Fagus sylvatica
recent genetic research

Gösta Eriksson

Fagus sylvatica recent genetic research

Gösta Eriksson

Cover photo: Beech trees close to Stenhammar castle at lat. 59.05ºN and 16.58ºE. These trees originate probably from beechnuts spread from beech trees planted adjacent to Stenhammar.
Photograph: Gösta Eriksson

Preface

European beech, *Fagus sylvatica*, is a widely spread forest tree in Europe. Several beech genetics studies were published. In agreement with previous overviews of recent genetics studies I have tried to summarize published reports on recent beech genetics research. There are several reports which cover research in the different chapters. They are presented in one chapter only. Several important reports were published in 2009 and this overview starts with publications from this year. I have used the term bud flushing even for the reports in which leafing out or bud burst were used.

As usual graphic illustrations are in focus in my overview. It should be noted that none of the illustrations were taken from the original papers. As in previous books, papers written in languishes that are not understood by the scientific society are not treated. An apology for missing of relevant literature in my search for beech genetic investigations

My sincere thanks to Björn Nicander and Christian Divander for for their willingness to swiftly solve any computer problems.

Uppsala December 2021

Gösta Eriksson

Contents

1.Population

Bresson et al. (2011) studied functional traits in populations from two Pyrenean valleys *in situ* and in a common garden trial in southern France. The traits analyzed *in situ* and in the common garden are shown in Table 1-1. The observed results were related to the elevation at the origin of the populations.

All traits observed *in situ* showed significant population differences and with one strong relationship with elevation origin; leaf size, L_s, $R^2 = 0.84$, and two moderately strong; maximum assimilation rate, A_{max} and nitrogen content, N_a, $R^2 = 0.61$ and 0.71. The population differences were attributed to adaptation to the different ambient conditions along the elevational gradient. This is experienced as a disruptive selection between populations, which is stronger than the gene flow among the geographically adjacent populations. In the common garden experiment only one strong relationship was noted for the relationship between population elevation and Leaf Mass Area, LMA, $R^2 = 0.78$. However, the populations did not differ significantly for LMA. Only leaf size showed a significant difference among the populations. It was speculated that a substantial population x elevation interaction might have resulted in non-significant differences among populations.

Much of the discussion in this report centered on physiological aspects of the traits studied as well as consequences for the future evolution of the species under the projected global warming. It was stated that the large within-population variation and large phenotypic plasticity noted in this investigation constitute good prospects for future evolution of beech. It should be noted than none of these parameters were studied in a classical genetic way. The plasticity is based on population means and variation within population on unreplicated recordings of trees.

One aspect, which this investigation offers to analyze, is the relationship between the performances *in situ* and *ex situ*. This was not reported. In Figs 1-1- and 1-2 I have plotted the leaf mass and leaf size in the common garden trial against the same traits in the parental populations. It
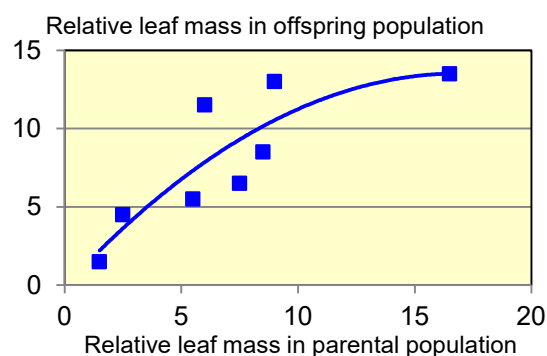

Figure Bresson1-1. The relationships between leaf mass in situ *of the parental population and the corresponding for offspring in a common garden trial. The populations were sampled along elevational gradients in two Pyrenéan valleys in France in 2009. Bresson et al. 2011.*
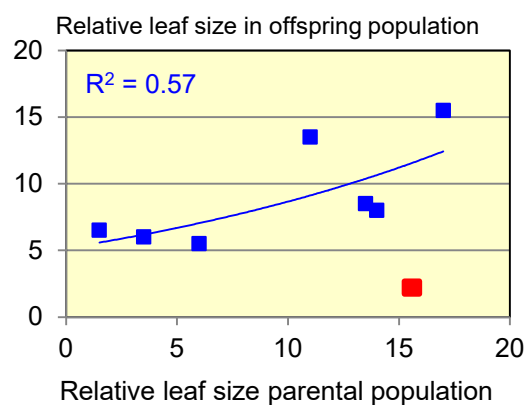

Figure 1-2. The relationships between leaf size in situ *of the parental population and the corresponding for offspring in a common garden trial. The populations were sampled along elevational gradients in two Pyrenéan valleys in France 2009. The population from 422 masl. (red) was excluded from the relationship. Bresson et al. 2011.*

is seen that the relationship for leaf mass is strong, $R^2 = 0.76$ while the corresponding for leaf size is absent, $R^2 = 0.01$. After exclusion of the population from 424 masl., the relationship becomes fairly strong, $R^2 = 0.57$.

