of global warming by the resultant increase of diversity.

Finally, it was noted that vegetative reproduction played a role at high altitudes.

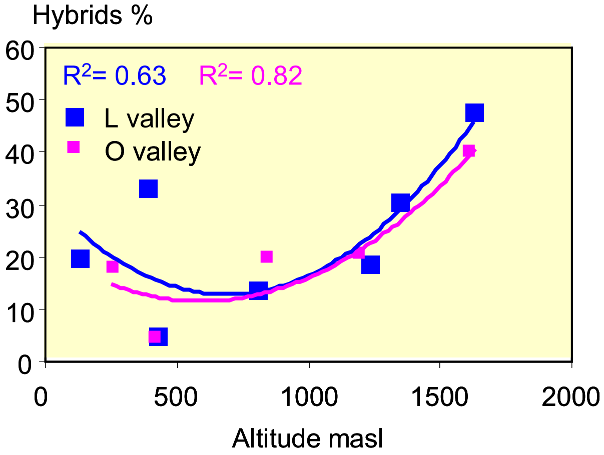


Figure 1-39. The percentage of Q. petraea *hybrids in two valleys in The Pyrenees. L = Luz valley, O = Ossau valley. Alberto et al. 2010.*

In the paper from 2011 (Alberto et al. 2011) germination and leaf unfolding were assessed in a nursery at Pierroton and in a field trial at Toulenne in south western France. Five populations from each of two valleys, Luz and Toulenne, were represented by a varying number of open-pollinated families. Leaf unfolding was assessed during three seasons, once in Pierroton and twice in field.

The Q_{ST} estimates for germination was 0.28 and the Q_{ST}s for leaf unfolding varied in the range 0.16-0.23. The heritabilities for the corresponding traits were 0.51 and 0.87-1.07, thus once surpassing the permitted level of 1.00. The heritability estimates for the individual altitude populations varied still more. It was stated that the family x block interaction was non-significant. A grouping of the material into low-altitude and high altitude populations resulted in lower heritability estimates for the high-altitude group than for the low-altitude group. It might be speculated that maternal effects contribute to the high estimates of heritability since the weight of acorns from high altitudes was much lower than the weights of acorns from low altitudes. However, high heritabilities were also obtained in the separate analyses of the different altitudinal groups. After effects were ruled out as explanation for the high heritabilities obtained since the estimates were of similar size whether obtained from the progeny trials or parent-offspring relationships. The large variation within populations was thoroughly discussed. It is obvious that there is a disruptive selection among the populations from different altitudes. Simultaneously, there may be changes in direction of natural selection owing to yearly changes in weather conditions. Both phenomena contribute to strong variation among populations as well as within populations. In addition to this, gene flow among populations will further increase within-population variation.

The leaf unfolding had been assessed in the female trees

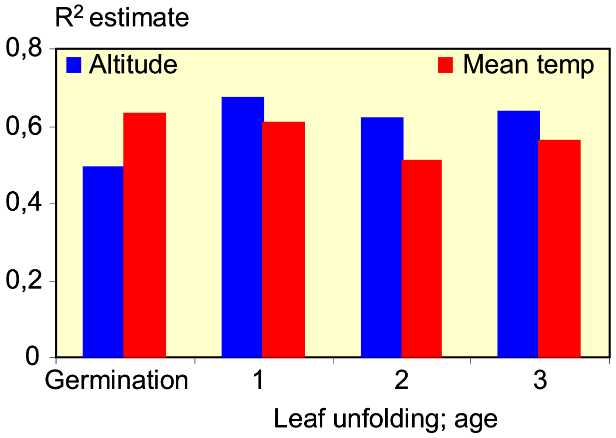


Figure 1-40. R^2 *estimates for the relationships between germination and* Q. petraea *population altitude or spring mean temperature at population origin. The corresponding estimates for leaf unfolding during three consecutive years. The populations originated from two altitude transects in two valleys in The Pyrenees. Alberto et al. 2011.*

included in this study. Heritability estimates were also obtained from the parent offspring relationships based on standardized values. (How this standardization was carried out was not fully explained.) Thus obtained heritabilities were all >0.90. However, it should be noted that the R_2 estimates did not exceed 0.30 in any case.

Strong relationships between population altitude and the traits were noted for the pooled material from the two valleys (Fig. 1-40). I made a separate analysis of the relationship between population altitude and leaf unfolding date for the two valleys (Fig. 1-41). This resulted in extremely strong relationships in both valleys. It was suggested that the late flushing of high altitude populations was an adaptation to avoidance of late spring frosts.

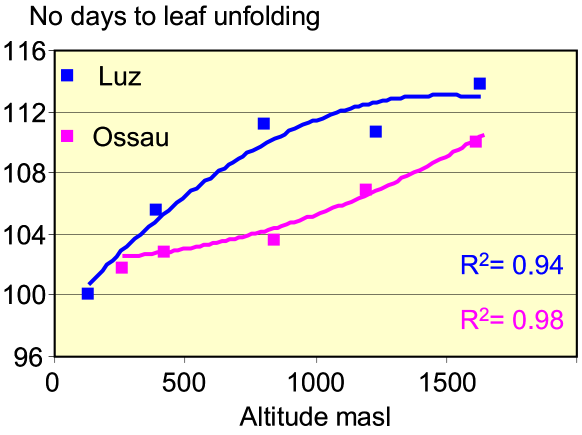


Figure 1-41. Leaf unfolding in a nursery in south western France of Q. petraea *populations from different altitudes in two valleys in The Pyrenees, Luz valley and Ossau valley. Days are counted from January 1st. Alberto et al. 2011.*

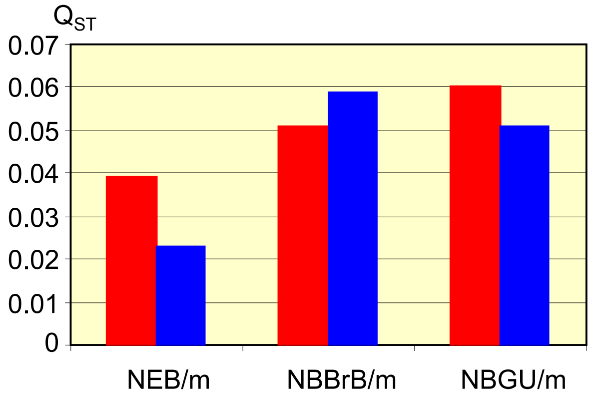


Figure 1-42. Q_{ST} estimates of total number of epicormic buds per meter (NEB/m), number of buds at branch base per meter (NBBrB/m), and number of buds at growth unit limits NBGU/m of five French , one Polish, and one Turkish population of Quercus petraea *studied in two French trials at Lat. 48.09 (red) and 48.99°N (blue). Collin et al. 2010.*

Tree heights at age 10 in seven populations growing in two French provenance trials were reported by Colin et al. (2010). This study focused on the influence of growth components on epicormics and no statistical evaluation of population differences in tree height was presented. Epicormics may cause serious damage to the trunk quality and has previously not been studied genetically. Three different traits related to epicormics were studied (Fig. 1-42):

total number of epicormic buds per meter (NEB/m)
number of buds at branch base per meter (NBBrB/m)
number of buds at growth unit (= distance between annual shoots) limits NBGU/m

As seen from Fig. 1-42 the differentiation among populations estimated as Q_{ST}s were rather limited. This suggests that these traits are of limited significance for adaptedness. Growth conditions had the greatest impact on epicormics.

The mean heights at the two test localities were almost identical, 319 and 318 cm. The data presented in Fig. 1-43 suggest that there might be a GxE interaction for tree height.

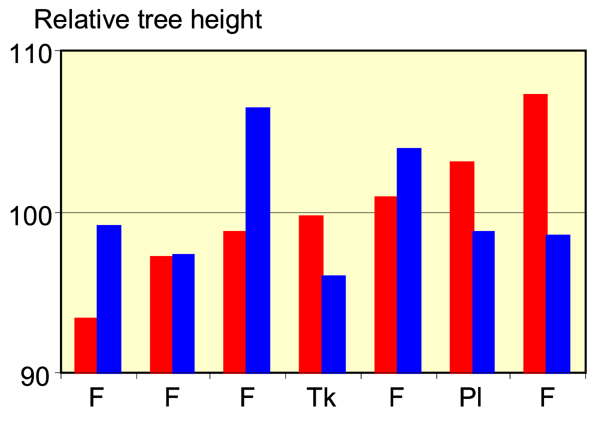


Figure 1-43. The relative tree height at age 10 of five French (F), one Polish (Pl), and one Turkish (Tk) population of Quercus petraea *studied in two French trials at Lat. 48.09 (red) and 48.99°N (blue). Collin et al. 2010.*

SNPs (single nucleotide polymorphism) of 106 candidate genes for bud flushing were studied in 758 trees in 32 autochtonous populations in two gradients in Western Europe (Alberto et al. 2013). One gradient was altitudinal, the other was latitudinal. The two valleys in the northern part of The Pyrenees that were included in previous studies were also included in this study; altitudinal range 131-1630 masl. The latitudinal range was 43.12-53.62°N. Open-pollinated progenies from individual trees were sown for establishment of one combined provenance and progeny test with 150 families. The trial was established in south-western France. Two additional populations with known performances were also included in this trial. Leaf unfolding was assessed at age 2 in the nursery and at ages 3-4 in the field trial. Based on bud flushing assessed at age 3 in a provenance test at Petite Charnie, 21 populations were selected for the latitudinal part of this study. Assessment of bud flushing was done at age 3 at one occasion. The data from the combined provenance and progeny trial were used to estimate the breeding values of the parental trees by use of BLUP (best linear unbiased predictor). These values were included in the search for SNP association with bud flushing.

The number of polymorphic SNPs that could be used for the latitudinal gradient was 157, of which six were insertion-deletions (INDs). A slightly larger number, 161, including the six INDs could be used for the altitudinal gradient. In addition to these SNPs 14 SNPS of the *GALA* (galactinol synthase) locus were included in the analyses. It should be noted that all SNPs do not involve changes in protein sequences.

F_{ST} estimates for SNPs were low in both gradients, ≈0.02. The pairwise F_{ST}s differed in the two Pyrenean valleys with the lowest estimate being 0.003 and the highest being 0.034. One of the latitudinal populations deviated from the other populations with a high F_{ST}, 0.050. These F_{ST} estimates are of the same size as obtained for isozymes. Such a low level of differentiation of the SNPs suggests that selection had not influenced the differentiation of them much.

Non-neutral pattern of differentiation (= outliers) was found for ten SNPs in the altitudinal gradient. The corresponding figure for the latitudinal gradient was 18 SNPs. Two SNPs were outliers in both gradients. One of them showed directional selection in both gradients while the other showed directional selection in the altitudinal gradient and balancing selection in the latitudinal gradient. In summary 15 SNPs showed directional selection and three showed balanced selection. It was noted that most of the outliers were related to responses to stress, biotic or abiotic.

The relationships between SNP allele frequency and geographic variables or climatic variables (spring and annual temperatures, spring and annual precipitation)

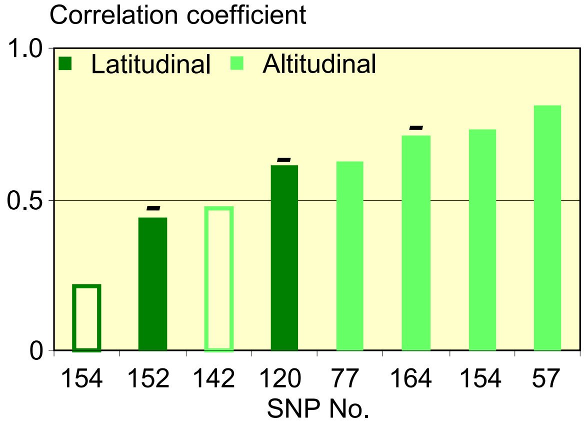


Figure 1-44. The correlation coefficients between SNP allele frequency and latitude or altitude for 8 SNPs. - = negative correlation, empty columns = non-significant correlation. Alberto et al. 2013.

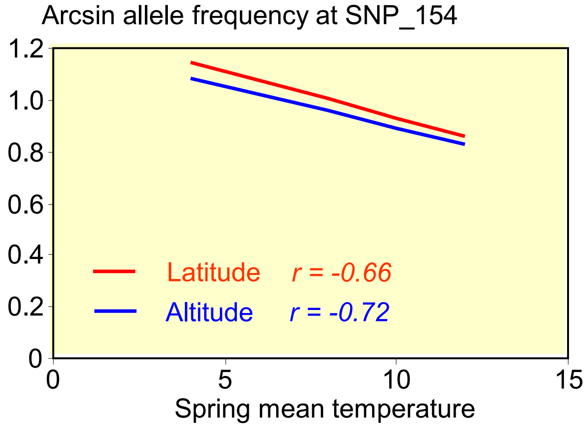


Figure 1-45. The relationship between spring mean temperature at population origin and the arcsine transformed allele frequency at SNP_154 for an altitudinal and one latitudinal gradient in Western Europe. Alberto et al. 2013.

were tested. As seen from Fig. 1-44 there were some significant relationships with geographic data both for the altitudinal material and the latitudinal material. Half of the relationships with climatic variables were non-significant. There was not much agreement between the two gradients although there is a similar temperature variation in altitudinal and latitudinal gradients. It was speculated that different environmental factors might have influenced the selection in the two gradients. Differences in genetic background were also suggested as an explanation for the lack of agreement between the two gradients. An agreement between the results from geographic variables and spring temperature would a priori be expected since bud flushing is probably linked to prevailing temperatures. In six out of eight cases there was an agreement as regards the significances. Fig. 1-45 reveals the strong agreement between the relationships between SNP_154 allele frequency and spring mean temperatures both for the altitudinal and latitudinal gradients. In contrast to these two significant relationships, the relationship with geographic data from the latitudinal gradient was weak and non-significant, r = 0.21. This difference between latitude and spring temperature was unexpected.

The test for association between SNPs and bud flushing data resulted in 14 significant associations; of them ten included the population or breeding values for bud flushing. Two SNPs, 98 and 112, turned out to be significant at all three years of assessment (Fig. 1-46). Only SNPs 114, 134, and 146 were involved in a change of protein sequence. SNP_112 originates from the *GST* gene. Proteins from this gene are involved in many processes in the cell; among others phytohormones. Thus, there is a connection to bud flushing.

In conclusion much genetic information was gained in this study but no conclusive information was obtained on the relationships of the molecular markers and the adap-

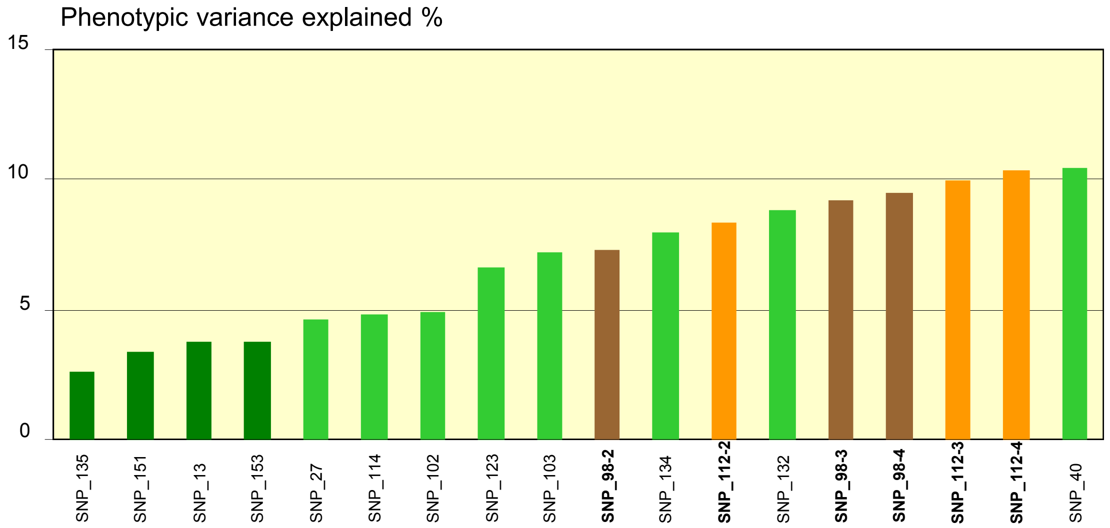


Figure 1-46. The percentage explanation of the phenotypic variance for bud flushing of individual SNPs in latitudinal and altitudinal gradients in Western and Central Europe. Dark green columns refer to the latitudinal gradient. SNPs detected at more than one age are shown in bold followed by age of detection; 2, 3, or 4. Alberto et al. 2013.

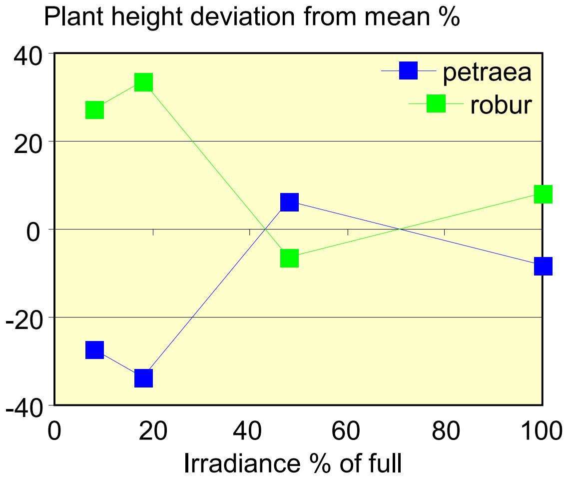


Figure 1-47. Relative plant height at age 2 of seedlings from one population of each of Q. petraea and Q. robur *originating from latitude 48.40°N and longitude 10.55°E in France. Seedlings were exposed to four different light regimes. Ponton et al. 2002.*

tively important trait bud flushing. There was a lengthy discussion of possible selection events that could explain the results without coming to a final conclusion. The polygenic inheritance of bud flushing date was suggested as one explanation for the meagre result. Difference in selection at the population and among-population levels was another suggestion. However, the low F_{ST} estimates for SNP variation suggest a minor role of natural selection for the observed differences. If the quantitative genetics theory of equal effects of many alleles in different loci is correct we may not even expect to find strong relationships between such a trait as bud flushing and any markers since a specific phenotype might have its origin in several different genotypes.

A brief report by Mather et al. (1993) with data from a provenance trial in Germany was presented as regards vessel area in wood of old trees of five populations of *Q. petraea* and *Q. robur* . As far as I can understand there was one replication at each of two sites. There was no species effect but population and population x locality were significant. This paper contains estimates of heritability but they were based on data from an unreplicated progeny trial. For this reason no results will be presented from this trial.

A study of one population of each of *Q. petraea* and *Q. robur* with a strong focus on physiology was reported by Ponton et al. (2002). The populations originated from two adjacent stands in north-eastern France at latitudes 48.60ºN and longitude 10.95ºE. Assimilation rate, stomatal conductance, δC^{13}, nitrogen content, and growth traits were assessed up to an age of two years with four light and two irrigation treatments.
Seedling heights at end of growth period 2 (GP2) are illustrated for the four light treatments in Fig. 1-47 as deviations from treatment means. Except for the 48% irradiance treatment the *Q. robur* population was tallest. In contrast to this, total biomass at the end of GP2 was higher in *Q. robur* in all treatments. This difference was attributed to the larger weight of the *Q. robur* acorns, 3.2 g versus 1.6 g for *Q. petraea*. It was noted that the difference between the species as regards growth traits dropped during the second growth period. Water use efficiency estimated by δC^{13} increased with increasing light intensity and most in *Q. petraea*. There were significant differences between the two species for stems and shoots but not for leaves.
There was a difference in net carbon assimilation but not in stomatal conductance. The species differed as regards stomatal conductance in the irrigation treatment.
It was suggested that the lower drought tolerance of *Q. robur* could be attributed to its lower water use efficiency noted in this study as well as in other reports.

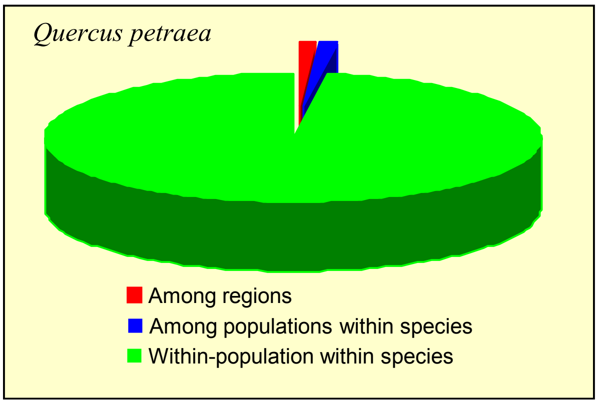


Figure 1-48. The separation of variation among regions, among populations, and within populations based on 11 isozyme loci. The study comprised 32 populations from western Europe, mainly French populations. Kremer et al. 1991

1.2 Associations between different types of trait

Kremer et al. (1991) studied eleven polymorphic isozyme loci to estimate the variation in 32 *Q. petraea* Western European populations; mainly of French origin. The variation was separated in the following way:
among regions,
among populations within regions,
among trees within populations
Almost 98% of the variation occurred within populations while there was an equal share of the remaining variation among regions and populations within regions (Fig. 1-48). The large within-population variation indicates that there was a strong gene flow among populations. Even if the among-population variation of isozymes was limited there was a geographic pattern. It might be added that the level of isozyme diversity in the two oak species is among the highest ever reported for wind pollinated tree species.
Kremer et al. also reported on variation of three cpDNA

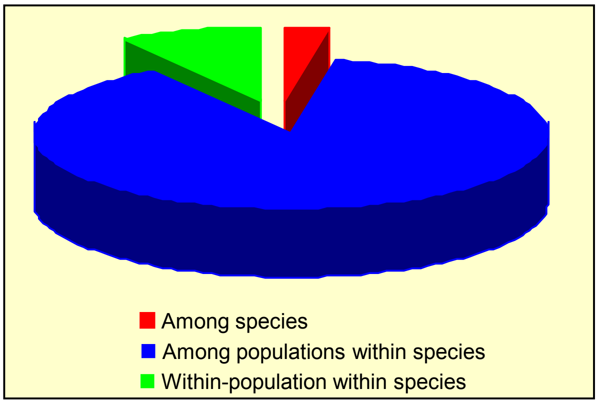


Figure 1-49. The separation of variation among regions, among populations, and within populations based on three haplotypes. The study comprised 32 populations from western Europe, mainly French populations. Kremer et al. 1991.

haplotypes in 27 *Q. petraea* and 24 *Q. robur* populations. The separation of variation based on cpDNA haplotypes shows a totally different picture (Fig. 1-49). For the haplotypes the among-population variation is responsible for almost 90% of the variation. The difference between nuclear (isozymes) variation and organelle variation was attributed to their different modes of inheritance. The latter are maternally inherited while there is a paternal contribution to the variation of isozymes. It should be remembered that the high degree of monomorphism with different haplotypes being monomorphic in different populations contributes strongly to large among-population variation for haplotypes.

Kleinschmit et al. (1995) compared morphological and molecular variation between *Q. petraea* and *Q. robur* in two stands, one in Germany and the other in France. Seven RAPD primers and ten isozyme loci were used as molecular markers. In the two stands 111 and 113 trees were assessed.

One prime objective of the study was to test whether any of the traits could be used to identify species hybrids in young seedlings. It was noted that the leaf morphology of leaves from seedlings differ from adult trees, which makes identification of individual hybrids problematic. As regards the molecular traits it was noted that the hybrid offspring resembled the females in the crosses. For 18 leaf morphology traits two distinct groups were observed, the first group consisted of progenies with *Q petraea* as female and the second with *Q. robur* as female. The mean distance between these two groups was estimated at 0.45.

It was pointed out that the technique for analysis of RAPD is very sensitive that calls for strict handling of the laboratory work. Eight of the RAPD fragments showed Mendelian inheritance. None of the fragments was species-specific. Discriminant scores were used for classification of the morphology traits in the German stand. The scores had a continuous distribution with two peaks, one with mainly *Q. petraea* trees and the other with mainly *Q. robur* trees. Hybrids occurred between the two peaks but were also parts of the two peaks. An estimated 55% of the morphology was common to the two groups. It was stressed that *no objective criterion exists for drawing limits between "pure species" and intermediate forms*. Since the RAPDs are dominant the authors preferred to refer to phenotypic difference between the offspring from *Q. petraea* and *Q. robur* females, which were estimated at 0.22. The total phenotypic differentiation in the *petraea* group was somewhat larger than in the *robur* group, 0.40 and 0.33, respectively. The corresponding figures for the ten isozymes studied were 0.28 and 0.25. The estimated distance between the *petraea* and *robur* groups was 14%. It was noted that the morphology traits that showed the largest difference between the two species types were the same in the German and French stand. However, different traits discriminated most strongly the difference between the German and French stands. The estimates of the phenotypic distances for RAPDs are illustrated in Fig. 1-50. The difference between *Q. robur* in the two stands is the smallest of all differences. The large difference between French *Q. robur* and German *Q. petraea* was expected while the largest difference, *petraea* and *robur* in the French stand, was less expected. The difference between the two *petraea* populations was also fairly large, 0.25. It was suggested that the 38 trees included in the French stand represented trees which morphologically were typically for the respective species. Thus, the selection of trees for analysis should be responsible for the results of

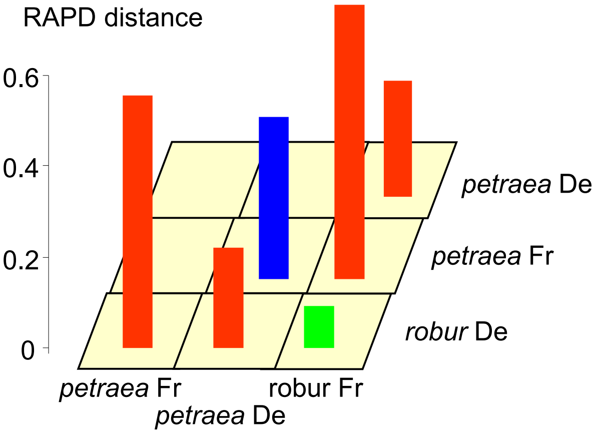


Figure 1-50. Eight RAPDs were used for estimation of all pairwise distances between Q.petraea *and* Q. robur *within two stands , one in Germany (De) and one in France (Fr) as well as differences between the stands. Kleinschmit et al. 1995.*

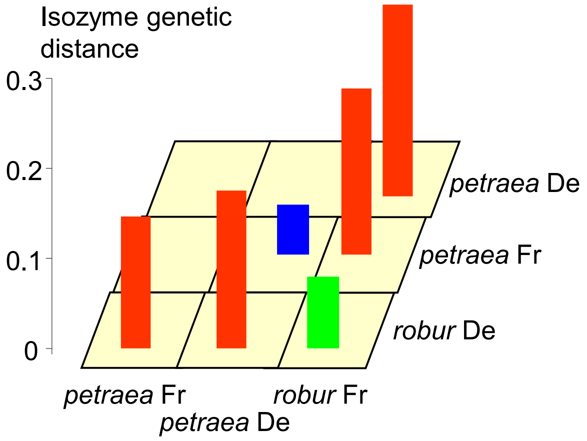


Figure 1-51. Eight isozymes were used for estimation of all pairwise distances between Q.petraea *and* Q. robur *within two stands, one in Germany (De) and one in France (Fr) as well as differences between the stands. Kleinschmit et al. 1995.*

the RAPD analysis.
The distances based on ten isozyme loci gave a partly different pattern (Fig. 1-51). The distances within species were smaller than all other distances in this case. The isozyme distances agreed better with morphology distances, r = 0.71 than the RAPD distances, r = 0.37. Based on this study, the authors preferred to regard *Q. petraea* and *Q. robur* as ecotypes of one species rather than two separate species since there is a continuous transition of traits between the two species. With such a continuous transition the variation is rather ecoclinal than ecotypic.

The differentiation in tree height and bud flushing (Q_{ST}) in 21 *Q. petraea* populations was estimated assuming different heritabilities (Kremer et al. 1997). The differentiation in these two quantitative traits was compared with F_{ST} for isozymes in the same populations plus another 60 populations from a broader range of origin (Fig. 1-52). Data from the 81 populations have been used for a series of analyses as is presented later in this chapter. There is a clear difference in differentiation as revealed by isozymes (F_{ST} = 2.4%) and the two quantitative traits for the normal range of heritabilities (Q_{ST} = 0.19-0.48). The low differentiation estimate for isozymes indicates a substantial gene flow among the *Q. petraea* populations. The authors also derived formulae to estimate multitrait Q_{ST}s with or without correlation of the two traits (Fig. 1-53). Different combinations of heritabilities were calculated. The estimates of differentiation is slightly higher when the traits are uncorrelated than when they are correlated, which agrees with the expectation.

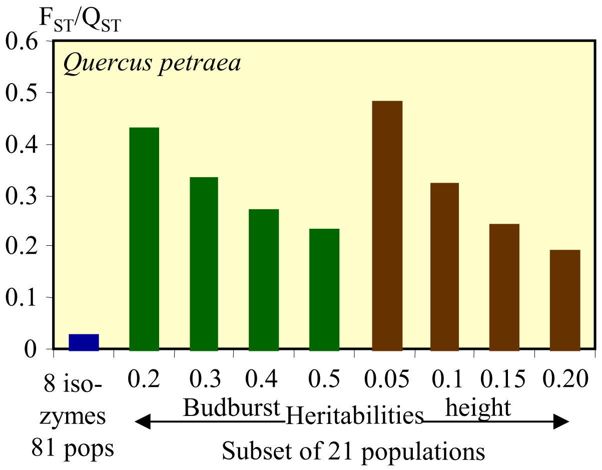


Figure Kremer1-52. Isozyme F_{ST}s for 81 Q. petraea *populations from a large part of the natural range of distribution and Q_{ST}s for bud flushing and tree height for different heritabilities of these two traits in a subset of Western European populations. Kremer et al. 1997.*

Principal component analyses were applied for a study of species differentiation of Q. petraea and *Q. robur* in pure and mixed populations, mainly from Germany (Hertel and Degen 2000). Leaf traits and isozyme loci were used for the analyses that also included published data. The three isozyme loci, *PGM-A, ACP-C,* and *IDH-B*, showed the largest differentiation between the two species. Therefore, the allele frequencies in these three loci were related to the observed variation in leaf traits. An astonishing strong agreement between the genetic and morphological data was noted. Thus, as an average for all populations 85% of all trees were classified in the same way with the two methods. A North-Rhine Westphalia population constituted an exception, with a moderate correspondence between the two methods, 49%. It was concluded that there was a close relationship between the two methods. This would allow a good species classification of young plants that usually do not differ morphologically to the same extent as adult trees. The findings in this investigation differ from most published results. The selection of

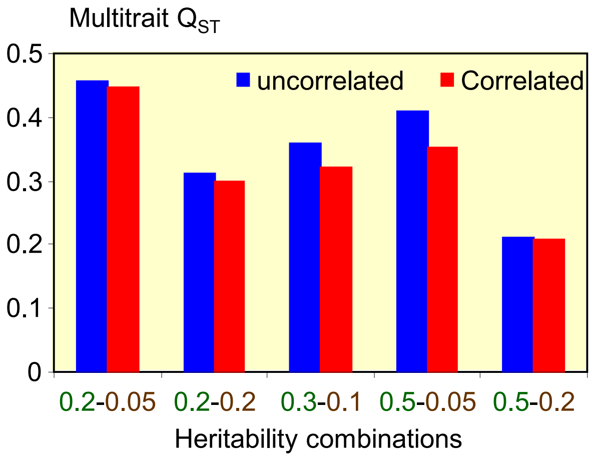


Figure 1-53. Multitrait Q_{ST}s for two traits combined with different heritabilities of the two traits, correlated or uncorrelated. The correlation coefficients are used to reflect the range of heritabilities shown for bud flushing and tree height. Kremer et al. 1997.

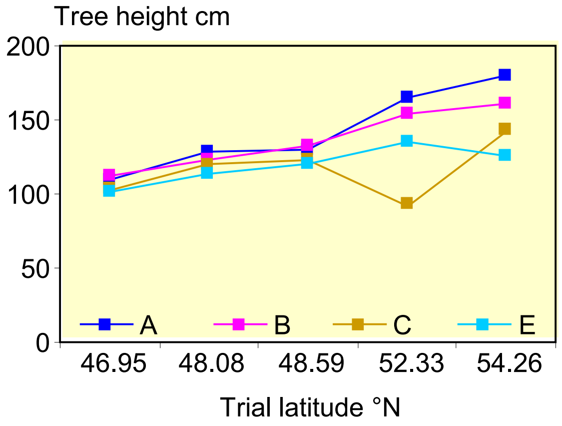


Figure 1-54. The mean height at age 7 or 8 of four haplotype lineages in three French (Lat. 46.95 – 48.59, age 7) and two British (Lat. 52.33 – 54.26 age 8) provenance trials with Q. petraea. *A = Balkan refugia B = Iberian refugia, C = Italian refugia, E = eastern origin. Latitudes of the trials are given. Kremer et al 2002b.*

isozyme loci included in the study might be one possible explanation for the discrepancies between this investigation and former ones. It was also suggested that the principal component analysis applied was very sensitive for detection of differences. It is hard to imagine that natural selection would influence allele frequencies in individual loci when most results suggest quantitative inheritance of fitness traits.

Tree height, breast height diameter, volume, trunk form, bud flushing, leaf retention in winter, damage, and survival were assessed in 16 provenance trials in France, Germany and Great Britain (Kremer et al. 2002b). One trial consisted of *Q. petraea* and *Q. robur* populations while all others contained only *Q. petraea* populations. In all 62 quantitative traits were included in the comparison with markers belonging to four lineages of haplotypes, lineages A, B, C, and E. Most assessments were carried out at age eight. Besides, eight isozyme loci with 18 alleles and 31 RAPD loci with 62 alleles were related to the haplotype lineages. A chloroplast genetic distance, CGD, *based on maternal lineages was calculated for all pairs of provenances within a provenance test. CGD was defined as the number of restriction fragments polymorphisms separating the two populations.*" A differentiation index, DI, *was computed*

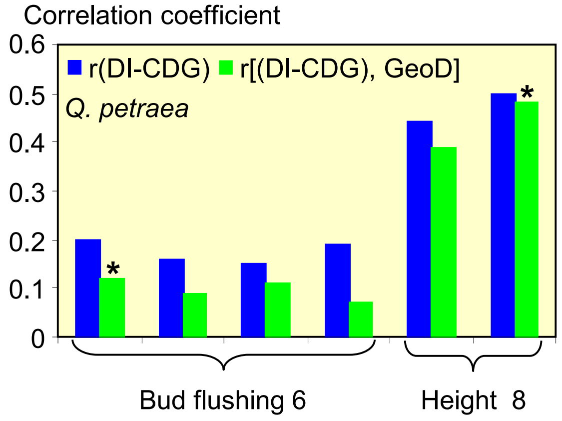


Figure 1-55. The correlation coefficients for significant relationships between differentiation index (DI) and chloroplast genetic distance (CGD blue) and the same relationships after correction for geographic origin of haplotypes (green). The two significant relationships after the correction are indicated. Kremer et al 2002b.

between all pairs of populations as the absolute value of the difference between mean values of phenotypic traits of two provenances." For each test locality matrices were constructed for the 13 haplotypes identified and for the phenotypic traits. Product moment correlations between CGDs and DIs were calculated. Besides, DGIs were corrected for geographic origin of the populations and thus corrected CGDs were correlated with DIs.

I have preferred to illustrate the difference in height of the four lineages occurring in the five trials that showed significant differences (Fig. 1-54). As seen from this figure the differences among the lineages were limited in the three French trials, latitudes 46.95-48.59, while more pronounced differences were noted in the two British trials. Leaf retention and survival showed significant differences in one trial each. Six of the 62 correlations between CGD and DI were significant. As seen from Fig. 1-55 the correlation coefficients were low for bud flushing and moderately high for tree height. When correction for geographic origin were included only two of the relationships remained significant. There were 13 significant correlations between quantitative traits and geographic variables. As expected the haplotypes do not have any great impact on quantitative traits while their geographic origin plays a greater role for the quantitative trait performances.

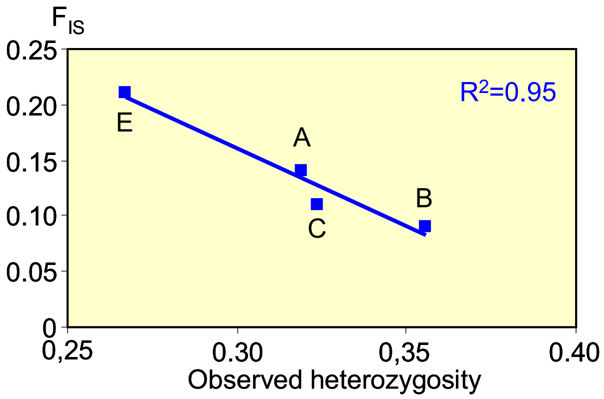


Figure 1-56. The relationship between observed heterozygosity in isozyme loci and the fixation index of four haplotype lineages. Q. petraea*. Kremer et al 2002.*

There were significant differences among lineages as regards, observed heterozygosity of isozymes, expected heterozygosity, and fixation indices while there was no significance for number of alleles per population. Similarly, there was no significant difference among the lines as regards the expected heterozygosity per population for RAPDs. There was a negative relationship between observed heterozygosity of isozymes and fixation index (Fig. 1-56). As for the quantitative traits, genetic diversity was also estimated for isozymes and RAPDs. For both types of marker there were significant relationships with CGD. However, the correlation coefficient for isozymes was only 0.08 and for RAPDs 0.28. Thus, in both cases a small part of the variation was explained by the relationships.

The authors presented a scenario for the absence of any association between phenotypic traits and haplotypes in four steps:

1. At the end of glaciation there were three main refugia with different haplotypes and different nuclear genes.
2. The haplotype differentiation remained even after that migration had taken place.
3. Once oak stands were established in central and northern Europe gene flow via pollen became an important evolutionary factor. Such a gene flow levelled to a great extent the allele frequencies among populations.
4. Natural selection at the local and regional level created considerable among-population variation for traits contributing to fitness while haplotypes were not affected by any selection.

I find this scenario highly likely.

Nuclear and plastid microsatellite and morphological variation in five Italian populations were studied by Bruschi et al. (2003). Three populations were classified as central Italian, one northern Apennines, and the fifth population originated from Sicily. Except for the latter population the others were mixed *Q. petraea* and *Q. pubescens* populations. Six microsatellite nuclear loci, three plastid microsatellite loci, and 36 morphological traits were studied. The among-population differentiation (R_{ST}) for nuclear microsatellites was estimated at 0.18 while the corresponding figure for plastid microsatellites was 0.39. Eight of the latter were population-specific. The Sicilian population had the lowest genetic diversity. It might be speculated that this could be attributed to its being a non-mixed and small population exposed to genetic drift or to its marginal status. It was stressed that the limited plastid diversity indicated an increased risk for extinction. This may be true if the plastid diversity is connected with adaptability in this population, which it is hardly expected to be. The relationship between geographic and genetic distance estimated by nuclear microsatellites was significant, r = 0.53. Contrary to this, the corresponding relationship with plastid microsatellites was non-significant. However, it is somewhat problematic to estimate these relationships with one population 6-7 degrees of latitude south of the other four populations which in turn occurred in a latitudinal range of 1.5 degrees.

An unmanaged Danish stand of *Q. petraea* with 339 trees was used for a study of spatial autocorrelation by aid of six microsatellite loci (Jensen et al. 2003). Additional information was gathered from three *Q. robur* and four *Q. petraea* stands. Nine morphological characters were used for discrimination of the two species.

Differences in basal leaf shape, number of leaf veins, hairiness, and petiole length showed the largest discrimination between the two species while other characters contributed marginally to the discrimination. For seven of the morphological characters the trees in the study stand were close to the four *Q. petraea* reference stands. Strangely enough, petiole length and hairiness that used to be good discriminators between the two oak species showed a large variation. No definite explanation for this deviation in this study could be given but hybridization might be one among several possible explanations.

There was no correlation between any of the nine morphological characters and microsatellite variation. The F_{IS} estimates for the six microsatellites varied between -0.033 and +0.018. Thus, inbreeding was limited in this stand. Moran's index based on individual microsatellites showed significant correlation up to 24 meters. A larger estimate of this index and a stronger significance was noted when data were combined from all six microsatellites. However, no figure for this estimate was given. The low estimates were explained by the limited dispersal of the heavy acorns of oaks. After a random selection of different numbers of trees for the spatial correlation it turned out that the larger the sample size the stronger the spatial correlation. This calls for large sample sizes in studies of autocorrelation. A comparison of two size classes of the trees resulted in somewhat larger spatial autocorrelation for the small trees (DBH <40 cm). It was speculated that selection may continue to reduce the number of young (small) trees leading to a levelling of the spatial pattern between the two size classes.

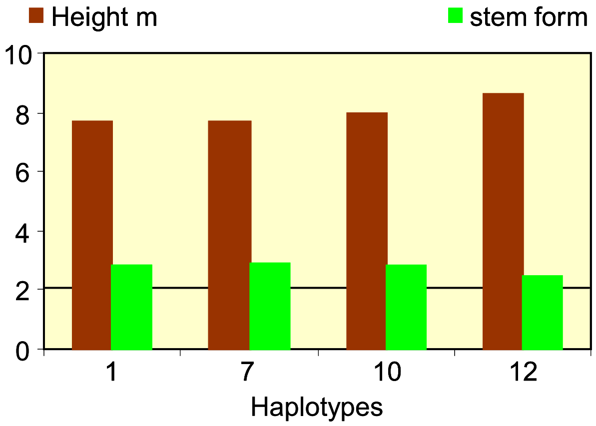


Figure 1-57. The association between haplotype and two quantitative traits, height at age 24 and stem straightness; "2 straight one plane, 3 not straight any plane". Jensen et al 2002.

In the study of 91 Nordic populations the 22 populations from the Baltic States stood out with a G_{ST} of 0.98 (Jensen et al. 2002) while G_{ST} for all 91 studied populations was estimated at 0.87. Most autochtonous populations were monomorphic, 77%, while planted stands had a lower degree of monomorphism, 54%. Several of the trees possessing haplotypes 10-12 in Denmark and Sweden were suspected to be of non-domestic origin.

Four haplotypes (1, 7, 10, and 12) of monomorphic populations (totally 37) in two field trials were associated with four quantitative traits, tree height, epicormics and stem form at 24 years, and bud flushing at age 14. For tree height and straightness there were significant correlations with haplotype in one of the trials containing 37 populations. The tallest trees had haplotype 12 while the shortest trees contained haplotype 1 (Fig. 1-57). The difference between the two was approximately one meter, 8.63 and 7.67 m, respectively. The haplotype 12 trees had also the best stem form. In the other field trial containing fewer populations (17) there was no association between haplotype and any of the four quantitative traits. It was suspected that the superior growth of haplotype 12 populations of Dutch and German origin might be attributed to a northwards transfer effect thanks to a longer growing season in Denmark. It is unlikely that one specific haplotype would confer higher fitness to its carriers than another haplotype.

An attempt to relate bud flushing to chloroplast markers was done by Gailing et al. (2004). They followed flushing in five German stands of *Q. robur* during three or four years. In addition data, were collected from additional stands in Germany but the focus was on the five stands mentioned. Flushing was scored in five classes. Four different haplotypes were identified. I have tried to summarize the essence of this paper in Fig. 1-58. This figure reveals that the populations kept the sequence in flushing over the three years of observation. It is also evident that there was a large difference in chloroplast association between the two early flushers and the two late flushers. The two latter populations were Croatian introductions to Germany during the late 18 hundreds. It was stated in free translation from German that these markers would enable a separation of early and late flushing genetic entries already in acorns or young seedlings. This is a too far-reaching statement based on only five populations. The haplotypes more likely reflect the origin of the populations rather than their specificity as regards flushing.

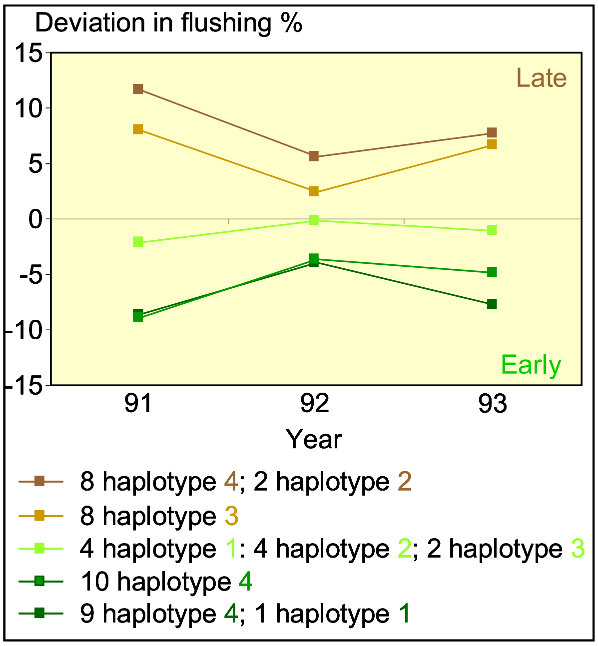


Figure 1-58. The percentage deviation from annual means in flushing in five Q. robur *German populations, each with ten (eight) individuals and their chloroplast haplotypes. Gailing et al. 2004.*

Oaks from twenty-four Irish locations were analyzed with respect to leaf morphology and AFLP by Kelleher et al. (2005). In all 123 trees were included in this study, which means that a low number of trees per location were analyzed. In total 147 fragments were available for the AFLP study. There was a clear morphological difference between trees classified as *Q. petraea* and as *Q. robur*. There was no agreement between morphological and molecular variation. The hierarchical analysis of molecular variance revealed that most of the variation was within populations (63.5%) while the variation attributed to differences among populations was 38.6%. There was a negative estimate for the difference between the two species, -2.1%. This analysis clearly shows that species differentiation was minute or non-existing. It was not surprising that no species-diagnostic AFLPs were detected.

Buds representing six stages (0 = quiescent bud, 5 = full leafing out with internode growth) during bud and shoot development were collected from one-year old seedlings originating from two *Q. petraea* stands in north-eastern

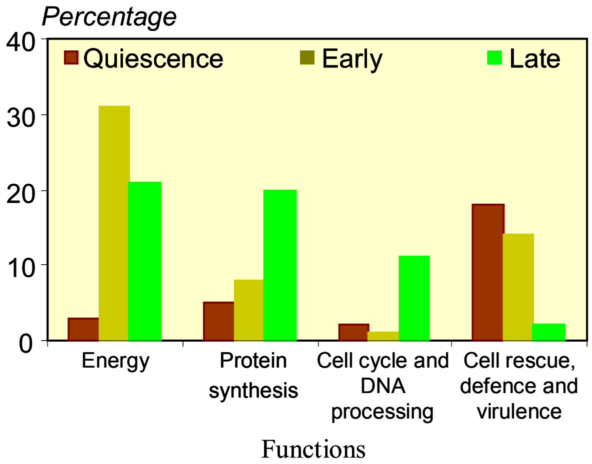


Figure 1-59. The percentage distribution of transcript to the four functions with highest percentages of transcripts in the quiescence, early, and late stages of bud development in Q. petraea. *Derory et al 2006.*

France for a study of transcriptome variation during the process of development (Derory et al. 2006). Different techniques were used in this study:

Suppression subtractive hybridization, SSH
High-density colony arrays
cDNA macroarrys

A combination of these three techniques means that it is possible to reduce the number of differentially expressed clones. As a corollary of this more focus can be on the remaining clones. Microscopic examination of quiescent buds was also done to secure that no mitotic activity had started.

More than 900 DNA clones were found to have at least a two-fold difference in expression between bud developmental stages. After discarding 99 of these 900, 192 quiescent, 403 early and 206 late DNA clones remained. Two hundred-thirty-three constituted unique transcripts. The functional role of each transcript was evaluated by comparison with known functions in the GenBank data base. A hierarchical clustering of the gene clones into three groups with some overlap between groups two and three could be done. All 70 genes of group I were induced during bud flushing stages 0-2 without any difference between these stages. They were up- or down-regulated during stages 3 and 4. Genes belonging to group II had different expressions in stage 0 on one hand and stages 1-2 on the other hand and they were mainly down-regulated during stages 3-4. This suggests that group II genes play a role in triggering of flushing. The group III genes were repressed during stages 0-4 and had energy-related functions. Energy, protein synthesis, cell cycle and DNA processing, cell rescue, defence and virulence were the functions with highest percentages among all functions attributed to transcripts. The pattern of these four functions varied between the three phases of bud development as seen from Fig. 1-59. To obtain a quantification of transcript accumulation a real-time PCR was used for ten of the most regulated clones. The results of this analysis may be summarised in the following way:

One strong peak of expression during stage 0; 2 cases
One strong peak of expression during stage 0 another peak at stage 4 that was a few times smaller; 4 cases
One strong peak at stage 4, lower expression at all other stages; 1 case
Strongest peaks at stages 4 and 0; 1 case
Strongest peaks at stages 0 and 4 with some expression during the rest of the stages; 2 cases

Thus, this quantification showed the possibility to identify functions that are specific to one or more stages. The authors concluded *This study has provided new insights in the understanding of gene expression during bud burst*, which is a good and concise summary of this publication. It might be added that physiological mechanisms of the different functions was thoroughly discussed.

Nine populations in four French provenance trials with differing time for bud flushing at age 3 (early, intermediate, and late) were included in a study of candidate genes for bud flushing (Derory et al. 2010). The populations represented a latitudinal and longitudinal span of 47.41-53.62°N and 2.33-16.57°E, respectively. They were classified as northern, central, or southern. The bud flushing had six classes from quiescent bud to fully developed leaves and elongated shoot. Q_{ST} for bud flushing was estimated earlier at a rather large value, 0.55. The candidate genes *were selected according to differential expression of ESTs before and after bud flush as assessed by cDNA-macroarray experiments and real-time reverse transcriptase-PCR*. Nine loci with candidate genes were studied. In addition microsatellites and QTLs were included in the study. The number of alleles identified in the nine loci varied between three and thirty. Almost 60% of the samples analysed contained coding sequences. It was noted that the diversity of the non-coding region was twice as large as in coding regions.

Both candidate genes and microsatellites showed limited population differentiation but with a large variation among loci, which was attributed to the small sample size of nine trees per population. There was no difference in F_{ST} whether the grouping was based on geographic origin or phenological differences. However, in the *GALA* locus the phenology grouping had 15 SNPs above 0.07 while the geographic grouping only had two SNPs above 0.07. A Bayesian F_{ST}-test did not reveal any locus-specific effect of any SNP. It was a disappointment that there was no association between candidate genes and flushing performance.

Nineteen female and 19 male QTL controlling bud flushing were identified. Since all of them were not located in homologous regions more than 19 QTL were detected. The total number of QTL pooled over years and test localities amounted to 286. The majority of QTL, > 90%, contributed little to the percentage of variation explained by QTL (Fig. 1-60). However, the QTL that contributed most to the variation were located in a few linkage groups. Moreover, five candidate genes were located in

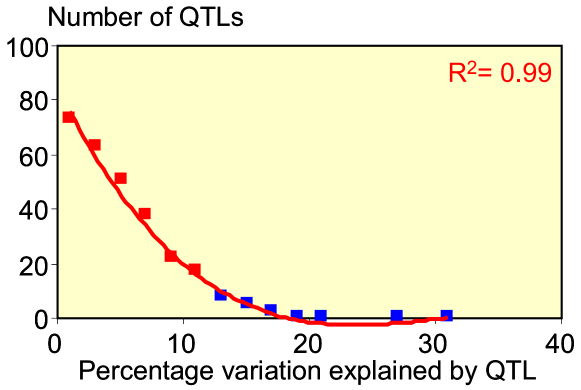


Figure 1-60. The number of QTL related to bud flushing in Q. petraea *populations from Austria, France, and Germany. The third-degree polynom with the best fit to the data is shown. Blue squares refer to QTLs explaining more than 12% to the variation. Derory et al. 2010.*

QTL regions that had the strongest impact on flushing.
A thorough discussion of the controversy of neutral variation of the candidate genes and the detection of candidate genes associated with QTL was carried out. The authors identified four reasons for discrepancy:

1. The selected candidate genes are not related to variation in bud set.
2. Imprecision of QTL mapping. It was stated that the region of a chromosome might be so large that it can house tens of loci related to bud flushing, many of them might be involved in the regulation of flushing rather than the candidate genes selected.
3. Inter-genic associations are responsible for the differentiation observed. From a theoretical point of view this result might be attributed to the multilocus nature of such a trait as bud flushing. It was argued that allelic associations (= coadaptation) rather than differences in gene frequencies should be responsible for differentiation among populations. Thus, associations should precede any change in gene frequency.
4. The genes regulating bud flushing are located outside the region explored in this investigation.

It was concluded that further studies are required to come to a final conclusion even if I find that the authors seemed to favour explanation 3.

In a mixed *Q. petraea* and *Q. robur* stand in western Switzerland leaf morphology and nuclear microsatellite variation was studied to find out whether there was any relationship between molecular and morphological variation (Gugerli et al. 2007). Fourteen leaf morphology and five microsatellite loci were studied; the latter with 15 to 53 alleles. A canonical discriminant analysis with all fourteen morphological characters resulted in seven misclassifications as regards species out the 414 trees investigated. It was suggested that these trees were outliers of the two species rather than hybrids. They were surrounded by trees of the species, to which they were molecularly assigned.
The molecular among species (*petraea, robur*, and unclassified trees) variance was estimated at 10.3% and the within species at 89.7%. At the multi-locus level an assignment test strongly supported the *a priori* grouping with less than two percent misclassifications. There was an extremely high congruence between morphological and molecular data with few putative trees in this stand of old trees. It was concluded that *These findings are taken as clear indication that gene flow between taxa has little effect on the genetic composition in the adult stage.*
According to the authors the observed results might be explained by:

Mating within species only

Limited seed dispersal

Micro-site induced selection

The latter should mainly be attributed to the different requirement of water availability of the two species. It was assumed that hybrids that may have arisen are selected against at early age.
In conclusion the results of this study strongly speak against the nuclear capture hypothesis for explanation of the genetic structure of *Q. petraea* and *Q. robur* (Petit et al. 2003).

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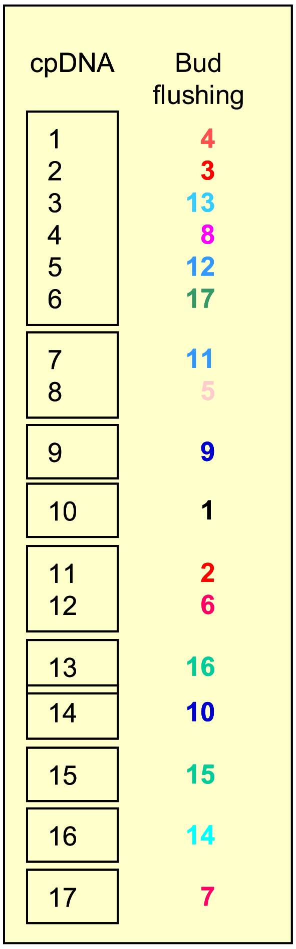

cpDNA	Bud flushing
1	4
2	3
3	13
4	8
5	12
6	17
7	11
8	5
9	9
10	1
11	2
12	6
13	16
14	10
15	15
16	14
17	7

Figure 1-61. The clusters of 17 Q. robur *populations analysed with respect to chloroplast DNA. Populations within a frame are closely related. Populations No 1 and No 17 are furthest apart. The clustering of the 17 populations with respect to bud flushing are shown with different colours. The cpDNA population 1 had position 4 in the bud flushing clustering, cpDNA population 2 had position 3, cpDNA population 3 had position 13 etc. Pliura et al. 2009.*

Pliura et al. (2009) studied chloroplast DNA and bud flushing in 28 and 17 Lithuanian populations of *Q. robur*. Bud flushing was recorded at age seven in three Lithuanian field trials. Four haplotypes were detected, 2, 5, 6 and 7. The latter being the most common.

One focal point of this investigation was the comparison between cpDNA and bud flushing variation among the 17 populations. The result of this comparison is shown in Fig. 1-61. As seen from this figure the cpDNA clustering did not correspond closely to the variation in budburst.

1.3 Reproduction

In an article by Ducousso et al. (1993) the state of art of reproduction and related issues during the early nineties was reviewed. This article dealt with available information from all species of genus *Quercus* but here I have extracted some information from the *Q. petraea* and *Q. robur*. It was stated that:

Begin of flowering varies considerably from site to site

Flowering phenology shows large annual variation

Oaks have a high potential for pollen dispersal thanks to small pollen grains with low weights

Number of migrants between populations ranged between 2.6 and 10.6, preventing differentiation between populations as regards molecular markers

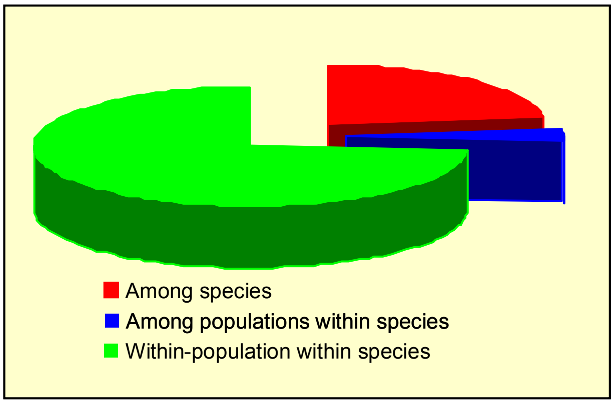

Figure 1-62. The separation of variation among species, among populations within species, and within populations within species based on nuclear and organelle markers. A summary of the state of the art in 1993. Kremer and Petit 1993.

There is a large individual tree variation in fruit set

There is a *relationship between acorn size and dispersal ability, their tolerance to parasite attacks and vigor of young seedlings*.

Dispersal by acorns was estimated at 300 m per year that approximates to seven kilometers per generation

Jays spread acorns over considerable distances

Oak species are highly self-incompatible with high outcrossing rates.

1.4 Markers

Thirteen isozyme loci were used to study the diversity within and among five German juvenile populations of *Q. petraea* and *Q. robur* (Müller-Starck and Ziehe 1991). The observed heterozygosity was 21.9 and 21.3% in *Q. petraea* and *Q. robur*, respectively. The differentiation among populations according to Gregorius and Roberds (1986) was 8.5 and 5.5% for the two species.

The state of the art as regards marker differentiation in the *Quercus* species studied so far was presented by Krémer and Petit (1993). Among-population within *Q. petraea* and *Q. robur* was very low, G_{ST} varying in the range 0.02-0.04. The species differentiation within subsections of genus *Quercus* was estimated at 23% while the corresponding estimate for within-population was 74% (Fig.1-62).

Petit et al. (1993c) analyzed 48 *Q. robur* individuals (adults and seedlings) and 24 *Q. petraea* individuals (adults and seedlings) with respect to chloroplast DNA and 29 individuals of *Q. robur* and 41 individuals with respect to ribosomal RNA. All individuals were growing in the Petite Charnie stand in northwestern France that contains both species. The authors concluded *Overall, very little differentiation was found between species using both markers*. It should be remembered that the number of markers available for study were limited during the early nineties.

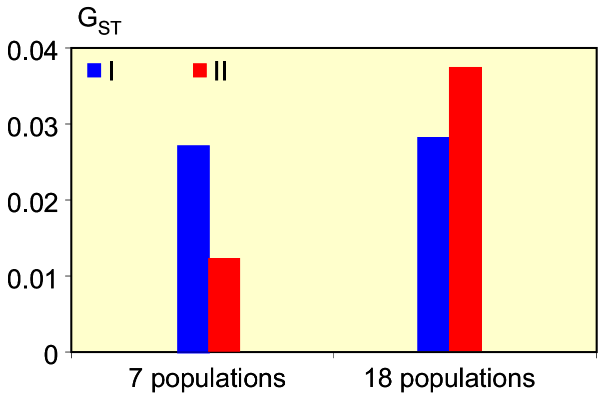

Figure 1-63. The mean G_{ST} estimates for two types of isozymes (I and II) of 18 Q. petraea *populations from Central Europe and 7 populations from the centre of distribution. Type I refers to isozymes involved in primary metabolism and II to isozymes involved in secondary metabolism. Zanetto et al. 1993.*

Petit et al. (1993a) developed a *Finite island model for organelle and nuclear genes in plants*. Some oak results were discussed in relation to the model developed. In essence it was shown that maternal and paternal inheritance of cytoplasmic genes is asymmetric even if the gene migration is of the same magnitude for these two types of organelle. In cases of larger pollen migration rate than acorn migration rate, which probably is the case for many oak species, the population differentiation will be much higher for maternally inherited genes than for paternally inherited genes. It was stated that the large differentiation observed for chloroplast genes in contrast to the differentiation based on isozymes could be attributed to the outcome of the model. However, many monomorphic populations containing different haplotypes would equally well explain the difference between nuclear and chloroplast genes.

Genetic variation among and within 18 *Q. petraea* Central European populations were studied for two types of isozymes (Zanetto et al. 1993), Three isozymes involved in primary metabolism (I) and three involved in secondary metabolism (II) were included in this study. Higher heterozygosities were noted for type II enzymes than for type I. Populations from the eastern part and southwestern part of the distribution range had lower levels of within-population variation. Separate estimate of genetic distances among populations was carried out for all 18 populations and for seven central populations (Fig. 1-63). The differentiation was larger of type II enzymes in the entire material while there was no difference between the two groups of material for type I enzymes. It was suggested that the differentiation of type 1 was a result of a balance between genetic drift and gene flow. In addition to these two evolutionary factors, it was suggested that natural selection also might be involved in differentiation of type II enzymes.

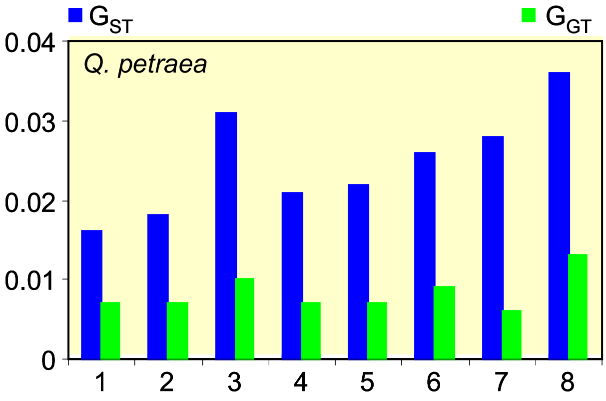

Figure 1-64. The population differentiation among 81 Q. petraea *populations, G_{ST}, and the differentiation among 14 regions in Europe, G_{GT}, for eight isozymes. 1-3 are isozymes involved in primary metabolism while 4-8 are involved in secondary metabolism. Zanetto and Kremer 1995.*

The above study was extended to comprise 81 *Q. petraea* populations with a broader origin, from southern France to Norway and from Ireland in Western Europe to Turkey in east (Zanetto and Kremer 1995). Eleven polymorphic loci were examined in this study.

The allelic richness and observed heterozygosity were lower for primary metabolism isozymes than for secondary metabolism ones. Significant regional differences were noted for both of these parameters and a west-east clinal variation was noted in both cases. Populations at the edges of distribution had lower within-population heterozygosity than central populations while the allelic richness showed the opposite trend. One reason for this is a higher occurrence of rare alleles in edge populations. Population bottle-necks were given as an explanation for such a pattern.

The genetic differentiation among all populations for eight isozymes studied in all populations varied in the range 0.016-0.036 (Fig. 1-64). The mean value for all eight isozymes was 0.025. In spite of the large geographical area studied the differentiation is extremely limited. Similarly, the differentiation among 14 regions in Europe (G_{GT}) was also low 0.007-0.013. Many alleles showed a clinal variation, mainly a longitudinal cline. A detailed discussion of the causes of such clines was carried out. Natural selection might be one explanation with different alleles favored along the gradient. Migration along a gradient is an alternative explanation. Natural selection should lead to different clines for different loci. Since this was not observed, this explanation was ruled out. Since seven of the eight isozymes showed concordant correlations of allele frequency and longitude, migration was the likely cause of the clinal variation in allele frequencies.

The same data were analyzed with respect to occurrence of linkage disequilibrium and its effects on gene diversity (Kremer and Zanetto 1997). All pairwise combinations of data from eight loci were tested with respect to linkage

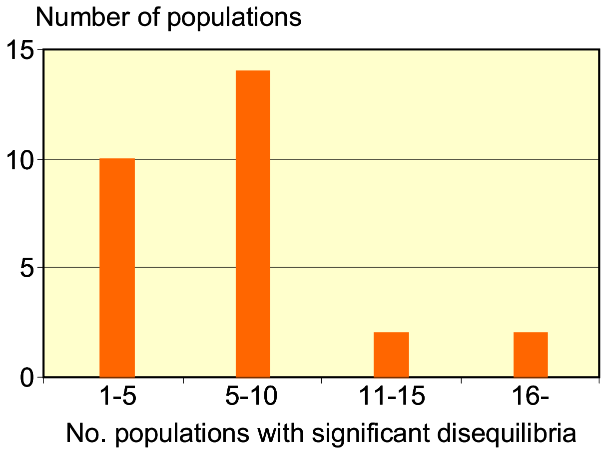

Figure 1-65. The number of populations showing varying numbers of significant disequilibria of 28 pairs of isozyme loci in Q. petraea. *Kremer and Zanetto 1996.*

disequilibrium. For each pair of isozyme loci there were at least one significant disequilibrium. The number of populations showing significant disequilibria varied considerably (Fig. 1-65). The majority of the pairs showed significances in 1-10 populations; the two in largest class deviated strongly from the rest with significances in 68 (*Mr-A/Dia-A* loci) and 80 cases (*Aap-A/Lap-A* loci). Nineteen of the 28 pairs showed significant heterogeneity among the populations. For these two loci the same allele associations occurred in all populations. It should be noted that the loci in each of these pairs are metabolically tightly linked. As regards population differences significant disequilibria were found in two populations, one French and one German. One eastern French and one Norwegian population constituted the contrast to these two populations with 7 and 9 disequilibria, respectively. Generally, populations at the margins of distribution had higher number of significant disequilibria than central populations. It was speculated that genetic drift was more important in marginal populations than in central populations, which should lead to more linkage disequilibrium. For the two loci mentioned above significant correlations with longitude were found but the coefficients explained less than 10% of the variation. A smooth clinal variation was observed indicating abundant gene flow via pollen. No significant relationships were found with latitude or altitude.

Thirteen isozyme loci were analyzed in seven pairs of populations of *Q. petraea* and *Q. robur* from similar climatic regions (Zanetto et al. 1994).
The mean number of alleles, the effective number of alleles, and gene diversity were almost identical in the two species. Generally, the isozymes involved in primary metabolism showed higher heterozygosities than secondary enzymes. For ten isozyme loci most of the variation was within populations, 94%, while among populations and between species contributed with three percent each (Fig. 1-66). The large within-population variation was attributed to substantial gene flow. There was no geographic pattern as regards interspecific differentiation. The largest differentiation was found in Serbia and Slovenia. In Serbia the two populations were from different altitudes, 80 and 450 masl, which might explain the large difference in this case. However, the Slovenian populations differed in altitude with less than 50 m. Estimates of the genetic distance of a specific population and all populations of the other species were calculated to find out whether the differentiation between the two species could be attributed to:

divergence of one species from the other
or to
common divergence.

The estimates obtained suggested that common divergence was the most likely reason for the observed differentiation.

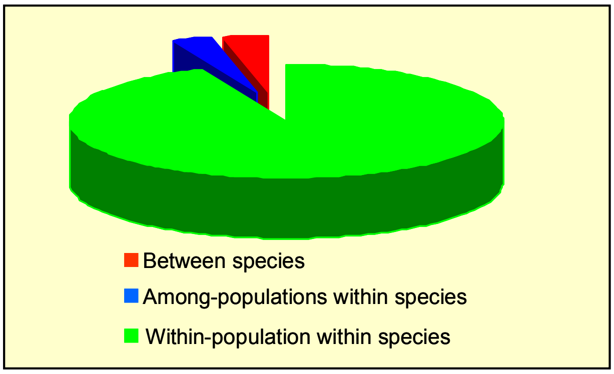

Fig. 1-66. The separation of variation within populations, among populations within species, and between species in pairwise sampled populations from seven different climatic regions in Europé. Ten isozyme loci were studied. Zanetto et al. 1994.

Still another report was based on the data from the 81 *Q. petraea* populations (Le Corre et al. 1998). Twelve additional populations from Eastern Europe were included in estimates of variograms. In a variogram it is assumed that the difference in allele frequency between two populations is due to the difference in distance between the two. Variograms can be calculated in any direction of space but in case of anisotropy, only the populations along a given direction in space are taken into account. *The variogram usually increases, then reaches a constant value called the sill. The distance at which the sill is attended, called the range, represents the distance beyond which two values may be considered as independent.* The variogram may take different shapes, exponential, Gaussian, or spherical. If the variogram is exponential it means that a range was reached while a linear shape means that no limit was found for the material under study. Flat variograms characterize random patterns of variation.
Eleven alleles with allele frequencies in the intermediate range were included in the estimates of variograms. Four of these alleles had exponential shapes with ranges varying between 565 and 995 kilometers with small

standard errors. Thus, the ranges are extremely wide. The variograms of the other seven alleles fitted better to a linear model. For five of these seven alleles there was a weak clinal variation, but random variation was suggested for them. A separate analysis of the western populations only did not give any dramatically different results. As disclosed in previous papers the east-west cline was observed for most of the alleles for variograms. Two alleles showed a south-north cline. Kriging was used to predict values at unsampled localities. In this way maps can be obtained that predict allele frequencies covering the range of the studied area. It was stated that even if the differentiation among populations is limited ($F_{ST} = 2.4\%$) it is possible to get further information on differentiation by variograms and kriging. However, the use of such information might be questioned, knowing that variation in other traits is several times larger.

The geographic distribution of five chloroplast genotypes was studied in 722 individuals from 107 populations of *Q. petraea, Q. pubescens, Q. pyrenaica,* and *Q. robur* (Petit et al. 1993b). The majority of the populations originated from France and other western European countries. The G_{ST}s were estimated at 0.905 and 0.925 for *Q. petraea* and *Q. robur*, respectively. Excluding all populations with less than four individuals the percentage of polymorphic populations amounted to 18%. The differentiation among species was low and contributed only to 3.2% of the total variation.
Spatial autocorrelation analysis was carried out between individuals in different 20 km classes of geographic distances apart. This analysis showed strongly significant correlations for limited distances, 40 km, between the pairs while there was no correlation or even negative correlations at larger distances, 300 km. It was stressed that the use of chloroplast DNA variation could be most useful for tracing past migration routes of the oak species.

Thirteen polymorphic isozyme loci in five populations in each of *Q. petraea* and *Q. robur* were used to estimate population differentiation and allelic set-up (Müller-Starck et al. 1993). Some of the populations constituted mixes from several stands while others were obtained from individual stands. The genetic set-up was almost identical without any private alleles in the two species. The observed heterozygosities did not differ much, 21.3 and 21.7%, in the two species. Pairwise genetic distances between the populations were estimated according to Gregorius and Roberds (1986). For two of the marker loci these distances were presented. In Fig. 1-67 the mean distances for each population within species (4 comparisons) and between the two species (5 comparisons) are shown. As seen from this figure the mean distances for the *SKDH-A* locus in *Q. robur* are small while they are several times higher in *Q. petraea*. Besides the estimates for the *SKDH-A* locus in *Q. robur* all estimates were extremely large for isozymes. As regards the *AP-A* locus part of this could be attributed to two deviating *Q. petraea* populations, Nos. 2 and 3 from the right in Fig. 1-67. The distance between these two populations was estimated at 0.08.

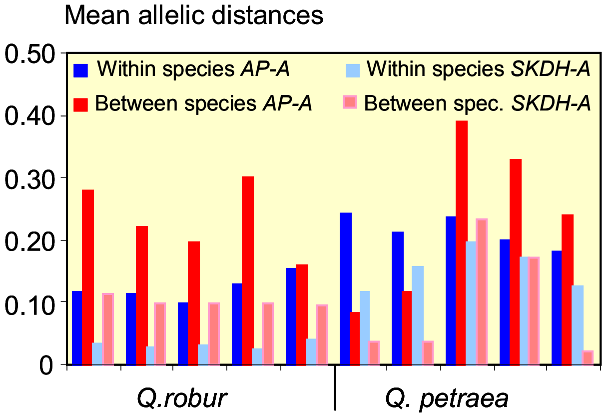

Figure 1-67. The mean allelic distance for the AP-A *and* SKDH-A *isozyme loci between five* Q. petraea *and five* Q. robur *populations. Blue columns refer to distances within species and red columns refer to distances between species. Müller-Starck et al. 1993.*

Besides a comment that the results showed *remarkable*"differentiations compared to other studies, the results concerning differentiation were not discussed. It is evident that analysis of individual loci might indicate larger differentiation than obtained when data from several loci are pooled. However, the remarkable differences would deserve a thorough discussion.

Eleven isozyme loci were used to study genetic variation within and among five southwestern Finnish populations (Mattila et al. 1994). The genetic distance estimated according Gregorius and Roberds (1986) was estimated at 5.5%. It was concluded that the Finnish *Q. robur* stands are more differentiated than central populations and that they have many rare alleles. From a gene conservation point of view it was stressed that collection of material must comprise many stands.

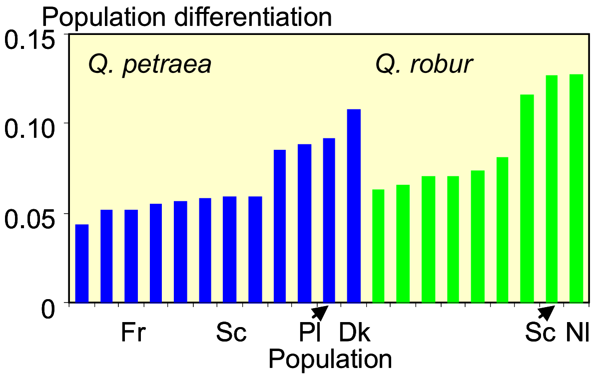

Figure 1-68. The population differentiation δ_j *according to Gregorius and Roberds (1986) of twelve* Q. petraea *and nine* Q. robur *populations estimated by 14 polymorphic isozyme loci. Most populations originated from Germany. Populations from Denmark (Dk), Poland (Pl), Scotland, (Sc), and The Netherlands (Nl) are indicated. Herzog 1996.*

Fourteen polymorphic isozyme loci were used in a study of population differentiation of 12 *Q. petraea* and nine *Q. robur* populations mainly from Germany (Herzog 1996). The gene pools of the two species were rather similar. The differentiation within species, δ_j, according to Gregorius and Roberds (1986) were estimated (Fig. 1-68). There was a fairly large variation among the populations in both species. However, no geographic pattern could be revealed according to the author. The overall population differentiation according to Gregorius and Roberds (1986), δ, was estimated at 0.067 and 0.088 for *Q. petraea* and *Q. robur*, respectively. Thus, most of the isozyme variation was found within populations.

Barrenche et al. (1996) reported on two-dimensional electrophoresis of 23 oaks from a large part of the distribution areas of *Q. petraea* and *Q. robur* in Europe. In all 101 polypeptide spots could be analyzed without finding any spot unique to one of the species. However, three spots showed frequency differences between the two species.

Trees in six stands affected by oak decline in Brandenburg, Germany, were classified with respect to size of decline in six morphological traits by Hertel and Zaspel (1996). Two stands were pure *Q. robur* stands. One stand was pure *Q. petraea*, the other three stands were mixed stands of these two species. Based on the morphological assessment and following a wewighting of the traits, a vitality index of the trees was calculated.

The vitality indices of *Q. petraea* were somewhat lower than for *Q. robur*. Eleven isozyme loci were analyzed to detect any genetic difference between trees with different vitality indices. No species specific alleles were detected. The fixation index (1 –Ho/H_e; H_o = observed heterozygosity, H_e = expected heterozygosity) for each vitality class reveals that the sensitive group has the highest fraction of homozygotes in both species (Fig. 1-69). Whether this pattern can be attributed to natural selection or to sampling cannot be evaluated based on these data.

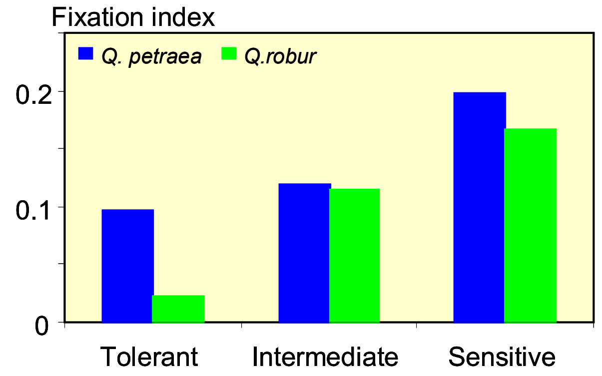

Figure 1-69. The fixation index based on eleven isozyme loci in three classes of vitality in six stands of oak in Brandenburg, Germany. Herzog and Zaspel 1996.

Dumolin-Lapègue et al. (1997) studied differentiation by 23 haplotypes within and between eight oak species. In all 1412 trees in 345 populations throughout Europe were analyzed. They found that haplotypes generally were restricted to some regions in Europe. Rarely occurring haplotypes were usually found in a smaller geographic region. The estimated G_{ST}s were high, 0.83. The following reasons for the high G_{ST} estimates were given:

1. Limited gene flow via acorns owing to maternal inheritance of cp haplotypes
2. Genetic drift is twice as large for the haploid genome
3. A low number of females contributes to the progeny.

Several populations have just one haplotype and different haplotypes occur in different populations. This is probably the most contributing factor for the high G_{ST} estimates.

A strongly significant correlation between G_{ST} and geographic distance was reported but the r-estimate of 0.24 tells that only a minute portion of the observed variation could be attributed to this relationship.

Certain genotypes had their refugia in Spain and others in Italy: From these countries one western and two central migration routes could be discerned. Besides, there was spreading westwards from the Italian refugia. The less dense sampling in Eastern Europe and neighboring regions of Asia makes the identification of migratory routes complex in this region.

Eleven pairs of populations of the two species *Q. petraea* and *Q. robur* from a large part of the distribution area in Europe were analyzed to detect genomic regions that might differentiate these two species (Bodénès, et al 1997a). A special type of Principle Component Analysis was used with 36 informative fragments out of the total number of 412 fragments available. The analysis resulted in a clear classification of the two species. There was

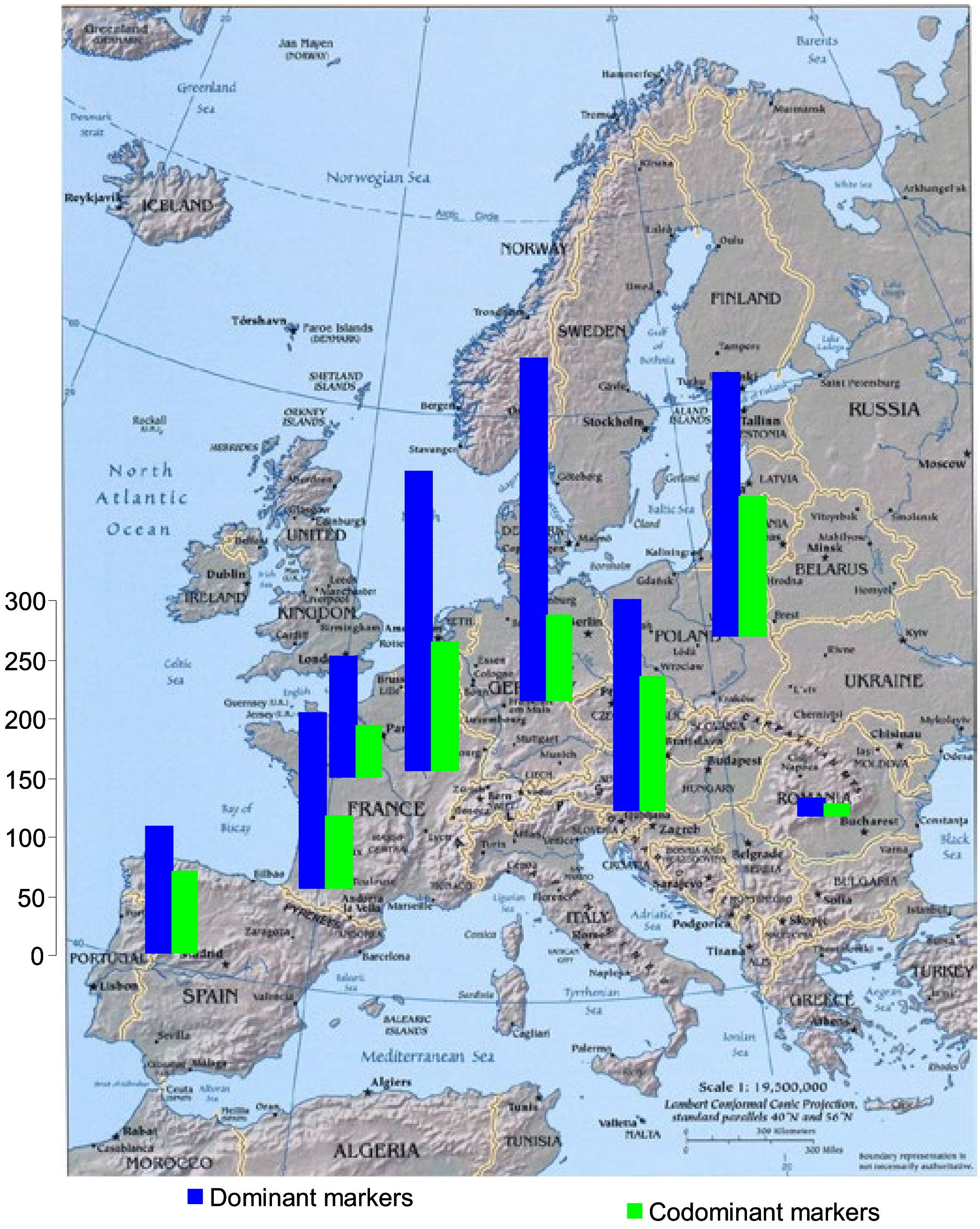

Figure 1-70. The local differentiation between Q. petraea *and* Q. robur *in eight European regions estimated by dominant and codominant markers. Bodénès et al. 1997b.*

ten times higher diversity between the two species when the 36 informative fragments were compared with the resting 376 fragments. Twenty-three of the informative fragments were partly cloned and sequenced to obtain Sequence Characterized Amplified Regions (SCAR). The remaining 13 informative fragments were discarded, partly owing to technical problems. Out of the 23 sequenced fragments three showed nucleotide similarities with known sequences in data banks.

Nine SCARs (sequence characterized amplified regions) that discriminate between *Q. petraea* and *Q. robur* were used for a study of local versus general differentiation between these two species (Bodénès et al. 1997b). For three of the SCARs there were two loci identified. Pairs of populations of the two species at eight localities from southern and central Europe were included in this study. Both single-stranded and double-stranded markers were used. The former are codominant, the latter being dominant.

The differentiation between the two species at the regional level was larger for dominant than for codominant markers (Fig. 1-70). It should be noted that the two Iberian populations were growing far apart, central Spain and coastal Portugal. For the dominant markers it seems as the differentiation between the species is more pronounced at northern localities. This might be attributed to a later colonization of these regions. The strong differentiation of the German populations might also be attributed to mixed origin (migration from different refugia after the latest glaciation) of the German oaks. The codominant markers do not show such a clear pattern as the dominant markers. The low differentiation at the Romanian locality is striking. Far-reaching intercrossing might explain the low differentiation in this case.