Table 1-1. Leaf functional traits analyzed and the R^2s for their relationship with elevation at population origin. Empty boxes: Weak relationships without any R^2 estimates presented in the report. Bresson et al. 2011.

| Trait	R^2 in situ	R^2 common garden
L_s leaf size cm^2	0.84 decreasing with elevation	
LMA leaf mass kg m^{-2}	0.56 increasing with elevation	0.78 increasing with elevation
SD stomata density St mm^{-2}	0.41 increasing with elevation	
G_{smax} maximal stomatal conductance mmol m^{-2} s^{-1}	0.32 increasing with elevation	
A_{max} maximum assimilation rate μmol m^{-2} s^{-1}	0.61 increasing with elevation	
N_a leaf nitrogen g m^{-2}	0.71 increasing with elevation	0.48 increasing with elevation
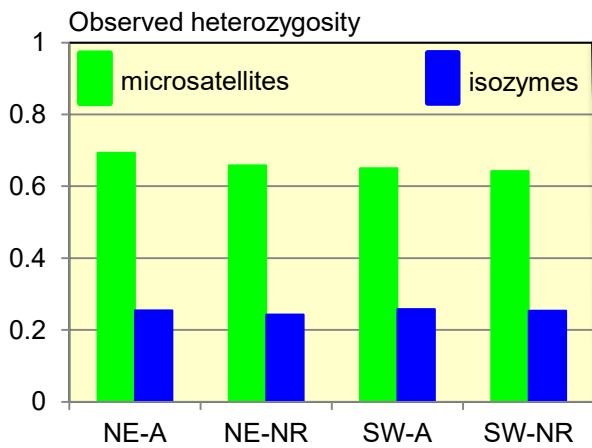
Figure 1-3. The observed heterozygosities by 5 microsatellite loci and 16 isozyme loci in two adult (A) and their two naturally regenerated (NR) beech populations on two slopes in southern Germany; NE = northeastern slope, SW = southwestern slope. Bilela et al. 2012.

A scrutiny of the graphs for all traits in the common garden trials reveal that the 424-masl-population deviates from trends for other traits as well. Whether there was an adaptation to the microsite condition of the 424-masl. locality, which caused a deviation from the elevational trend would be worthwhile to analyze.

From one locality in southern Germany (Lat.47.99ºN, 740-800 masl.) one south-west and one north-east adult beech population, naturally regenerated after clear cutting 77-87 years ago, were analyzed together with their regenerated populations to evaluate the effect of microclimate on genetic parameters (Bilela et al. 2012). The soil conditions were identical in the SW and NE slopes. From each population 80-100 individuals were sampled at a minimum distance of 50 m for analysis of 16 isozyme loci and five microsatellite loci.

The effective number of microsatellite alleles varied in the range 5.76-7.12. It was stated that the observed excess of homozygotes suggests that null alleles occurred in the range 0.12-0.21. The clear difference between isozymes and microsatellites with respect to observed heterozygosity is visualized in Fig. 1-3. The differences between the populations for observed heterozygosity were marginal for both types of marker.

The structure analysis based on microsatellites clearly indicated that the SW-A (southwest adult) population was most differentiated from the other three populations.
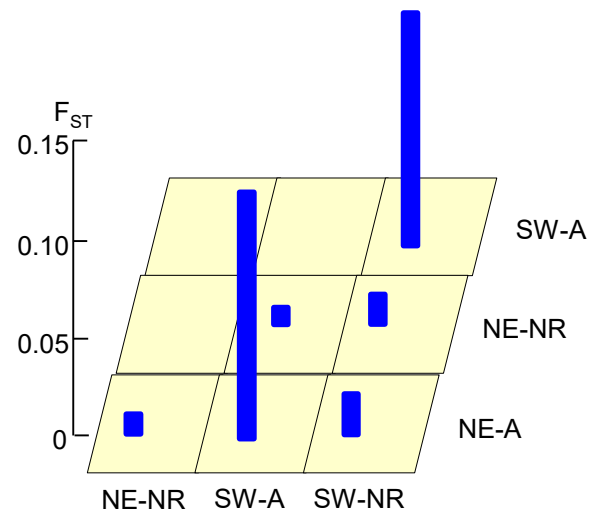

Figure 1-4. Genetic distances, F_{ST} between two adult (A) and two naturally regenerated (NR) beech populations on two slopes in southern Germany; NE = northeastern slope, SW = southwestern slope. Five polymorphic nuclear microsatellites were used for the estimations of F_{ST} Bilela et al. 2012.

There were two F_{ST} estimates for the microsatellites that reached levels above 0.12; SW-A versus NE-A and SW-A versus SW-NR (southwest natural regeneration, Fig. 1-4). Strangely, the F_{ST} between SW-A and NE-NR was only 0.007. Moreover, the SW-NR population was closer related to the two NW populations than to its parental (SW-A) population. Three possible reasons for this were presented.

1. Different origins of the materials
2. Different adaptation processes in the two microclimates
3. Extensive gene flow from the NE-A population to the SW-A population

Only the second explanation seems reasonable, which does not necessarily mean that the markers contribute to fitness themselves. However, this explanation disagrees with the limited pollen and gene flow estimated in other studies. It should be noted that the F_{ST} estimates for the pairwise differences between the four populations did not exceed 0.004 for the isozymes. It would be useful to analyze a potential differentiation between the progenies from the SW and NE slopes for metric traits.