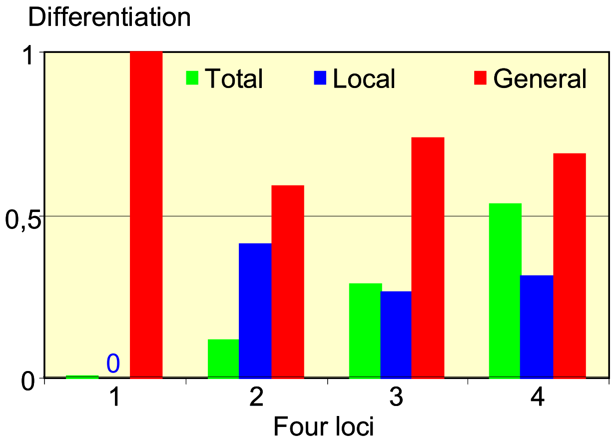

Figure 1-71. Total, local, and general differentiation in four (SCAR) loci in a study comprising eight pairs of populations of Q. petraea *and* Q, robur. *Bodénès et al 1997b.*

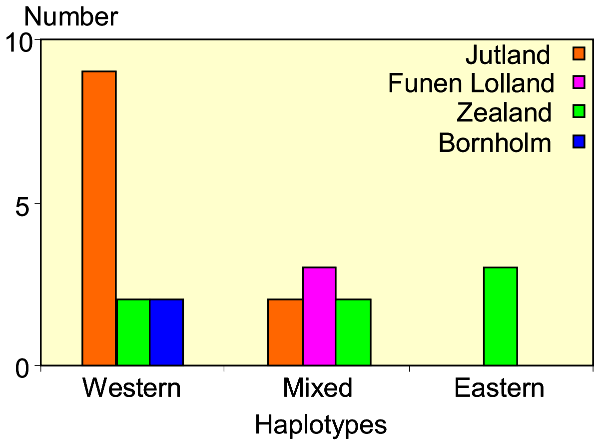

Figure 1-72. The number of populations with eastern and western chloroplast haplotypes in populations from different regions in Denmark. Jøhnk and Siegismund 1997.

Estimates of the differentiation at the local level were calculated marker by marker (Fig. 1-71). As seen from this figure the whole range of variation from zero to 100% occurred for the dominant markers. This means that the estimates of variation are largely dependent upon the markers selected for a study. It was speculated that selection for some of the markers might have occurred *because the markers used in this study were not anonymous but selected after a strong screening procedure.*

Another test of the variation among loci as regards differentiation was done by plotting the allele frequencies of the most common allele in the two species against the mean allele frequency of this allele in the two species. The following three patterns were observed:

The general differentiation in the two species was low (one locus)

One species with low differentiation and the other highly differentiated (eight loci)

Similar levels of differentiation across regions with local differentiation lower than general differentiation (three loci).

It was concluded that the observed differentiation might be attributed to population history, gene flow, and selection. Contrary to this, genetic drift did not contribute to the differentiation in this case.

Autochtonous Danish populations of *Q. petraea* (3) and *Q. robur* (17) were analyzed with respect to eastern and western chloroplast haplotypes by Jøhnk and Siegismund (1997). In addition two putative hybrid populations of the two species were included in this study.

In Fig. 1-72 I have tried to summarize the results without consideration of the species or hybrids. The western haplotype was dominating in Jutland populations while three of the Zealand populations were monomorphic for the eastern haplotype. All three populations from the islands between Zealand and Jutland had both haplotypes. The existence of monomorphism of both haplotypes explains the relatively high estimate of population differentiation, $G_{ST} = 0.60$. This estimate is lower than many others for haplotype differentiation. This was explained by the migratory routes after the latest glaciation, most of the Danish oaks came from southwest and they met oaks coming from south/southeast in Denmark. This would explain the relatively large number of populations with both haplotypes on Zealand and Funen. This explanation does not hold for Bornholm populations, which were monomorphic for the western haplotype in spite of their easternmost location of all populations. It might be added that the *Q. petraea* populations contained with one exception only the western haplotype. The Bornholm *Q. petraea* population was also monomorphic for the western haplotype.

Nine haplotypes in southern French populations were compared with two mitochondrial haplotypes (Dumolin-Lapègue et al. 1998). It was found that all 663 trees that belonged to cpDNA lineage A had the same mtDNA haplotype. All trees with other cpDNA haplotypes were associated to the other mtDNA. In all, twelve associations of cpDNA-mtDNA were identified. A clear geographic division of the lineages, A-C, of the haplotypes was noted. cpDNA haplotypes of lineage A occurred in eastern France, B lineage haplotypes dominated in western France. Finally, haplotypes of lineage C were confined to the south-eastern corner of France. It was concluded that no paternal leakage of cpDNA or mtDNA had taken place. The obtained data were used to identify colonization routes after the latest glaciation. This will be discussed further below with a more geographically comprehensive material.

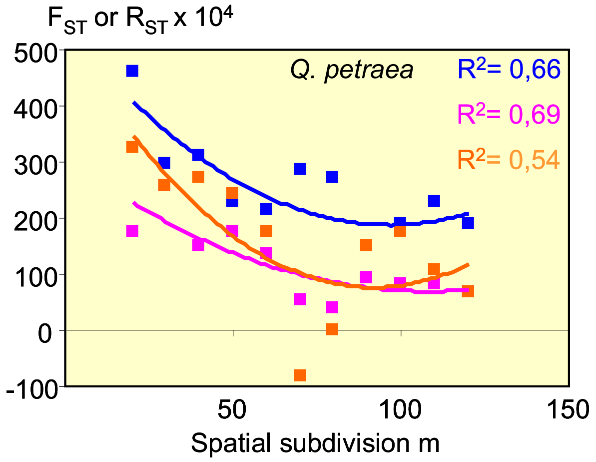

Figure 1-73. Q. petraea F_{ST} estimates x 10^4 for 4 isozyme loci (blue) and 6 microsatellite loci (purple) as well as R_{ST} estimates (orange) for the six microsatellite loci for varying spatial subdivisions in a stand with Q. petraea *and* Q. robur *in north western France. Streiff et al. 1998.*

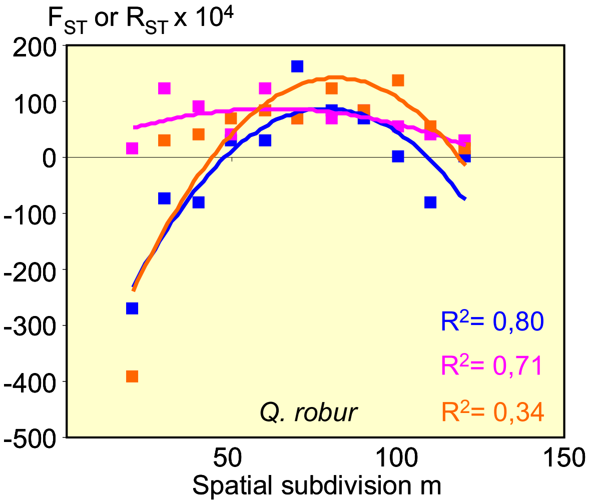

Figure 1-74 Q. robur *F_{ST} estimates x 10^4 for 4 isozyme loci (blue) and 6 microsatellite loci (purple) as well as R_{ST} estimates (orange) for the six microsatellite loci for varying spatial subdivisions in a stand with* Q. petraea *and* Q. robur *in north western France. Streiff et al. 1998.*

The spatial distribution of isozyme and microsatellite alleles in a mixed *Q. petraea* and *Q. robur* stand in north western France was studied by Streiff et al. (1998). The stand, 240 x 240 m, was subdivided into squares of different sizes. The smallest square had a side of 20 meters and the largest 120 meters. This means that there were 144 squares of the smallest size and four squares of the largest size. For each subdivision F_{ST} or R_{ST} (only microsatellite loci) was estimated.

Generally the estimates, independent of marker used, were low (Figs. 1-73 and 1-74). There was a tendency to larger estimates for the small squares in *Q. petraea* while a corresponding pattern was not disclosed for *Q. robur*. There was also a slight tendency for larger subdivision for isozymes than for microsatellites in *Q. petraea*. This could be attributed to larger number of alleles per microsatellite locus. The reason for the difference between the two species was at least partly attributed to longer dispersal of acorns in *Q. robur* than in *Q. petraea*. Jays are distributing the acorns in the former species. As in many other cases the expected heterozygosity was significantly higher for the microsatellites than for the isozymes.

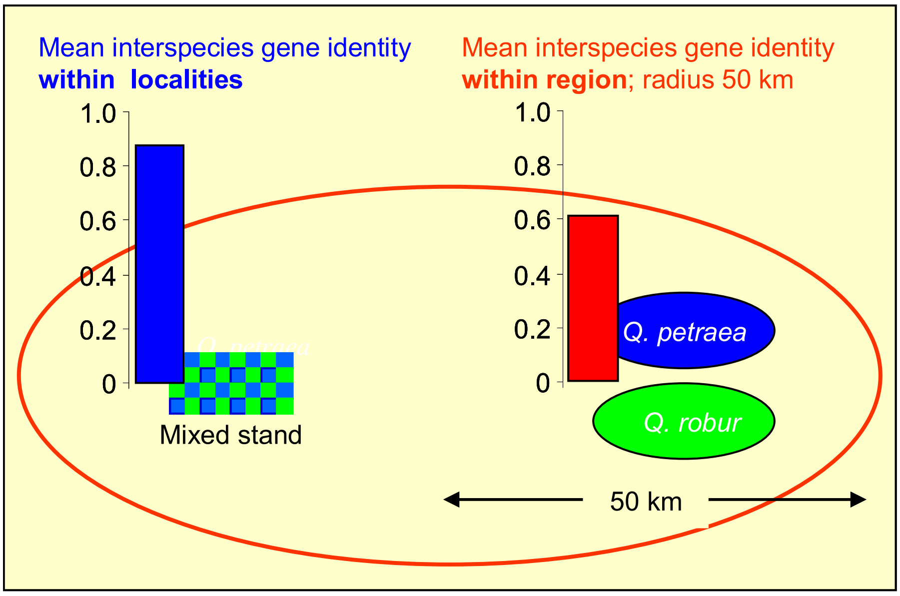

Figure 1-75. Estimates of the mean interspecific gene identities of haplotypes within localities (blue column) and mean of gene identities within a radius of 50 km. Dumolin-Lapègue et al. 1999.

The similarities in haplotype occurrence (chloroplast and mitochondria) between species from the same locality were studied by Dumolin-Lapègue et al. (1999). These estimates were compared with gene similarities between the species within a region with a radius of 50 km. This report was the first including a quantitative validation of such gene similarities. Three species combinations were included, *Q. petraea/Q. pubescens, Q. petraea*/*Q. robur*, and *Q. pubescens*/*Q. robur*.

The results of this comparison for the species pair *Q. petraea*/*Q. robur* are illustrated in Fig. 1-75. The difference for the two other species pairs were approximately of the same size, 25%. All differences were strongly significant. It should be added that the single-population diversity was significantly larger than the diversity in mixed *Q. pubescens* populations while the difference in the two other species was non-significant.

The difference illustrated in Fig. 1-75 clearly shows that two species growing together are more similar to each other than to populations within a region with a radius of 50 km. This indicates a great sharing of cytoplasm among species. It was concluded that *recent hybridization and introgression events* were responsible for the results obtained. It was further stated that introgression is not complete and that it varies with pair of species. The co-occurrence in localities may influence the introgression. In spite of the relatively large introgression in mixed stands of *Q. petraea* and *Q. robur* these species remain distinct. This suggests that this distinctness prevails thanks to strong disruptive selection. It was hypothesized that the asymmetric gene flow from *Q. petraea* to *Q. robur* followed by repeated back-crossing would gradually substitute the robur genome for the petraea genome. This would eventually result in *Q. petraea* trees with the same haplotypes as *Q. robur*.

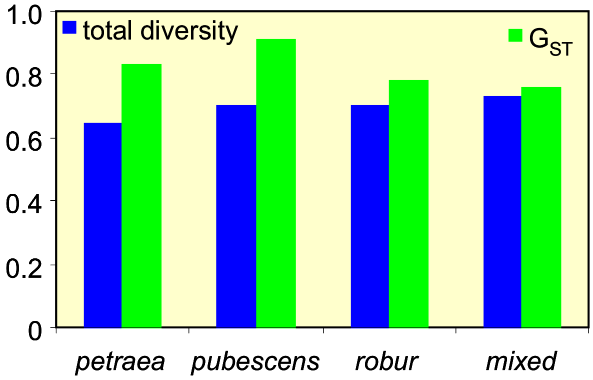

Figure 1-76. The total genetic diversity and G_{ST}s in pure species and mixed oak populations. Dumolin-Lapègue et al. 1999.

The total genetic diversity in pure species populations as well as G_{ST}s in pure and mixed populations was rather large (Fig. 1-76). It is seen that the G_{ST} for *Q. robur* is lower than for the other two species. Part of this might be attributed to more human material transfer in *Q. robur*.

In a brief report Muir et al. (2000) presented a dendrogram of five populations from each of *Q. petraea* and *Q. robur* based on 20 microsatellite loci. The dendrogram showed a clear distinction between the two species but also a differentiation of populations within species. By use of these microsatellite loci it was tested whether it would be possible to assign individual oaks to proper species (based on morphological traits). In 78% of the cases the assignment was correct, in 15% of the cases a hybrid status was indicated and the final 7% were not informative.

Five *Q. petraea* and two *Q. robur* trees were used for an analysis of the internal transcribed spacer ITS2 and 5.8S of ribosomal rDNA to detect any possible species differences (Muir et al. 2001). No species specific differences were detected. Three divergent rDNA were found in both species, which suggests that they originated before speciation took place.

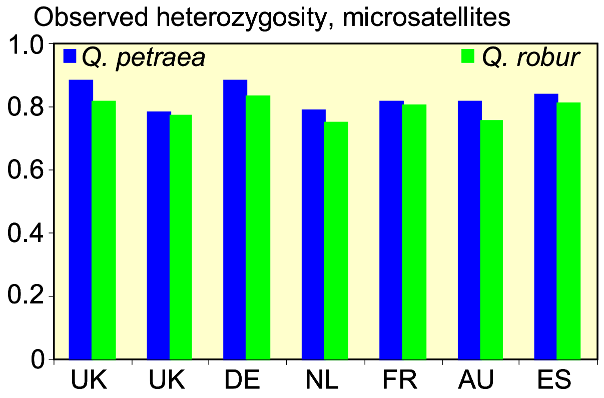

Figure 1-77. The observed heterozygosity in seven European mixed stands of Q. petraea *and* Q. robur *estimated by six highly polymorphic microsatellite loci. The countries of origin are indicated. Mariette et al. 2002.*

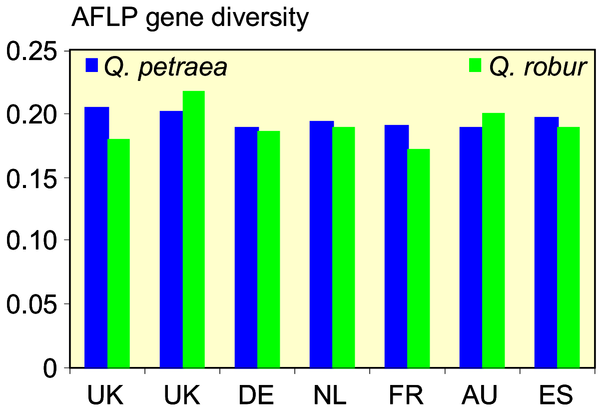

Figure 1-78. The gene diversity in seven European mixed stands of Q. petraea *and* Q. robur *estimated by 155 AFLP markers. The countries of origin are indicated. Mariette et al. 2002.*

Morphological and molecular variation in four Italian populations of each of *Q. petraea* and *Q. robur* were used to study differentiation between the two species (Bruschi et al. 2000). The focus in this study was species differentiation. No figures on population variation were given but it was stated that most of the variation was within species both for morphological and molecular markers.

The level of genetic diversity based on microsatellites or AFLP in seven mixed *Q. petraea* and *Q. robur* stands, mainly from Western Europe, was studied by Mariette et al. (2002). Six microsatellites and 155 dominant AFLP markers were used. The genetic diversity for microsatellites was estimated in several ways, allelic richness, expected and observed heterozygosity. Besides, the fixation index F_{IS} was estimated. Phenotypic diversity and two different estimates of gene diversity were presented for the AFLP part of the study.

I preferred to illustrate one estimate of diversity for microsatellites and one for AFLP in Figs. 1-77 and 1-78. Generally, the estimates were somewhat higher for *Q. petraea* than for *Q. robur* (Fig. 1-77) as was the case for the other parameters as well. However, only 13 of 24 estimates were significant. The number of significant genetic differences between the two species was much lower for the AFLP markers, three out of 36. As regards microsatellites, there was good agreement between the performance of the two species within stands, r = 0.77. The fixation index was significantly higher in *Q. robur* than in *Q. petraea* in two stands (Dutch and Austrian), 0.128 versus 0.086 and 0.142 versus 0.071.

The genetic differentiation between the two species in the same stand was limited for microsatellites, range 0.005 – 0.024. The corresponding range for AFLP was 0.016 – 0.096. The G_{ST}s (microsatellites and AFLPs) for differentiation among populations within species was slightly higher in *Q. petraea* than in *Q. robur*, but never significant. The results obtained with the two types of parameters were uncorrelated in both species. The high mutation rate in microsatellites was suggested as one explanation for the difference between the two types of marker. Since the number of AFLPs was several times higher than the number of microsatellite loci it was speculated that some of the AFLPs were linked to loci for adaptive traits. Some AFLPs from chloroplasts and mitochondria might be another contributing factor since they are maternally inherited, which in itself contributes to stronger differentiation. G_{ST} estimates for individual AFLP markers showed an L-shaped distribution for the individual species as well as for the differentiation between the two species. The percentage of high G_{ST}s was higher within species than when the species were compared. Thus, four loci had estimates higher than 0.10 in the species comparison while the number of loci exceeding this limit was 36 for *Q. petraea* and 23 for *Q. robur.* This shows that there is stronger relationship between the two species in a stand than between the same species at different localities.

The authors concluded that *If differences in the level of diversity have to be assessed on the whole genome, the number of loci rather than the number of individuals should be as great as possible.* This is of significance in case of studies done for gene conservation purposes.

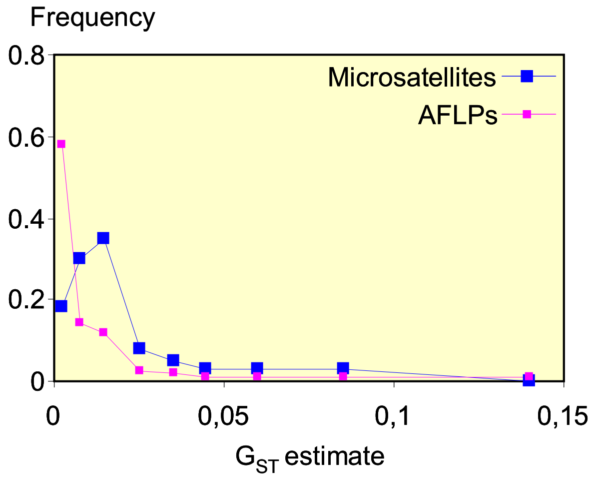

Figure 1-79. The distribution of G_{ST} estimates of individual loci for microsatellites, and one type of AFLPs for differences between Q. petraea *and* Q. robur *in neighbouring populations. G_{ST} estimates above 0.10 are arbitrarily set at 0.14. Scotti-Saintagne et al. 2004.*

A review of G_{ST} estimates between pairwise growing *Q. petraea* and *Q. robur* populations based on 389 markers was presented by Scotti-Saintagne et al. (2004b).

Figure 1-79 shows that the distribution of G_{ST}s for microsatellites and AFLPs differ. The latter showed an L-shaped relationship. It should be added that the two other types of AFLPs presented in this paper showed the same type, of L-shaped curves. Contrary to this, the highest frequencies for microsatellites were noted for the classes 0.005-0.01 and 001-0.02. It was reported that 12% of the markers that exceeded the expectation from neutrality, *i.e.* markers with high G_{ST} estimates. These markers were suspected to be influenced by natural selection. The deviating pattern for microsatellites was attributed to the way microsatellites were developed. Thus, only loci that were polymorphic were included. Theoretical investigations (Le Corre and Kremer 2003) showed that not all loci participating in the regulation of an adaptive trait will show up as outliers. Therefore, it was speculated that the L-shaped curves resulted from two curves, one corresponding to markers undergoing selection and another for neutral markers.

The location of so called outlier loci, *i.e.* loci with significant G_{ST} estimates, was determined. The 20 outlier loci were distributed over nine linkage groups. It was noted that loci separated by less than 2 cM showed significant correlations between their G_{ST} estimates. Most of these loci occurred in linkage group 12. In spite of this clustering it was stated that loci responsible for species differentiation was widely spread over the genome. It was assumed that the linkage of outliers might be a result of hitchhiking of neutral genes at selection for genes contributing to increased fitness.

This study with a large number of markers (389) confirmed results from earlier investigations of low species differentiation. The location of loci contributing to species differentiation to different linkage groups is one important result of this investigation.

Twenty or twenty-one microsatellites were used to study the differentiation between *Q. petraea* and *Q. robur* by Muir and Schlötterer (2005). Material from the two species in eight regions in Central and Southern Europe was included. Besides, three localities with one of the species only were analyzed. The four Italian populations, two of each species, were included to disclose any possible specific alleles. The five loci with the highest F_{ST} estimates were analyzed in these four populations.

The F_{ST} based on a joint analysis of all microsatellite loci and populations was estimated at 0.05. There was a significant difference between the two species for all loci tested. The F_{ST}s of neighboring pairs were compared with F_{ST}s for more distantly located pairs to reveal the importance of gene flow. If gene flow is important F_{ST}s for neighboring populations should be lower than F_{ST}s for population pair from geographically distant localities. No difference was detected, which according to the authors suggested a limited role of gene flow. The fact that there were significant differences for all loci was interpreted as a genome-wide differentiation between the two species. The authors stressed that their results did not support high gene-flow between the two species. Nor did the results support species differentiation being dependent on a limited number of loci. It was also stated that the data obtained did not suggest any importance of interspecific crosses. It was concluded that *the high frequency of shared alleles in combination with a genome-wide genetic differentiation among the two species* points at shared ancestral variation as explanation for the results obtained. Alleles in the three loci with the highest F_{ST}s were sequenced to possibly detect adaptive variation between the two species. However, no difference between the two species was detected.

The observed and expected heterozygosities did not differ much between the two species; both were highest in *Q. petraea*.

The interpretation of genome wide differentiation between the two species by Muir and Schlötterer (2005) was challenged by Lexer et al. (2006). A scrutiny of the 20 loci showed that three of them contributed most the differentiation. Moreover, the same loci had the highest F_{ST}s in Scotti-Saintagne et al. (2004b) study as well. Therefore, Lexer et al (2006) claimed that instead of a genome wide differentiation there are a few loci that contribute to the differentiation by the two oak species. Selection is responsible for the larger differentiation at these three loci. The two loci with the highest F_{ST}s showed a reduced heterozygosity that indirectly supports the selection hypothesis at these loci.

As regards the question of gene flow, Lexer at al. (2006) pointed out that there are numerous studies of artificial studies of successful interspecific crosses, which supports a strong gene flow between the two species. As one example of this, they referred to data from Steihoff (1997) who carried out crosses during the period 1989-1996 at Escherode in Germany (Fig. 2-1). The general picture of higher success rate of the *Q. robur* x *Q. petraea* cross than the reciprocal was also noted in this case with a huge number of flowers pollinated, 9,177 and 11,680 respectively.

Seven isozyme loci and four microsatellite loci were used for a comparison of genetic variation within and between two planted *Q. robur* stands in northern Germany by Degen et al. (1999). Local acorns were used for the reforestation in both localities located 15 km apart.
The observed heterozygosity was approximately three times higher for the microsatellite loci than for the isozymes. This must be attributed to the higher allelic richness for the microsatellites. The pooled genetic distance estimated according to Gregorius (1978) was 0.151 for the microsatellites and 0.034 for the isozymes. The genetic distances according to Nei (1972) were minute 0.039 and 0.003 for microsatellites and isozymes, respectively. The variation among loci was larger for isozymes than for microsatellites. Thus the genetic distance according to Gregorius (1978) for individual isozyme loci varied in the range 0.006-0.105 as compared to 0.141-0.160 for the microsatellites. It was suggested that natural selection could explain this difference. However, it is hard to believe that any of the markers has any effect on adaptedness.

Fourteen populations of *Q. robur* from the middle region of Volga in Russia showed limited among population variation as estimated by nine polymorphic isozymes (Yakovlev 1999), F_{ST} = 0.025. This estimate corresponds to 9.7 migrants per generation. These data clearly show that there is a great gene flow among these Russian populations. The inbreeding coefficient, F_{IS}, varied between -0.104 and +0.086 for different isozyme loci with a low F_{IS} mean value, 0.001.

Six isozyme loci were used for a study of variation among seven Danish populations of *Q. petraea*, 17 *Q. robur* and two hybrid populations (Siegismund and Jensen 2001). In addition one Dutch population of *Q. robur* was included. Some rare alleles occurred in one species only but then at a very low frequency 0.01-0.03 suggesting that sampling might be responsible for the difference. Average allele number per locus and gene diversity did not differ between the three groups of material but with some differences at individual loci. Two loci contributed most to the F_{IP} estimate (Fig. 1-80; F_{IP} = deviation from random mating within populations). The differentiation among populations within species, F_{ST}, was low (0.022) as in most other studies of variation in isozyme loci. The low value indicates a large gene flow among populations. The relatively large estimate for differentiation between species, F_{SG}, was attributed to one locus that usually was not included in similar studies. No geographic trend for the differentiation among populations was found. The Dutch population with its deviating phenology and growth potential was located among the Danish populations in the geographic map constructed on the basis of allele frequencies.

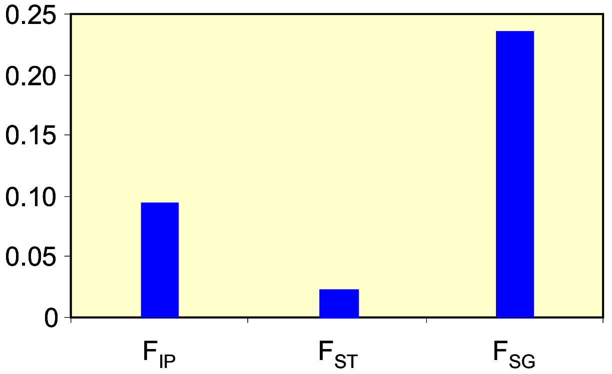

Figure 1-80. The deviation from random mating within populations, F_{IP}, differentiation among populations within species, F_{ST}, differentiation among species within the genus, F_{SG}. The analysis comprised 7 Q. petraea, *17* Q. robur, *and 2 hybrid populations from Denmark. Six isozyme loci were studied. Siegismund and Jensen 2001.*

RAPD variation in four *Q. robur* populations from a wide range of longitudes in Russia, 31.50ºE – 56.16ºE, was studied by Yakovlev and Kleinschmidt (2002). Each population was represented by 24 randomly selected trees growing at a distance of >50 meters apart. No population-specific DNA fragments were detected. However, some fragments had a good discriminating power. The estimates of genetic variability were based on 48 RAPD loci. The total diversity of the four populations varied in the range 0.202-0.245 while the within-population diversity varied between 22.6 and 27.1%. The G_{ST} was estimated at 0.098, which corresponds to a high gene flow of 4.6 migrants per generation. In spite of the isolation of these Russian populations this estimate of population differentiation (0.098) was somewhat lower than estimates from Central and Western Europe. This difference was not discussed.

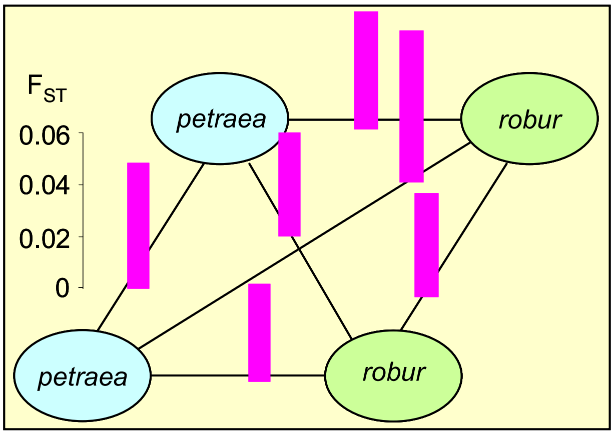

Figure 1-81. The differentiation of isozymes, F_{ST}, between two populations of each of the species Q. petraea *and* Q. robur *sharing the same localities in Italy. Belletti et al 2005.*

The isozyme variation in and between populations of four oak species, *Q. cerris* (2), *Q. petraea* (5), *Q. pubescens* (1), *Q. robur*(5), from the Piedmont region in Italy were presented by Belletti et al. (2005).

The study of eleven isozyme loci showed that the within-population variation was 86% of the total variation. Most of this variation among populations was attributed to the variation among species. Two of the *Q. petraea* and *Q. robur* populations shared the locality with the other species. This enabled estimates of differentiation between the two populations of the same species as well as differentiation between the species at the two localities. As indicated in Fig. 1-81 the differentiations did not differ much whether it was within a species, between species, or within a locality. When all populations of these two species were analyzed, the F_{ST} for species differentiation was estimated at 0.048. The mean estimates of the inbreeding, F_{IS}, were 0.04 for *Q. petraea* and 0.09 for *Q. robur*.

In a preliminary report (Gömöry 2000) a strong differentiation between *Q. petraea* and *Q. robur* amounting to 54% was observed for the *Gludh-A* isozyme locus. The within-population variation was estimated at 43.5%, which is a very low estimate for isozymes. It was suggested that the *Gludh* isozyme system, which is substrate-unspecific, is exposed to selection contrary to substrate-specific isozymes.

In an extended study (Gömöry et al. 2001) eight isozyme loci were used to study interspecific, inter-region, inter-population, and intra-population variation in 25 populations of *Q. petraea* (8 pops.) and *Q. robur* (17 pops.) from Slovakia, Bulgaria and eastern European Russia.

The allelic richness was slightly higher in *Q. petraea* than in *Q. robur* while the observed heterozygosity showed the opposite pattern. The Bulgarian *Q. robur* populations had the highest estimates of observed heterozygosity. The *Gludh-A* locus separated the two species. Therefore, separate genetic distances were estimated with inclusion or exclusion of this locus. Fig. 1-82 shows that the greatest impact of this locus was on the genetic distances in *Q. petraea*. However, the impact was considerable when the distance between the two species was estimated, 0.0208 and 0.1456. Generally, the among-population variation within the two species was extremely low considering the wide geographic area covered in this investigation.

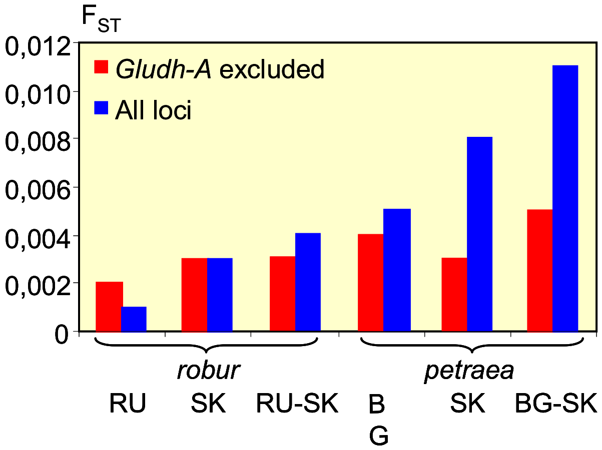

Figure 1-82. The genetic distances within regions and between regions for Q. petraea *and* Q. robur *populations in two eastern European regions, BG = Bulgaria, RU = South-eastern Russia, SK = Slovakia. Gömöry et al. 2001.*

Seventeen polymorphic isozyme loci were analyzed to estimate the differentiation between twelve *Q. petraea* and six *Q. robur* Swiss populations (Finkeldey 2001, Finkeldey and Mátyás 2003). Besides, three populations had a mixture of these two species. In the 2003 paper seven *Q. pubescence* populations were included in the investigation. The data from the isozyme analyses were compared with the haplotypes of the populations (Finkeldey and Mátyás 2003). With two exceptions the populations were monomorphic, either with a “western” or an “eastern” haplotype.

As regards isozyme variation 92.8% was found within populations and 5% among the three species. The F_{ST} estimates were 1.2% and 1.9% for *Q. petraea* and *Q. robur* populations, respectively (Finkeldey and Mátyás 2003). In the Finkeldey (2001) paper the among-population differentiation was estimated at 1.7% while the differentiation between the two species amounted to 4.3%. The four populations from province Ticino, south of The Alps, deviated most from the rest of the populations of the same species.

For two loci significant deviations were found between observed and expected heterozygosity in 14 and 9 of the 21 populations. For the rest of loci the agreement between expected and observed heterozygosity was significantly different in a few instances (Finkeldey 2001). The number was so low that the significances observed were attributed to chance.

The inbreeding coefficients for *Q. petraea* and *Q. robur* were estimated at 0.071 and 0.077, respectively. A slightly higher estimate, 0.116, was noted for the three mixed stands.

Only for two of the 75 isozyme alleles a connection to a specific haplotype was noted. These findings were attributed to chance events (Finkeldey and Mátyás 2003). The higher allelic variation was used as an argument for including mixed stands in gene conservation of the two oak species. A suggestion of inclusion of mixed stands in gene conservation is a rare case since most conservationists disfavor such stands in gene conservation. To support this suggestion Finkeldey (2001) stated that *The occasional formation of species hybrids may even add to the adaptive potential of progenies from "mixed" populations.* A statement that is easy to support.

One-hundred and seventy polymorphic AFLP markers were screened in a study of Belgian oak populations, six *Q. petraea* and four *Q. robur* (Coart et al. 2002). Besides the markers, several morphological traits were studied. As in many other studies no species specific markers were found. The vast majority of the variation was found within the populations, amounting to 97.9% in both species. However, the population differentiation in both species was significant. This significance was unexpected since outcrossing is common in these two oak species. Even if the differences were significant, estimates of F_{ST}= 0.021 in both species do not deserve any raised eye-brows over population differentiation. There was a clear separation of the two species as regards the morphological traits.

The nuclear capture hypothesis for evolution in *Q. petraea* and *Q. robur* was outlined in Petit et al. (2003). The meaning of it is that genome of one species is substituted by another species via pollen flow. In case of these two oak species the *Q. petraea* genome will eventually substitute the *Q. robur* genome. This hypothesis builds on the more pioneer character of *Q. robur*. The following steps might be distinguished in this hybridization:

1. Long-distance transfer of *Q. robur* acorns and establishment of a population during the recolonization after the glaciation
2. Long-distance transfer of *Q. petraea* pollen and formation of species hybrids. Back-crosses with these hybrids and selection within the stands against *Q. robur*, the pioneer species.
3. Further extension of *Q. robur* with long-distance acorn distribution
4. The process is repeated as a recurrent process.

The support for the capture hypothesis according to the authors is outlined below.

There are plenty of examples showing the far-reaching agreement in chloroplast haplotypes across species growing in the same locality/region. Thus, the nucleotide divergence between the two species amounts to a few percent only.

The several times wider pollen flow than acorn dispersal is another support of the hypothesis. Several reports state that acorn dispersal is wider in *Q. robur* than in *Q. petraea* thanks to the shape of the acorns and to jays. The latter preferentially select *Q. robur* acorns and transmit them.

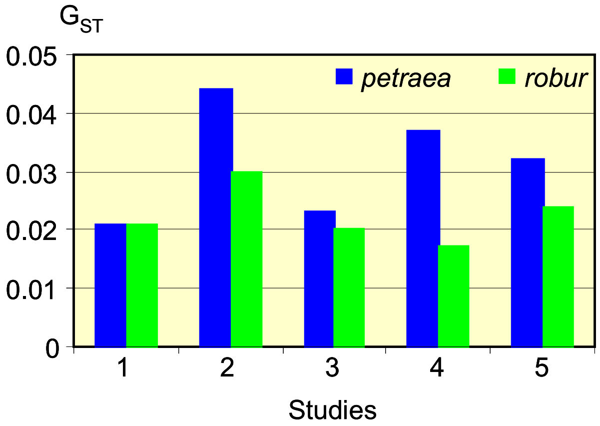

Figure 1-83. G_{ST} estimates in five investigations, in which populations of Q.petraea *and* Q. robur *diversities were studied by markers. Petit et al. 2003.*

The initial growth of young *Q. robur* seedlings is faster than *Q. petraea* seedlings. This probably is a favorable trait for a pioneer species that invades open ground.

There is limited palynological information of the colonization of the two oak species but there are indications that *Q. robur* in Britain constituted a first faster phase of colonization than *Q. petraea*. This was probably the case in Scandinavia as well.

A large gene flow via pollen in *Q. robur*, as anticipated in the nuclear capture hypothesis, should result in less differentiation in *Q. robur*. This was the case as is shown in Fig. 1-83. It was also stated that the haplotype differentiation was approximately 30 times larger than nuclear gene differentiation. This was interpreted as much larger pollen dispersal than acorn dispersal. However, with the limited number of haplotypes and the high occurrence of monomorphic populations with different haplotypes a high differentiation is expected even if the acorn dispersal is wide.

The lower competition ability and shade tolerance of *Q. robur* point at its character as more of a pioneer tree than *Q. petraea*. The latter is also characterized by good drought tolerance. All these ecological differences between the two species support the capture hypothesis.

In artificial crosses experiments asymmetric gene transfer was shown with a greater success of hybridization with *Q. petraea* as male than as female. In a mixed stand with 47% *Q. petraea* trees as many as 67% of the seedlings were *Q. petraea* or hybrids (Further data on this issue in Chapter 2).

Gene diversity according to Nei and Roychoudhury (1974) was estimated in two autochtonous *Q. robur* populations and two open-pollinated progenies by Bakker et al. (2003). Six microsatellite loci were used in this investigation. There was a small but significant difference

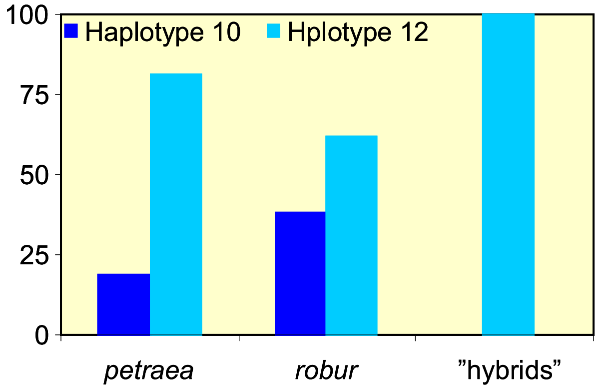

Figure 1-84. The percentage distribution of cpDNA haplotypes 10 and 12 from 26 localities in Ireland in Q. petraea *and* Q. robur *as well as in nine trees of presumed hybrid origin. Kelleher et al. 2004.*

between the two populations as regards gene diversity. This difference was smaller than the difference between the two OP-families. The F_{ST} estimates for individual loci varied in the range 0.00-0.02. In spite of this they were significant for two loci. Since the populations grew approximately 100 km apart it was suggested that there must be a large long-distance pollen-flow. It ought to be added that autochtonous populations of *Q. robur* are rare in The Netherlands. No indications of inbreeding were noted.

Ten cpSSR loci were used to study the variation between 48 *Q. petraea* and *Q. robur* populations in France as well as between these two species (Deguilloux et al 2004). The results were compared with earlier data from previous RFLP analyses.
The comparison did not reveal any great difference between the two methods. Neither did the analysis reveal any distinction between the two species. The G_{ST} and R_{ST} estimates were of similar size, 0.75 and 0.72 respectively. Most focus in this paper was on haplotypes and not on population differentiation.

The distribution of cpDNA haplotypes 10 and 12 in 149 oak trees in Ireland varied significantly between *Q. petraea* and *Q. robur* (Kelleher et al. 2004 and Fig. 1-84). Haplotype 10 did not occur in the northern part of Ireland while haplotype 12 was found all over Ireland although it was most frequent in peripheral localities. One tree carried haplotype 7 and it was suspected that this tree was planted. The distribution of the haplotypes supports the hypothesis that the oaks migrated from south to north and not as believed previously from east to west, *e.g.* Dumolin-Lapègue et al. (1997). The present study contained more populations than in previous reports, which can explain the difference in interpretation of haplotype variation. It was pointed out that the total gene diversity of cpDNA in Ireland, 0.374, is twice as low as found in France or Spain. This was partly attributed to the limited dispersal of the heavy acorns and thus a small founder population. Eighty percent of the populations were monomorphic. The G_{ST}s were almost identical for the two species, 0.713 and 0.718 respectively while the total diversity was almost twice as large in *Q. robur* as in *Q. petraea*, 0.574 and 0.286, respectively.

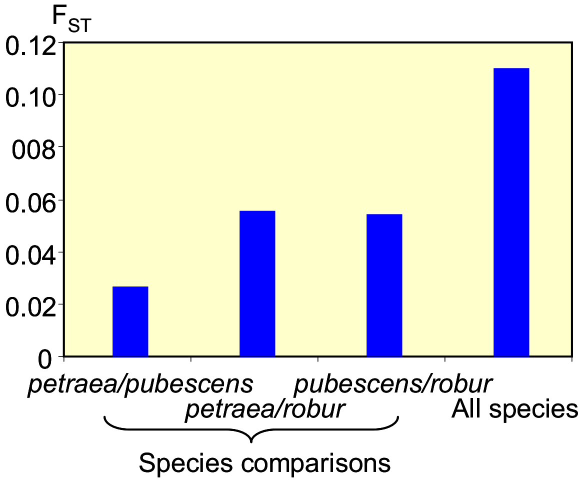

Figure 1-85. The pairwise F_{ST} estimates between the three oak species Q. petraea, Q. pubescens, *and* Q. robur *in 23 populations from Slovakia. Nine polymorphic isozyme loci were used. Gömöry and Schmidtova 2005.*

Ten *Q. petraea,* two *Q. pubescens*, nine *Q. robur*, and two mixed populations from Slovakia were analyzed with 13 isozymes (Gömöry and Schmidtová 2007). The species pairwise F_{ST} calculations showed that *Q. petraea* and *Q. pubescens* were less differentiated than *Q. robur* and the two other species (Fig. 1-85). This was somewhat unexpected since the habitat demands of *Q. pubescens* differ most from the demands of the two other species. Except for extremely rare alleles no species-specific alleles were detected. The pattern of allelic sharing was studied by a clustering procedure according to Pritchard et al. (2000). Three groups of sharing were identified and the means for the three species in each group are illustrated in Fig.1-86, which reveals a clear difference among the species. In spite of the clear morphological separation of the species there is evidently a large similarity as revealed by isozymes. The authors suggested that there is a substantial gene flow but also disruptive selection to form this dichotomy of these oak species.

The hypothesis that fragmentation leads to high genetic differentiation among the fragmented populations and low genetic diversity within fragments was addressed by Vakkari et al. (2006). They analyzed isozyme data from 33 Finnish populations of *Q. robur* in Finland. This species reaches its northern distribution limit in southern Finland.
Allelic richness, expected and observed heterozygosity, fixation index and pairwise genetic distances based on twelve polymorphic isozyme loci were calculated. A series of correlations were estimated (Fig. 1-87). As seen from this figure some of the correlations were significant

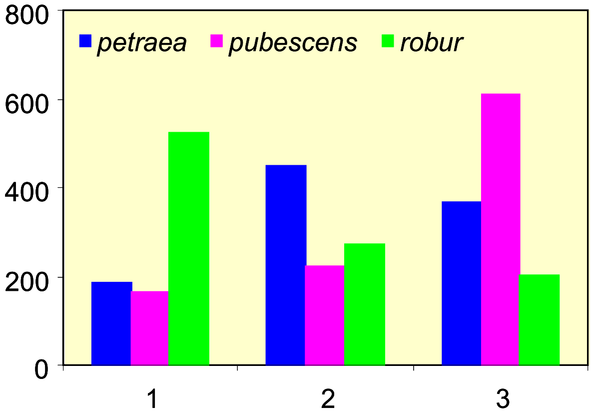

Figure 1-86. The proportion of membership to three groups of population allelic structure of Slovakian populations belonging to three oak species Q. petraea, Q. pubescens, *and* Q. robur. *Gömöry and Schmidtova 2007.*

but the explanation of the variation did not exceed 35% in any case. The expectation of a larger genetic differentiation among fragmented populations is supported by the negative correlation (red column) between population size and population differentiation (Fig. 1-87). Moreover, F_{ST}s for the Finnish populations were estimated at 0.066 while the corresponding estimate for Central Europe was a few times lower (Zanetto et al. 1994). The expected heterozygosity supported the above hypothesis with estimates of 0.164 in the Finnish populations as compared to 0.254 for Central European populations. The Gulf of Finland and The Baltic Sea cause a geographic isolation. As corollary of this it was concluded that *the location at the margin of the species distribution enhances the effect of fragmentation*.

A U-shaped relationship between the age of the population and genetic distance was noted, which called for a study of two separate relationships between age of stand (4,000 years as border between the two) and genetic distance. As seen from Fig. 1-87 there was a negative relationship in the young stands and a positive in the old stands. This might be attributed to a larger differentiation among newly colonized populations than in old populations. In the latter, gene flow among populations might have leveled the allele frequencies.

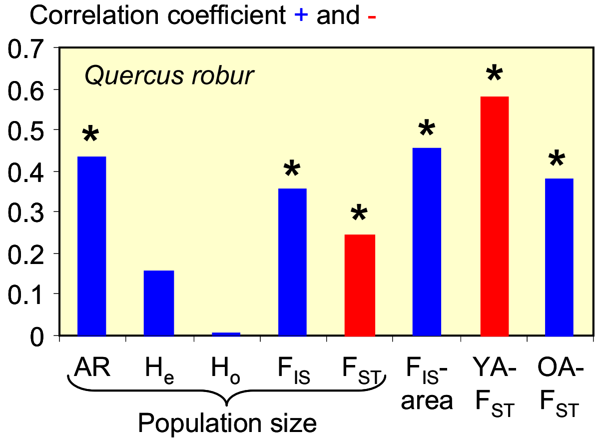

Figure 1-87. The correlation coefficients for relationships between: Population size and genetic parameters AR- F_{ST}, F_{IS} and stand area, young stand age (<4,000 years YA) or old stand age (OA) and F_{ST}

AR = allelic richness, H_e = expected heterozygosity, H_o = observed heterozygosity, F_{IS} = fixation index, F_{ST} = genetic distance. Estimates were based on 12 polymorphic isozyme loci in 33 Q. robur *populations in Finland. Red column = negative correlation. Significant relationships are indicated. Vakkari et al. 2006.*

The positive relationship between F_{IS} and population size was attributed to cryptic sub-structuring of the large populations and a Wahlund effect. It might also be attributed to the existence of null alleles but this explanation was ruled out as unlikely.

There were positive but non-significant correlations between geographical and genetic distances. It may be concluded that the results obtained support the hypothesis about larger differentiation of fragmented populations.

Curtu et al. (2007b) studied the variation within and between five oak species (*Q. cerris,* and the four white oak species *Q. frainetto, Q.petraea, Q. pubescens,* and *Q. robur*) at a western Romanian locality. Only results for the four white oak species will be treated here. Seven

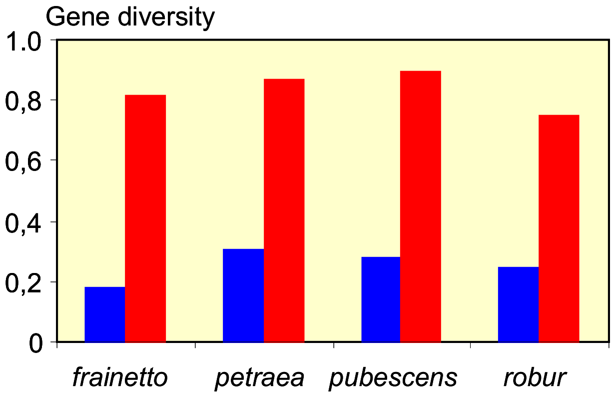

Figure 1-88. The gene diversity estimated by isozymes (blue) and microsatellites (red) in four white oak species growing in a locality in western Romania. Curtu et al. 2007.

isozyme loci, six microsatellite loci, and five chloroplast haplotypes were used. The number of alleles at the microsatellite loci was high and varied between six and 30. The microsatellite loci showed 2-3 times higher gene diversity than the isozyme loci (Fig. 1-88). It should be added that the level of variation within each species differed markedly among the seven isozyme loci.

Q. frainetto and *Q. pubescens* had two haplotypes which were shared with *Q. petraea* and *Q. robur* while the latter had three haplotypes. The third haplotype was not the same in *Q. petraea* and *Q. robur*.

The pairwise differences (F_{ST}) between the four species were somewhat larger for isozymes than for microsatellites (Fig. 1-89) and they were uncorrelated. It is somewhat hard to explain why the estimates are so large in a mixed forest of the four species with several possibilities for hybridization among the species. Moreover, the estimates were larger in this study than in many other studies of similar character.

Dering and Lewandowski (2007) studied the species composition in two Polish mixed stands of *Q. petraea* and *Q. robur* and in seven plantations with material originating from these two stands. Natural regeneration in the stands was also assessed. Five leaf morphological traits were used for classification of species.

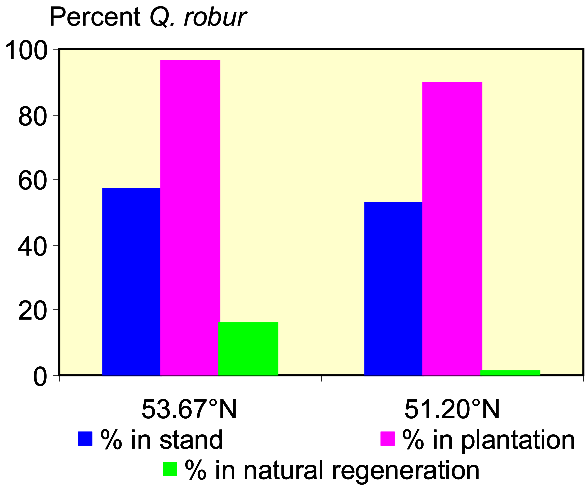

Figure 1-90. The percentage of Q. robur *in two Polish stands, in plantations and in natural regeneration in the two stands. Mean values for five and two plantations are given. Dering and Lewandowski 2007.*

Fig. 1-90 reveals a striking difference in species composition between the original stands on one hand and the plantations and natural regeneration on the other hand. The rapid decline of *Q. robur* acorn viability was suggested as one explanation for the low percentage of *Q. robur* in the natural regeneration. Although there should be no discrimination between *Q. petraea* and *Q. robur* acorns it cannot be excluded that the larger acorns of *Q. robur* were preferred during collection. The juvenile growth of *Q. robur* seedlings is faster than in *Q. petraea* seedlings. This means that *Q. robur* seedlings might have been selected for planting in the nursery. The fast germination and even vivipary of *Q. petraea* acorns may have been a gateway for infections of germinating acorns. This might have reduced the *Q. petraea* contribution to the plantation populations. The difference in fruiting behavior of the two species might also cause differential regeneration of them; with *Q. robur* having mast years every 4-8 years while *Q. petraea* flowers more regularly. The asymmetric pollen flow in species hybridization might also contribute to a higher percentage of *Q. robur*

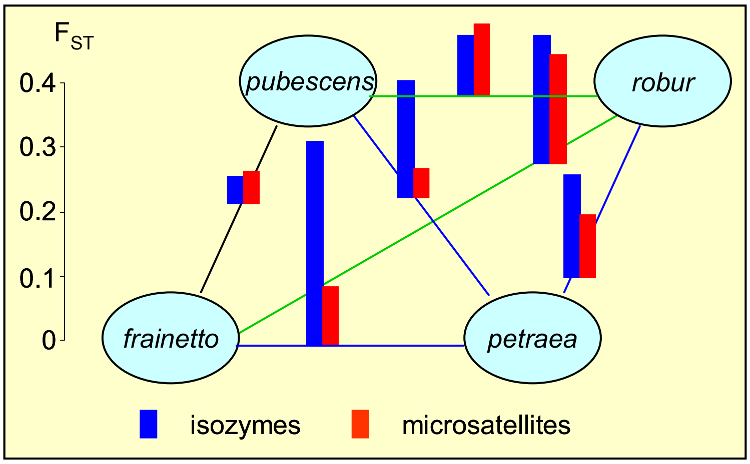

Figure 1-89. F_{ST} estimates for differences among four oak species in a western Romanian locality. The estimates were based on analysis of seven isozyme and six microsatellite loci. Curtu et al. 2007.

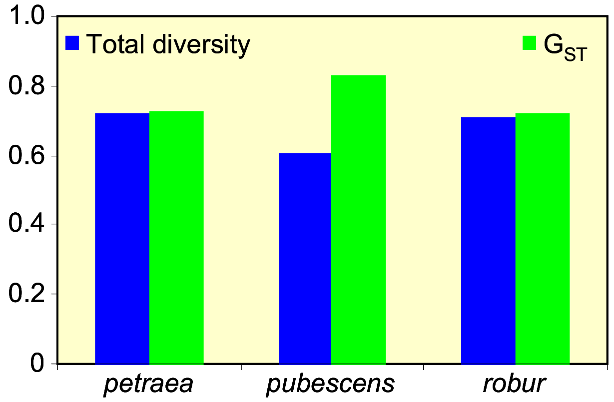

Figure 1-91. The total genetic diversity and G_{ST}s in oak populations from The Balkans. Slade et al. 2008.

in the progeny plantations.
It should be added that the percentage of putative hybrids in the plantations from both stands was low, 0.2 and 2.5, while it was higher in the naturally regenerated population from the northernmost stand, 8%.

The genetic diversity within populations, the total diversity, and genetic differentiation among Balkan populations for haplotypes were reported by Slade et al. (2008). The total diversity was lowest in *Q. pubescens* and genetic differentiation was highest in this species (Fig. 1-91). This difference was attributed to absence of human impact on *Q. pubescens*. The southernmost localities of haplotypes 4 and 31 were observed. This study must be regarded as complementary to previous studies on the phylogeography of oaks.

Microsatellites in four loci were used for pairwise estimates of F_{ST} among 12 populations of *Q. robur* in Bosnia and Herzegovina. (Ballian et al. 2010).
The means varied in the range 0.025-0.075 (Fig. 1-92). There was no tendency to differences in F_{ST} between the high-altitude populations and low-elevation populations. The differentiation observed was attributed to variation in site conditions among the populations as well as strong anthropogenic factors. According to the authors *Populations have virtually been brought to the verge of extinction.* This might have led to poor gene flow among populations, which in turn have resulted in genetic drift and thereby population differentiation. Still the largest variation was obtained within populations, 92.5%, while only 7.5% was found for variation among populations according to the AMOVA. Extremely high inbreeding coefficients of the individual populations were noted, 0.07-0.36 (Fig. 1-92). These results suggest that genetic drift might have been strong in most of the populations. This would have caused a stronger differentiation than observed. There was no relationship between the mean F_{ST}s and the inbreeding coefficients. Thus, the observed inbreeding coefficients are not compatible with the observed population differentiation. Finally, the authors warned for too far-reaching interpretation of their results.

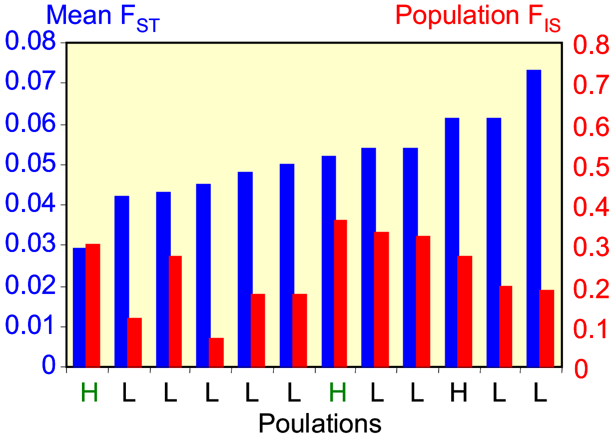

Figure 1-92. The mean F_{ST} from pairwise comparisons of 12 Q. robur *populations from Bosnia and Herzegovina and their inbreeding coefficient, F_{IS}, estimates. Four microsatellite loci were used. H = populations from altitudes above 600 masl, L = populations from altitudes below 600 masl.. Ballian et al. 2010.*

Borovics and Matyas (2013) studied the relationship between ambient climate factors, such as temperature and precipitation, and genetic parameters in 12 isozyme systems in 15 Hungarian *Q. petraea* populations. A focus was on populations growing under xeric conditions. Spearman correlations were calculated for allele and genotype frequencies and several components of precipitation and temperature data for the population localities.
The F_{ST} of these 15 populations was estimated at 0.056 without any relationship to geographic distances between populations. Thirty-three significant relationships between allele frequencies of individual loci and climatic variables were obtained. The corresponding figures for genotype frequencies were 30. It should be noted that total numbers of correlations estimated were not given and that there are dependencies between some of the climatic variables. As regards observed heterozygosity there were several significant relationships with precipitation variables. One important information emerged; precipitation seemed to be more important than temperature for the pattern of genetic parameters. The mean F_{IS} was high, which was attributed to homozygosity for fitness-contributing alleles and not to inbreeding.
In conclusion, an important contribution for the understanding of the evolution of a tree species under harsh conditions thanks to the approach to clarify the genetic effects caused by individual components of the ambient climatic conditions. As stated by the authors more studies along these lines are worthwhile to carry out.

Guichoux et al. (2013) investigated the utility of outlier and non-outlier SNPs (single nucleotide polymorphisms) for correct assignment of individuals to *Q. petraea* and *Q. robur*. In all 855 individuals from six northern French stands were genotyped. Outliers were defined as loci with

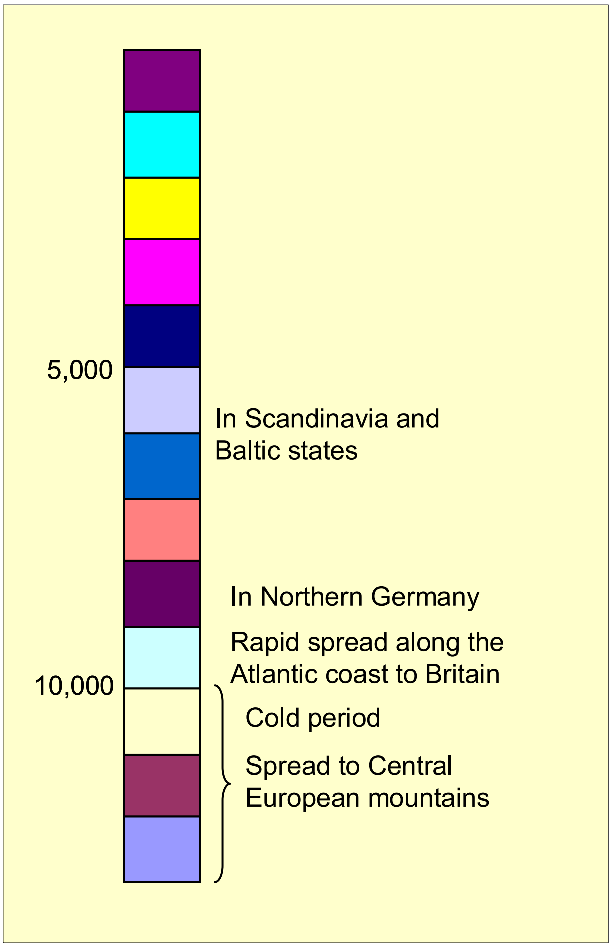

Figure 1-93. Approximate before present time spread of oaks from their refugia in Southern Europe. Brewer et al 2002.

alleles of frequencies above 0.50 in one species and absent or at very low frequency in the other species. The use of all 262 SNPs led to correct assignments in > 95% of the cases. One of the major results was the finding that there was a larger genetic diversity in outlier loci in *Q. petraea* than in *Q. robur.* This result fits the hypothesis that a species of later succession harbors a larger diversity than a more pioneer-type of species as a result of the larger gene flow from the later successional species to a pioneer-type of species. No difference in the diversity between the species was noted for non-outlier loci.

1.5 Phylogeography

The spread of the two oak species after the latest glaciation was discussed based on fossil pollen data (Brewer et al. 2002). There were three main primary refugia in Southern Europe, southern Iberian Peninsula, southern Italian Peninsula and southern Balkan Peninsula. The spread started between years 13,000 and 11,000 before present time and the oaks reached the second refugia in Central European Mountains during this period (Fig. 1-93). A cold millennium followed that halted the future spread. Even the few populations that had managed to reach beyond the Central European mountains became extinct. This period was followed by a warmer climate with a rapid spread along the Atlantic coast. A slower migration northwards was noted for the spread from the Italian and Balkan refugia. Thus, the British Isles were reached before northern parts of Germany and Poland were reached. Oaks had reached latitude 60°N in Scandinavia and The Baltic states at about 6,000 years before present time.

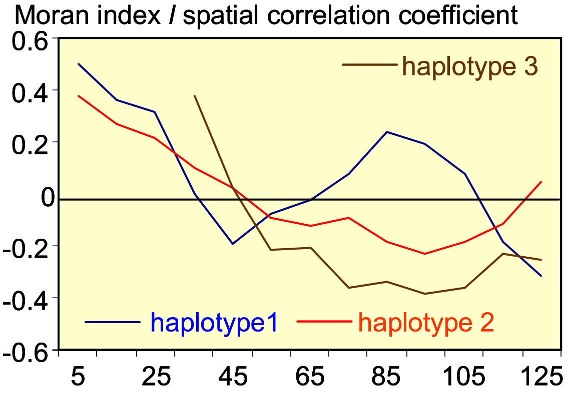

Figure 1-94. The spatial correlation coefficients for haplotypes 1, 2, and 3 with distance classes of 10 km in a region in central France . Petit et al 1997.

Several studies on the relationship between oak species and phylogeography have been carried out by use of chloroplast DNA.

Ferris et al. (1993) described a sequence change in the highly conserved chloroplast tRNALeu1 intron in *Q. robur* and *Q. petraea*. There was a divide in Central Europe between the two types of sequence occurring parallel in both species. It was speculated that there were two refugia of these two species during the latest glaciation.

This study was extended to trace the origin of British oaks (Ferris et al. 1995). The duplication in the chloroplast tRNALeu1 intron was found only in 22 out of 28 East Anglian populations and nowhere else in Britain or Europe. Furthermore, this duplication only occurs in *Q. robur* and no other related oak species. It was hypothesized that the duplication had arisen in Britain after the colonization over the land bridge between The Continent and Britain approximately 8,000 years ago.

A thorough discussion of postglacial migration of oaks was carried out by Petit et al. (1997). The observed patchy structure within a geographically limited region in France was discussed. The authors tried to explain this structure by use of spatial autocorrelations. For this purpose the so called Moran index I (Moran 1950) was computed for each 10 km class of distance for each of the four most common haplotypes identified in this study. Figure 1-94 reveals that the relationship between the spatial correlation coefficients according to Moran (1950; Moran index

I) and the 10 km distance classes drops rapidly from a high level at low distances for all three haplotypes. This drop was followed by a second peak that was most pronounced for haplotype 1. The reason for the second peak was not discussed but it must be attributed to two geographically widely separated areas that happened to have haplotype 1 with all intermediate populations lacking this haplotype. Haplotype 4 showed a rather flat relationship, mainly within the range -0.1 and +0.1 of the Moran index I. The authors concluded that the patchy distribution could be a result of long-distance acorn dispersal during the early postglacial colonization into open ground and a contemporary diffusion (short-distance) into such areas. Below some arguing in favor of this conclusion is presented.

The large size of the patchy areas suggested that 30-40 km long and rare dispersal events had taken place during the colonization of open areas. It was argued that such long-distance dispersals are required to account for the speed of postglacial colonization. Long-distance are required to match the observed postglacial speed of oak colonization, which was estimated to 500 m per year. It should be noted that oaks rarely flowers before an age of 20 years. This means that it takes a considerable time until one tree becomes a new spreading point for acorns. Moreover it was shown by simulations (le Corre et al. 1997) that: *dispersal distances rather than the number of dispersal events per generation are the variable to which migration rates are most sensitive*.

The acorn production within the newly established stand will supply any still empty spots with acorns. The spread of acorn within an established stand will be more limited than dispersals to open ground. Both of these conditions prevented the establishment of newcomers resulting in a freezing of the haplotype structure in the various patches. Selection for different haplotypes in different patches owing to differences in site conditions among the patches was ruled out as unlikely since there is a high degree of heterogeneity within patches with respect to site condition variables. Besides, I do not think that any of these haplotypes contributes to fitness, which is required if natural selection should influence their geographic distribution.

Ferris et al. (1998) analyzed the haplotypes of 47 trees originating from 42 localities with a focus on Finnish localities. They identified four haplotypes, three of them had separate extensions from south to north while the fourth was found in East Anglia. The results were interpreted as a migration from three separate refugia in southern Europe during the last glaciation. The haplotype in the Åland archipelago and the south-western corner of Finland shared the haplotype with eastern Swedish localities. In more eastern Finnish localities another haplotype occurred, which also occurred in Estonia and the St Petersburg area in Russia. This suggests two migration routes of oak into Finland one eastern via Baltic States and another from Sweden.

To study migratory routes of oaks in The Alps a comprehensive collection of material from Switzerland was carried out by Mátyás and Speriesen (2001).

The detection of a series of populations along a latitudinal gradient in western Switzerland with the western haplotype (A7) surrounded by the eastern haplotype (C1) was conspicuous. This indicates that oaks migrated independently of each other and that mixing by acorn dispersal was low.

To test whether haplotypes were randomly distributed spatial autocorrelations were calculated for different distance classes. The correlations were strongly significant up to distances of 40 km. G_{ST} was estimated at 0.88 indicating a strong differentiation among populations. A detailed discussion of possible migratory routes within Switzerland was carried out. Contrary to other hypotheses about oak migration it was suggested that west-east migration might have taken place of haplotype C7. This would require refugia in southeastern France of this haplotype that is assumed to have its refugia in The Balkans according to other studies. Such a location does not seem to be rather likely.

In a series of papers chloroplast haplotypes in certain regions of Europe were used to study the variability and the postglacial distribution of oaks (Bordács et al. 2002, Cottrell et al. 2002, Csaikl et al. 2002a and b, Fineschi et al 2002, Svejgaard Jensen et al. 2002, König et al. 2002,

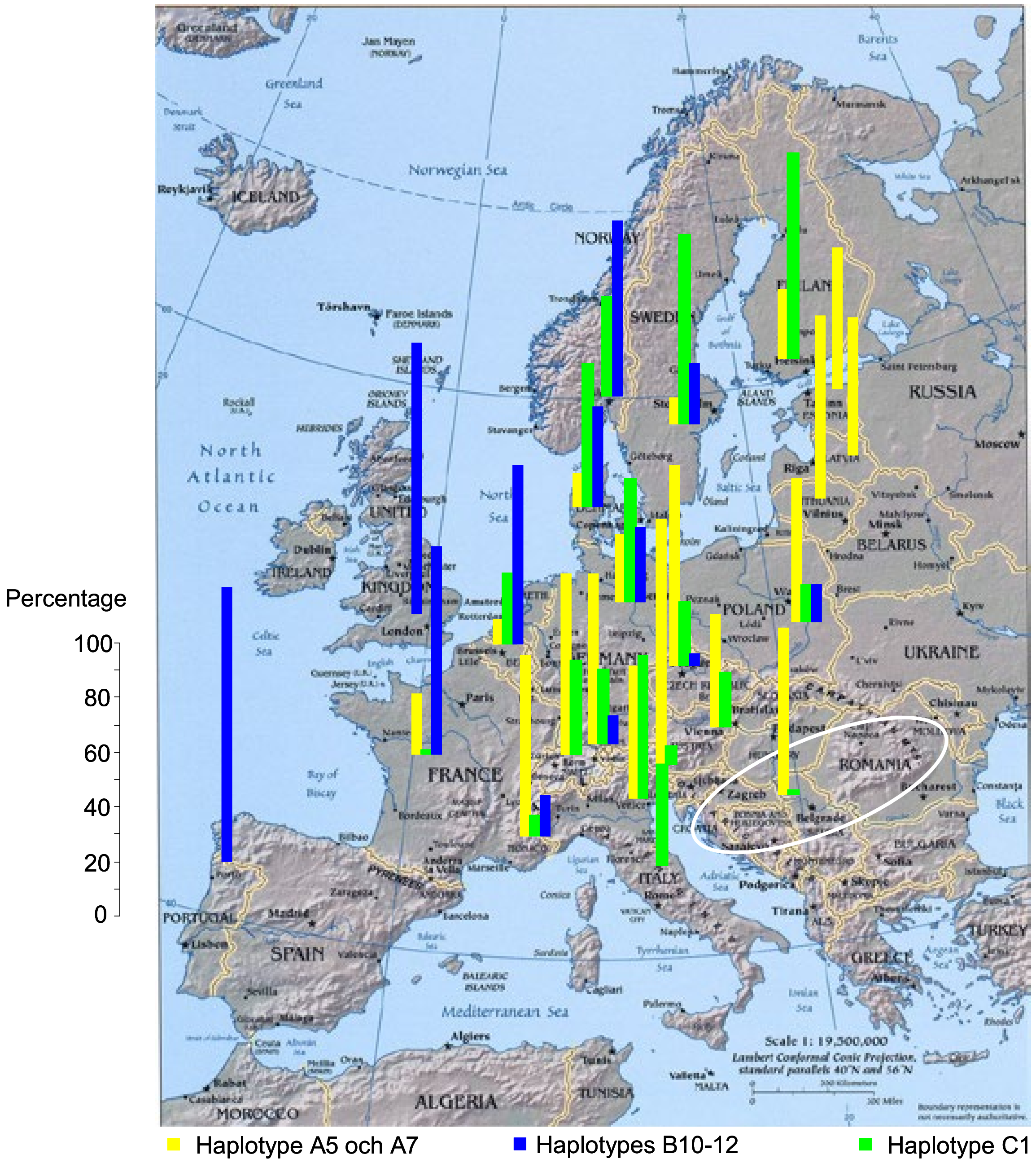

Figure 1-95. The percentage occurrence of common haplotypes in Q. robur *in different countries/regions in Europe. Owing to inconsistency of reporting the percentages are in a few cases mixed with data from* Q. petraea. *The Balkan report did not specify percentages for individual countries. Bordács et al. 2002, Cottrell et al. 2002, Csaikl et al. 2002a and b, Fineschi et al 2002, Svejgaard Jensen et al. 2002, König et al. 2002, Olalde et al. 2002, Petit et al 2002c.*

Olalde et al. 2002, Petit et al 2002c). The major trends in these reports are summarized in the European map in Fig. 1-95, in which the percentages of the most common haplotypes are illustrated for different regions. It should be noted that the results were not reported in a consistent way. In some papers the percentages were pooled for *Q. petraea* and *Q. robur*, in other cases percentages were not reported at all. In these cases the percentages were obtained from maps with occurrence of haplotypes. Since the difference between the two species is not too great, the trends of the geographic distribution of the haplotypes will not be much distorted. It should also be noted that transfer of acorns in Europe has occurred to large extent, which means that the authenticity of the analyzed trees are not guaranteed.

With all these limitations in mind it is clear that:

1. Haplotypes B10-B12 strongly dominate in Western Europe
2. Haplotype A5 and A7 dominate in eastern Europe and is almost absent in the Iberian peninsula and United Kingdom

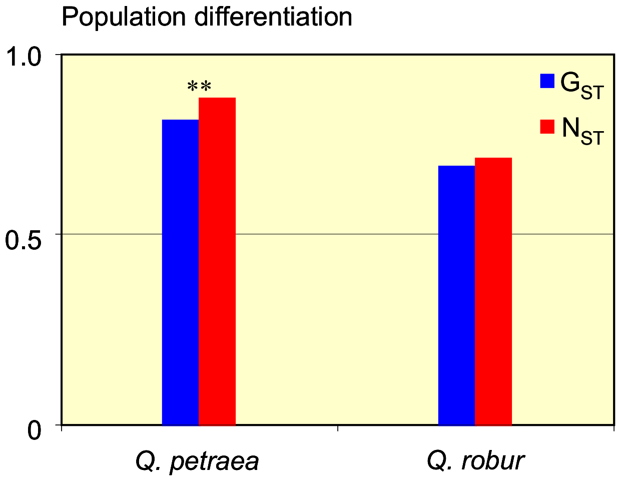

Figure 1-96. The G_{ST} and N_{ST} estimates for eastern European populations of Q. petraea *(98 populations, 11 haplotypes) and* Q. robur *(88 populations, 10 haplotypes). Bordács et al. 2002.*

3. Geographically haplotype C1 takes an intermediate position to the previous ones. It is also almost absent in the Iberian peninsula and United Kingdom

These results constitute the basis for the hypothesis that there were three refugia during the latest glaciation, Iberian Peninsula, Southern Italy, and Balkan Peninsula. – In most papers it was concluded that cp haplotypes are useful tools for identification of seed sources.

Below some details from some of the individual studies are presented.

Eleven or ten chloroplast haplotypes were used for a study of differentiation among 98 *Q. petraea* and 88 *Q. robur* populations from Eastern Europe (Bordács et al. 2002). The number of trees per population was low with harmonic means of 4.59 and 4.18 in *Q. petraea* and *Q. robur,* respectively.

None of the haplotypes was species-specific. Five of them were rare. Most of the populations had just one haplotype. As a consequence of this the genetic differentiation was substantial (Fig. 1-96). Some of the haplotypes occurred in restricted areas. It might be remarked that it was not discussed whether the low number of trees per population might have resulted in lack of detection of another haplotype. There was a significant difference between N_{ST} and G_{ST} estimates in *Q. petraea* but not in *Q. robur*.

Csaikl et al. (2002b) analyzed 394 samples from 68 Baltic countries and Polish populations with respect to their haplotypes. In all 13 haplotypes were found, four of them were rare. As seen from Fig. 1-97 the A lineage with the origin in The Balkans was dominating in this part of Europe. The haplotypes with Iberian origin were only found in Polish populations. The rare haplotype E22 belonging to lineage A was found in three populations, one in Latvia and two in Estonia. Seventy-five percent of the populations were monomorphic.

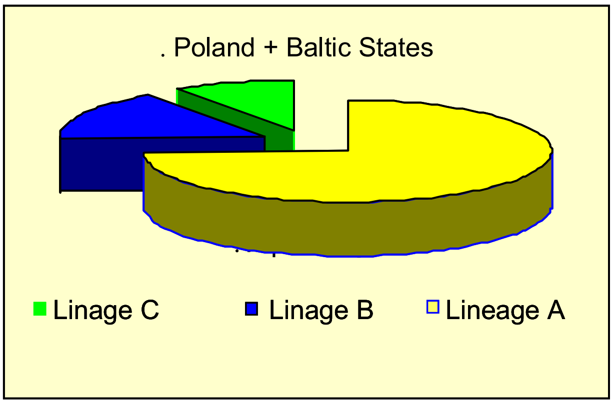

Figure 1-97. The separation of the origin of the haplotypes found in Poland and the Baltic countries. Csaikl et al. 2002b.

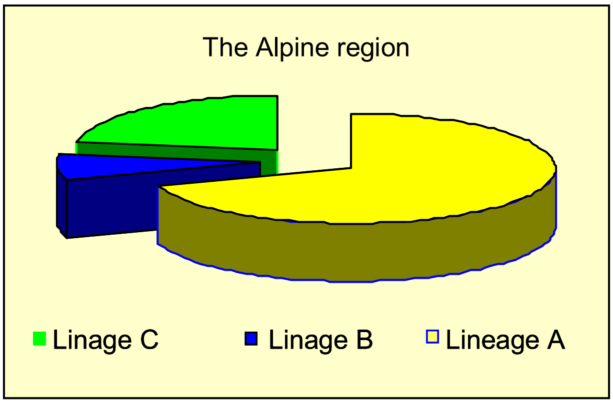

Figure 1-98. The separation of the origin of the haplotypes found in The Alpine region. Csaikl et al. 2002a.

Csaikl et al. (2002a) analyzed 1375 samples from 292 populations from The Alpine region. In all, eleven haplotypes were found. Haplotype 7 of Balkan origin was the most frequent haplotype found over the whole region (Fig. 1-98). Haplotype 1 of Italian origin was the dominating haplotype in the southeastern corner of France but occurred in scattered populations in The Alpine region. Haplotype 11 of Iberian origin was only found in French samples.

Haplotypes in a country-wide study of more than 200 oak stands (1,076 trees) in Great Britain were carried out by Cottrell et al. (2002). Stands of pure *Q. petraea* or *Q. robur*, as well as a number of mixed stands of these two species, were included in this study.

Three haplotypes, which are common in southwestern

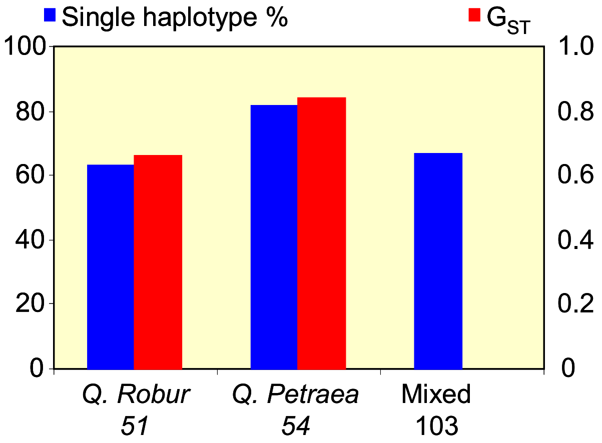

Figure 1-99. The percentage of British stands with a single haplotype in Q. petraea, Q. robur, *and in mixed stands of these two species. The population differentiation estimated as* G_{ST} *is illustrated. The number of species in the three categories is also given. Cottrell et al. 2002.*

Europe, occurred in 98% of the oaks. It was speculated that the exceptional 2% might be attributed to human transfer of acorns. A majority of stands, whether pure species or mixed ones, had just one haplotype (Fig. 1-99). The G_{ST} estimate for *Q. petraea* is particularly high, amounting to 0.84. This is a reflection of the high percentage a stands with just one haplotype. The high percentage of monomorphism in the mixed stands, 66.8%, might be attributed that crosses between the two species and back-crosses are common. Even if a majority of stands was monomorphic, there was a significant difference in prevalence of haplotypes in the two species

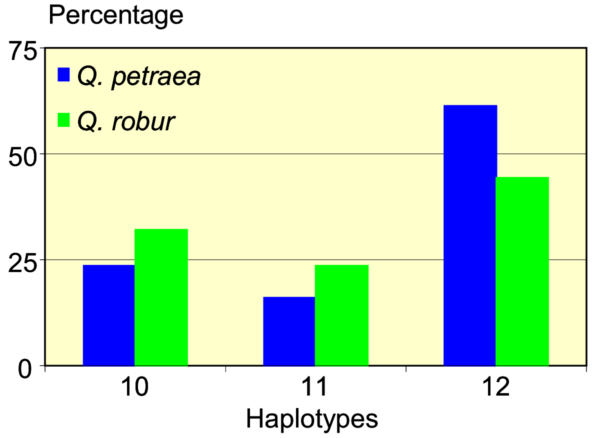

Figure 1-100. The percentage of the most common haplotypes in 383 Q. petraea *and 439* Q. robur *trees in pure stands of these two species in Great Britain. Cottrell et al. 2002.*

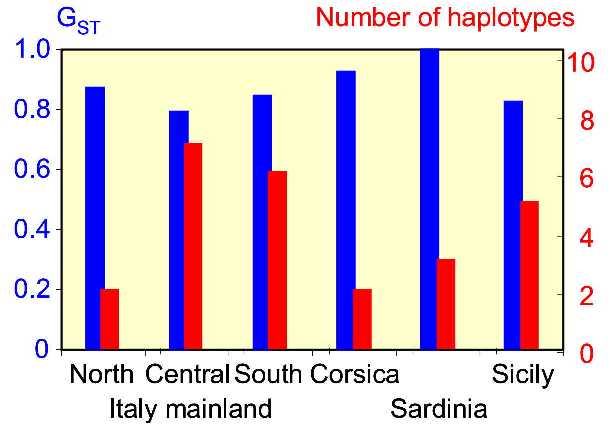

Figure 1-101. G_{ST} *estimates for haplotype differentiation in six different regions of Italy. The number of haplotypes detected in each region is given. Fineschi et al 2002.*

(Fig. 1-100). Haplotype 12 dominated in *Q. petraea* while its occurrence was less than 45% in *Q. robur*.
Certain regions of Great Britain had higher percentages of polymorphic stands than others. In some parts of the country a few stands were surrounded by stands with deviating haplotypes. Long-term forest management was suggested as a reason for this.

Fineschi et al. (2002) reported high haplotype G_{ST} estimates in five regions of Italy and Corsica (Fig. 1-101). As far as I understand G_{ST} was estimated based on all four oak species in this study, *Q. frainetto, Q. petraea, Q. pubescence*, and *Q. robur* . As seen from Fig. 1-101 in Sardinia G_{ST} reached its maximum limit 1.0. This is a consequence of monomorphism in each of the five populations sampled. Corsica had the next highest G_{ST} and only one out of 22 populations was polymorphic. G_{ST} for all Italian populations amounted to 0.879 and the corresponding N_{ST} was slightly higher 0.890. It was stated that the G_{ST}s did not differ much among the species. The 60 Central Italian populations had the highest number of haplotypes, 7, and the lowest G_{ST}. It was stated that there was a clear divide between the northern part of the Italian peninsula and central and southern parts of the peninsula. However, based on Fig 1 in the paper the divide does not seem too clear.
A test of the human impact on population differentiation was carried out by König et al. (2002) in their study of haplotype variation in 426 western Central European populations. For this purpose G_{ST} was estimated in four regions with different human impact: from roadside Dutch populations with a great human impact to Bavaria

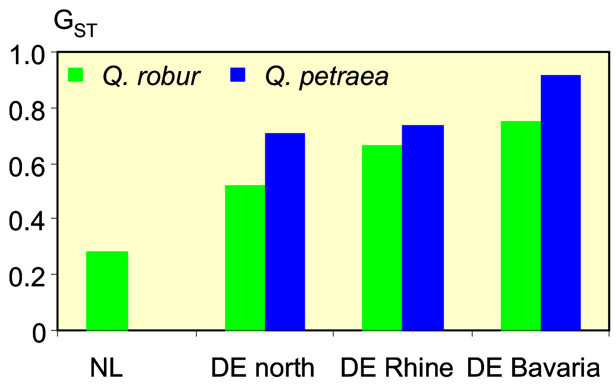

Figure 1-102. G_{ST} estimates in four western Central European regions. NL = Roadside populations in The Netherlands, DE north = North German lowlands, DE Rhine = Rhine region in Central Germany, DE Bavaria = Bavarian populations. König et al 2002.

in southern Germany with limited human impact (Fig. 1-102).

For oaks the roadside plantations in The Netherlands had the lowest ever estimated G_{ST}. This was attributed to a mixture of material from different nurseries, which led to high within-population diversity, h_s = 0.53, as compared to 0.17 for the Bavarian populations. Fromw Fig. 1-102 it is seen that the lowest human impact has led to highest differentiation. The differentiation was higher in *Q. petraea* than in *Q. robur*, which also was attributed to greater human impact on *Q. robur* than on *Q. petraea*. This explanation is supported by the percentage of German populations classified as autochtonous seed stands, which is 42 for *Q. robur* and 84 for *Q. petraea*. Thus, many more transfers of acorns have taken place with Q. robur than with *Q. petraea*. Some of the populations of *Q. petraea* may even be converted coppice stands that have existed for hundreds of years.

Petit et al. (2002b) summarized the differentiation of oak populations in seven regions in Europe (Fig. 1-103). As seen from this figure the G_{ST} estimates for *Q. petraea* are in most cases much larger than the estimates for *Q. robur*. In both species N_{ST} estimates were in some cases larger than the G_{ST}s but usually there was no dramatic difference between G_{ST} and N_{ST}. The G_{ST} and N_{ST} for the British populations of *Q. robur* are lower than all other estimates. As suggested above (Cottrell et al 2002) the low estimates were attributed to the human impact on forest management such as mixing of seed sources and transfer of acorns.