The title of the article promises more than what is supported in this study, although it gives useful information on genetic differentiation among adjacent populations of beech.
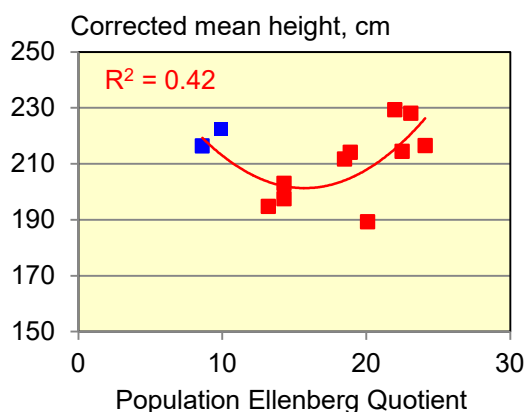
Figure 1-5. *The relationship between population Ellenberg quotient and corrected height (explained in text) at age 8 of 12 populations studied in three provenance trials in Hungary, Slovenia, and Slovakia. The blue squares refer to high elevation populations, >1,000 masl. Matyas et al. 2009..*

1.2 Provenance trials

Several reports from assessments in an international series of provenance trials in Europe were published; mostly treating data from just one trial. I have not found any joint evaluation of all trials.

Matyas et al. (2009) studied the performance of 12 populations in three eastern European provenance trials close to their limit of distribution. They focused on the so called ecodistance which was described as follows: *The ecodistance (ecological distance) concept is based on the idea that if populations adapted to certain ecological (climatic) conditions are transferred to a new environment, and all other site factors are kept equal or disregarded, their phenotypic response to climate depends not only on the climatic conditions where the population is actually grown or tested, but also on the ecodistance of transfer, i.e., on the magnitude and direction of environmental change experienced due to the transplanting to the test site, related to the macroclimate they had been adapted to originally*. The localities of population origin were classified with Ellenberg's quotient $EQ = 1,000 \times T_{07} \times P_{ann}^{-1}$, in which T_{07} is July mean temperature and P_{ann} is the annual precipitation. Tree height at age 8 in one trial in each of Hungary, Slovenia, and Slovakia was used in this study. Measured average heights of provenances at the three tests were corrected with the test site effect, to make them directly comparable.

The corrected tree height varied between 185.2 cm in the Slovak trial and 228.3 cm in the Slovenian trial. The corresponding value for the Hungarian trial was 218.9 cm. The relationship between EQ and corrected mean height for the three provenance trials shows that high elevation populations >1,000 masl (blue squares), on an average performed well in these three trials (Fig. 1-5). Exclusion of them as done by the authors resulted in a linear relationship with a moderately high R^2, 0.48. However,
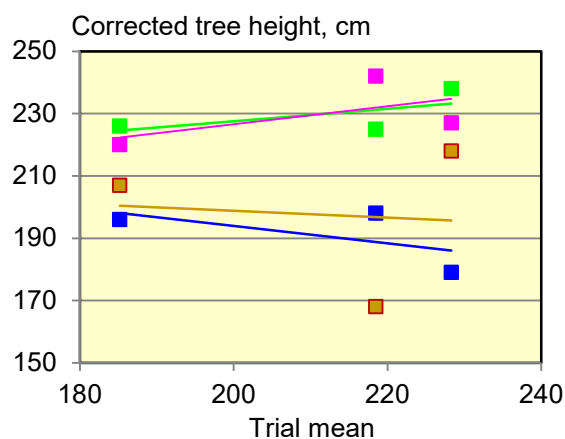

Figure 1-6. *A so called Finlay-Wilkinson diagram for the relationship between trial mean corrected tree height and population tree height in three trials. The populations are: Tarnawa, PL, (green), Aarnink, NL (purple), Plateaux, FR (brown), Grasten, DK (blue). Matyas et al. 2009..*

another high-elevation population from Czechia, Horni Planá, from 990 masl. had a poor growth with a mean value for the three trials, 194.8 cm. With the large variation in height means for the three trials it would have been useful to have the relationships: EQ – corrected height of the individual trials. The relationships between trial EQ and corrected height were given for four populations; for two low elevation populations there was an increased height with increasing EQ while there was a decline for two populations, one French and one Polish. I have used the information in Fig. 3 and Table 1 of the paper to make a so called Finlay-Wilkinson diagram (Fig. 1-6). The number of trials should ideally be larger but this figure gives some principle information. The Tarnava and Aarnink populations are examples of populations with good growth at all three test localities while the Danish Grasten population shows poor growth at all test localities. The growth of the French Plateaux population shows an erratic pattern.

These trials are three of the trials belonging to a series of trials in several countries. It will be interesting to have a joint analysis of all trials in this series of trials.