The results from a country-wide study in France containing 878 populations of *Q. petraea, Q. pubescens, Q. pyrenaica*, and *Q. robur* showed three main regions as regards haplotype variation (Petit et al. 2002c):

1. The south-eastern corner of France; haplotype 1 dominating
2. A region situated east of a line Toulouse-Strasbourg; haplotype 7 dominating
3. The remaining part of France; haplotypes 10-12 of lineage B dominating

The 367 populations with more than one species were used for a study of cytoplasmic exchange. It was found that *Q. robur trees found in mixture with Q. petraea are not more similar to each other than to Q. petraea trees*. The existence of more than one oak species at one place should indicate that such a forest is of ancient origin. Under such conditions, the *Q. petraea* trees are assumed to carry the local haplotype and thus be one possible explanation for this result. One the other hand it was also stated that transfer of oaks mainly involves *Q. robur*, which would increase the variation within *Q. robur* and the variation between *Q. robur* and *Q. petraea*. It is hard to come to a final explanation without a detailed knowledge about the history of the individual populations.

As in other studies the G_{ST} estimates were somewhat higher in *Q. petraea* than in *Q. robur* (Fig. 1-104). The differentiation of *Q. robur* was significantly lower in the northern region than in the other regions.

The human impact on the haplotype structure was studied by comparing the haplotypes in different size of sur-

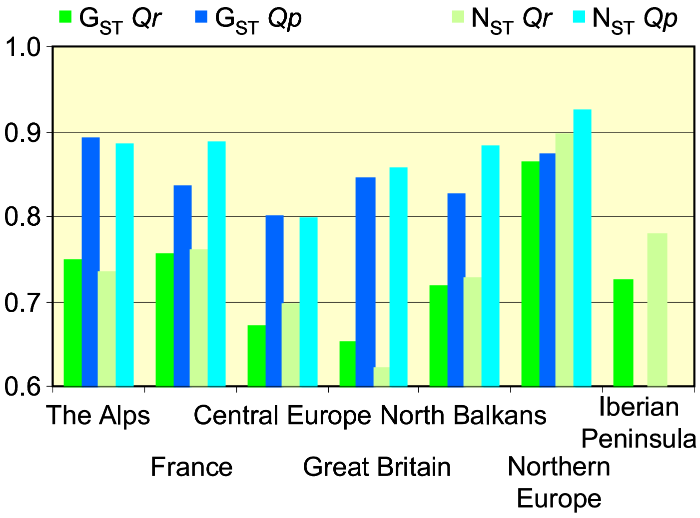

Figure 1-103. Haplotype differentiation, G_{ST} and N_{ST}, in Q. petraea *and* Q. robur *in seven regions in Europe estimated by haplotypes. Petit et al. 2002b.*

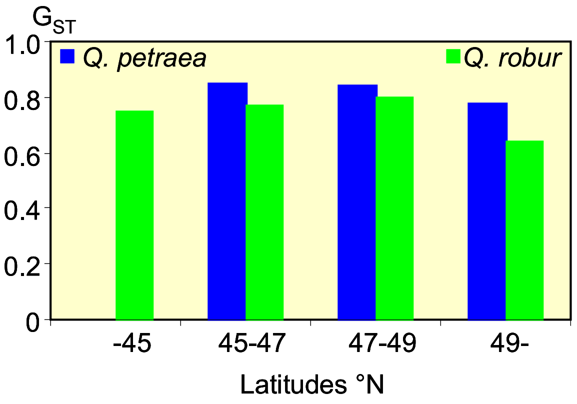

Figure 1-104. G_{ST} *estimates based on haplotypes in four latitudinal regions in France. Petit et al. 2002c.*

rounding populations such as isolated populations and populations surrounded by large forests. For *Q. petraea* there were no significant relationships with any of these variables or with geographic variables. One significant relationship was noted for *Q. robur*, local forest area/ total forest area. With 322 populations studied it is highly likely that some relationships turn out to be significant. However, the correlation coefficient was only 0.14, which means that an extremely low part of the variation could be attributed to this factor. It was concluded that only minor changes in the populations are expected following human impacts other than seeding or plantation with non-local material.

A detailed discussion of migratory routes was carried out in Petit et al. (2002a), in which migration from the three main southern European refugia was treated separately and lineage by lineage. Simultaneously, support from pollen fossil data were presented.

Haplotype 12 belonging to lineage B had *an almost continuous distribution from northern France to eastern Germany*. According to the authors this result demonstrated that migration was not only south – north but also from west to east. This must be regarded as unexpected since the climate in this low-altitude part of Europe does not change dramatically from west to east and should not provoke a west-east migration. Contrary to this, an east-west migration of lineage A haplotype 5 within Poland was suggested.

The oak migration across The Alps could not take place until the post-glacial warm period. It was concluded that three haplotypes of lineage C could be traced to a southern Italy refugia. It was suggested that a migration from northern Italy took place to Catalonia via France during the cold period 11,000 to 10,000 years ago. This migration was mainly restricted to *Q. petraea*.

It was suggested that refugia existed in Corsica and Sardinia.

Low temperature and limited precipitation during the glaciation restricted the distribution of oaks to mid-altitudes in mountainous regions in southern Europe. These refugia never consisted of wide-spread forests.

The leading edge hypothesis says that populations at the edge of distribution contribute most to the colonization. In the case of European oaks some populations might for this reason be trapped in southern regions. Thus, populations in Corsica and Sardinia have certain haplotypes that do not exist in the European continent.

A full understanding of the migratory routes require according to the authors *to better integrate climatic and ecological data, so that the processes that have led to the observed patterns of recolonization can be better identified.*

Haplotypes and microsatellite variation in seven latitudinal (51.75 – 55,12°N) Irish *Q. petraea* populations were studied by (Muir et al. 2004). These populations were disjunct with the exception for two populations growing at a distance of six kilometers. Two populations from France and one from Spain were reference materials for this study.

The three haplotypes occurring in these populations belonged all to the Iberian refuge. Five of the populations were monomorphic for haplotype 12. G_{ST} for the haplotypes was estimated at 0.50. The differentiation based on 13 microsatellite loci was low $F_{ST} = 0.008$. Only two of the 21 pairwise calculated F_{ST}s were significantly different. There was no correlation between pairwise F_{ST}s and geographic distance, which rules out significant roles of genetic drift and limited gene flow in these Irish populations. Most likely gene flow was strong among the populations, which had large effective population size. The low average inbreeding coefficients in the populations support this interpretation. All Irish populations were significantly different from the eastern France reference population. This supports the hypothesis of a western migration route from the Iberian peninsula into Ireland.

The observed heterozygosity of the Irish populations was slightly higher than in the three reference populations, 0.69 versus 0.64. It was stressed that the high diversity occurred in spite of marginal location of the Irish populations and the large clearing of oak forests in Ireland. However, it was pointed out that the effect of such a clearing might not have come to effect yet owing to the long generation time in oaks. It was speculated that hybridization with *Q. robur* also contributed to the large microsatellite variation.

It wasstated that grazing in existing populations constitutes a threat for the regeneration of oaks in Ireland and measures should be taken to guarantee the continued existence of the oaks in Ireland.

The haplotypes of 297 oaks from 65 Polish oak stands with an age over 200 years were determined. Six haplotypes were found, one of the Iberian type, two of the Italian, and three of the Balkan type (Dering et al. 2008). The Balkan haplotypes were most common, 71%, and the Iberian least common, 5%. The majority of stands with the Balkan haplotypes were fixed for one haplotype, 84%. All stands with the Iberian haplotype were monomorphic and they occurred in the northern part of Poland. The finding of Iberian haplotypes in Poland was discussed thoroughly since stands with this haplotype might be suspected to be non-autochtonous. Moreover, these stands seemed to be isolated from other populations with Iberian haplotypes further west. One reason for this isolation might be that they by chance were missed during sampling. The authors suggested that these stands were autochtonous. The high age of the trees supported this interpretation.

1.6 Host-parasite relationship

To study whether there is a synchrony between hatching of *Operophtera brumata* pupae and bud flushing van Dongen et al. (1997) studied these traits in five Belgian stands of *Q. robur* during three years. Copulating pairs were collected at different times and hatching of formed pupae was recorded indoors (1993) or outdoors (1994).

There was a good agreement between the flushing of individual trees between years. R^2 estimates were presented for two stands and amounted to 0.56 and 0.67, respectively. The maximum difference among trees within one stand was almost four weeks. The variation in flushing was dependent on the weather conditions the different years. Similarly, there was a large variation in time for hatching. Several significant relationships between bud flushing and hatching time were obtained. However, the degree of explanation of the relationships was low, the R^2 estimates were lower than 0.40.

The significance of the results was discussed from an evolutionary point of view. It was stated that there was a tendency that female oviposition took place on the tree from which it was hatched. This would eventually result in an adaptation to the phenology of this tree and an improved synchrony between insect and host tree. However, as stated above the degrees of explanation for the relationships were weak or moderate, which speaks against a strong narrowing down of the variation in hatching points of time. Rather, there might be an advantage of keeping a large variability in hatching time since there is large variation in flushing among the trees within each stand. Keeping a large variation means that there will always be suitable trees for emerging larvae.

The matching of the insect *Operophtera brumata* to *Q. robur* leaf flushing in Scottish *Q. robur* was studied by Tikkanen and Julkunen-Tiito (2003) in feeding experiments. Out of 28 young oak trees, ten representing the total range of bud phenology were selected for this study. Neonate larvae were placed in vials after six different degree days (DD) of starvation (0, 5.5, 11, 22, 27.5, and 33DD). The decline over time of the leaf quality for larvae feeding was done by exposing larvae to leaves of different ages. The time to pupae formation was recorded. The growth rate of larvae was estimated by dividing pupae weight by DD needed to complete the larvae period.

As expected there was a strong relationship between DD of starvation and larvae mortality; after 30 DD the mortality was > 50%. Similarly, there was a significant relationship between mortality and DD time after bud flushing (R^2=0.46). This means that the quality of the food for the larvae decreases with development of leaves. This R^2 estimate is reflected in the large variation at individual DD. Only at the later stages when the mortality reached almost 100% the variation became smaller for obvious reasons. This study clearly shows that timing of larvae development and host leaf development is critical for the success of this herbivore.

Erysiphe alphitoides is a parasitic fungus attacking all life stages of *Q. petraea* and *Q. robur* with a "narrow" window during which the oaks are susceptible for infection (Desprez-Loustau et al. 2009 and litt. cit.). One of the Pyrenean slopes (131-1630 masl) included in several other studies were used by Desprez-Loustau et al. (2009) for a study of the host-parasite relationship and specifically if any coadaptation can be proven. Young disinfected oak seedlings were used as traps in the *in situ* populations to assess the spore release. Every ten days during the period early April – end of June new seedlings were put into the forests. Spore-derived infections were determined after incubation in growth chamber at a photoperiod of 16 h light and 22/18°C. Strongly infected leaves from 4-5 scattered trees in the populations were collected in late September for a study of chasmothesia phenology.

In agreement with the assessments in 2005 by Vitasse et al. (2009a) there were strong relationships between flushing and population elevation origin (Fig. 1-105). In the low elevation populations flushing of the oak trees occurred before appearance of *E. alphitoides* spores in these populations (Fig. 1-106 and 1-107). The opposite was the case for the population from the highest elevation

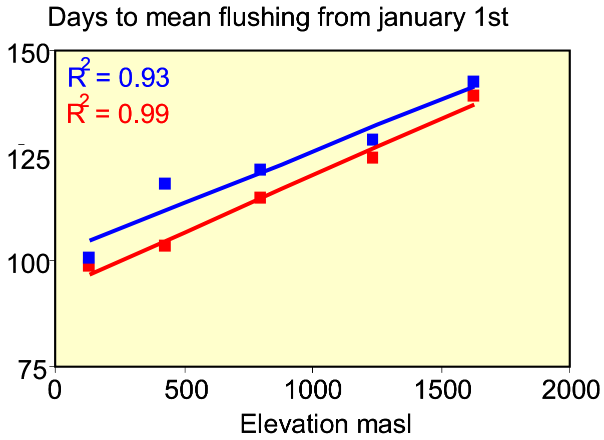

Figure 1-105. The mean flushing days from January 1st in five Q. petraea *populations along an elevation gradient in The Pyrenees in France. Red and blue refer to assessments in 2006 and 2007, respectively. Desprez-Loustau et al. 2009.*

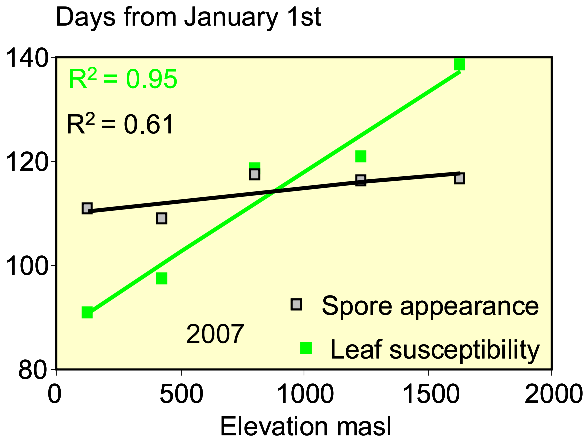

Figure 1-107. The beginning of susceptibility of Q. petraea *leaves and appearance of first spores of* Erysiphe alphitoides *in five populations along an elevational gradient in French Pyrenees in year 2007. Green refers to leaf susceptibility and grey to spore appearance. Desprez-Loustau et al. 2009.*

with flushing after the spore appearance. The difference in slope between the two regression lines for flushing and spore appearance was particularly different in 2007. These patterns resulted in the strongest infections at intermediate elevation with 20-30% of the leaf area infected while at low and high elevations less than 10% of the leaf area was infected. It should be remarked that the occurrence of *E. alphitoides* was low at high elevations, which might explain the low rate of infection at this altitude. It was evident that there was no matching of the fungal phenology to the host phenology.

The release of ascospores from the chasmothesia of material collected at different elevations did not differ much, which suggests limited genetic variation of the fungus. Phenotypic plasticity was suggested as the explanation for the variation observed. It was stressed that the large variation in flushing time in individual stands would lead to weak natural selection for phenology matching of the fungus. In most instances there will be host trees with "the receptive window" open for infection. It was noted that the early flushing trees in the low-elevation population were less infected than the late flushing trees from the same population. In conclusion, with the difference in response of host and parasite to temperature (Fig. 1-106 and 1-107) there does not seem to be any strong coadaptation in this case.

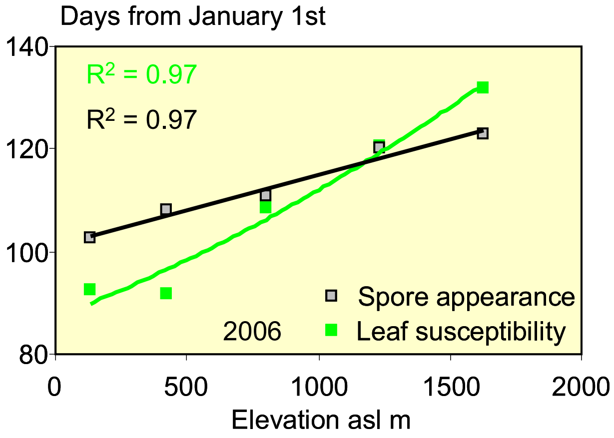

Figure 1-106. The beginning of susceptibility of Q. petraea *leaves and appearance of first spores of* Erysiphe alphitoides *in five populations along an elevational gradient in French Pyrenees in year 2006. Green refers to leaf susceptibility and grey to spore appearance. Desprez-Loustau et al. 2009.*

Studies on the relationships between oak hosts and fungi infections or herbivores have been carried out but without inclusion of genetic differences of the host trees (*e.g.* Visser and Holleman 2001, Roslin et al. 2007).

1.7 Summary

1.7.1 Quantitative traits

Northern and eastern European trials. The results from northern provenance trials revealed large differences among populations for many traits. Owing to acorn transfers during previous centuries geographic trends in the population performances were assumed to be masked. Bud flushing took place earliest in northern populations while their growth was poorest. Fairly high Q_{ST} estimates were noted for phenology traits. In a Lithuanian series of provenance trials significant population x test locality interactions were noted for bud flushing and leaf coloring. An irrigation treatment experiment revealed much larger population effects than population x treatment effects. One experiment did not show any relationship between growth and acorn length or width.

Central European trials. Large difference in juvenile growth was noted in a German series of trials at three elevations with a fairly limited number of ranking changes among the trials. There was no impact of tree height on frost damage.

Western Europe. Moderately strong relationships between latitudinal or elevational origin and bud flushing were noted in a Scottish nursery. Southern and high elevation populations had the earliest bud flushing. Freezing tests revealed that LT_{50} estimates were moderately strongly correlated with days to bud flushing. The late flushers had the lowest LT_{50} temperatures. Similarly, populations with early growth cessation had the lowest LT_{50} estimates. A considerable superiority in tree height of the best population over the trial means was noted in two British provenance series. Large Q_{ST} estimates were noted for several growth, phenology, and quality traits in French trials with over 100 populations. There were relationships between latitudinal or altitudinal origin and flushing. Southern and high elevation populations had the earliest flushing.

Populations in two Pyrenean valleys with populations in the altitudinal range of 131-1630 masl were studied extensively by French geneticists. Very strong relationships were noted between altitudinal origin and bud flushing or growth cessation after assessments *in situ*. Low elevation populations had the earliest flushing and the latest growth cessation, which must be attributed to adaptation to the local climatic conditions. Bud flushing had the largest variance components of the two phenology traits. The relationship between population altitude and plant height in nursery was moderate while the corresponding for bud flushing was very strong, $R^2 > 0.94$. The differentiation of populations within valleys and between valleys was estimated with haplotypes and microsatellites with limited differentiation in all comparisons for the microsatellites while it was several times higher for haplotypes. There was a strong clinal variation for two microsatellite alleles, one increasing with altitude and the other decreasing with altitude. The same pattern was noted for both valleys. There was a larger gene flow from low elevation to high elevation than in the opposite direction and the highest frequency of interspecific hybrids at high elevations. Significant correlations between SNP allele frequencies in populations from the two Pyrenees valleys and populations from a latitudinal gradient were noted for some of the SNPs. Generally the F_{ST}s based on SNPs of different altitudinal and latitudinal origins were low suggesting that they were not influenced by natural selection. A lower water use efficiency in *Q. robur* than in *Q. petraea* was noted.

From an evolutionary point of view the finding that northern populations have the earliest flushing in trials in northern Europe while southern populations have the earliest flushing in southern European trials suggests that natural selection operates in different ways in north and south.

1.7.2 Comparison of different types of trait

A significant relationship between geographic origin and microsatellite allele frequency was noted for Italian populations of *Q. petraea*. Early and late flushing German populations differed with respect to their haplotypes. However, the different origins of the populations could as well explain the observed difference in population performances. A study of transcriptome variation during bud development of young seedlings of two *Q. petraea* populations was summarized well by the authors in the following way: *This study has provided new insights in the understanding of gene expression during bud burst.* Candidate genes and QTL for bud flushing were studied in early, intermediate, and late flushing populations in a French provenance trial. No association between candidate genes and flushing was noted. As many as 286 QTL were detected; 90% of them explained a limited part of the observed variation in bud flushing. Leaf morphology and microsatellite genotyping was compared for trees in a Swiss stand with a mixed population of *Q. petraea* and *Q. robur*. There was a good agreement in the classification with both types of traits. It was concluded that the gene flow between the two species had little impact on the adult population. Bud flushing and cpDNA showed different geographic patterns in a Lithuanian study.

1.7.3 Markers

Many studies on the genetic differentiation were carried out. When isozyme markers were used limited variation among populations was found. In the majority of studies there was no geographic trend of the variation but in a few cases a geographic pattern was revealed. Large among-population variation was found for cpDNA. N_{ST} estimates were usually somewhat larger than G_{ST} estimates but no dramatic differences were noted. The differentiation between *Q. petraea* and *Q. robur* populations from the same stand were usually smaller than differences between populations of the same species growing at different localities. Most results suggest that there is high gene flow in both species but in some cases limited gene flow is claimed.

Sophisticated discussions of the causes of high estimates of genetic differentiation for haplotypes occurred in many papers. However, the occurrence of monomorphic populations of different haplotypes leads automatically to large estimates of population differentiation. Why there is monomorphism of different kinds is another matter.

1.7.4 Phylogeography

A comprehensive study of the migratory routes following the latest glaciation was carried out jointly by many laboratories in Europe. The major finding in this study can be summarized in the following way:

1. Haplotypes B10-B12 dominate in Western Europe
2. Haplotype A5 and A7 dominate in eastern Europe

and is almost absent in the Iberian peninsula and United Kingdom

3. Geographically haplotype C1 takes an intermediate position to the previous ones. It is also almost absent in the Iberian peninsula and United Kingdom

It can be concluded that there were three refugia during the latest glaciation, one Iberian, one in southern Italy, and one in The Balkans. See also Fig. 1-95.

1.7.5 Host parasite relationships

A few studies were carried out on this topic. Significant relationships between flushing and hatching time of *Operophtera brumata* were obtained but the degree of explanation of the relationship was less than 40%.

Spore appearance of the fungus *Erysiphe alphitoides* was related to bud flushing of populations from a Pyrenean valley. Matching between spore appearance was strongest at intermediate elevations. It does not seem to be a question of coadaptation between this fungus and the host tree.

2 Species hybrids

In 1993 Rushton published a paper entitled *Natural hybridization within the genus* Quercus *L.* in which he analysed ways to certify the existence of oak hybrids. Morphological intermediacy has been the most common method to identify hybrid existence. Moreover, in most cases it was the only means to identify them. However, this method has its drawbacks since morphological traits are influenced by the site conditions and the position in the crown when leaves are used. The doubts on the morphological classification of hybrids and pure species were also stated by Elsner (1993) in his work on certification of pure seed tree stands of *Q. petraea* and *Q. robur* in Germany. He concluded that *An exact classification is not possible.* Pollen vitality is expected to be reduced in hybrids and it was shown that hybrids between *Q. petraea* and *Q. robur* had reduced vitality. As regards isozymes Rushton concluded that *it is unlikely that isozyme investigations will generally provide accurate estimates of the levels of natural hybridization*. He also concluded that use of phenolic compounds was not useful for hybrid identification. He was more optimistic as regards DNA techniques, even if they were in their infancy in the early nineties. He stressed that hybrids might occupy habitats lacking the parental species. It was hypothesized that existence of hybrids was rather a question of habitat availability than anything else. Examples from Great Britain were referred to.

Early reports on much successful artificial hybridization were dismissed owing to incomplete data. Rushton concluded that analysis of an F_2 generation would be *a most useful tool in establishing parentage.* Finally, he treated the levels of hybridization in natural populations. Based on published data he stated that it was impossible to generalize on the levels of hybridization in nature. Examples of geographic clines in hybrids between *Q. petraea* and *Q. robur* in Great Britain were presented.

Kleinschmit et al. (1995) carried out four interspecific crosses; two with *Q. petraea* females and two with *Q. robur* females. Besides, two intraspecific *Q. petraea* crosses and three *Q. robur* intraspecific crosses were included. One prime objective of the study was to test whether any of the traits could be used to identify species hybrids in young seedlings. It was noted that the leaf morphology of leaves from seedlings differ from adult trees, which makes identification of individual hybrids problematic. For 18 leaf morphology traits two distinct groups were observed, the first group consisted of progenies with *Q petraea* as female and the second with *Q. robur* as female. The dendrogram based on leaf morphology showed two distinct groups separating offspring from petraea and robur females, respectively. Thus, it was noted that the hybrid offspring resembled the female parents to a large extent.

Steinhoff (1993) carried out a huge number of intra- and inter-specific crosses in Germany between the two oak species. In one year 12,647 flowers were isolated; in addition selfing of 2,348 flowers was carried out. The success rate estimated as number of acorns produced per isolated flower did not exceed 20% for any type of cross (Fig. 2-1). The cross *Q. robur* x *Q. petraea* had a higher success rate in 1989 than the reciprocal while the opposite was the case in 1990. The number of seedlings per isolated flower was higher for *Q. robur* x *Q. petraea* cross than the reciprocal, 3.1 versus 1.8%. For the 1989 crosses the percentage survival of the acorns was given; also in this material there was a higher percentage for the *Q. robur* x *Q. petraea* hybrids than the opposite combination. It was speculated that the greater success for the *Q. robur* x *Q. petraea* could be attributed to some of the presumed *Q. robur* females being hybrids. This was supported by large variability of the "*Q. robur*" females. Selfing success rate was low, less than two percent in both species. Seedling height was found to be strongly dependent on acorn weight.

A mixed *Q. petraea* and *Q. robur* stand 30 km west of Le Mans in France with an area of 250 x 250 m has been used in several investigations, partly with the same empirical data (Bacilieri et al. 1993, 1995, 1996a and b, Streiff et al. 1998). The stand, called Petite Charnie, PC, is located on a slope at a mean elevation of 140 masl. The soil varies from a humid clay soil at the bottom of the slope to a silty and sandy soil at the highest part of the stand. The stand contains 426 trees with an age of about 120 years.

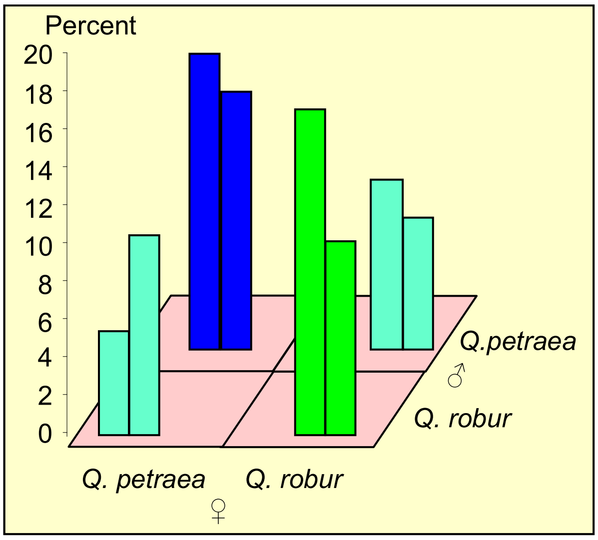

Figure 2-1. Percentage acorns per pollinated flowers in intra- and inter-specific crosses of Q. petraea *and* Q. robur. *The left columns in each pair refer to crosses carried out in 1989 and the right columns refer to crosses carried out in 1990. Steinhoff 1993.*

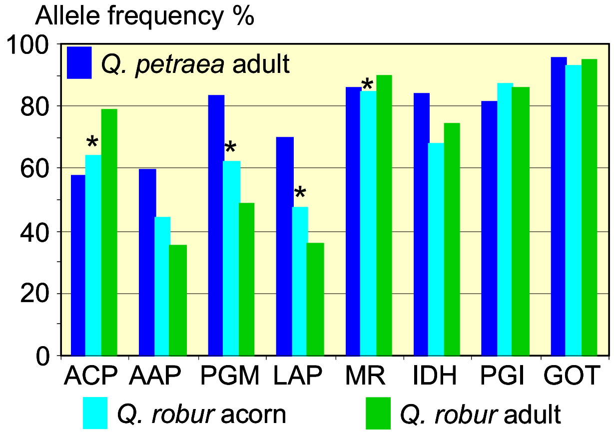

Figure 2-2. The allele frequencies of the most common allele in eight isozyme loci in adult trees of Q. petraea *and* Q. robur *in an oak stand in north-western France. The allele frequencies in acorns from* Q. robur *are also given. The significance for the shift towards a more* Q. petraea *profile of the* Q. robur *acorns are indicated. Bacilieri et al. 1993.*

Bacilieri et al (1993) studied the isozyme profiles of trees in the PC stand. In Fig. 2-2 I have shown the percentage of the most common allele in eight isozyme loci for the adult populations as well as for the acorn population of *Q. robur*. It is evident that the isozyme profile of the *Q. robur* acorns approach the frequencies in the *Q. petraea* adult population. The shift in allele frequency of the *Q. robur* acorn population was significant in four loci while it was significant in two loci for the *Q. petraea* acorn population. In four loci the allele frequency of the acorn population was intermediate to the frequencies in the two adult populations (Fig. 2-2). It is somewhat surprising that significance was noted for the *MR* locus but not for the *AAP* locus. It should be added that it is hard to prove a shift in the acorn population when the most common allele occur in high frequencies in both species. The shift in the *Q. robur* acorn population was attributed to fertilizations with *Q. petraea* pollen.

The trees in the PC stand were also used for a comparison of morphological, ecological and molecular markers in hybrids between *Q. petraea* and *Q. robur* (Bacilieri et al. 1995 and 1996b). Eleven isozyme loci were included in this study. More than 25 morphological characters were included, among them flowering phenology that was recorded during three years. Recordings were carried out every three, seven, or 14 days. Soil samples were taken to characterize the ecological variation in the stand.

I have illustrated the peak time for receptivity and pollen dispersal in Fig. 2-3 for the two years with most frequent recordings. Since there was total agreement between the two species no separate illustration of the species performances are shown. This figure reveals that the peak of pollen dispersal occurs earlier than the receptivity and that the peak appears at different times the two years. This suggests that the weather conditions during the two years influence the start of flowering.

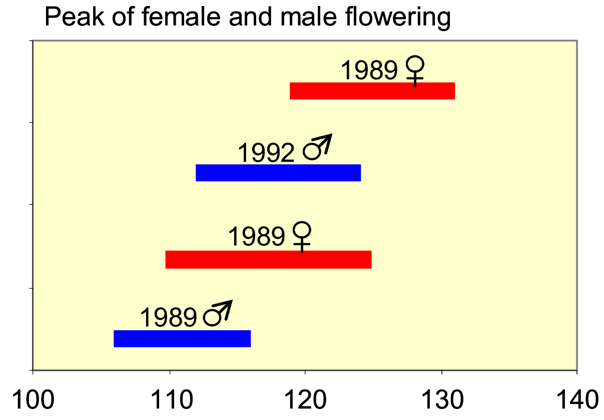

Figure 2-3. The extension in time of the peak in female and male flowering in Q. petraea *and* Q. robur *in a north-western French mixed stand of these two species. Observations from 1989 and 1992 are illustrated. Bacilieri et al. 1995.*

As seen from Table 2-1 the morphological traits supported the findings of an asymmetric contribution to the progeny. A little less than half of the morphological traits showed significant differences between the parental species and their hybrids.

The gene frequencies in pollen, seedlings, and adult populations for seven isozyme loci were again presented in Bacilieri et al. (1996b). In this paper two different methods were used to estimate the parental contribution to the progeny. As seen from Table 2-1 and Fig. 2-4 there was a considerable contribution from *Q. petraea* to *Q. robur*. It was hypothesized that the reason for the introgression of *Q. petraea* to *Q. robur* might be attributed to the stages in the ecosystem of the two species; the latter being a pioneer species and the former being a climax species. There should be a trend that *Q. petraea* gradually substitutes *Q. robur*. The notion that the two species occupy different site conditions is not totally in line with this interpretation; *Q. petraea* grows on somewhat drier sites than *Q. robur*.

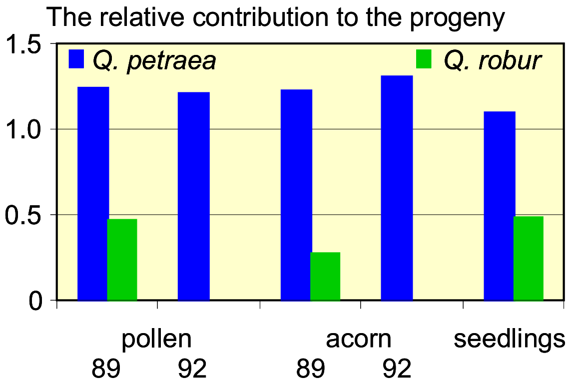

Figure 2-4. The relative contribution of Q. petraea *or* Q. robur *in pollen pool, acorn population, and seedlings two years 1989 and 1992 (only* Q. petraea*) in a mixed* Q. petraea *and* Q. robur *stand in northwestern France. Seven isozyme loci were used. Bacilieri et al. 1996b.*

Table 2-1. A summary of studies on the hybridization between Q. petraea *and* Q. robur *in a French stand containing these two species.*

	Study of	Methods	Results
Bacilieri et al. 1993	Mating pattern	8 isozyme loci in stand and in the offspring	The hybridization was assymetric; introgression of *Q. petraea* into *Q. robur.* Significant difference between stand and offspring populations found for 4 isozyme loci.
Bacilieri et al. 1994	Spatial organization of flowering phenology and 7 isozymes	Phenology observations every 3 days. Spatial structures at 10 - 240 m analyzed by F_{ST}	F_{ST} for 4 isozyme loci was positive and significant in *Q. petraea* and *Q. robur* up to classes 100 and 120 m. Early, intermediate, or late flowering trees did not show any difference in isozyme profiles.
Bacilieri et al. 1995	Isozyme pattern, flowering phenology, morphological differentiation, edaphic impact on the populations	Flowering phenology 3 years, every 3, 7, or 14 days. 11 isozyme loci, > 25 morphological traits, soil samples along the gradient in the stand	No species difference in flowering phenology. No association between isozymes and the other types of traits.
Bacilieri et al. 1996a	Comparison of RAPDs and morphological markers in hybrids	31 morphological characters that differed between the two species	RAPDs showed assymetric introgression into *Q. robur* from *Q. petraea*. Support was obtained from morphological traits. 14 of the 31 morphological traits showed a significant difference between *Q. petraea* and the hybrids. The corresponding for *Q. robur* was 12.
Bacilieri et al. 1996b	Mating pattern	7 isozyme loci in seedlings. 2 different methods to estimate contribution to the progeny; admixture model and 2-species mixed mating model. *Q. petraea* 46 families, *Q. robur* 15 families	The *Q. petraea* contribution to the *Q. robur* population varied in the range 17 - 48% while *Q. petraea* was "preferentially" fetrilized with *Q. petraea* pollen.
Streiff et al. 1998	Spatial distribution	Differentiation: 4 isozyme (F_{ST}) and 6 microsatellite (F_{ST} and R_{ST}) loci. *Q. petraea* 46 families, *Q. robur* 15 families	The larger the spatial distribution the lower the differentiation estimates. In *Q. petraea* R_{ST} gave the largest differentiation

One conclusion from all these reports is that outcrossing is prevalent in both species.

The occurrence of introgression in four oak species, *Q, petraea, Q. pubescens, Q. pyrenaica,* and *Q. robur* was studied in 377 oak populations (Dumolin-Lapègue et al. 1999). Twelve haplotypes were used in this study. It was noted that all haplotypes occurred in all four species. It was also noted that trees within populations showed greater similarity within populations than with trees from other populations. This was the case even if they belonged to different species. There does not seem to be any strong crossing barriers between these four species, even if *Q. robur* trees within a mixed population with *Q. pubescens* had a higher similarity with each other than with the trees of the other species. The estimated G_{ST} values were high whether individual species or mixed populations were studied, 0.76-0.91. This must be attributed to frequent occurrence of monomorphic populations with different haplotypes in different populations.

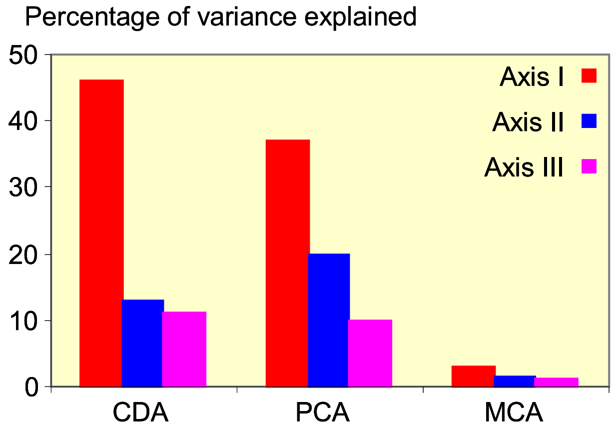

Figure 2-5. Fourteen morphological traits were used for a classification of Q. petraea *and* Q. robur *trees in mixed stands. The percentage of variance explained by the first synthetic variable in canonical discriminant analysis (CDA), principle component analysis (PCA), and Multiple correspondence analysis (MCA) Kremer et al. 2002.*

Fourteen leaf characteristics were used for classification of oak trees in mixed stands of *Q. petraea* and *Q. robur* in the latitudinal range 42.98- 56.68°N by Kremer et al. (2002a). At least five leaves from the last flush were examined with a total number of 16,055 leaves assessed from 3,025 trees. Ratios between traits were used to avoid differences related to the varying growth conditions over the large latitudinal and longitudinal range of sampling. Analyses were carried out separately for each stand as well as a pooled analysis of all stands. Canonical discriminant analysis (CDA), principle component analysis (PCA), and multiple correspondence analysis (MCA) were used at both levels; individual stands and all stands pooled.

Pooled material. There was a good agreement among these three methods. Thus, the first synthetic variable contributed most to the total variation (Fig. 2-5). Moreover, there were strong correlations between the first three synthetic variables. The first two axes resulted in bimodal distribution for all three test methods. There was always a continuous variation with an overlap of two tails of the bell-shaped curves. The overlap was taken as an indication of variation at the individual level. Correlations between the synthetic variables and the original variables were estimated to reveal the biological significance of the synthetic variables. Independent on the statistical method used (CDA, PCA, or MCA) the highest correlations were noted for the same traits: petiole length, intercalary venation, pubescence, and sinus width. It was noted that these traits are commonly used for separation of the two oak species. In summary, seven traits were strongly correlated with the first synthetic variable, three were correlated with the second synthetic variable while the remaining four were distributed among the rest of synthetic variables.

One purpose of this investigation was to develop a simple method for species classification. The following equation with a good discrimination between the two species was obtained:

ID = 357 + (205 x NV) – (97 x PL), in which NV = number of intercalary veins and PL = petiole length.

A discussion of the inheritance of the morphological traits that discriminate between the two species was carried out but the available data cannot give any conclusive evidence for the mode of inheritance.

Individual stands. In each stand the first synthetic variable resulted in a bimodal distribution. However, the bimodality was less pronounced in the two northernmost stands in Denmark and Scotland. The correlations between the CDA, PCA, and MCA from the individual stands and their pooled counterparts were strong with exception for the Danish stand, the correlation coefficients varying between 0.58 and 0.76.

A mixed Spanish oak stand with *Q. petraea* and *Q. pyrenaica* was used for a study of gene dispersal and hybridization (Valbuena-Carabaña et al. 2005). This stand is located in the core area of *Q. pyrenaica* distribution. *Q. petraea* shows a scattered distribution in the region, in which the stand is located. In all 176 adults and 96 seedlings were genotyped with five microsatellite loci with a high number of alleles resulting in unique genotypes of each individual.

There was short distance of dispersal in *Q. petraea* and the dispersal was largely restricted to 75 meters. However, some long-distance distribution (>200 m) occurred (12%). Gene flow from other stands was estimated at 37% for *Q. petraea* and slightly lower for *Q. pyrenaica*, 34%. In spite of the difference in distribution pattern there was no difference between the two species as regards remote pollen flow.

The F_{ST} and R_{ST} estimates for the differentiation between the two species were 0.081 and 0.109 and they did not differ significantly.

The percentage of introgressed trees was low among the seedlings, 1.2%, and fairly low among the adults, 10%. At most five of the adult trees were F_1 hybrids.

Olrik and Kjær (2007) used five microsatellite loci to study the success of *Q. petraea*, *Q. robur*, and *Q. petraea* x *Q. robur* pollinations with two pollen mixes with equal parts from each type of male. An additional mix contained only pollen from the pure species. One female of each category was included in this study. A germination test of pollen viability was carried out and it showed that there was no reduced viability of the hybrid pollen.

The percentage of aborted acorns was highest in *Q. robur* and lowest in the hybrid with only a few aborted acorns. The differences in number of acorns per isolation bag, germination percentage, and seedlings per isolation bag were strongly significant. Fig. 2-6 reveals that this hybrid tree does not display any restrictions to back-crossing with either of the parental trees. Even selfing occurred to some extent, eight percent. Except for one selfed acorn the *Q. petraea* crosses had exclusively the hybrid as male

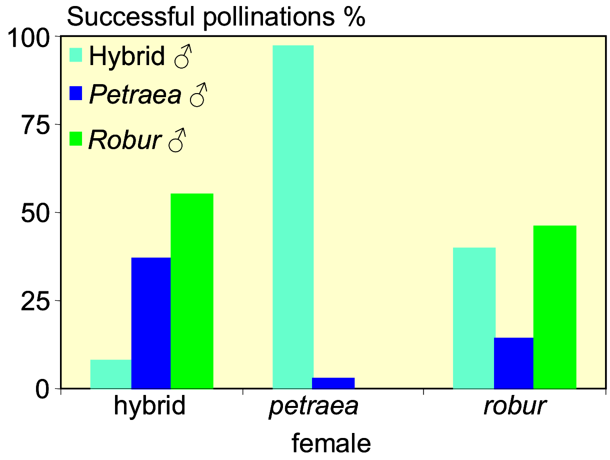

Figure 2-6. The percentage of successful pollinations on one hybrid, one Q. petraea*, and one* Q.robur *female with hybrid pollen,* Q. petraea*, and* Q.robur *pollen. The percentages for hybrid x hybrid and* petraea *x* petraea *were the result of selfing. No selfing was successful in* Q. robur*. Olrik and Kjær 2007.*

parent. Contrary to this the *Q. robur* female had high percentages of offspring both for the own species and the hybrid pollen. The low success rate for *Q. petraea* pollen on the *Q. robur* female suggests that there is some preventive mechanism against *Q. petraea* pollen for this type of cross. One important conclusion from this study was that this hybrid produced viable acorns as well as viable pollen. Although one third of the pollen was *Q. petraea* in the cross with the female *Q. petraea* tree, these pollen grains did not give rise to any offspring. It was suggested that *the results may be due to some kind of allelic based incompatibility system operating.*

The authors stated that more trees of each category are needed before any general conclusions about introgression between these two species can be drawn.

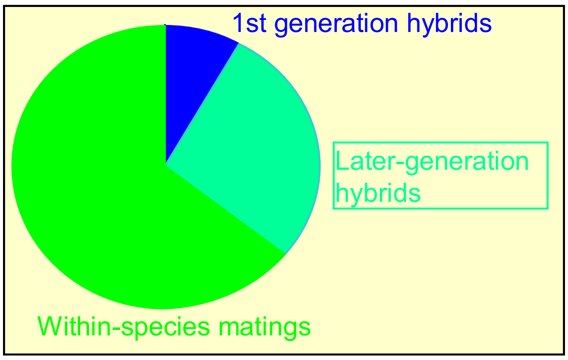

Figure 2-7. The percentage of first generation, later-generation hybrids and matings within species in a mixed oak stand with four species Q. frainetto, Q. petraea, Q. pubescens*, and* Q. robur*. The stand is located in Romania. The asssessments were done by microsatellites. Curtu et al. 2009.*

A Romanian stand 45.85ºN and 22.88ºE with five different oak species, *Q. cerris, Q. frainetto, Q. petraea, Q. pubescens*, and *Q. robur* has been used in a series of investigations (Curtu et al. 2007a, 2009). To a large extent the four species occupied different niches in this stand. *Q. cerris* belongs to another section of *Quercus* than the four other species and is not further treated here.

Several morphological traits were used to identify the species and potential hybrids. The data from this classification of the trees was compared with the marker pattern of the trees. Four chloroplast haplotypes, six microsatellites, and seven isozyme loci were used for determination of the marker pattern. The marker pattern was a blind test without any consideration of the species belonging. The agreement between the marker and morphological methods was remarkably good. The haplotype pattern of *Q. frainetto* and *Q. pubescens* was identical and different from the two other species, which in turn differed strongly from each other. The percentage of intermediate types was estimated at 6% of which approximately half of them were classified as F_1 hybrids. In spite of the great overlap in pollen dispersal and receptivity of *Q. petraea* and *Q. robur* the introgression was mainly from *Q. petraea* to *Q. robur*. The interspecific hybrids were mainly found in the contact zone of their parental species. It was concluded that *Our study provides no evidence for a breakdown of species pairs into a hybrid swarm and indicates the existence of reproductive barriers among species.*

The mating pattern was assessed by analysis of microsatellites (Curtu et al. 2009). Progenies from four trees of *Q. petraea* and one tree of *Q. robur* were genotyped. As expected the matings within each species dominated, approximately 64% (Fig. 2-7). The recent generation hybrid matings amounted to approximately 8%. A higher percentage of hybrid matings was detected in the acorn population than in the stand. This was interpreted as a result of reduced fitness of the hybrids. There was a large variation in the percentage of within-stand matings of the five *Q. petraea* and *Q. robur* trees (Fig. 2-8). The low percentage of within-stand matings of the *Q. robur* tree could at least partly be attributed to the low number of this species within the stand. A similar explanation might be true for its higher mean pollination distance.

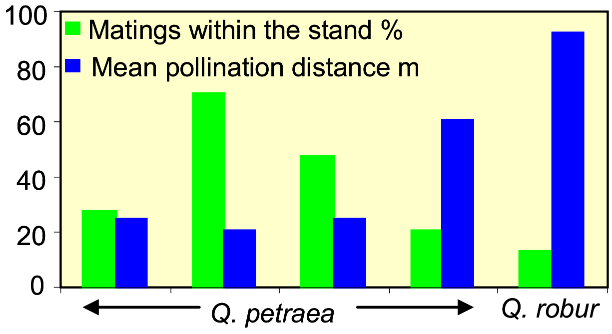

Figure 2-8. The number of matings with males in the stand and mean mating distance in four Q. petraea *trees and one* Q. robur *tree. The stand is located in Romania. The asssessments were done by microsatellites. Curtu et al. 2009.*

A western Danish oak stand with 365 trees of *Q. petraea* and *Q. robur* was used in a study of hybridization between the two species (Jensen et al. 2009). Nine morphological leaf traits were used for a classification of species belonging. Flushing and male flowering were recorded during two years. Eight microsatellites were used for genotyping of these trees as well as 343 seedlings growing in this stand. Acorns were collected directly under 22 trees. The germination of the seed crop was poor, less than 20% for both species.

There was no difference in DBH of the two species, nor was there any difference in flushing time. Flushing dates showed a strong correlation between years. The canonical discriminant analysis based on leaf morphology revealed a clear separation of the two species. For around 20% of the trees there was disagreement between the morphological classification and the molecular data. Thus, some of the trees with "petraea" leaves were classified as robur with the molecular markers. Expected and observed heterozygosities were almost identical in the two species. The mean F_{IS} was 0.039 in *Q. robur* while a slightly negative estimate was noted for *Q. petraea*, -0.017. The spatial autocorrelation showed a strong relationship up to 30 meters for *Q. petraea* and slightly lower estimate for *Q. robur*.

It should be noted that the eight microsatellite loci give a highly reliable estimate of true parentage. Many males contributed to the filial generation; one year 140 males participated in 262 crosses while the corresponding figures for the second year were 120 males and 198 crosses. A total number of 199 trees were identified as males during the two years.

The percentage of interspecific hybrids was much higher in *Q. robur* females than in *Q petraea*. There was a good agreement between years. To avoid that the estimated hybridization was biased upwards owing to pollinations from interspecific hybrids morphologically transition phenotypes were excluded from the estimates, which did not result in any substantial changes of the percentages of hybrids. It was speculated that the higher number of *Q. petraea* females might have contributed to a lower number of interspecific hybrids. The coexistence of the two species may according to the authors be attributed to different preference at micro-site level resulting in a strong disruptive selection between the two species.

The percentage of exterior pollinations was much higher in *Q. robur* than in *Q. petraea*. The stand was relatively isolated, which might explain the comparatively low contribution from exterior pollinations. It was also remarked that oaks have light pollen grains that may fly over large distances.

Lepais et al. (2009) determined the percentage of oak trees belonging to four species, *Q. petraea, Q. pubescens, Q. pyrenaica*, and *Q. robur*, and their hybrids in six French oak forests. The assignment to each category was based on alleles in ten microsatellite loci. One conspicuous result was the high frequency of hybrids. Even hybrids with species not occurring in the stand were detected (Fig. 2-9). The pooled hybrid percentage was estimated at 19.1 percent in the western French Petite Charnie stand, which means that approximately every five individual is a hybrid. The occurrence of 13.4% hybrids with *Q. pubescens* and *Q. pyrenaica* in the Petite Charnie stand was remarkably large and can only be explained by long-distance pollen transfer. The mean percentage of hybrids in all six oak forests was also high, 22.7%. Also in another material, in which *Q. pyrenaica* was missing, 5.8% of the individuals were hybrids with *Q. pyrenaica* and the other three species.

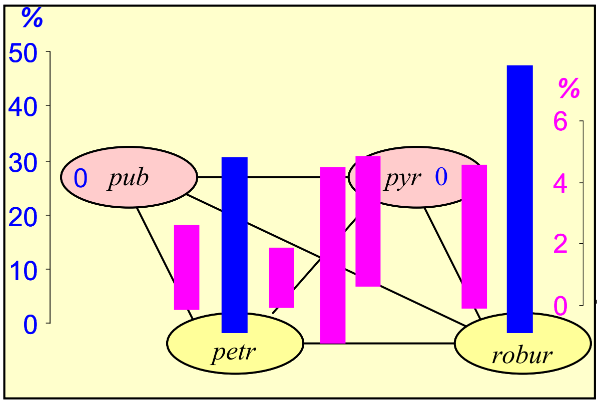

Figure 2-9. The percentage of trees of four oak species (blue columns) and their interspecific hybrids (purple columns) in a western French stand at Petite Charnie. Q. pubescens *and* Q. pyrenaica *do not grow in this stand. Ten microsatellite loci were used for genotyping of the 262 trees. Note the different scales. Lepais et al. 2009.*

One objective of this study was to estimate the impact of tree frequency on the contribution to the hybrid progeny. As seen from Fig. 2-10 the species with lowest occurrence in the Briouant stand contributed more to the hybrids than expected according to their representation

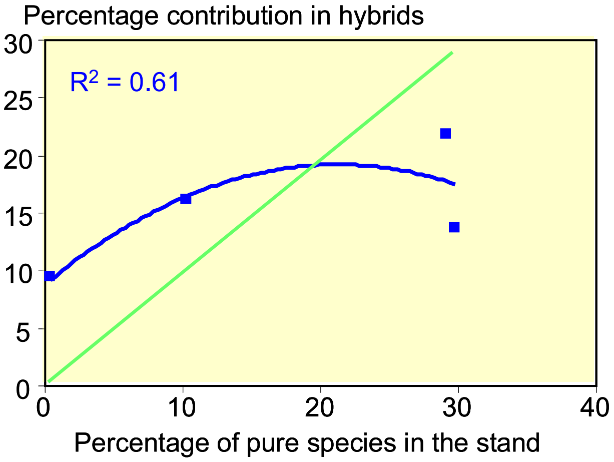

Figure 2-10. The pooled percentage of hybrids with the four species plotted against the percentage of the pure species in the Briouant stand in south-western France. The ideal relationship if the pure species would contribute to hybrids according to their occurrence in the stand is shown with the green line. Lepais et al. 2009.

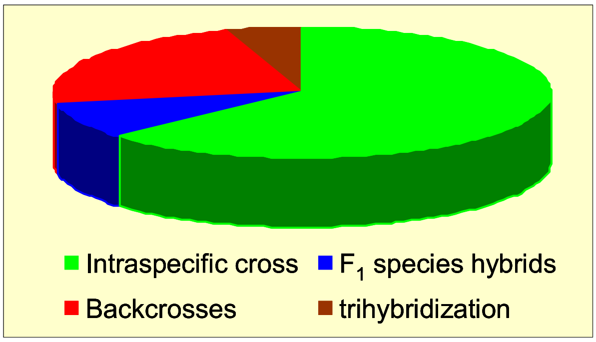

Figure 2-11. The observed percentage of different types of progeny from pure species females in an oak stand with Q. petraea, Q. pubescens, Q. pyrenaica*, and* Q. robur *at Briouant in south-western France. Trihybridization means a cross between a hybrid male tree and a female not included in the male. Lepais and Gerber 2010.*

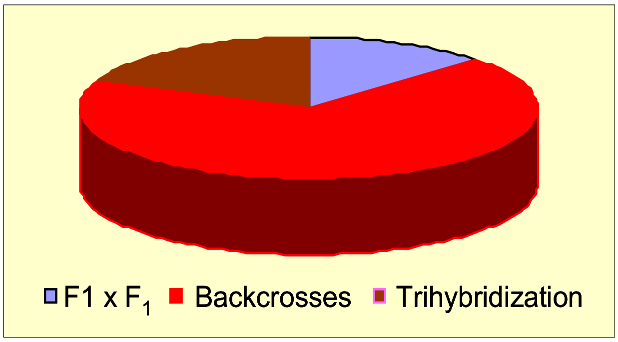

Figure 2-12. The observed percentage of different types of progeny from species hybrid females in an oak stand with Q. petraea, Q. pubescens, Q. pyrenaica*, and* Q. robur *at Briouant in south-western France. Trihybridization means a cross between a hybrid male tree and a female not included in the male. Lepais and Gerber 2010.*

in this stand. This was a general observation in this investigation. A simulation study was carried out to ascertain that assignment errors did not cause the large contribution to the hybrid offspring by the low-occurrence species. Based on this study, assignment errors were excluded as responsible for the observed results in this respect. However, it is likely that there are not only pure species and first generation interspecific hybrids in the stands but also different generations of back-crossing occur, which renders a proper assignment of the trees difficult. Unequal frequencies of the pure species were suggested as one reason for the unexpected high frequency of hybrids.

The parentage of 623 acorns from 16 trees and 208 seedlings in the Briouant stand was analyzed in detail by Lepais and Gerber (2010). The seedlings selected were evenly distributed in the stand. The same ten microsatellite loci as used in the previous study were used for genotyping in this investigation. The allele frequency of the microsatellite alleles in the stand were used for estimation of exclusion probabilities of male parents in each acorn. Acorns that had equal probabilities of two males were excluded from the study. Analogous to this, both female and male parentage was determined for the seedlings. Two models were used for calculation of the expected outcome of the mating within the stand. The first was called the full panmixia (FP) model and the second constrained panmixia (CP). The first assumes a random mating between each studied female and all other trees within the stand. In the CP model pure species trees are allowed to mate randomly with conspecific trees and with their hybrids and hybrids mate randomly with hybrids of the same type (producing F_2) as well as backcrosses with the pure species trees.

Before the results are presented it is important to consider that the authors pointed out the difficulties to discriminate between pure species and different generations of backcrosses. There were no significant differences in acorn weight or germination rate between pure species and interspecific hybrids. A male parent could be assigned to 167 of the 623 acorns. In all, 126 males were identified and only 15 of these trees sired more than one acorn. One tree sired as many as ten acorns.

The percentage of different types of offspring from pure females and hybrids is illustrated in Fig. 2-11 and 2-12. The largest percentage of offspring was the intraspecific cross (Fig. 2-11) followed by backcrosses. Back crosses dominated in the offspring from the hybrid trees (Fig. 2-12).

Table 2-2. Significant deviations between obsserved matings according to two models of mating in a mixed stand of four oak species, Q. petraea, Q. pubescens, Q. pyrenaica, *and* Q. robur. *This stand, Briouant, is located in south-western France. Higher stands for higher than expected according to full panmixia or constricted panmixia models. Lepais and Gerber 2010.*

Acorns pure species	Full panmixia	Constrained panmixia
Intraspecific crosses	higher	NS
Interspecific crosses	lower	NS
Backcross	NS	NS
Trihybrization	NS	NS
Hybrid acorns		
F_1 x F_1	NS	NS
Backcrosses	higher	lower
Trihybridization	lower	higher
Seedlings pure species		
Intraspecific crosses	higher	NS
Interspecific crosses	lower	NS
Backcrosses	higher	Ns
Trihybridization	lower	NS
Hybrid seedlings		
F_1 x F_1	NS	NS
Backcrosses	NS	NS
Trihybridization	NS	NS

In two cases there was a significant difference between observed percentage and the CP model while there were eight cases out of fourteen that showed significant differences for the FP model (Table 2-2).

Although the percentage of *Q. pyrenaica* trees in this stand was 42%, the representation of this species in the pollen pool was low. In contrast to this, *Q. pubescens* contributed much to the pollen pool in spite of its low percentage in the stand, 15%. It was pointed out that pollen dispersal in *Q pyrenaica* occurs approximately three weeks later in this species than in the other species. This might explain the results obtained.

Comparisons of the results between acorns and seedlings inform about whether or not postzygotic barriers to hybridization exist. Since there were minor differences between the results from these two life stages it was concluded that prezygotic barriers are mainly responsible for limitation of interspecific hybridization.

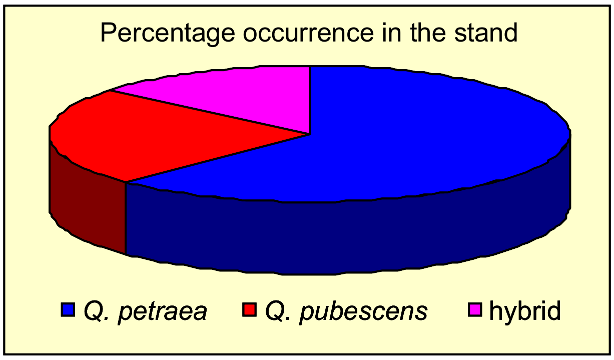

Figure 2-13. The percentage occurrence of Q. petraea, Q. pubescens, *and their hybrid in a central Italian stand at latitude 43.35°N and longitude 10.94°E. Salvini 2009.*

The authors concluded that:

There was an unequal contribution of fertilizations from the different species and it did not reflect the species occurrence in the stand

Hybrids appeared to be fully fertile

Species maintenance was kept in spite of interspecific hybridization estimated at 9% for acorns and 3% for seedlings.

Hybrids were preferentially mated with one of their parental species.

It was pointed out that pollen dispersal resulting in interspecific fertilizations followed by backcrosses is a means for a species to expand into an area already occupied by a sister species. Its impact of the obtained results for coping with global warming was discussed.

A study of spontaneous hybridization between *Q. petraea, Q. pubescens*, and their hybrid was carried out in a central Italian stand (Lat. 43.35ºN, Long. 10.94ºE) by Salvini et al. (2009).

The composition of the stand is illustrated in Fig. 2-13. Acorns from 30 trees (23 *Q. petraea*, 5 *Q. pubescens*, and 2 hybrids) among the 295 trees in the stand were collected and genotyped with respect to parentage. A fluoro-chromatic reaction method was used to test the pollen viability immediately after pollen harvest.

Fig. 2-14 reveals that the percentage of heterospecific matings was lowest in *Q. petraea* (blue) with approximately four percent for each of *Q. pubescens* and the hybrid. The low occurrence of hybrid trees explains partly the high percentage of heterospecific matings of the hybrid females. Of these matings little less than two thirds were with *Q. pubescens* as males.

The number of successful heterospecific matings with *Q. petraea* pollen was largest but not in proportion to its occurrence in the stand (Fig. 2-15 and 2-13). There was a clear asymmetry in the pollen flow with more than five times higher from *Q. petraea* to *Q. pubescens* than vice versa. The higher number of *Q. petraea* trees in the stand than *Q. pubescens* trees might have contributed to this

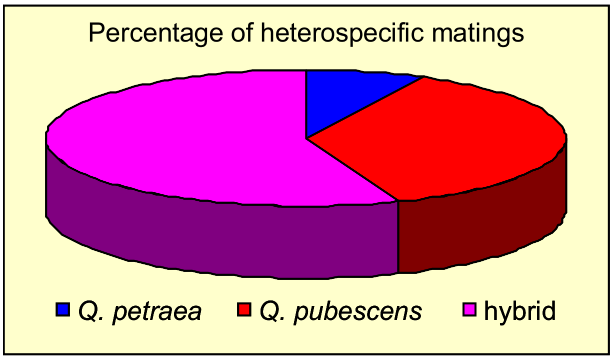

Figure 2-14. The percentage of heterospecific matings of Q. petraea, Q. pubescens*, and hybrid females in a central Italian stand. Salvini 2009.*

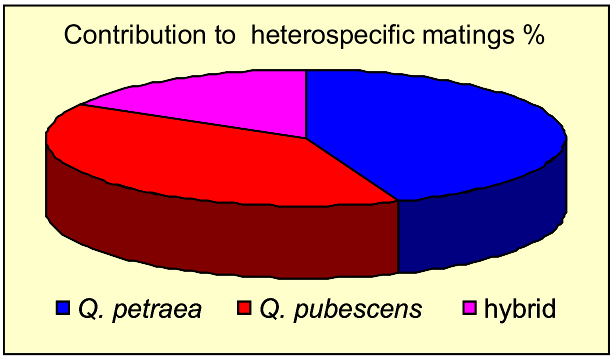

Figure 2-15. The percentage contribution to heterospecific matings of Q. petraea, Q. pubescens, *and their hybrid in a central Italian stand. Salvini 2009.*

asymmetry. The difference in flowering phenology between the two species might also contribute to the asymmetry observed.

Pollen viability was higher in *Q. pubescens* and the hybrid (approximately 95%) than in *Q. petraea*. Similarly, the *Q. pubescens* pollen showed the highest germinability and the drop of germinability over time was much lower than in *Q. petraea*. These results would suggest an asymmetry of the pollen flow in opposite direction to what was observed.

The high percentage of heterospecific matings of the hybrids constitutes a good platform for introgression between the two oak species and particularly from *Q. petraea* to *Q. pubescens*.

Spontaneous hybridization in two Polish stands was estimated by Boratýnski et al (2010). The frequency of the three types, *Q. petraea, Q. robur*, and intermediate phenotypes in the adult populations and in the offspring population was assessed. Besides, the number of the three types of offspring was assessed under the canopy of the adult trees.

For one of the localities the frequency of seedlings of different types is illustrated in Fig. 2-16. It is clear that the frequency of intermediate types (= hybrids) is higher under *Q. robur* trees than under *Q. petraea* trees. This suggests that there is an asymmetry in the hybridization between these two species with more successful *Q. petraea* than *Q. robur* pollinations. The similar trend was noted for the other test locality. The percentages of intermediate adult trees at the two localities were 1.8 and 2.1 while the percentages of intermediate seedlings were 15.7 and 10.0. This shows that several hybridizations must have taken place. During the course of development the individuals of the pure species are favored. Besides the higher rate of *Q. robur* x *Q. petraea* hybridization than the reciprocal cross, it was discussed that the rapid germination after acorn fall during autumn of *Q. petraea* in contrast to the dormancy of *Q. robur* acorns might result in higher regeneration rate of *Q. petraea* than *Q. robur*.

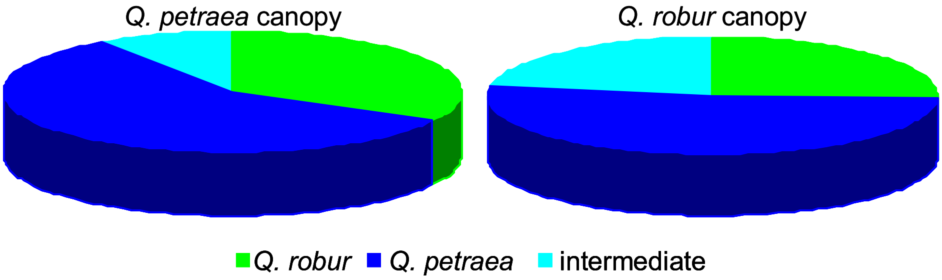

Figure 2-16. The frequency of seedlings under the canopies of Q. petraea *and* Q. robur *trees in a Polish mixed oak forest at Lat. 53.68°N and 18.88°E. Boratýnski et al. (2010).*

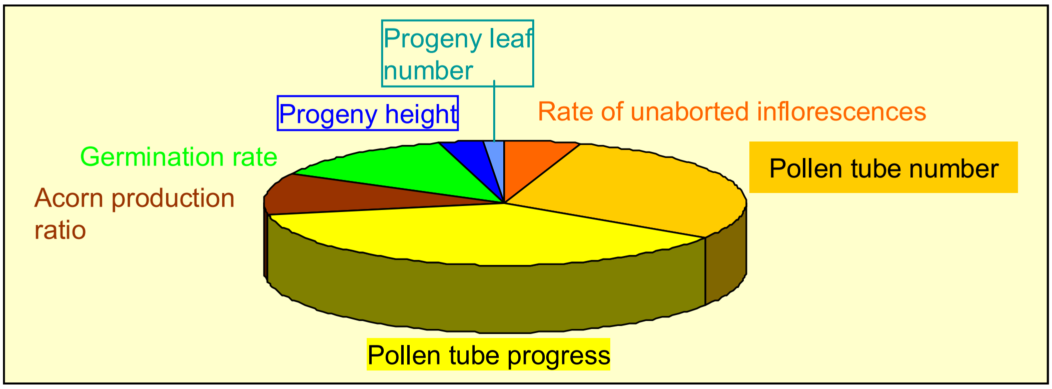

Figure 2-17. The partitioning of the contribution of different traits to the reproductive isolation between Q. robur *and* Q. petraea *based on interspecific full-sib and open-pollinated families. Abadie et al. 2012.*

Abadie et al. (2011) carried out a study with the following objectives:

To characterize and quantify the reproductive isolation between *Q. petraea* and *Q. robur* at different developmental stages during the reproductive process

To estimate any genetic variation in the reproductive process.

Two-hundred-seven full-sibs from an intraspecific mating between two *Q. robur* trees were vegetatively propagated and planted in a field trial in south-western France. A pollen mix of four *Q. petraea* trees from a natural population outside Orleans was used for interspecific crosses with 87 ramets from 27 genotypes of this family. Acorns were also collected after open pollination from 68 of the 87 ramets. Ten different traits related to reproductive isolation were assessed.

Prezygotic traits:

1. unaborted inflorescences
2. pollen tube numbers
3. pollen tube progress

Traits related to acorns:

4. acorn weight per female
5. acorn production ratio = number of acorns divided by number of inflorescences

Later stages traits:

6. germination rate per female
7. germination survival
8. progeny height
9. number of leaves on the offspring year 2.

Traits 1-4, 6, and 8 showed significant differences between the two types of mating with higher values for the open-pollinated material. Progeny height of the interspecific seedlings was lower in most families, which suggests a reduced fitness of the hybrids. It was remarked that the growth traits were obtained from seedlings growing under non-limiting environmental conditions, which all hybrid families might have benefitted from. Thus, the fitness differences may be larger under natural growth-limiting conditions. A large variation among families within crossing type was noted in many cases. The total reproductive isolation was estimated at 0.719, which agreed with expectation based on the number of interspecific hybrids obtained. The relative contribution to the total reproductive isolation of the different traits is illustrated in Fig. 2-17. The two pollen tube traits and germination rate contributed most to the isolation. It should be remarked that acorn weight had a negative estimate for total reproductive isolation. Only three significant correlations among the traits were noted; traits 4 – 5, 6 – 8, and 7 – 8. Worth mentioning is the weak and non-significant relationship between pollen tube number and pollen tube progression. This suggests independent mechanisms of the prezygotic reproductive isolation.

Significant genotypic effects were noted for:

Pollen tube progress
Acorn production ratio
Acorn unit weight
Germination rate
Progeny; height
Progeny; number of leaves
Pollen tube number; but only with height or height of neighbors as covariates in the analyses

The genetic component of the reproductive isolation was larger than 50% for pollen tube progress, acorn production ratio, and acorn unit weight (Fig. 2-18). When covariates were included in the analyses the strength of the significances increased for some of the other traits as well.

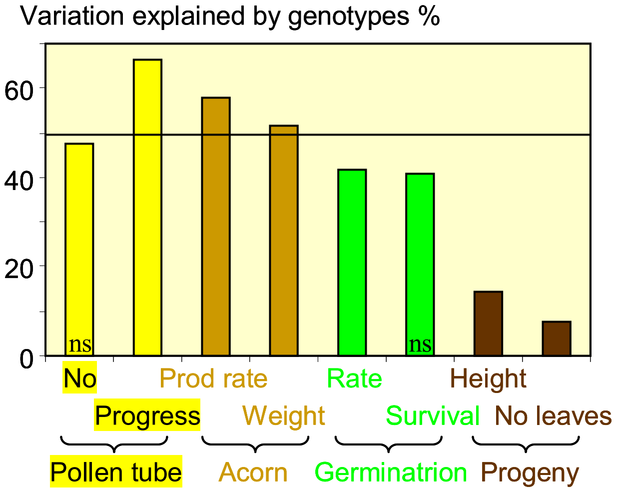

Figure 2-18. The genetic component in % for the expression of traits related to reproductive isolation between Q. robur *and* Q. petraea*. The data emanate from interspecific full-sibs and open-pollinated families. Except for pollen tube number and germination survival all other traits showed significance. Abadie et al. 2012.*

It means that the micro-environmental conditions have an impact on reproductive isolation. This in turn means that these traits show a large phenotypic plasticity. No significant male effects were noted. The use of only four males from a single population might be one explanation for the non-significant variation among the males. Limited variation in interspecific cross compatibility was suggested as another reason for this observation.

It was concluded that:

The reproductive isolation was stronger in the early reproduction steps than in post-zygotic stages

Most of the studied traits were uncorrelated

Genotypic effects were proven for the majority of traits and that the ambient conditions of each tree play a great role.

Lepais et al. (2013) carried out a study with the objectives to estimate:

The relative proportion of interspecific hybrids produced after pollination with a different species

Pollen competition after pollination with a pollen mix containing equal amounts of pollen from *Q. petraea, Q. pubescens, Q. pyrenaica*, and *Q. robur*.

Crosses with *Q. petraea* and *Q. robur* as females were carried out in a clone archive while the crosses with the two other species were carried out in stands. Each acorn was labelled and followed during the germination treatment. Seedlings obtained were grown in 4L-pots and followed during two years to establish final survival. Crosses with all three *Q. pubescens* females largely failed. The genotyping of the 1,640 acorns obtained was carried out by alleles in five microsatellite loci. Fifty-nine of the acorns were the result of selfing with one exceptional tree having 46 selfed offspring. Reproductive isolation was separated into gametic incompatibility, pollen competition, germination, and survival. The gametic reproductive isolation (RI_G) was simply estimated as:

RI_G = 1 – (Interspecific paternal contribution/intraspecific contribution); a value of 1.0 means total reproducetive isolation.

It may be remarked that a higher number of interspecific hybrids than intraspecific offspring results in a negative estimate of RI_G. This was the case for *Q. petraea* x *Q. pubescens* cross. The calculation of reproductive isolation related to pollen contamination RI_C was done according to Box 2-1. However, *interspecific paternal contribution in monospecific cross* sounds strange. I assume that it should be individual interspecific cross rather than monospecific cross.

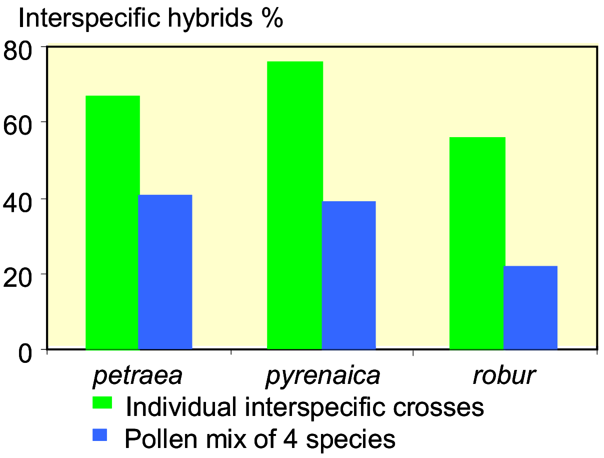

Figure 2-19. The observed percentage of interspecific hybrid acorns in the offspring after two types of crosses between four oak species Q. petraea, Q. pubescens, Q. pyrenaica, *and* Q. robur. *Individual interspecific cross means that the species are mated individually while the other type is a cross with a pollen mix with equal proportions of the four species. Crosses with* Q. pubescens *as female largely failed. Crosses with one* Q. petraea *female yielded only two acorns and data from this female were omitted. Lepais et al. 2013.*

I have preferred to illustrate the percentages of interspecific crosses separately for the individual interspecific matings and for the pollen mixes in Fig. 2-19. The percentages are the pooled data from all three interspecific hybrids out of the total number of acorns obtained for a specific female species. It should be remarked that the number of acorns obtained was low in some cases making the estimated percentages somewhat uncertain. However, this figure clearly shows that interspecific hybrids may easily be obtained following controlled crosses. An especially large percentage (25 out of 37 acorns) was obtained with *Q. pyrenaica* as female. Mixed pollen resulted in a drop of interspecific hybrids suggesting that interspecific pollen suffered from competition with conspecific pollen. It is worth mentioning that all *Q. petraea* females failed to produce any hybrids with *Q. robur* or *Q. pyrenaica* pollen in the individual crosses. In one of the crosses with the pollen mix one *Q. petraea* x *Q. pyrenaica* acorn was detected among 43 acorns.

Box 2-1

$$RI_C = 1 - \frac{\left(\dfrac{\text{Interspecific paternal contribution in crosses with mixed pollen}}{\text{Interspecific paternal contribution in monospecific cross}}\right)}{\left(\dfrac{\text{Intraspecific paternal contribution in crosses with the pollen mix}}{\text{Intraspecific paternal contribution in monospecific cross}}\right)}$$

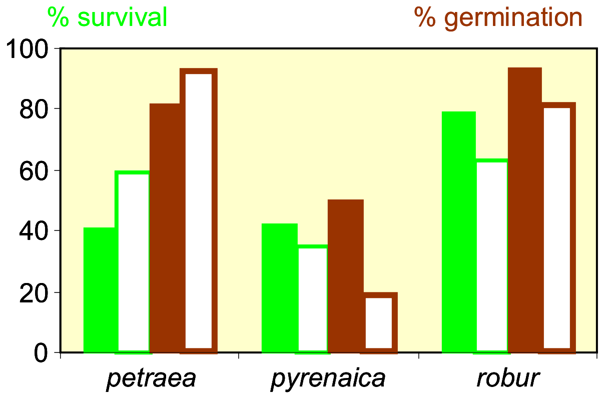

Figure 2-20. The percentage of survival (green) and germination (brown) of intraspecific (filled columns) and interspecific crosses (open columns). Four oak species, petraea, pubescens, pyrenaica *and* robur *were included in this mating but the* pubescens *females failed to give rise to any acorns but this species functioned as male. Lepais et al. 2013.*

When it comes to the later developmental stages, survival and germination, the results for *Q. pyrenaica* and *Q. robur* are analogous to the results for acorn percentages; *i.e.* the interspecific hybrids showed lower percentages than the intraspecific families (Fig. 2-20). The opposite trend was noted for *Q. petraea*. As regards the *Q. robur* x *Q. pyrenaica* hybrids, the percentages for survival and germination rate were significantly lower than in the pure *Q. robur* families. The *Q. pyrenaica* x *Q. pubescens* hybrid had a significantly lower survival than the pure *Q. pyrenaica* families while the other two interspecific hybrids did not differ from pure *Q. pyrenaica* with respect to survival.

In Fig. 2-21 I have illustrated the reproductive isolation for one component, pollen competition, for the interspecific families obtained. The large column for the cross *Q. petraea* x *Q. pubescens* is conspicuous and originates from the high percentage of this hybrid in the individual cross between these two species, 72.4%, and the lower percentage following the mixed pollination of *Q. petraea*, 54,4%. It should be noted that the number of acorns for *Q. robur* x *Q. pyrenaica* was based on one single acorn after pollination with the pollen mix. Except for the cross *Q. pyrenaica* x *Q. pubescens* the data on survival and germination did not contribute much to the total reproductive isolation, RI_T. The RI_T of the nine families with three females of each species of *Q. petraea, Q. pyrenaica*, and *Q. robur* varied in the range 0.91 – 1.00. The latter value was valid for the *Q. petraea* x *Q. pyrenaica* and *Q. petraea* x *Q. robur* matings, which did not give any seeds. Such high estimates are hard to understand with the high percentages of hybrids both after individual and mixed pollen matings.

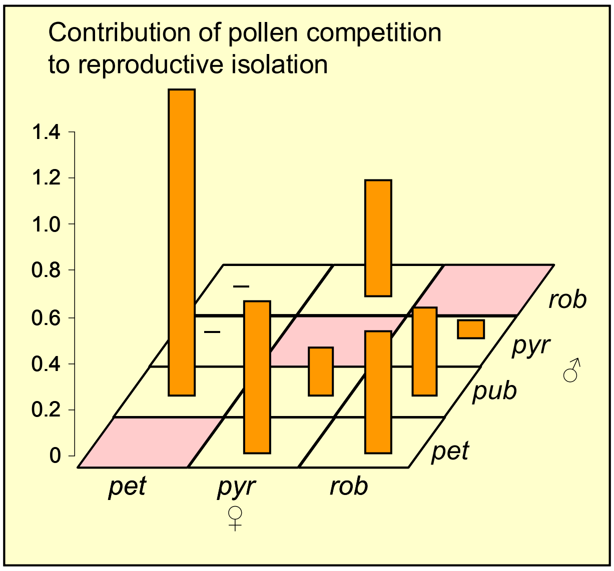

Figure 2-21. The contribution of pollen competition on reproductive isolation in interspecific oak hybrids among the four oak species Q. petraea, Q. pubescens, Q. pyrenaica*, and* Q. robur. *Crosses with* Q. pubescens *failed and no* Q. petraea *x* Q. pyrenaica *or* Q. petraea *x* Q. robur *hybrids were obatained. Lepais et al. 2013.*

A study of male fecundity difference between *Q. petraea* and *Q. robur* was carried out in the Petite Charnie stand by Lagache et al. (2014). Offspring from 3,046 acorns were genotyped by twelve highly polymorphic microsatellite markers. The acorns originated from 22 *Q. petraea* and 26 *Q. robur* trees evenly distributed in the stand. In all 260 adult trees in the stand were genotyped. The trees in the stand were assessed with respect to phenology of male and female flowering as well as several growth traits. Rather complex models were derived to estimate the parameters of fecundity and mating pattern within the stand.

The following differences between the two species were noted:

- the influence of tree size on male fecundity was larger in *Q. robur* than in *Q. petraea*
- phenologically the first onset of male and female flowering occurred a little earlier in *Q. robur* than in *Q. petraea*
- the effective number of siring males within the stand was significantly lower in *Q. petraea* than in *Q. robur*; eleven as compared to 45
- mean pollination distance; 97 meters in *Q. petraea* and 137 meters in *Q. robur;* main pollination direction: east to west in *Q. petraea* and northeast to southwest in *Q. robur;* influence of phenology on pollen dispersal: none in *Q. petraea*, late flowering *Q. robur* trees had a much longer dispersal (193m) than early and intermediate trees (95 and 114m, respectively)
- the hybridizing rate was 37 times higher in *Q. robur* than in *Q. petraea*
- fertilization by trees outside the stand was twice as high in *Q. robur* as in *Q. petraea*
- the dependence of location in the stand on immigration rates was larger in *Q. petraea* than in *Q. robur*

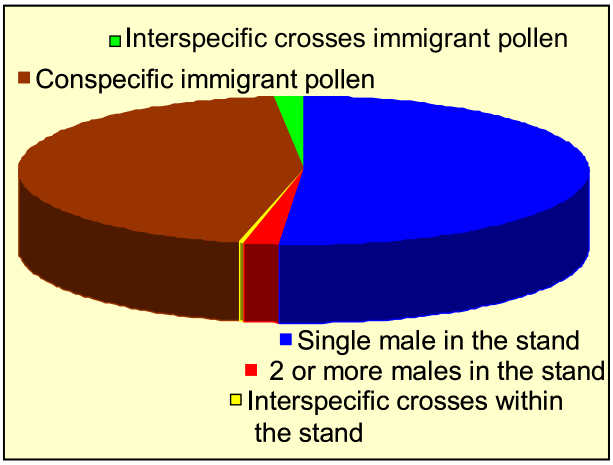

Figure 2-22. The separation of matings in a mixed Q. petraea *and* Q. robur *stand in western France. Matings in the stand was separated into matings with a single male, two or more males, and interspecific crosses. Matings with immigrant pollen was separated into crosses within species and between species. Lagache et al. 2013.*

> It was found that the distance between trees had the greatest impact on the mating pattern. Increasing overlap between male and female flowering increased the mating between trees.

It was speculated that the results reflect the difference between the two species with respect to their stage in the ecosystem, *Q. robur* being more of a pioneering species and *Q. petraea* a climax species. Moreover, difference in site preference, *Q. robur* being more tolerant to moist habitats than *Q. petraea*, might also affect the male fecundity of the two species.

In an earlier study of the same material Lagache et al. (2013) reported estimates of mean pollen dispersal distances of 69 and 151 meters for *Q. petraea* and *Q. robur*, respectively. With the large number of matings with immigrant pollen, 46.5%, it was assumed that the two estimates of pollen dispersal within the stand underestimated the real pollen dispersal. The number of adult trees and genotyped offspring used for estimates of mating pattern were 48 and 3,046, respectively. The selfing rate was estimated at 0.2% in this earlier study. The hybridizing rate was lower than in other studies and amounted to less than two percent (Fig. 2-22). The large number of seedlings studied should guarantee that the estimates of hybridization were reliable. Post-zygotic selection was ruled out as an explanation for the low hybridization rate. The difference in hybridization between the two species was in this case less than in the above report, 6.7 versus 36.8 times. The number of hybrids was as in the previous report higher in *Q. robur* than in *Q. petraea*.

A larger hybridization rate with immigrant pollen than with stand pollen was noted for both species (Fig. 2-22). It was suggested that the stand was exposed to a large swamping by pollen from outside sources.

An import outcome of this study was summarized in the following way: *....we found that due to the nonrandom distribution of the trees in the stand, the average hybridization rate of the population differs from the hybridization rate of a tree exposed to the average pollen environment.*

Chybicky and Burczyk (2013) summarized the objectives of their study on mating pattern in a Polish mixed stand of *Q. petraea* and *Q. robur* in the following way *We attempted to account for all major factors influencing paternity, including non-random pollen dispersal, uneven male fecundity and non-random interspecific mating.*

The genotyping was done by alleles in five microsatellite loci. The mean fertilization with immigrant pollen was almost identical with within-stand fertilizations, 49.7 and 49.8%, respectively. There was a large variation among trees with respect to fertilizations with stand or immigrant pollen, approximately a range of 12 – 88% for both categories. The fertilizations with immigrant pollen were significantly higher in *Q. robur* trees than in *Q. petraea*. In Fig. 2-23 I have illustrated the mean hybridizations of the 13 *Q. robur* and 57 *Q. petraea* female trees. As is evident from this figure the *Q. robur* females are much more prone to species hybridization than the *Q, petraea* female trees. It was also observed that proximity between males and females did not influence mating pattern to any large extent. The estimates of mean (3366 m) and median pollen dispersal (283m) had extremely large confidence intervals. The high percentage of fertilizations with immigrant pollen (49.7%) supports the estimates of long-distance pollen dispersal. In conclusion, pollen dispersal seemed to be leptokurtic with a heavy-tailed distribution.

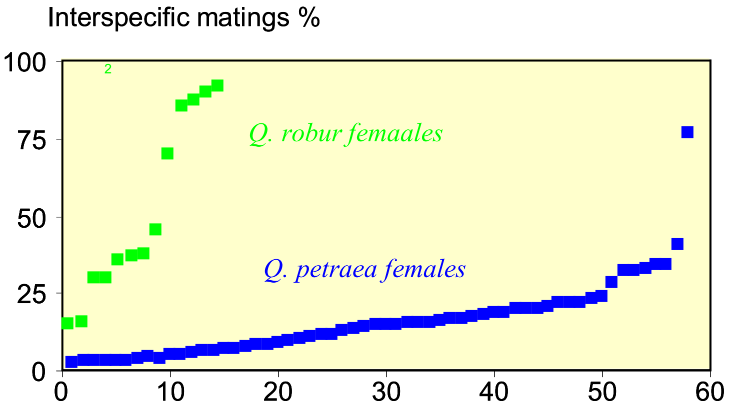

Figure 2-23. Percentage of interspecific matings in 13 Q.robur *trees and 58* Q. petraea *trees in a Polish mixed stand of the two species. The estimates were based on alleles in five microsatellite loci. Chybicki and Burczyk 2013.*

One *Q. petraea* tree showed an exceptionally high selfing percentage, 7.4%. Another tree in a previous investigation showed a high selfing rate too, but this tree did not have a high selfing percentage in the present study. Therefore, the authors were keen to warn for far-reaching conclusions as regards selfing rates.