Gömöry and Paule (2011) studied growth and phenology traits at ages nine and ten in 32 populations growing in a Slovak provenance trial at lat. 48.64ºN and 810 masl. The populations originated from a large part of the distribution of beech in Europe except for southern Europe. The latitudinal range was 41.64 – 55.54ºN and the range in elevation was 33 – 1,450 masl. Bud flushing was recorded at 3-5 days intervals in a seven-point scale during April 1 to June 1 at age nine. The corresponding dates for age 10 were April 10 to May 27. Autumn leaf coloring was recorded from September 5 to November 1 at six to ten days interval in a six-point scale. The corresponding dates for age 10 were August 29 to October 27. Besides, tree height, breast height diameter, root collar diameter, and frost damage (age 10) were recorded.
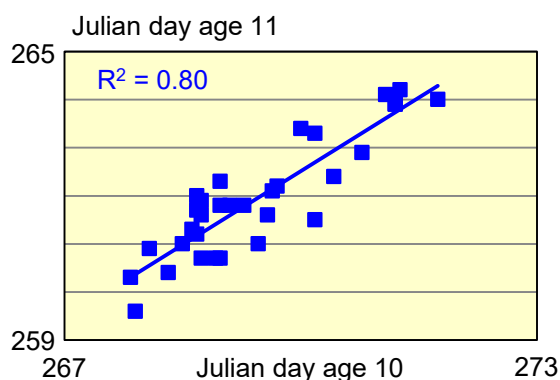
Figure1-7. *The relationship between .leaf coloring dates at ages 10 and 11 in a Slovak provenance trial at lat. 48.64ºN and 810 masl. including 32 populations from a wide range in Europe. Gömöry and Paule 2011.*

As expected with such a wide origin of the populations most traits showed strongly significant population effects. Bud flushing took place somewhat later at age 11 than at age 10 and was more contracted, 11 days versus 16 days for age 10. It was stated that the correlation between bud flushing the two years was strong, which agrees with the calculation I carried out based on data in Table 1; R^2 = 0.96. It was stated that agreement between leaf coloring the two years was less correlated. However, I found that R^2 = 0.80, which is still a strong agreement (Fig. 1-7). The tree height varied between 1.34 and 2.34 meters while DBH varied between 14 and 23 mm. It should be noted that DBH was based on fewer trees since all trees had not reached 1.3 m.
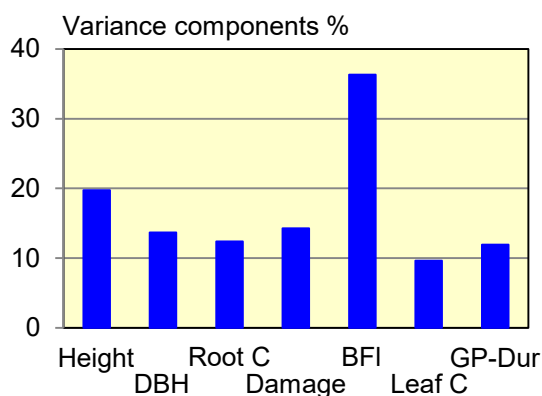

Figure 1-8. *Mean population variance components for seven traits assessed during two years in a Slovak provenance trial with 32 populations from a large part of distribution area for beech in Europe. DBH = breast height diameter,, Root C = root collar, BFl = bud flushing, Leaf C = autumn leaf color, GP-dur = growth period duration. Gömöry and Paule 2011.*
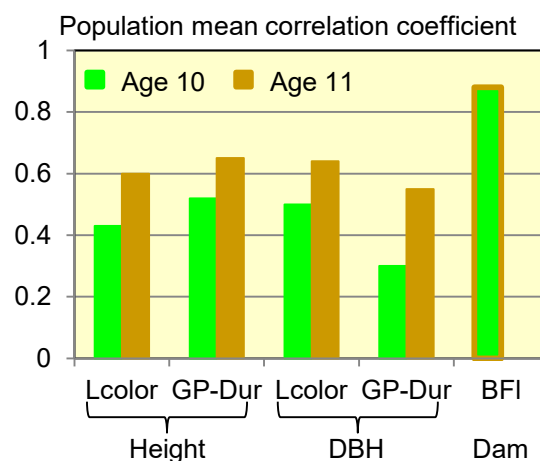

Figure 1-9. *Significant population mean correlation coefficients between height, DBH, and frost damage on one hand and three phenological traits; Lcolor = autumn leaf coloration, GP-Dur = Growth period duration, BFl = bud flushing. The brown framed column refer to a negative relationship, which was estimated at age 10. Data were obtained from a Slovak provenance trial located at lat. 48.64ºN and 810 masl. including 32 populations. Gömöry and Paule 2011.*

I preferred to illustrate the mean variance component estimates for the two ages in Fig. 1-8. This figure shows that most variance components were higher than 10%. Especially, the bud flushing component was substantial, 36.3%. This indicates that bud flushing is strongly genetically regulated.

Relationships between the traits and geographic variables were tested with generally weak correlations. Only twice the correlation exceeded 0.40. The relationships between population elevation and leaf coloring duration; R^2 = 0.63 (age 10) and 0.56 (age 11) were the two relationships that exceeded 0.40. With a difference in latitude of almost 14 degrees and difference in elevation of more than 1,400 meters of the included populations it is highly unlikely that correlations with just one geographic variable at a time will result in any strong relationships. In this situation a stepwise regression would have been worth testing. In Fig. 1-9 I have illustrated significant relationships between the traits studied. Most of the correlations between traits were weak. One really strong and negative relationship was found between bud flushing and frost damage at age 10. This means that early flushing can lead to comprehensive frost damage. Time for flushing seems to be more important for fitness than time for growth cessation, which is one of the most important results from this investigation.
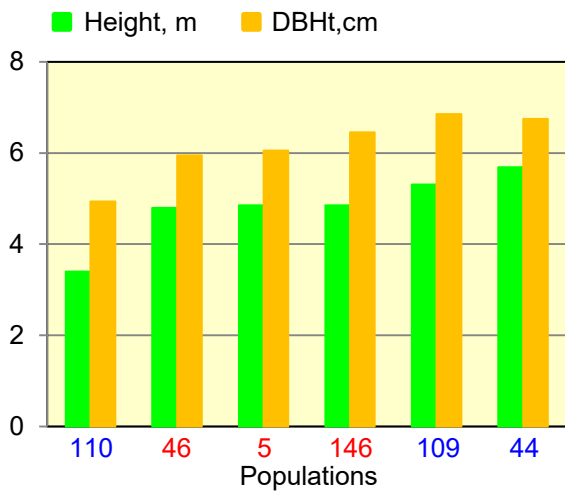
Figure 1-10. Tree height and breast heigt at age 15 in three populations from cold (blue) and mild (red) regions respectively. Liesebach 2012.

Liesebach (2012) presented a detailed study on tree growth, phenology, and stem quality of three populations from a cold climate and three populations from a warm climate. These populations grow in a northern German provenance trial at lat. 54.30°N, long. 10 28°E, and 38 masl., which is a part of the European series of provenance trials with beech. Three replications with plot size of 10 x 10 meters and 2 x 1 m spacing were used in this trial. Bud flushing was assessed in a 7-degree scale and leaf coloring was classified in 5 stages. Three classes were used to assess stem form, straight, crooked, and very crooked. The highest survival, 66%, was noted for the German population (44) from Lower Saxony and the lowest, 55% was noted for the German Brandenburg population (146). The mean tree height varied between 3.4 m of a Czech population, 110, and 5.7 m for a Lower Saxony German
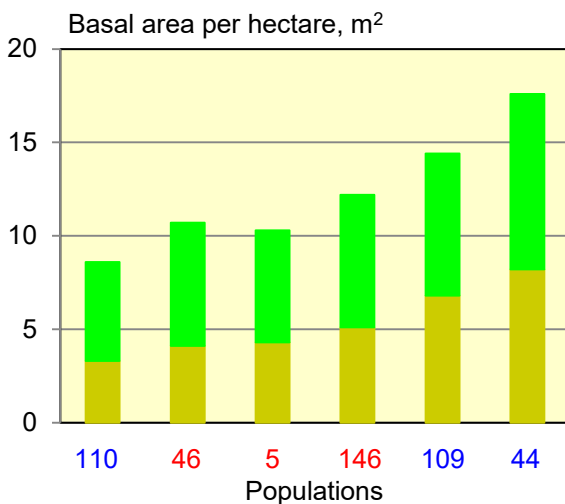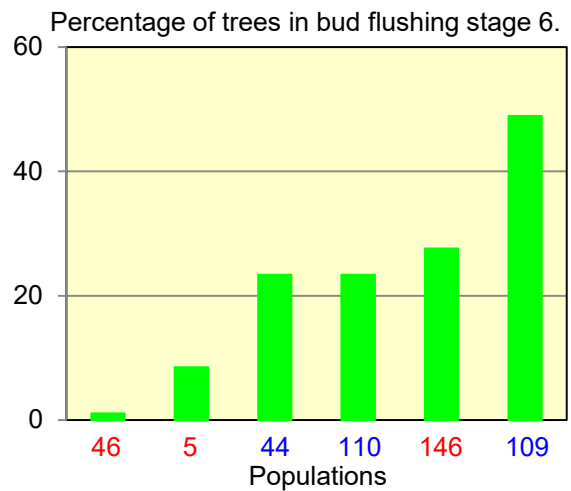

Figure 1-12. Percentage of trees in bud flushing stage 6 in three populations from cold (blue) and mild (red) regions, respectively. The scoring took place on May 10. At this date the largest resolution in bud flushing was noted Liesebach 2012

population, 44, (Fig. 1-10). The Tukey test revealed two overlapping groups of populations. As seen from this figure there was a strong agreement between tree height and DBH. The juvenile – mature relationships were nonsignificant. The basal area per hectare showed a larger differentiation among the six populations (Fig. 1-11) owing to differences in survival among the populations. There were only two significant relationships between growth and climatic data at sites of population origin. Regarding the large number of relationships tested it is not surprising that a few of them will turn out as significant for random reasons

The variation in bud flushing is considerable as is illustrated in Fig. 1-12 with the earliest development in the Austrian population, 109, and the latest development in the German Gransee population, 46, from Brandenburg. At scoring dates with the largest resolution in bud flushing significant differences among the populations were noted. There was fairly good relationships between the ranking of the populations among years. No significant relationships were noted between bud flushing and climatic data.

The leaf coloring did not differ much among the populations. At day 305 the differences among the populations reached its maximum with the German population No 46 as the latest with a score of 3.8 and the Czech population 110 the earliest with a 4.8 score. The other four populations clumped close to a score of 4.2. In spite of the more limited variation in leaf coloring, several significant relationships with climatic variables were noted such as annual temperature, growth period temperature, number of days >10°C, and Ellenberg quotient (presented above in Matyas et al. 2009).