The authors summarized their results in the following way *we have demonstrated that mating patterns in oaks are far from being random. This applies to all mating components: self-fertilization; outcross mating within a local population; and mating with distant males through pollen immigration.*

2.1 Summary

Studies of hybridization between *Q. petraea* and *Q. robur* 20 + years ago had to rely on morphological traits, which was regarded as unsatisfactory. Thanks to development and use of molecular markers species hybridization studies have been more sophisticated over time, which has enabled a better understanding of hybridization between *Q. petraea* and *Q. robur*. These species hybridize easily and adult hybrids do not seem to suffer from any reduced vitality with respect to growth or pollen fertility. However, one study showed a reduced growth of the hybrid seedlings compared the seedlings of the pure species.

Unequal frequencies of the two species in mixed stands might contribute to a high frequency of hybrids. However, some studies suggested that unequal frequencies of occurrence had no impact on hybrid formation.

Hybridization is easier with *Q. robur* as female partner than with *Q. petraea* as female. Large differences in crossability exist. It was speculated that some mechanism is working in *Q. petraea* to prevent interspecific hybridization in this species owing to its more specific site requirements than *Q. robur*. The coexistence of these species in the same stands must be attributed to strong disruptive selection at the micro-site level.

The detailed studies of the reproductive isolation between the two species showed that this isolation must to largest extent be attributed to prezygotic processes.

Interspecific hybridization with species that does not grow in the stand was noted in several cases.

With a few exceptions selfing occurred in low frequency in the majority of trees.

3.1 Quantitative traits and QTL identification

A brief report on vessel area in grafts growing in a German seed orchard was presented by Kanowski et al. (1991). In the two sets of material analysed the broad-sense heritabilities were estimated at 0.87 and 0.93.

Jensen et al. (1997) reported growth data at age 14 and quality data at age 17 in a combined progeny and provenance trial in The Netherlands with 37 open-pollinated *Q. robur* families from six Dutch populations. In addition three O-P families of *Q. petraea* were included. The populations were selected alongside roads in The Netherlands. In such a selection it might be suspected that part of the progeny trees are full-sibs. Therefore, individual heritabilities assuming the progeny to be half-sibs, full-sibs, or a mixture of half- and full-sibs were presented. In Fig. 3-1 two heritability estimates for each of the four traits are presented. Both quality traits, which were scored in nine classes, had higher estimates than the growth traits. Especially breast height diameter had low heritabilities. The heritabilities for stem straightness, 0.79 and 0.59, were unusually high even for a quality trait. The mean tree height and DBH of the three *Q. petraea* families were somewhat lower than the mean values of the *Q. robur* families.

As expected the family mean correlation between the two growth traits was moderately strong, $R^2=0.56$, while the correlation between the two quality traits was weak, $R^2=0.14$ (Fig. 3-2). Only the correlations between tree height and the three other traits were significant.

The genetic gains following mass selection or backward selection at a selection intensity of 1.0 are not impressive for the quality traits but somewhat better for the growth traits (Fig. 3-3). From a breeding point of view it was suggested that selection for stem straightness and the ratio height/DBH would be the best option. It was also pointed out that the moderate family mean correlations suggest that it might be possible to combine traits in different ways to obtain trees useful for different conditions/products.

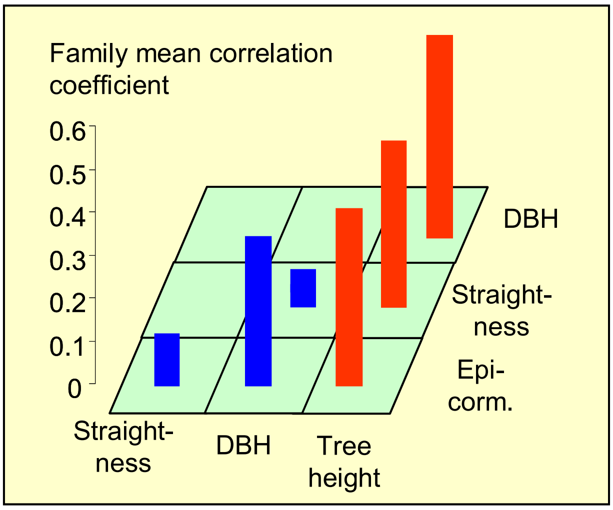

Figure 3-2. Family mean correlation coefficients between four traits in a Dutch Q. robur *combined progeny and provenance trial. Growth data from age 14 and quality trait data from age 17. Red columns refer to significant correlations. Jensen et al. 1997.*

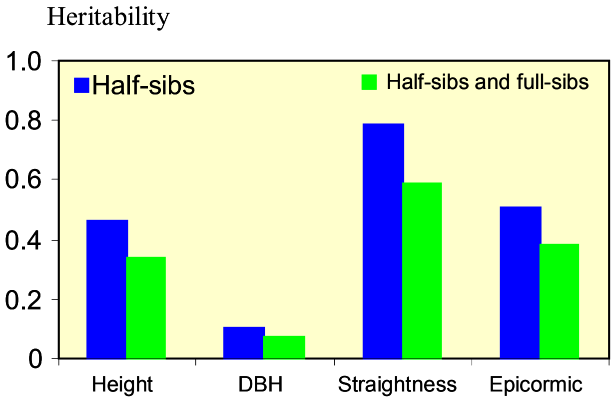

Figure 3-1. Individual heritability for four traits based on open-pollinated families assumed to be either half-sibs or equal portions of full-sibs and half-sibs in a Dutch Q. robur *combined progeny and provenance trial. Growth data are for age 14 and quality traits are for age 17. Jensen et al. 1997.*

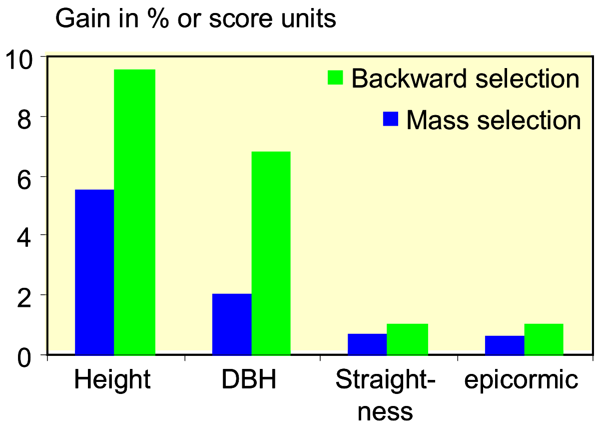

Figure 3-3. The genetic gains following mass selection or backward selection. Gain is shown as percentage for tree height and breast height diameter and as score units for straightness and epicormic shoots. Selection intensity was set at 1.0. Growth data are for age 14 and quality traits are for age 17. Jensen et al. 1997.

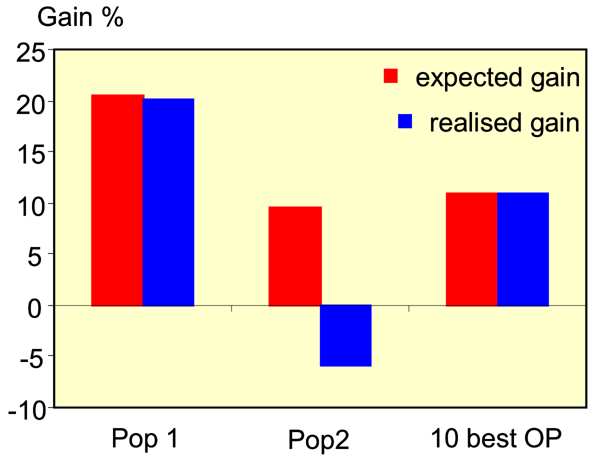

Figure 3-4. The expected and realised gains at age 9 in two Croatian populations of Q. robur *and the additional gain by selection of the 10 best performing of the 21 OP-families tested. The gains were compared to the performance of standard lots from the two populations. Vidakovic et al. 2000.*

Vidakovic et al (2000) reported extremely high heritabilities, 0.70-0.90, for plant height at ages 2-9 years in a progeny trial with 21 open-pollinated families from two populations of *Quercus robur* in Croatia. The estimates may be inflated by the population effect that was significant. A possible maternal effect might also have contributed to the high heritability estimates. The trial contained standard lots from the two populations. The theoretical gains based on the heritabilities were compared to the performance of the standard lots. As seen from Fig. 3-4 the realised gain in population 1 agreed with the expected while the progenies from population 2 did not reach the heights of the standard lot. By selection of the ten best performing OP-families an additional gain of ten per cent might be obtained.

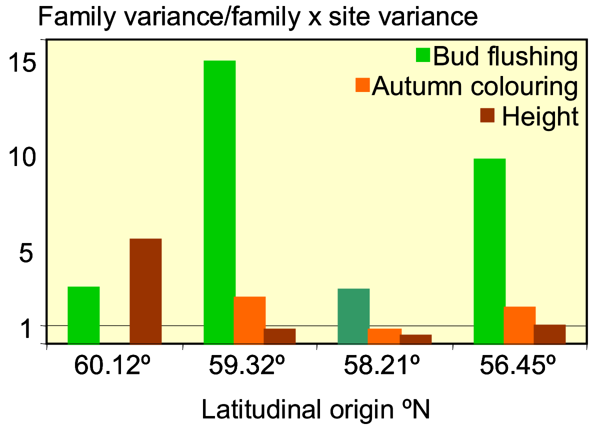

Figure 3-5. The ratio family variance/family x site variance for budflushing, autumn leaf colouring, and tree height in four Q. robur *populations from Sweden studied in two trials in southern Sweden, latitudes 56.54 and 59.67°N. The family x site interaction for autumn colouring in the population from 60.12° was = 0. Baliuckas et al.2001.*

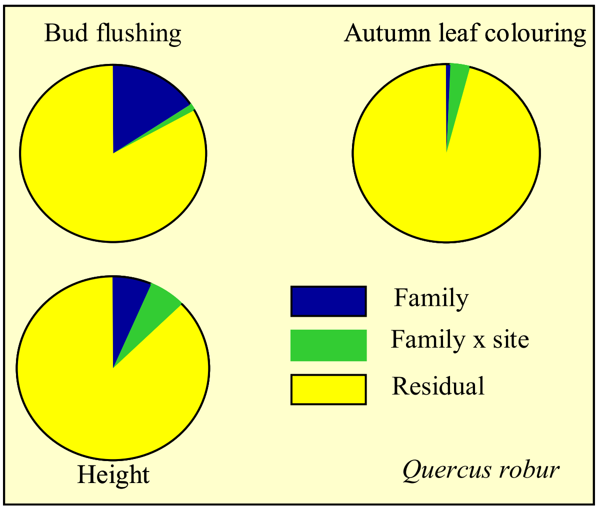

Figure 3-6. The partioning of variance components for bud flushing, autumn leaf colouring, and tree height at age 6 in three Lithuanian combined provenance and progeny trials with Q. robur. *The significances are indicated. Baliuckas and Pliura 2003.*

Open-pollinated families from four southern Swedish populations of *Q. robur* were studied with respect to bud flushing, autumn colouring and plant height in two nurseries in southern Sweden at latitudes 56.54ºN and 59.67ºN (Baliuckas et al. 2001).

The plant height in the southern nursery varied between 72 and 90 cm in the four populations. The family variance in per cent of the total was large for all traits in both trials. Especially, the family variance was large for bud flushing in the southern trial. To give an illustration of the importance of family x test locality interaction the ratio of the family variance over the family x locality interaction are presented in Fig. 3-5. As seen from this figure this ratio was highest for bud flushing in all four populations while the interaction was larger or equal to the family variance for height in three of the four populations. Autumn colouring took an intermediate position. It is evident that bud flushing is strongly genetically regulated. In the 58.35ºN population the interaction was largest and significant for all three traits.

The genetic correlations between years of the same trait were:

Moderate to strong for bud flushing
Moderate to strong for autumn colouring
Very strong for height

These results indicate that there are good possibilities to select families early to get satisfactory results especially for plant height. The between trait genetic correlations varied from strongly negative to strongly positive. This result was somewhat surprising since the ranking of the families with respect to each of these two phenological traits was almost identical over years. The explanation was that the families ranked in different ways for these two traits. The absence of strong between trait correlations makes breeding more cumbersome.

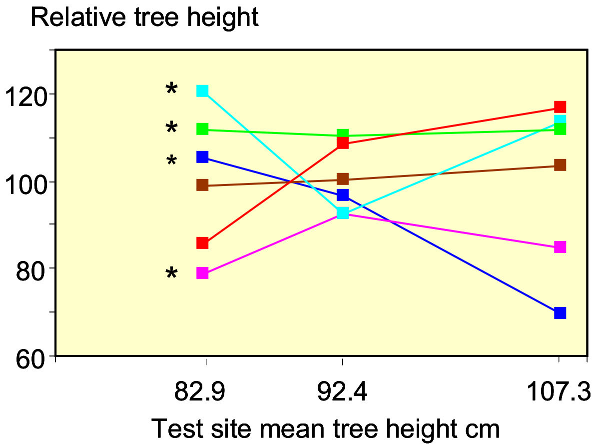

Figure 3-7. The relative tree height of six open-pollinated Q. robur *families from one population plotted against the trial mean heights at three field trials in Lithuania. An * means a family contributing significantly to the family x site interaction. Baliuckas and Pliura 2003.*

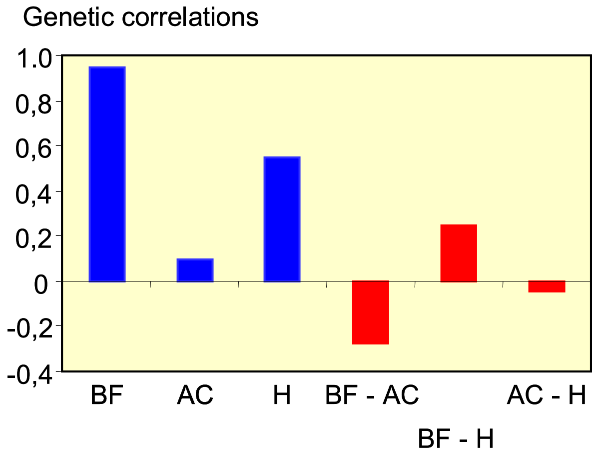

Figure 3-8. The mean genetic correlations for bud flushing (BF), autumn colouring (AC), and height (H) based on data from three field trials with Q. robur *in Lithuania. The mean genetic correlations betweeen the three traits within each of the three trials are also shown (red columns). Baliuckas and Pliura 2003.*

In a Lithuanian series of combined provenance and progeny trials with *Q. robur* juvenile growth, bud flushing, and autumn leaf colouring were studied (Baliuckas and Pliura 2003, see also Baliuckas and Pliura 2009).

As seen from Fig. 3-6 the family variance component at age 6 for bud flushing was several times larger than the component for family x test site, which means that there are good possibilities for these oak populations to respond to changes in climate by a change in time of bud flushing. The family variance component was extremely low for autumn colouring of leaves. There are limited possibilities for change of autumn colouring in these populations. The family x site interaction was significant for autumn colouring and tree height. Thirteen % of the OP-families contributed significantly to ecovalences for autumn colouring while the corresponding number for tree height was 33%. The relative tree height of the six OP-families of the population with largest proportion of families contributing significantly to the G x E interaction is illustrated in Fig. 3-7. The most pronounced ranking shifts occurred between the two test localities with slowest growth. The blue family had the highest ecovalence estimate. It is surprising that the green family contributed significantly to the interaction while the red family did not. Could it be a mislabelling in Fig. 2 in the paper?

The genetic correlations of bud flushing between trials were consistently strong (Fig. 3-8) while they were weak for autumn colouring. Tree height took an intermediate position. All genetic correlations between types of trait within each of the trials were weak.

The relationship between bud flushing of parents and their progeny was presented, the slope of which should be ½h^2. The regression coefficient was estimated at 0.436 suggesting heritability for bud flushing of 0.87, which is an extremely high value. There was a large uncertainty of this high heritability since the R^2 estimate explained less than half of the relationship. However, it is important to have such relationships in long-generation tree species.

In conclusion bud flushing was stable over trials with limited family x test site interaction. Contrary to this strong family x test site interactions were noted both for tree height and autumn colouring.

Jensen and Hansen (2008) reported on growth and phenology of four open-pollinated families from each of five Danish *Q. robur* populations tested in a greenhouse experiment. Seedlings were grown in large containers (16 x 36 x 3 dm) and placed in an unheated greenhouse. Except for flushing the other traits were assessed during the first growth period.

Fig. 3-9 reveals that the coefficients of additive variance were large, especially for height and yellowing.

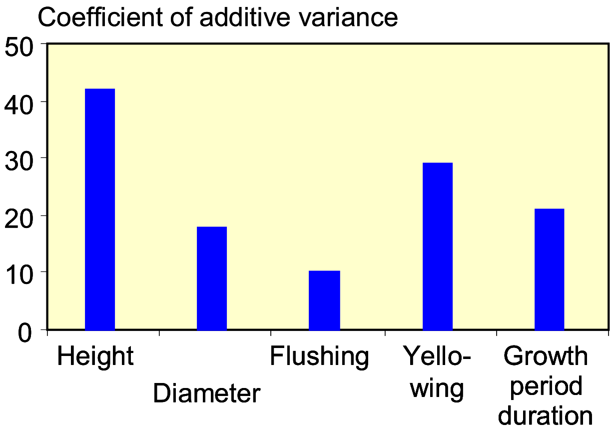

Figure 3-9. The coefficient of additive variance based on four open-pollinated families in each of five Danish populations of Q. robur. *Jensen and Hansen 2008.*

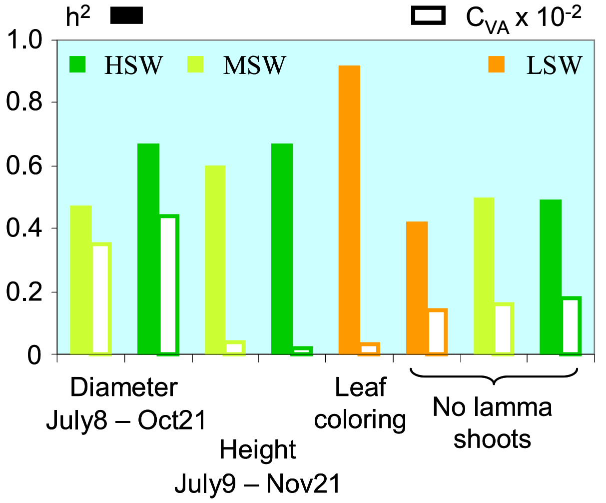

Figure 3-10. Heritabilities and the coefficiens of additive variance, CV_A *divided by 100,, for growth traits and leaf coloring following three treatments with different irrigation regimes, HSW = high soil water MSW = medium soil water LSW = low soil water content of four open-pollinated families from each of five Danish populations. Filled columns refer to heritabilities while open columns refer to* CV_A*.* Q. robur. *Jensen and Hansen 2010.*

The seedlings in this study were included in an irrigation experiments during the second growth period (Jensen and Hansen 2010) of four open-pollinated families from each of five Danish *Q. robur* populations. Seedlings were grown in large containers (16 x 36 x 3 dm) and placed in an unheated greenhouse. From July 8 three irrigation regimes were applied, 38% (LSW), 55% (MSW), and 80% (HSW) of field capacity. Recording of height growth was carried out three times and leaf coloration was recorded on October 8.

The families stopped the growth in the LSW treatment which prevented the estimation of genetic parameters for growth traits. The variation among families in diameter, plant height, and lamma shoots was significant for the MSW and HSW treatments. In addition there was a significant effect for lamma shoots in the LSW treatment. Heritability and coefficients of additive variance component estimates for these traits were presented (Fig. 3-10). Both heritabilities and CV_As were high for diameter growth while the rest of the traits showed moderately low or low estimates of CV_A. The high heritabilities must be attributed to the uniform conditions in the greenhouse that reduces the phenotypic variance. The heritability for diameter growth was somewhat higher for the HSW treatment than for the MSW treatment but this was not accompanied by a higher CV_A. There were limited changes in rank of the families between treatments as illustrated for diameter in Fig. 3-11. Owing to the limited variation in the LSW treatment the deviations from the mean value are probably exaggerated. It was concluded that selection for genotypes with good growth under high water availability and high temperature might be possible. However, the correlation between seedling performance and adult tree performance was questioned. Similarly, concern was raised for the use of material with long growth periods owing to risks for autumn frost damage.

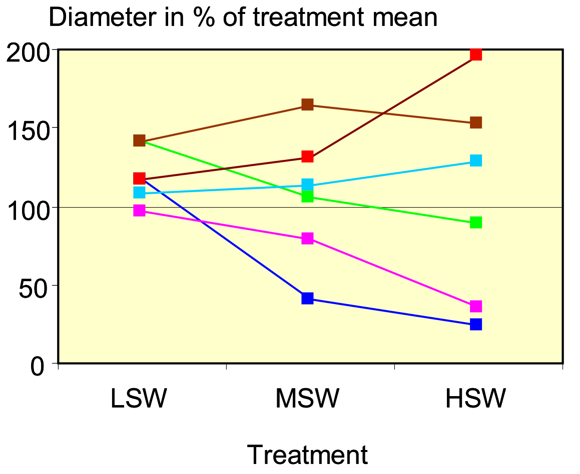

Figure 3-11. The diameter growth in percentage of treatment mean for six representative open-pollinated Q. robur *families in three irrigation regimes, LSW = low soil water MSW = medium soil water HSW = high soil water content. Jensen and Hansen 2010.*

The effect of acorn weight on seedling growth and competitive ability within the three oak species *Q. petraea*, *Q. pubescens*, and *Q. robur* was reported by Landergott et al. (2012). Sixty acorns per female tree were collected. Two randomly selected acorns from the same open-pollinated family were placed in pots (8 x 8 x 20 cm) for germination and further growth in greenhouse. Percentage of germination and seedling height were assessed. Three categories of seedlings were identified; those with superior growth of the two seedlings of the pair and growth of inferior seedlings, as well as growth of solitary seedlings in cases when there was just one seedling per pot.

The mean acorn weights differed considerably among the species. *Q. pubescens* had the lowest acorn weight, 2.82g; *Q. robur* had the heaviest acorns, 5.44g, while *Q. petraea* took an intermediate position with 4.63g.

There was no significant difference in germination percentage among the species. Nor did acorn weight affect germinability in a significant way. The effect of acorn weight on height was significant in the following cases:

Acorn weight as covariate
Species
With versus without competition
Superior versus inferior competitors
Acorn weight x competitive situation
Female parent within population and within species

The effect of population within species did not turn out as significant. The mean heights of the three categories of seedlings in the three species are shown in Fig. 3-12. This figure illustrates the large difference between superior competitors and inferior competitors as well as the large differences among the species. However, acorn

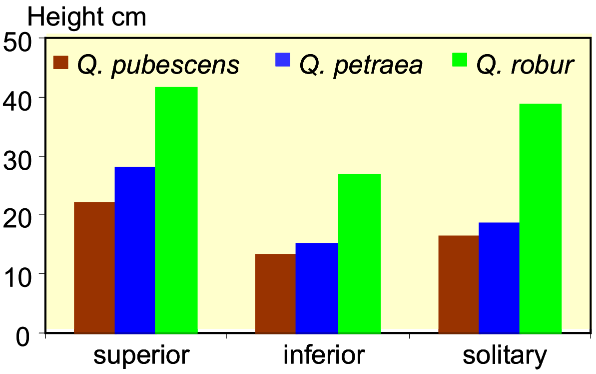

Figure 3-12. Seedling heights at age 1 of the three categories of seedlings in each of Q. petraea, Q. pubescens, *and* Q. robur *after cultivation of pairs of seedlings from the same open-pollinated family in pots in greenhouse. Seedlings in pots with just one seedling after germination are referred to as solitary. Landergott et al. 2012.*

weight did not play that dominating role as regards the percentage of cases, in which the heavier acorn gave rise to superior seedlings under competition (Fig. 3-13). This observation suggests that genetic constitution also played a central role for a successful competition. The large difference between superior and inferior seedlings (Fig. 3-12) was to a large extent attributed to competition for light under the experimental conditions. It was suggested that still stronger competition may occur under natural conditions.

The assignment of an oak tree to *Q. petraea* or *Q. robur* is based on morphological differences. Therefore, Saintagne et al. (2004) carried out a study to identify regions of the genome that differentiate between these two species. In a mixed forest in north-western France with the two species 15 morphological traits were studied in detail. A hybrid family consisting of 278 seedlings was used for the study of any QTL for the morphological traits. As far as I can understand 30 full-sibs were used in vegetative propagation for establishment of a clone trial with 36 cuttings per clone that was referred to as experiment 1. A second experiment comprising ≈ 7 cuttings from each of 216 seedlings of the full-sib family was also used for QTL identification. In both experiments two leaves from the young cuttings were assessed with respect to the morphological traits.

The ratio between species variance/total variance for the morphological traits was calculated to identify which traits are most discriminating between the two species. The following traits had high ratios and were focused on in the QTL identification:

1. Petiol length	0.81
2. Pubescence on central part of lamina	0.77
3. Basal shape of lamina	0.72
4. Number of intercalary veins	0.67
5. Sinus width	0.59

The linkage map used for QTL mapping consisted of 84 AFLP markers, 34 microsatellites, one SCAR, and nine RAPDs. For 13 of the 15 traits one or two significant QTL were detected. The percentage of the clonal mean variance explained by the QTL varied in the range 10-23%. I have illustrated the clonal mean variance explained by those QTL that showed significance in both experiments in Fig. 3-14. This figure illustrates the strongly varying degree of explanation of QTL estimates in different experiments. Ideally there should be a good fit to a linear relationship of the two data sets in the figure. However, the R^2 was as low as 0.03. It was pointed out that the five most discriminating traits between the two oak species had their QTL distributed over six linkage groups.

It was pointed out some QTL might be fixed within one species which means that they cannot be detected in a full-sib family from just one of the species. It was also acknowledged that increased family sizes are required to get satisfactory statistical power for QTL detection.

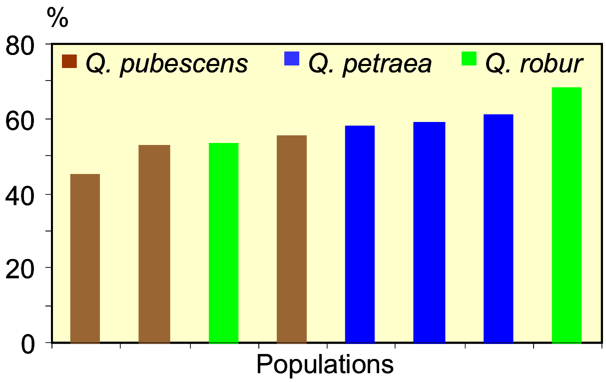

Figure 3-13. Percentage of two-seedling-pots in which the heavier acorn gave rise to a superior seedling after cultivation of pairs of seedlings from the same open-pollinated family in pots in greenhouse. Eight populations of Q. petraea, Q. pubescens, *and* Q. robur *were included in this study. Landergott et al. 2012.*

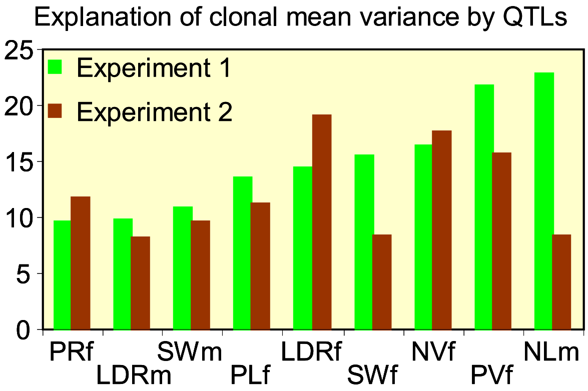

Figure 3-14. The explanation of clonal mean variance in two expertiments with clonally propagated individuals from one Q. robur *full-sib family in two experiments. PR = petiole length/lamina length + petiole length, LDR = lobe depth ratio, SW = sinus width, PL = petiole length, SW = ; NV = number of intercalary veins, PV = percentage venation, NL = number of lobes; f = female and m = male. Saintagne et al. 2004.*

A German *Q. robur* tree was used as female in crosses with three *Q. robur* and two *Q. petraea* trees as males to generate 144 seedlings for a QTL study of flushing date (Gailing et al. 2005). Based on three recordings of bud flushing at ages 3 and 6, the within-year correlations for flushing date were 0.56 and 0.71 for ages 3 and 6, respectively. The between-year correlations were weaker, 0.38. Sixteen early and 16 late flushing seedlings were used to identify association between flushing and AFLP fragments, which resulted in association with ten fragments. Only one fragment showed significant association at both ages 3 and 6. Two other fragments were weakly but significantly associated with flushing at age 3. Two formerly identified QTL loci in linkage groups 2 and 7 (Scotti-Saintagne et al. 2004a) revealed 12 AFLP fragments with significant correlations with flushing in the earlier study. Two of these fragments were significantly associated with flushing in the five German families with a total of 144 seedlings. However, two of the markers from the earlier study (Scotti-Saintagne et al. 2004a), which showed strong association with early flushing showed weak association with late flushing in the German material. Three marker loci were located to each of linkage group 2 and 7 while two marker loci were not linked to groups 2 or 7. Two of the markers had a strongly significant effect on bud flushing. There were strong associations between the two alleles in a marker locus, early and late, and flushing date at age 6, but only a weak association at age 3. Another microsatellite marker, whose early and late alleles were significantly associated with flushing, explained 6.7% of the phenotypic variance at age 3 and 22.8% at age 6. Considering all markers at linkage group 7, 10,9% of the phenotypic variance was explained at age 3 and 22.3% at age 6. It is disturbing that strong association with markers occur at one age and not at another age. The authors were aware of the problems involved in the search for QTL such as small families and absence of linkage disequilibrium. The authors also pointed out the polygenic inheritance of flushing date as one explanation for limited degree of explanation of the QTLs.

Ninety-two ESTs (expressed sequence tags), which were developed in oak were used for a comparison of QTL for three types of adaptive traits between *Q. robur* and *Castanea sativa* (Casasoli et al. 2006). Bud flushing, plant height, and carbon isotope discrimination were included in this study of colocalization. Available marker and linkage information in the two species were used for construction of consensus genetic maps for each species. The number of plants in the F$_1$-families used for QTL detection was 165 for *Q. robur* and 147 for *C. sativa*.
The study showed that there was a high degree of syntheny between the two species. The identification of colocalized QTL were confined to so called unique QTL, which were defined as *either QTL expressed in different years but located within the same interval defined by orthologous markers or QTL expressed in only one year*. Of the three types of traits studied, only bud flushing showed colocalization between the two species (Fig. 3-15). The colocalized QTL explained a considerable part of the phenotypic variance 57.9 and 67.1 in *Q. robur* and C.sativa (Fig. 3-16). However, it was suspected that the obtained phenotypic explained variances (PEVs) overestimate the true values owing to the limited number of individuals in the tested families. It was also warned for too far-reaching conclusions since the QTL were estimated in one family in each of the species. It was concluded that QTL for adaptive traits mostly have limited or moderate effect on these types of trait. The difference between the three types of traits as regards colocalization was discussed. One reason for this difference was the higher heritability for bud flushing than for the other traits since the QTL detection power increases with the increase of heritability.

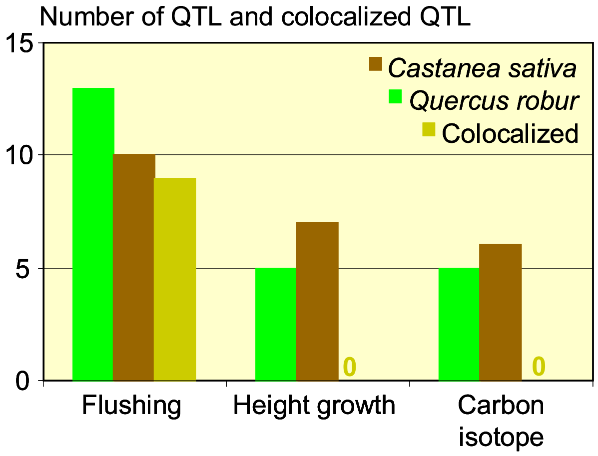

Figure 3-15. The number of QTL for three types of trait, bud flushing, plant height, and carbon isotope discrimination in Q. robur *and* Castanea sativa *as well as the number of colocalized QTL. Casasoli et el 2006.*

One family with 192 individuals from a cross between a *Q. robur* female and a *Q. robur* subspecies *slavonica* male was studied for growth and stomata density during three years in greenhouse to detect QTL for these traits (Gailing et al. 2008). Stomata density, height, root collar diameter, and their increments between years were assessed during three years. Fifty-seven QTL for growth traits and 18 for stomata density were noted. There was some overlap of QTL over the years. In four linkage groups QTL for growth were obtained on both female and male maps. The phenotypic variance explained (PVE) by QTL for growth traits varied in the range 2.1 – 15.6%. The corresponding range for stomata density was 3.6 – 15.9%. The authors regarded the PVEs as moderate and attributed this to polygenic regulation of the traits as well as phenotypic plasticity of the traits. In six cases the QTL for growth and stomatal density coincided, which was anticipated since growth and stomatal density were significantly correlated in this family.

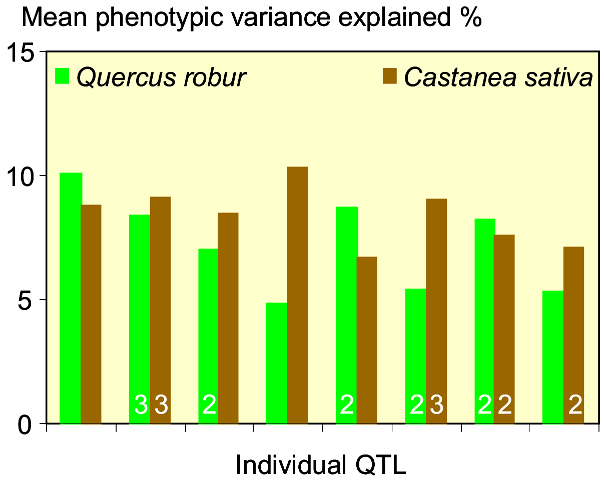

Figure 3-16. The mean phenotypic variance explained for bud flushing QTL in Q. robur *and* Castanea sativa*. The figures indicate the number of QTL for each chromosome segment. Casasoli et al. 2006.*

3.2 Molecular studies

Twenty-five isolated ESTs (expressed sequence tags) from an osmotic stress treatment of a *Q. petraea* callus were used in a study to identify genes in two full-sib families including four parental trees, one *Q. robur* family and one hybrid family between *Q. robur* and *Q. petraea* (Porth et al. 2005b). Eight of the 25 putative osmotic stress related genes had introns. Fourteen had segregating SNP sites. The number of segregating loci in the families was as follows:

3 in both full-sib families,
4 in the *Q. robur* family,
7 in the hybrid family

Thirty-four SNP sites were detected in these 14 loci but only six of them in coding regions.

In total 16 genes were located to linkage groups, nine of them from the hybrid family. Two of the three that were located in both families agreed with respect to location to linkage group. One problem for locating the genes properly to linkage groups is the low number of individuals. Much larger numbers are required to get a satisfactory mapping.

To get a better understanding at the molecular-physiology level of the difference in drought tolerance between *Q. petraea* and *Q. robur* a study with induced osmotic stress was carried out by Porth et al. (2005a). Seedlings from eight females and somatic embryo plants were grown for an acclimation period of 14 weeks in greenhouse and then transferred to a hydroponic system and exposed to osmotic stress. Samples were taken after 1, 6, 24, and 72h treatments. Seven of 33 ESTs had a known or putative connection to osmotic stress tolerance in oaks and were studied in detail. Four of these seven genes had a higher expression ratio after one hour of osmotic stress in *Q. petraea* than in *Q. robur*. They were

Thiazole biosynthetic precursor
Lipid transfer protein precursor; also a second higher expression ratio after 24 hours
Dc-3 promoter-binding factor-2
Betaine aldehyd dehydrogenase

Glutathione-S-transferase and oxygenase/oxidoreductase had a higher expression rate at 24h and 72 h, respectively. The seventh gene, heat-shock cognate protein, did not show any significant difference in expression rate between the two species. The results from this investigation gave additional support to the hypothesis that these two species differ with respect to preferences on water availability. Thus, *Q. petraea* is more tolerant against drought, while *Q. robur* is more tolerant to high soil water content.

An investigation attempting to detect differences in drought tolerance between *Q. petraea* and *Q. robur* at the gene level was presented by Provost et al. (2012).

Experiment 1. The objectives of this experiment were to reveal discrimination time points of gene expression between the two oak species after increasing time of hypoxia and to sequence candidate genes. Cuttings from six genotypes of each of *Q. petraea* and *Q. robur* were used in this study. The *Q. robur* cuttings were obtained from a water availability gradient in the Petite Charnie stand in north-western France. The *Q. petraea* cuttings originated from a certified seed tree stand. The cuttings were exposed to 6, 12, 24, 48 h of hypoxia; each treatment had six cuttings. After each treatment white roots were collected and 12 candidate genes were analyzed to monitor the level of these candidate genes. To accomplish this, subtractive suppressive hybridization was used. The authors stressed that the results obtained are limited to white roots and different results may be obtained if older roots were analyzed.

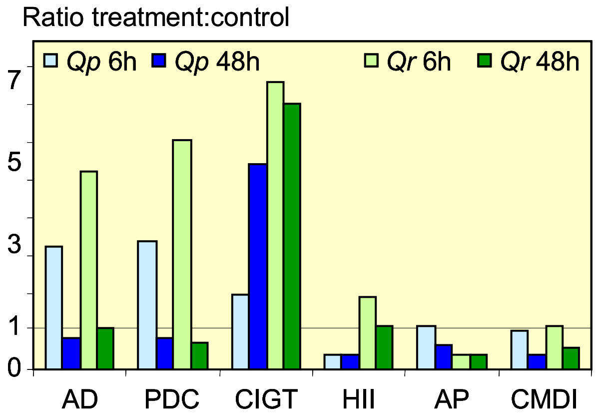

Figure 3-17. The ratio for treatment over control of some candidate genes after six and 48 hours of hypoxia treatment. AD = aldehyde dehydrogenase, PDC = pyruvate decarboxylase, CIGT = cold induced glycosyl transferase, HII = hemoglobin II, AP = ascorbate peroxidase, CMDI = calmodulin-I. Provost et al. 2012.

Results. Two of the twelve candidate genes failed to amplify. One of the remaining candidate genes was not differentially expressed. Three groups of response were identified:

Early response that declined over time; AD and PDC in Fig. 3-17

Early and longtime response; CIGT and HII; the control of *Q. petraea* had a strong expression

Decline in expression over time, especially in one of the species, AP and CMD-I.

It should be noted that the ratio is strongly dependent on the expression in the control, which to some extent masks absolute differences between the two species. Thus, for PDC there was a stronger expression in *Q. petraea* at the 6h treatment than in *Q. robur* while it was the opposite situation for AD. The 6-hour treatment showed substantial discrimination between the species for some of the candidate genes.

Discovery of expressional candidate genes using subtractive suppressive hybridization (SSH). Four SSH libraries were generated (6 h treatment in each species and 24 + 48 treatment in each species). A total number of 1315 unigenes were found; 411 contigs and 904 singletons. The number of ESTs per contig varied strongly with a mean value of three but 80% of the contigs contained less than three ESTs. Annotation to the Swiss-prot database was carried out, which resulted in 59% similarities of the contigs and 42% similarities for singletons. As many as 762 sequences did not match any database used in this study. This means that new genes were identified in this investigation. The functional categorization resulted in high expression in all four libraries:

Protein synthesis; more represented in *Q. robur* in the short-term libraries

Glycolysis and glycogenesis; more represented in *Q. petraea* in the short-term libraries and more represented in *Q. robur* long-term library

C-compound and carbohydrate metabolism; more represented in *Q. petraea* in the long-term libraries

Cellular transport.

More than 70% of the ESTs corresponded to unclassified proteins or no hit proteins. It was stated that far reaching conclusions cannot be drawn owing to the high proportion of *hypothetical and unknown proteins found in the libraries*.

Experiment 2. The objective was to quantify transcript accumulation after hypoxia for 6 and 48h. Cuttings from ten other genotypes belonging to open-pollinated families were included in this experiment, which had five cuttings per treatment. After three and a half months of growth the cuttings were exposed to the two hypoxia treatments. White roots were collected for RNA extraction and analysis.

Results. Forty-five of the unigenes were selected and their expression profiles were monitored by real-time polymerase chain reaction (qPCR). Six of them had to be discarded owing to failure of amplification. Eleven genes showed a significant species x treatment effect. Two of

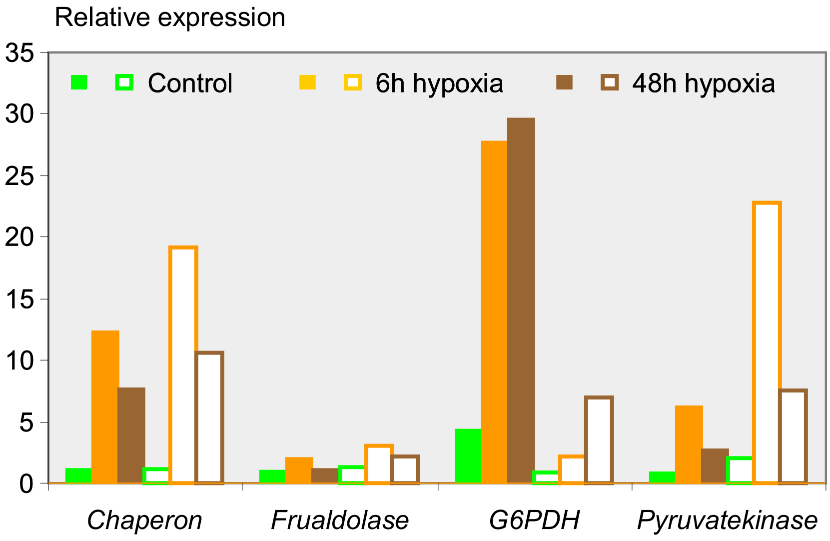

Figure 3-18. The relative expression of transcripts for four compounds in control and after two treatments of hypoxia, six hours and 48 hours treatment. Filled columns = Q. petraea and empty columns = Q. robur. Provost et al. 2012.

the genes did not show any significant effect while 24 of the genes showed a treatment effect but no species effect. Significant differences both for treatment, species, and interaction were noted for two genes. The expressions for one of them, frualdolase, are shown in Fig. 3-18, which was drawn to illustrate different patterns of response. Two cases with stronger expression in *Q. robur* than in *Q, petraea* are shown in this figure, Chaperon and Pyruvatekinase, while the reverse was noted for the *G6PDH* gene in Fig. 3-18. The obtained results were discussed in relation to the metabolic pathways that may confer tolerance to hypoxia in *Q. robur*.