Figure 1-11. Basal area per hectare at ages 12 and 15 in three populations from cold (blue) and mild (red) regions, respectively. Liesebach 2012
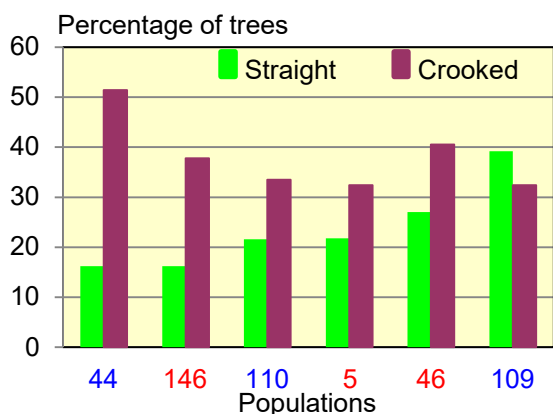
Figure 1-13. Percentage of trees with straight stems and severe crookedness at age 19 in three populations from cold (blue) and three from mild (red) regions respectively. Liesebach 2012.

The Lower Saxony population (44) had the lowest percentage of straight stems and the highest percentage of severe crookedness (Fig. 1-13). The high-elevation Austrian population, 109, constituted a contrast to the former population with the highest percentage of straight stems and lowest percentage of severe crookedness. It was pointed out that this result agrees with the general trend of better straightness of high-elevation populations than of low-elevation populations. Much of the poor stem quality was attributed to late spring frost damage. However, this interpretation is not supported by the data as regards bud flushing with the Austrian population as the most advanced in bud flushing (Fig. 1-12). One interpretation might be that this population had passed the most frost sensitive stages at frost occurrence.

With the experimental design used it is hard to prove significant differences even with large variability of the traits studied.

Results from a Spanish trial belonging to the same series of provenance trials were reported by Robson et al. (2013). This trial is located at 1,340 masl. in northern Spain at a locality with summer drought, only 137 mm precipitation during May – September. Growth and phenology were recorded in 2008, 2010, and 2011. The design was three complete blocks with 50-tree plots of each population. Bud flushing was recorded in a 7-degree scale. Curves for individual trees were plotted and reaching of 2.5 in 7-degree scale was defined as bud flushing date. A principle component analysis was calculated.

According to the text there was a large variation in survival among the populations, 34 – 98%, with the highest mortality in high elevation populations. These figures do not agree with the mortality for 2011 listed in Table A3 of the paper, in which the range of mortality was 5 – 71%. The mean mortality according to this table was 34.4% and the mean for populations originating from 800 masl and higher was 28.1%. There was a large variation in tree height among the populations, 65 – 173 cm. It was noted that bud flushing was correlated between the three years

of observation (no correlation coefficients were presented). Mean bud flushing date for the 32 populations was illustrated by six shadings in grey for bud flushing stages 2 – 4.5 on a map with the geographic position of the populations given. The elevation of the populations was also indicated in this map. Similarly, five shadings (80 – 160 cm in 20 cm classes) in grey were used for classifying of mean tree height of the 32 populations. There is certainly a possibility to distinguish the extreme scorings of flushing or height classes but intermediate performance is impossible to identify, which is regrettable. Two trends in bud flushing were observed:

  Northern populations flushed late while southern and eastern populations showed early flushing,

  Low elevation populations flushed later than high elevation populations.

It was remarked that it is problematic to disentangle the effect of latitude and elevation since all northern populations originate from low elevation and the high elevation populations originate from southern latitudes. In Fig. 1-14 I have illustrated a representative sample of populations, which illustrates these general trends. The Bulgarian, the Italian, and the local Spanish populations originate from elevations 1,000 – 1,450 masl, and all have an early flushing (green columns) and are characterized by good growth (brown). The early flushing of the high elevation populations must be attributed to a lower heat demand for bud flushing than in low-elevation populations.

The principle component analysis resulted in two significant axes:

  1. Mainly associated with latitude, elevation, temperature at population origin, and duration of the growth period explaining 32% of the variability

  2. Mainly associated with precipitation during the growth period, longitude, and winter temperature at population origin explaining 29% of the variability.

Plant height and mortality during the first four years after establishment of the trial was correlated, $R^2 = 0.60$. Contrary to this, the correlation between growth increment and survival was weak, $R^2 = 0.17$. Nor was there any strong correlation between early growth and growth increment (2001 – 2008), $R^2 = 0.22$.

There was a significant and negative correlation between early bud flushing and tree height at age 12. However, the R^2 was only 0.24. It was admitted that most of the variability could not be attributed to geographic differences but to other reasons. There were no significant relationships between bud flushing and senescence at October 1 ($R^2 = 0.068$) or between bud flushing - survival ($R^2 = 0.039$).

The trade-off between early flushing and growth was discussed and particularly the risks for late spring frost damage. Owing to the summer drought at the test locality it is important for the trees at this locality to capitalize on the good growth conditions before summer drought appears. This means that the window for good growth of the late flushing low-elevation populations from northern latitudes is limited, resulting in a poor growth of these populations.
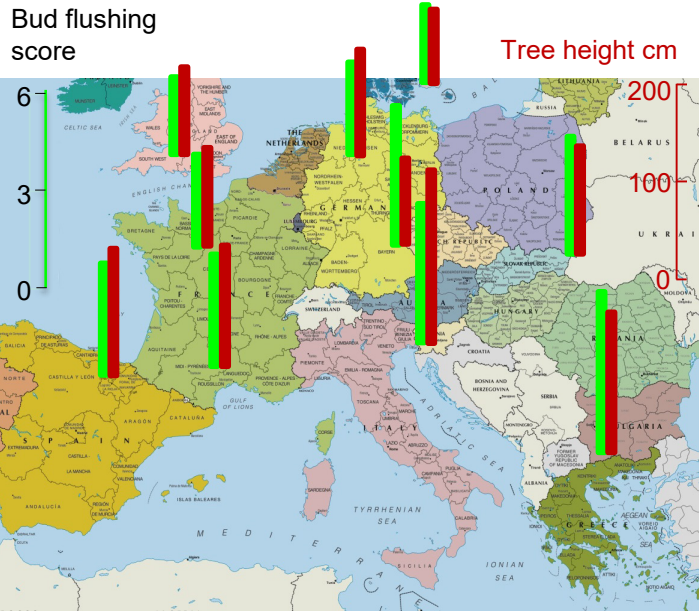
10

Figure 1-14. Mean bud flushing score (green) and tree height (brown) in nine populations in a provenance trial at latitude 42.30ºN and 1,340 maslc in Spain. Bud flushing was scored in a 7-degree scale during three years; 2008, 2010, and 2011.

A representative sample of six populations in an international series of provenance trials were studied in a trial at 1,340 masl. in northern Spain by Robson et al. (2012). This locality is characterized by dry summer and a short growth period. Besides phenology and growth, several physiology traits (see Table 1-2) were studied in this trial at two occasions, early summer, July 9-11, and midsummer, August 13-15. Bud flushing was recorded at three occasions from April 25 to May 8. Leaf senescence, estimated as proportion of discolored leaves, was estimated once on October 1-2. One population from each of Bulgaria, Czechia, Germany, Poland, Spain, and Sweden were selected for this study. The physiology measurements included 12 trees from each of three blocks. (A demanding task.).

Table 1-2 reveals that population differences were significant for all traits except C/N ratio. The focus in this paper was on plant physiology. My focus will be on relationships between physiology traits and tree growth. First a discussion of the relationship between phenology and tree growth will be presented.

Table 1-2. Traits studied and the significances of population differences at early summer and mid summer. Robson et al. 2012.

| Trait	Early summer	Mid summer
A_{area}, photosynthetic rate $m^{-2}s^{-1}$	significant	significant
A_{mass}, photosynthetic rate $CO_2 g^{-1}s^{-1}$	significant	significant
g_{wv}, stomatal conductance mol H_2O mol^{-1}	significant	significant
C_i, internal CO_2, μmol mol^{-1}	significant	significant
IWUE, Instantaneous water use efficiency $A_{satmass}/g_{wv}$	significant	significant
Leaf $\delta^{13}C$, water use efficiency	not analyzed	significant
SLA, specific leaf area $m^2 kg^{-1}$	significant	significant
Leaf size, cm^2	significant	significant
N_g, nitrogen % g^{-1}	significant	significant
N_{area} nitrogen mg m^{-2}	significant	significant
C/N ratio	non-significant	non-significant
P_{NUE}, photosynthetic nitrogen use efficiency A_{mass}/N_g	significant	significant
Ψ_{pd}, predawn water potential	significant	significant
Ψ_{mid} midday water potential	significant	significant
Ψ_{diff}, Ψ_{mid} - Ψ_{pd}	significant	significant
KL, soil–leaf hydraulic conductivity	significant	significant
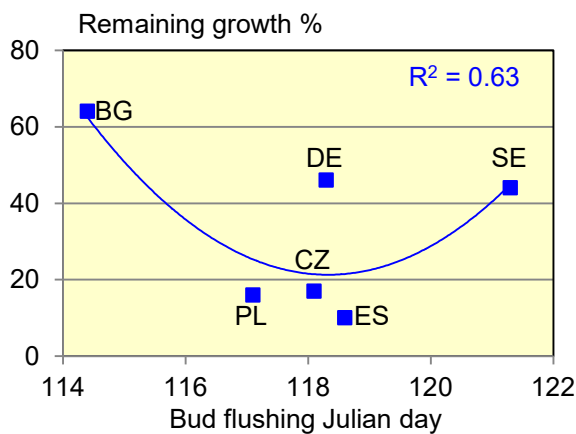
Figure 1-15. The relationship between bud flushing and remaining growth, % (100 – senescence %). in six beech populations of wide origin studied in a provenance trial at 1,340 masl in northern Spain. BG = Bulgaria, CZ = Czechia, DE = Germany, ES = Spain, PL = Poland, SE = Sweden. Robson et al. 2012.