This investigation is one of the first investigations, in which tree seedlings were exposed to limiting growth conditions and the responses at the transcript level were analyzed. This is certainly a useful approach that hopefully will be followed by many more studies of relationships between trait development and expressions at the gene level in forest trees.

3.3 Maps and inheritance

In 1997 a linkage map of *Q. robur* was presented by Barreneche et al. (1998). This linkage map was based on 271 RAPDs, 10 SCARs (Sequenced Characterized Amplified Regions), 18 microsatellites, one minisatellite, 5S rDNA and six isozyme markers. The principle of the two-way pseudo testcross was used for the mapping of the 94 individuals in a full-sib family. The separate maternal and paternal maps have both 12 linkage groups with approximately 85% of the genome mapped. The total length of the female and male maps was estimated at 1192 and 1235 centiMorgan, respectively. Two small linkage groups were noted for both parental groups.

Kampfer et al. (1998) developed 32 microsatellites from fresh leaf tissue of *Q. robur*. Of the 122 clones containing microsatellite motives 32 primers amplified PCR products of the expected size. Twenty-eight of them were informative in a mapping cross in *Q. robur*.

In 1996 two reports were published with information about inheritance of isozyme markers in *Q. petraea* (Zanetto et al. 1996) and *Q. robur* (Müller-Starck et al. 1996). The information was based on full-sib families.

For *Q. petraea* 15 polymorphic loci were identified and the expected segregations, 1:1 or 1:2:1; were confirmed. In case of rare alleles deviations from these segregations were noted.

For *Q. robur* 17 polymorphic loci were identified and also for this species some significant deviations from the expected segregations were observed.

There was an agreement in mode of inheritance in the two species. The same loci showed linkage in the two species. The segregation in four interspecific crosses, *Q. petraea* x *Q. pubescens*, and one cross *Q. robur* x *Q. pubescens* were studied. In all 17 segregating progenies could be analysed with two of them deviating significantly from the expected segregations. In the light of these results Müller-Starck et al. (1996) challenged the botanical classification of *Q. petraea* and *Q. robur* as separate species.

Dumolin et al. (1995) described for the first time a polymorphism in a mitochondrial gene of *Q. robur*. This polymorphism was used to study the inheritance of a mitochondrial genome. A similar approach was used for the inheritance of the chloroplast genome. Five crosses of six *Q. robur* trees from south-western France were included in this study comprising 143 individuals. With the limited number of offspring it was estimated that the paternal contribution at most could amount to 2% for mitochandria and chloroplasts 2.5%.

Eleven crosses between *Q. robur* trees with different microsatellite markers were reported by Steinkellner et al. (1997a). For five of the six microsatellites a Mendelian inheritance was proven. The microsatellite loci contained 6-16 alleles per locus.

Q. petraea nuclear microsatellite markers were used to test possible counterparts in seven other Fagaceae species among them *Q. robur* (Steinkellner et al. 1997b). In all 17 loci were compared. Two loci were polymorphic in all five other *Quercus* species included in this study; *Q. cerris, Q. palustris, Q. pubescens, Q. robur*, and Q. rubra.

Segregation of single-strand conformation polymorphism (SSCP) was studied in three *Q. robur* full-sib families by Bodénès et al. (1996) containing 26, 26, and 48 seedlings. Both parents and the six families were analysed. The same male clone was used in two of the families. Four of the six pairs of primers (length varying between 250 and 790 base pairs) showed polymorphic SSCP fragments among the five parents tested. The bands observed in the parents were with two exceptions always found in their offspring. Moreover, none of the parental bands was missing. Similarly there were no extra bands in the offspring. No significant deviation from the expected segregation according to Mendelian inheritance was observed. However, for one of the fragments two of the crosses showed *unexpected features*". Thus, one of the male alleles was missing in the offspring, which was interpreted as a result of mutation such that a null allele existed in this male parent. It was concluded that SSCPs would be useful in studies of diversity in oak populations but it was recommended that testing of segregation should precede any use of this method.

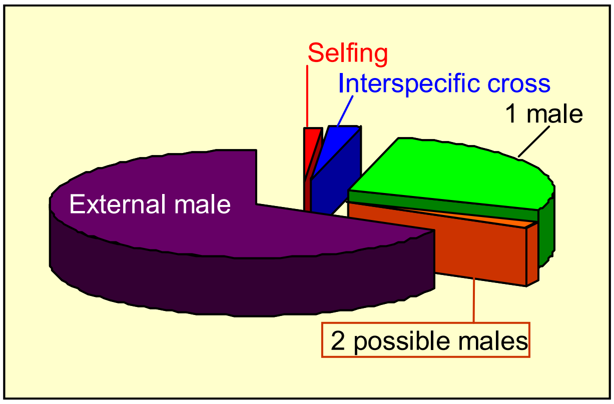

Figure 3-19. The percentages of different matings, selfing, interspecific crosses, one male, two possible males, and males outside the stand. Streiff et al. 1999.

3.4 Tracing parentage

Several aspects of pollen migration in the mixed stand of *Q. petraea* and *Q. robur* at Petite Charnie in north-western France were studied by Streiff et al (1999). In all 296 adult trees and 984 open-pollinated offspring were genotyped with six microsatellite loci. Thanks to the large number of alleles at each locus, identification of the individual offspring could be unambiguously done in 967 of the 984 offspring. The G_{ST} for the difference between the two species based on the six microsatellites was extremely low. 0.01.

Fig. 3-19 reveals that a majority of the siring males grew outside the stand, 66.8%. A low percentage of seedlings had two potential fathers, 1.7%, while male parents to 31.5% were identified and grew within the stand. Selfing and interspecific hybridization were low; below 3%.

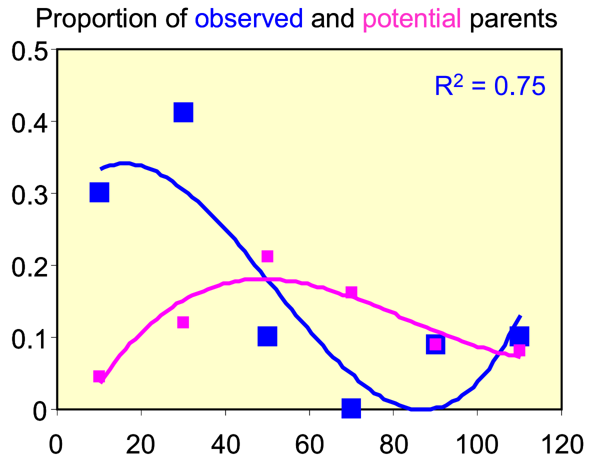

Figure 3-20. The proportion of observed and potential parents in the open-pollinated offspring from one female Q. petraea parent tree in a French stand with 296 Q. petraea *and* Q. robur *trees. For further explanation see text. Streiff et al. 1999.*

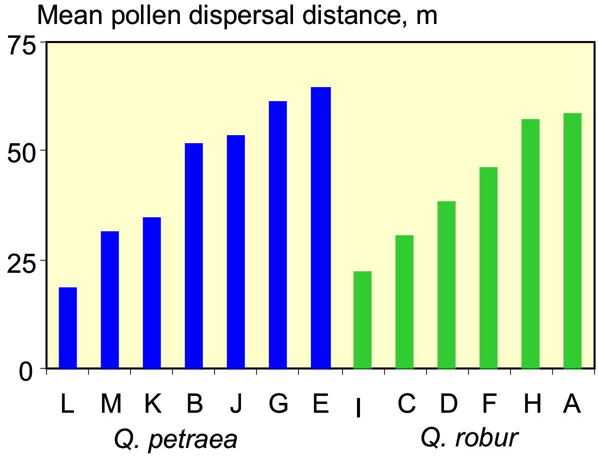

Figure 3-21. The mean pollen dispersal distance in meters for seven open-pollinated Q. petraea *families and six* Q. robur *families. Six microsatellite loci were used in this investigation. The investigation was carried out in a mixed stand of* Q. petraea *and* Q. robur *in North-western France. Streiff et al. 1999.*

After separation of the material into distance classes of 20 meters, the mating success as a function of distance between parental trees was estimated in the following way: *First, the number of pollinating male parents was compared with the census number of trees (potential number) within each distance class. ... Tests were performed for less than 120 meters because of the sampling design.* The number of trees in larger distance classes was too low for obtaining reliable estimates. *Second, the frequency distribution of intermate distances was plotted as a function of distance. Data were bulked over all parents and fitted to a dispersion curve f(x), where f(x) is the contribution (in relative frequency) of male parents as a function of distance to the mother tree*. In Fig. 3-20 I have illustrated the results for one *Q. petraea* female tree with all offspring being the result of intraspecific matings. The curve for observed matings differs strongly from the straight line for potential matings. In eight of the 13 trees there was a significant difference between the observed and potential curves. It was evident from this study that pollinations preferentially took place within short distance. It was noted that at short distances, <20 m, one to fifteen trees within this range sired the offspring while the corresponding for distances of 120-200 meters was just one male. The mean pollen dispersal distance varied strongly among the open-pollinated progenies (Fig. 3-21). A rather limited dispersal was noted with no mean value exceeding 100 meters. An exponential equation, $f(x)=ae^{-bx}$, explained more than 90% of the variation for pollen dispersal within distances up to 100 meters. There was no difference between the two species. It should be noted that this equation is not compatible with the large contribution of external pollen.

The pollination direction was studied by separating data on observed and potential matings from eight sectors around the females. For seven of the 13 females there was a significant difference between observed and potential pollinations. There was an excess of pollinations from east for six of these seven females.

The allele frequencies of the pollen inside the stand and the external pollen cloud participating in the fertilizations within the stand did not differ much with G_{ST}s of individual loci varying in the range 0.003 to 0.046.

In conclusion a high level of diversity is maintained in this stand, which partly can be attributed to a large proportion of pollinations taking place with pollen from other stands. Heterogeneity of pollen flow owing to differences in fecundity and in flowering phenology contributes also to high variability.

In a pilot study nine microsatellites were used for genotyping a *Q. robur* parental tree and 28 filial seedlings (Lexer et al. 1999). This tree was growing in the Arboretum at Grosshansdorf in northern Germany. In addition open-pollinated (OP) seedlings from six Austrian trees were genotyped. The objectives of this study was

1. to reconstruct the parent tree genotype based on OP seedlings from the tree,
2. to verify the half-sib character of OP seedlings from an individual parent tree.

Seven of the nine microsatellite loci showed, with one exception, a close 1:1 segregation in the progeny while two of the loci were homozygous. At these seven loci none of the alleles was found in all seedlings, which indicates that this female tree was heterozygous at these loci. It was concluded that it would be possible to identify the maternal genotype by analysing an OP progeny from that tree.

A few of the seedlings from the Austrian trees could not be assigned to their parent trees under which the acorns were collected. Collection of acorns on the ground is obviously not a satisfactory handling in genetic studies. However, the importance of this finding was that it is possible to identify "illegitimate" seedlings in OP families.

A relatively high estimate of F_{ST}s among the Austrian OP progenies was noted, 0.16. I assume that such a low number as five seedlings per entry influences the F_{ST} estimate. However, with increasing number of individuals per family the probability for pollen contribution from the female trees increases. It was pointed out that the type of analysis carried out in this investigation might be useful in certification of materials.

Eighty-nine trees from the "famous" Petite Charnie stand in north-western France were used for calculations of correct identification of parentage for varying number of markers and varying allele frequencies (Gerber et al. 2000). Six microsatellite loci were used with a varying number of alleles per locus, 14 – 31. One hundred fifty-nine polymorphic AFLP loci were also included in this study. The focus of the paper was on the exclusion probability, which ... *can be defined as the average capability of any marker system to exclude any given relationship.* The authors referred to three types of exclusion probabilities:

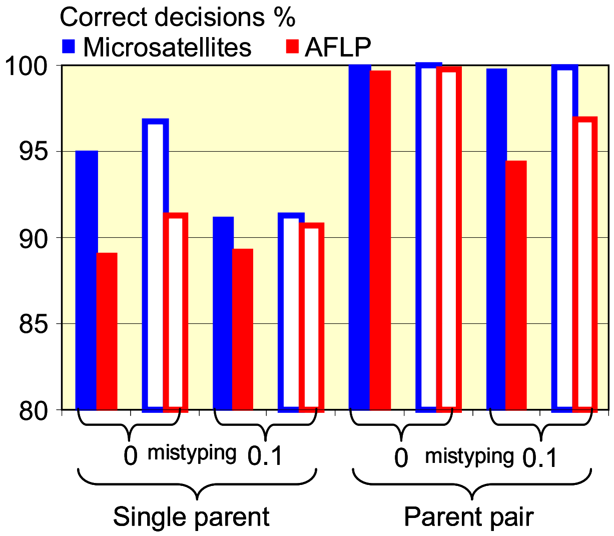

Figure 3-22. Results from a simulation with no mistyping or 0.1% mistyping for correct decision of a single parent or a parent pair at two population sizes, 500 (filled columns) or 1,000 (open columns) individuals. Six micro-satellite loci and 159 AFLPs were used for genotyping. Gerber et al. 2000.

1. Mother-offspring is compared with a potential father,
2. An individual parent compared with offspring and no information of the other parent is available,
3. A pair of potential parents is compared with an offspring.

In a first step 30 AFLPs were used to estimate exclusion probabilities for varying frequencies. There was a maximum of this probability at frequencies in the range 0.1 – 0.4. The paternity and pair exclusion probabilities for subsets of the 159 AFLP loci were estimated. At 45 AFLPs with their frequencies in the range 0.1 – 0.4 both parental and pair exclusion probabilities were high, 0.97 and 0.997, respectively. In the whole range of allele frequencies with the full set of 159 AFLPs the two exclusion probabilities were almost 100%.

Fig. 3-22 reveals that correct classification with microsatellites is much higher than with AFLPs. This is valid both for single pair and parent pair identification. Mistyping with AFLPs caused a considerable drop in correct classifications of parent pairs.

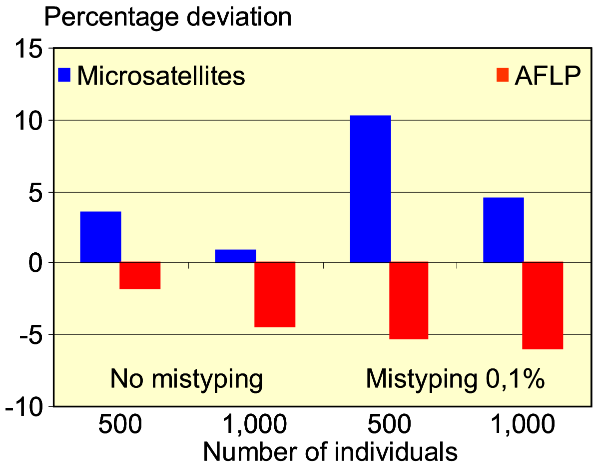

Figure 3-23. The percentage deviation from the true estimate of contamination in a stand for two different population sizes, 500 and 1,000 and for correct genotyping and for 0.1% mistyping. Gerber et al. 2000.

In Fig. 3-23 the percentage deviation from the true contamination of pollen from sources outside the stand is presented. It shows that for all cases studied microsatellites give an overestimation of contamination while AFLPs underestimate the true contamination. Both for microsatellites and AFLPs, mistyping leads to a stronger deviation from the true contamination than at perfect genotyping. The number of individuals influences the size of the deviation in different ways. At higher number of individuals, the deviation is reduced for microsatellites while the opposite is the case for AFLPs. However, the impact of population size is limited at a mistyping of 0.1% for AFLPs.
In conclusion microsatellites give in most cases better estimates of correct classifications than AFLPs. However, the high number of AFLPs compensate for this. It was stated that the cost are similar for microsatellites once they are available for use. The cost to develop microsatellites is higher than AFLP.

The mating pattern in two Polish stands (designated L and J here) with *Q. petraea* and *Q. robur* was studied by Chybicky and Burczyk (2010). The number of adult trees genotyped with microsatellites was 290 and 421. In the center of each stand a subplot containing 295 seedlings one to five years old were genotyped. The possibility for exclusions of parents in the offspring was high in both stands and particularly in the stand with largest number of trees studied. No selfed seedling was detected in the L stand while two percent selfing was detected in the J stand. The pollen dispersal was found to be approximately four times higher than the dispersal of acorns. It was estimated that 90% of the matings took place between trees separated up to 800 meters. The corresponding figure for the L stand was 81%.

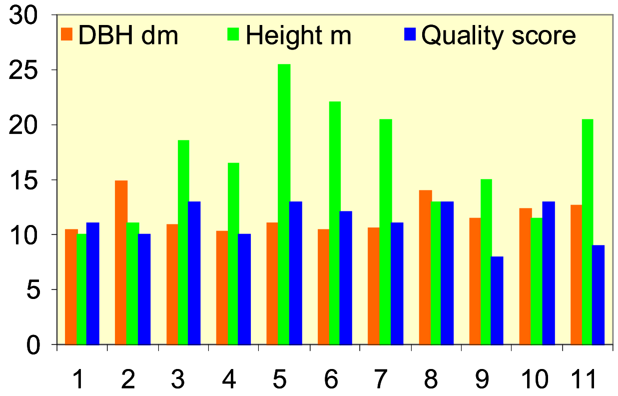

Figure 3-24. The breast height diameter in dm, tree height in m, and the pooled quality score for butress, stem form, epicormic shoots, and apical dominance. Each quality trait was scored 1-4, with 16 as the maximum quality value. Lefort et al. 2000.

3.5 Certification of material

Identification of traded material may become more important in future. Therefore, the potential of molecular methods for identification purposes ought to be investigated. Especially tracing of illegal logging is already of great importance. Finkeldey et al. (2010) gave a few examples of such studies, of which one dealt with oaks. In one case several new haplotypes that did not occur in the original stand were found in the material after transport and storage of the acorns. Thus, suggesting that mislabelling or any other mistake was responsible for the false identity of the material

3.6 Miscellaneous

Eleven selected oak trees at Tullynally Castle in Ireland were measured and their exterior traits were scored from a breeding point of view (Lefort et al. 1998). Buttress form, stem form, apical dominance, and epicormics shoots were scored from 1-4. The ideal tree should have a score of 16. The trees in this study scored from 8 to 13. The tallest tree scored 13 while the other three trees with a score of 13 varied strongly with respect to tree height and DBH. (According to the text the heights of trees 3 and 5 should be 34.5m and 26.5m, respectively. However, in Table 1 the heights are 18.5 and 25.5m. Thus, a dramatic difference for tree No. 3). If the figures given in Table 1 are correct there was a negative relationship between DBH and tree height (Fig. 3-24).
Nine microsatellite loci were analysed in five of the eleven trees with the objective to detect any relationship between the selected trees. The reason for selection of five of the trees for this analysis was not given. There was no close relationship among the five trees analyzed. There were three-band patterns for trees No 4 and No 11 suggesting that they might be triploids. No chromosome counting was carried out.

In another report by Lefort et al. (2000) the microsatellite profiles of 17 selected oak trees was presented, five of them were described in Lefort et al. (1998). The 17 trees originated from 11 different sources. Also in this case nine microsatellite loci were included. Five of the trees showed three-band patterns for one to three loci suggesting triploidy or aneuploidy. The results were compared with Austrian data (Steinkellner et al. 1997) without finding any major differences.

In his attempt to develop predictions for the outcome of global warming Kramer (1995) used data collected from yearly observations in phenological gardens in many countries. Two clones of *Q. robur* are included in these gardens. Even if no satisfactory models for predictions of bud flushing and growth cessation were reached it was evident that the two clones showed a large phenotypic plasticity.

3.7 Summary

There are few progeny trials old enough to get genetic parameters for growth and quality traits. The few studies carried out suggest large genetic variation for growth traits and especially for quality traits. In spite of the higher heritabilities for the quality traits the predicted gains were higher for the growth traits than for the quality traits. In one study tree height was found to be relatively strongly correlated with two quality traits.

The results as regards phenological traits are conflicting. In one study bud flushing was found to be more strongly genetically regulated than growth cessation traits. In another study an extremely high heritability was noted for leaf colouring. The family variance component was several times higher for bud flushing than for the family x test locality component for bud flushing. The genetic correlations for bud flushing among trials were above 0.90 while the correlations for autumn colouring were weak.

The acorn weight had a great impact on juvenile seedling height.

QTL for a series of morphological traits were estimated in two experiments with low correlations between the results in the two experiments. The explanation of the variance was high for some traits, > 15%. In another experiment a large number of QTL were found for bud flushing (10), and somewhat lower number for height and carbon isotope discrimination (5). It was pointed out that the family sizes were too small to obtain satisfactory precision in the estimates.

To get a better understanding of the metabolism as response to different treatments, efforts were made to determine the expression of genes (RNA transcripts) after hypoxia treatment of varying duration. There was a focus on the difference between *Q. petraea* and *Q. robur* since these species prefer different habitats with respect to water availability. RNA transcripts for some compounds were more expressed in *Q. robur* than in *Q. petraea* in treatments with hypoxia for 6 or 48 hours while the vice versa was noted for other compounds. This kind of research will certainly be followed by many more to enable understanding of plant responses at the molecular level.

Microsatellites and AFLPs were compared in estimates of fertilizations with pollen originating outside the stand. The precision was better with microsatellites but the high number of AFLPs compensates to some extent for their drawback. Since the costs for development of microsatellites are high it was argued that microsatellites should be used when microsatellites are at disposal.

It was again confirmed that siring with males outside the studied stands occurred to a great extent.

4 Breeding

4.1 Propagation

Six or four acorns from 11 late flushing *Q. robur* trees were used to start a micropropagation experiment (Meier-Dinkel et al. 1993). *In vitro* cultures were initiated from the embryos of the acorns. Four weeks after the initiation developing shoots were cut into nodal segments and shoot tips for further subculturing. After the first subculture explants of three different types were obtained; shoot tips, nodal segments, and basal segments with callus. After the fourth or fifth subculture *ex vitro* rooting was started with commercial rooting-promoting substances, IBA and IAA. In all 925 microcuttings from 24 clones were included in a first experiment. In a second experiment 45 clones were included. *Ex vitro* rooting was used to test if propagation of superior trees could be simplified. A large variation among the clones was noted with respect to number of microcuttings per clone, 11- 409. Even the percentage of surviving plants per clone of the individual female trees was noted (Fig. 4-1). Subcultured basal segments gave the highest number of surviving plants.

Q. robur cuttings 10-20 cm long with developed leaves were harvested during the period May 20 to July 20 for studies of rooting after soaking the cuttings in 200mg/l of IBA (indole-3-butyric acid) and other root stimulating media (Chalupa 1993). Cuttings were collected from material of different ages and in one case the cuttings originated from hedged 6-year old plants.

As seen from Fig. 4-2 the rooting percentage dropped drastically with age. It is also seen that hedging improved the rooting capacity. The higher percentage of rooting was also combined with a somewhat larger number of roots formed. The cuttings from hedged *Q. petraea* plants had several times higher shoot formation than cuttings from ordinary ortets. Also cuttings from hedged *Q robur* plants developed a substantially higher number of shoots. Cuttings that rooted rapidly with fast shoot growth turned out to be most vigorous.

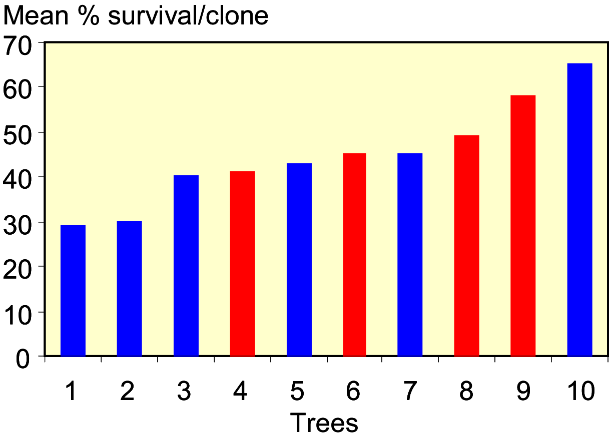

Figure 4-1. The mean percentage of surviving plants per clone of ten trees from two stands shown with different colours. Meier-Dinkel et al 1993.

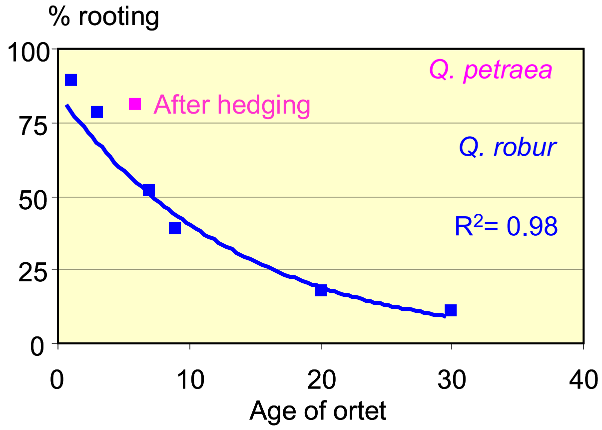

Figure 4-2. The relationship between rooting percentage and the age of the Q. robur *ortets. Hedging at age 6 (purple square) improved the rooting percentage. Chalupa 1993.*

Cuttings of *Q. petraea* seedlings exposed to continuous light had a higher rooting percentage than cuttings from plants growing under normal light conditions, 92 versus 76%.

Actively growing shoots were used to get nodal segments and shoot-tips for tissue culture propagation. Significantly higher shoot formation than in the control (Fig. 4-3) was obtained on low-salt media + low concentrations of the cytokinins, 6-amylaminopurine (BAP) or N-benzyl-9(2-tetratetrahydropyranyl) adenine (BPA). Microshoots from adult trees had a lower multiplication rate than cultures from juvenile material.

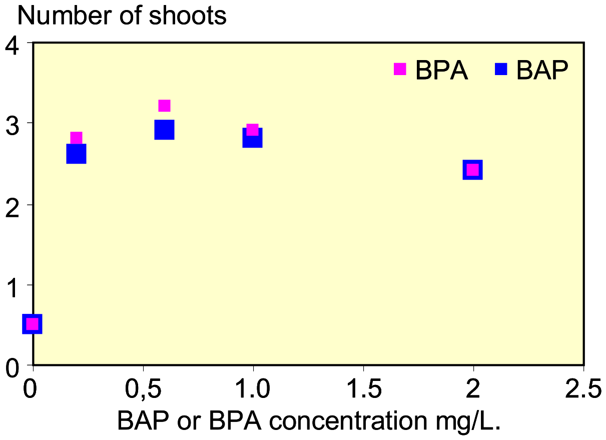

Figure 4-3. Te impact of BAP (6-amylaminopurine) and BPA N-benzyl-9(2-tetratetrahydropyranyl) adenine treatments on shoot formation in tissue cultures of Q. petraea. *Chalupa 1993.*

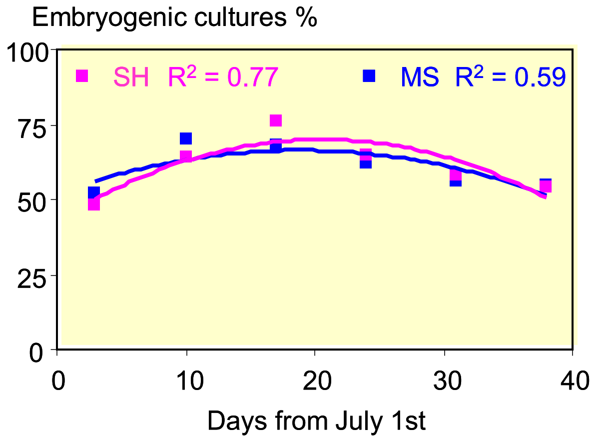

Figure 4-4. The percentage of embryogenic cultures from cultured immature embryos of Q. petraea *from July 3 to August 7. MS = Murashige and Skoog and SH = Schenk-Hildebrandt media, respectively. Chalupa 1993.*

Auxin treatment resulted in root formation of more than 50% of the microshoots after 2-3 weeks. There was limited difference in rooting between *In vitro* and *in vivo* treatment.

Embryos were excised from immature *Q petraea* acorns in July and early August for production of somatic embryos. Two different culture media were used, Mirashuge and Skoog (MS) and Schenk and Hildebrand (SH). After 7-9 weeks on initiation media embryogenic cultures were obtained. Fig 4-4 reveals that the highest percentage of embryogenic cultures was obtained in mid-July. Medium containing cytokinins kept the embryogenic potential of the cultures as long as three years. Plantlets were obtained following exposure for 2-3 weeks to low temperatures, 2-3°C, and desiccation in sterile sealed dishes. After desiccation the somatic embryos were transferred to a medium containing a low concentration of cytokinin (BAP 0.1mg/L) and cultivated under continuous light. Only 12-18% of the embryogenic cultures produced germinating somatic embryos.

This paper reveals that rapid vegetative propagation of juvenile material might be obtained. This is especially the case after hedging of the ortets.

In five *Q. petraea* OP-families from parents with different growth patterns five clones were selected for a study of the effect of decapitation on scion production during first and second flushes by Harmer and Baker (1995). The ortets were cultivated under outdoor conditions and two temperature conditions, 20/15 and 15/10°C. Bud expansion, first visible leaf, leaf expansion, and end of flushing were assessed on original shoot, first-flush shoots and second flush shoots.

According to the analysis of variance there were significant clone effects for the below traits:

Shoot length; 1st and 2nd flushes

Active buds; original plants (Fig. 4-5)

Proportion of buds becoming branches; original plants and 1st flushes

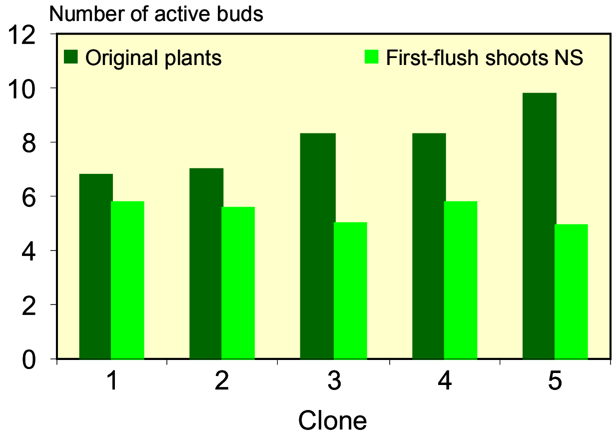

Figure 4-5. The number of active buds in five clones of Q. petraea *in original plants and in first-flush shoots. Harmer and Baker 1995.*

Number of branches; original and 1st flushes

Temperature conditions were only significant in a few of the treatments while the two-way or three-way interactions (clone x decapitation x temperature) were significant for the first flush assessments. Two statements from the paper that highlight important results of this paper are quoted below:

> *In most cases control trees were less branched than corresponding decapitated plants under the same growing conditions*
>
> *There was no obvious relationship between branchiness of the experimental plants and the mother trees*

A study of methods for vegetative propagation of *Q. petraea* was carried out by San-Jose et al. (1990). Small explants, 5 mm, were taken at different occasions from juvenile plants grown in growth chamber or greenhouse. Explants were also taken from stump sprouts of adult trees, 55 years old. Cultivation on a variety of media took place in small test tubes (15 ml) or in somewhat larger jars (50 ml). Rooting was studied after treatment with dipping for various times in indole-3-butyric acid (IBA) of different concentrations.

For explants from the seedlings the best result was obtained from the collection in May and at an age of five months. The differences between growth media were non-significant. In a second experiment with cultivation on a growth media according to Gresshoff and Doy (1972) six clones were included. There was a clonal difference in the multiplication coefficient (= proportion of explants forming axillary shoots x mean number of new segments per explant, Fig. 4-6). Rooting in two of them was determined with a large difference in rooting ability. The rooting ability of explants from the adult material was lower. However, the concentrations of IBA differed between the two experiments. This study showed the potential for rapid multiplication of juvenile explants but there was a substantial variation among clones.

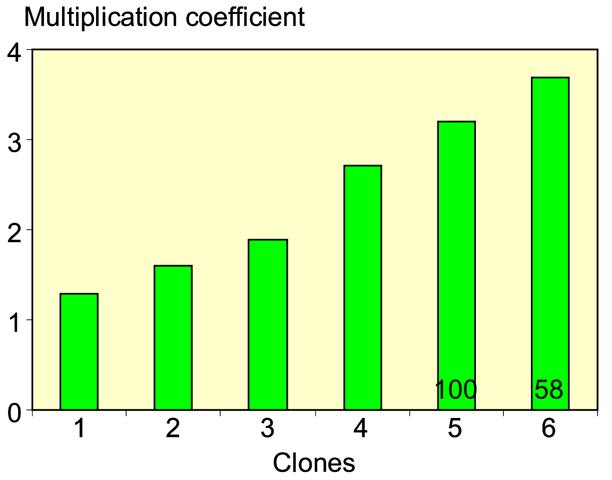

Figure 4-6. The multiplication coefficient (= proportion of explants forming axillary shoots x mean number of new segments per explant) for six Q. petraea *clones cultivated on a medium developed by Gresshoff and Doy (1972). Percentages of explants with roots are shown for the two juvenile clones in the rooting experiment. Dipping for six minutes in 0.5g/ litre indole-3-butyric acid. San-Jose et al. 1990.*

Burley and Kanowski (2005) published a paper entitled *Breeding strategies for temperate hardwoods*. It is a synthesis of breeding in general terms without any explicit outline of breeding strategies for oaks. It was stressed that breeding objectives must be clearly defined. Financial resources to employ skilled staff over time are required. Self-evident was the statement that the best genetic resources should be included in the breeding population.

4.2 Pollination pattern within a seed orchard

Buiteveld et al. (2001) carried out an analysis of paternity in a Dutch seed orchard of *Q. robur*. The seed orchard consists of 57 clones planted in 1978 with 10-year old grafts; 1-15 per clone. Twenty-one year after planting 60 acorns from each of three grafts were collected for an analysis of paternity by aid of six microsatellite loci. All 57 clones in the seed orchard were also genotyped.

The expected heterozygosity was higher in the parental generation, 0.84, than in the three progenies, 0.64-0.73. The expected heterozygosity in the seed orchard was almost identical with the estimates in two indigenous Dutch populations, 0.84 and 0.86. The differentiation between the parental and the progeny populations (F_{ST}) was limited, 0.023. Twelve of the alleles in the seed orchard were not found in the progeny population. They were all low frequency or rare alleles. The progeny had seven alleles that were not present in the seed orchard population. The F_{ST} for the difference between the seed orchard and the two stands was estimated at 0.013. The fixation indices were close to zero in the parental (0.038) and in the progeny population (-0.049).

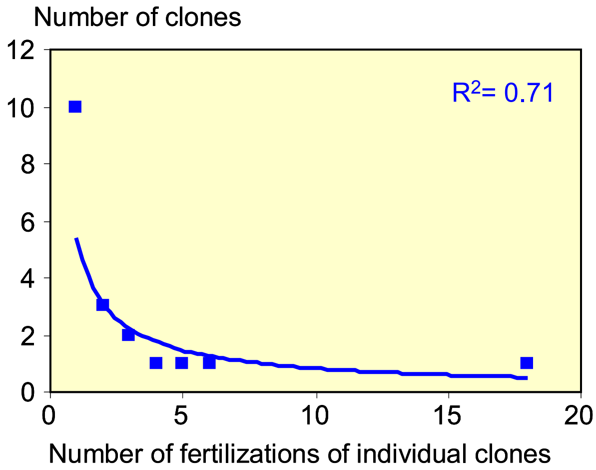

Figure 4-7. The number of clones with 1 to 18 detected fertilizations in a Dutch Q. robur *seed orchard with 57 clones, of which 19 were found as contributors to the progeny from three seed orchard grafts. Buiteveld et al. 2001.*

The pollen cloud originating from outside the orchard did not differ much from the orchard pollen cloud, G_{ST} = 0.013. A somewhat higher differentiation was noted for the pollen clouds of the progenies of the three grafts, G_{ST} = 0.043.

Only 19 of the 57 clones in the seed orchard had served as males in the progeny. Ten of them were found only in one progeny while one male had participated in 18 fertilizations (Fig. 4-7). In spite of this it was found that random mating could not be ruled out. It was stated that the number of fertilizations correlated fairly well with the number of grafts of the individual males.

The fertilization with pollen from outside the orchard was at least 64%. A correction owing to uncertainties led to an estimate of 70% background fertilizations with a large variation among the three trees, 62-82%. It was speculated that mislabeling of orchard grafts might have taken place. However, even if this was the case a mislabeling could only contribute to a marginal increase of the estimated background pollinations. Since there were only scattered oak trees within a radius of five kilometers around the seed orchard it was concluded that pollen dispersal over wide distances takes place in *Q. robur* in The Netherlands.

It may be concluded that the genetic diversity was not much affected in the progeny population. The high contamination in this seed orchard reduced the expected genetic gain in the progeny. However, it was stated that it is hardly possible to locate seed orchards of such a wind pollinated species as *Q. robur* to have total isolation from outside pollen sources.

4.3 Summary

Propagation via microcuttings was tested and resulted in a large variation among clones with respect to number of microcuttings. Subcultured basal segments gave the highest number of surviving plants. The rooting dropped drastically with age of the scions and resulted in less than 15% rooted cuttings at age 30. Hedging of the ortets improved the rooting percentage substantially. Tissue culture experiments were used to produce microcuttings and auxin treatment resulted in formation of microshoots in 50% of the cuttings. Somatic embryos were initiated from excised embryos from acorns during the summer months with the highest success rate for mid-July. Less than 20% of the embryogenic cultures produced germinating somatic embryos. The effect of decaptiation on scion production in five clones revealed clonal effects for proportion of buds becoming branches. There are possibilities for rapid multiplication of juvenile explants but with a substantial variation among clones.

One study of the pollination pattern in a seed orchard showed a high percentage, > 60, of fertilizations with pollen from outside sources. The differentiation, F_{ST} between the seed orchard population and the progenies from three grafts was limited and estimated at 0.023. Selfing was low.

In the German study of 12 *Q. petraea* and nine *Q. robur* populations Herzog (1996) recommended minimum gene resource population sizes of 30 to 50 hectares. It was stated that on one hand gene resource populations should be free from introgression on the other hand introgression might lead to improvement of adaptability that is particularly important in the light of global climate change. It was concluded that gene conservation might be accomplished within the frame of current silvicultural regimes in Germany. The author also advocated for the use of the Multiple Population Breeding System concept of gene conservation even if this concept was not mentioned in the text.

Bruschi et al (2003) in their study of five Italian *Q. petraea* populations discussed gene conservation. Since the marginal Sicilian population in this study presumably is one population from which migration took place after the latest glaciation it should be of particular value for gene conservation according to the authors. Therefore, focus was on efforts to conserve a marginal Sicilian population that had a lower diversity than the other four populations. Besides, *logging disturbance, fire and heavily grazed pastures* constitute serious threats. Especially the grazing might prevent a satisfactory regeneration of this population. The Sicilian plus three of the other four populations are legally protected. The authors also suggested that *ex situ* conservation might be carried out. *Restoration of extirpated populations should also be considered.*

Baliuckas and Pliura (2008) addressed gene conservation based on the ecological character of tree species and summarised results from field trials in Lithuania with *Q. robur, Alnus glutinosa,* and *Betula pendula*. Owing to the large variation among *Q. robur* populations in Lithuania they concluded that several gene resource populations should be included in a conservation effort. They advocated for a dynamic gene conservation

5.1 Miscellaneous

A few papers reporting on deviating trees that deserved preservation or protection were published.
Two morphological deviating *Q. robur* trees in the Voronezh region in Russia were classified as triploid but cells in the meristems were not truly triploids but aneuploids (Butorina 1993). The meiosis in the pollen mother cells was severely disturbed as could be expected for a triploid.

During an investigation of oak decline in Germany 400 trees were analyzed with respect to isozyme pattern Naujoks et al. (1995). In this survey one tree with deviating isozyme profile was detected. The study comprised eleven isozyme loci, stomata measurement, and counting of chromosomes. At sampling it was detected that the leaves of the suspected triploid had *thick, nearly stiff, leather-like leaves…..*
In the suspected triploid nine of the isozyme loci were monomorphic while two loci revealed three bands. At the aminopeptidase locus three bands corresponding to three of the four common alleles in this locus were detected. At the isocitrate dehydrogenase locus three bands were also detected, one of them with a pronounced strong staining. The stomata length was estimated at 24.5µm while the mean value for the five reference trees was below 20µm. The chromosome counting of metaphase nuclei resulted in a varying number of chromosomes in the control tree as well as the suspected triploid, 19-27 and 27-36, respectively. It should be remembered that counting of the tiny chromosome is a delicate task. Fifty cuttings were collected in July and treated with 3-indolyle butyric acid with a low success rate for root formation, only two of the cuttings produced roots. The cuttings sprouted during the second year, which showed that such old trees may be vegetatively propagated.

Owing to their unusual microsatellite banding pattern two Polish oaks were suspected to be polyploids (Dzialuk et al. 1995). One tree was classified as *Q. petraea* and the other as *Q. robur*. To verify the ploidy level three different methods were used:
microsatellite analysis,
stomata length measurements,
determination of nuclear DNA content by flow cytometri.
Six microsatellite loci were used and four of them showed three alleles. Sampling was done of leaves and wood at different levels in the crowns and at different times. In all cases the same result was obtained. The stomata length of the suspected *Q. robur* triploid was 30.6µm as compared to 27.7µm for two reference trees. The corresponding values for the *Q. petraea* tree were 30.4µm and 25.8µm, respectively. For both species the differences were significant. Both suspected triploids contained 50% more DNA than their diploid reference materials. Determination of chromosome number would be a more direct approach to verify the ploidy level. However, the tiny chromosomes make the counting cumbersome.

5.2 Summary

Gene conservation of *Q. petraea* and *Q. robur* has as far as I have found not attracted much focus in scientific litterature. A suggestion on large gene resource populations in Germany was presented. An Italian paper discussed the many threats of gene resource populations, which called for active protection of the resources. Dynamic gene conservation was suggested.

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