Coming from a part of Europe where duration of growth period is decisive for tree growth it was tempting to analyze the relationship between duration of the growth period and growth of the six beech populations in this investigation. Unfortunately, estimates of the duration of the growth period were not presented. Indirect, an idea might be obtained from Fig. 1-15, in which remaining growth in percentage is plotted against bud flushing. Remaining growth in % = 100 – senescence in % at October 1-2. It is evident that the Bulgarian population has the longest growth period; early flushing and less than 40 % senescence. The Spanish population had the highest senescence percentage and intermediate bud flushing, which means a short growth period. This characteristic is shared
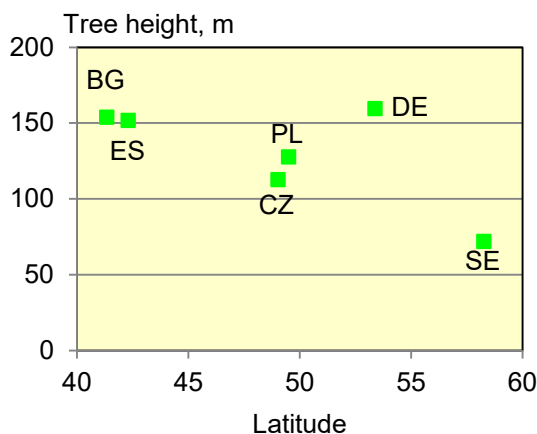

Figure 1-16. The relationship between population latitude and mean tree height at age 12 in six populations studied in a provenance trial at 1,340 masl in northern Spain. BG = Bulgaria, CZ = Czechia, DE = Germany, ES = Spain, PL = Poland, SE = Sweden. , Robson et al. (2012).
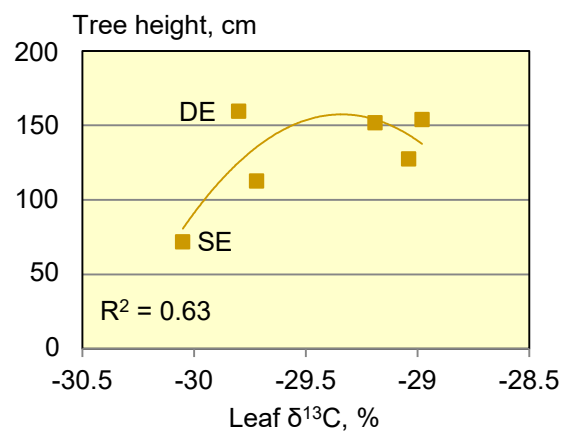

Figure 1-17. The relationship between population latitude and mean tree height at age 12 in six populations studied in a provenance trial at 1,340 masl in northern Spain. BG = Bulgaria, CZ = Czechia, DE = Germany, ES = Spain, PL = Poland, SE = Sweden. Robson et al. (2012).

by the Czech and Polish populations. A comparison of thus derived rough estimates of the duration of the growth with population tree heights reveals that the duration of the growth period does not explain the observed variation in tree growth. If the German population is excluded from the latitude - tree height relationship a smooth south-north cline is emerging.

One of the important questions related to this report is the relationship between various physiology traits and tree growth. Based on population mean values presented in the paper I have selected to illustrate relationships for some traits with a substantial variation among the six populations. Since drought is a limiting growth factor at the test locality I have also included water use efficiency in one relationship with tree growth even if the variation in this trait was limited, -28.98 – -30.05. I have only used tree height in these relationships but basal diameter would give the almost the same strength of the relationships since the R^2 for tree height – basal diameter was 0.96.

The R^2 for the polynomial relationship between carbon isotope discrimination and tree height was moderately strong, 0.63. However, the Swedish population has a great impact on this relationship (Fig. 1-17). Without this poorly growing population there is virtually no relationship, $R^2 = 0.08$. Therefore, this trait does not give good prediction of tree growth. Nor did instantaneous water use efficiency result in any strong relationships with growth. Both at early summer and midsummer strong relationships were noted for net photosynthesis – tree height relationships, $R^2 = 0.87$ and 0.78 (Fig. 1-18. The two relationships between soil-leaf hydraulic conductivity and tree height were both fairly strong (Figs. 1-19), 0.68 and 0.89. Also for this trait the estimates from mid summer resulted in higher R^2s. None of the two relationships between photosynthetic nitrogen use efficiency and

Net photosynthesis μmol CO_2 g^{-1} s^{-1}
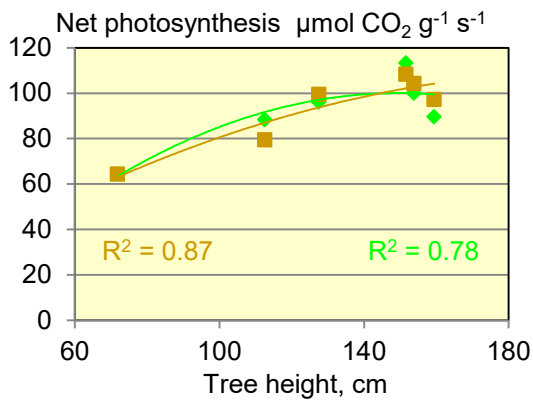
$R^2 = 0.87$ $R^2 = 0.78$

Figure 1-18. The relationship between tree height and net photosynthesis per mass unit in early summer (green) and mid summer (brown) in six populations from a wide distribution area in Europe studied in a provenance trial at 1,340 masl in northern Spain.. Robson et al. (2012).

Soil-leaf hydraulic conductivity, mmol m^{-2} Mpa^{-1} s^{-1}
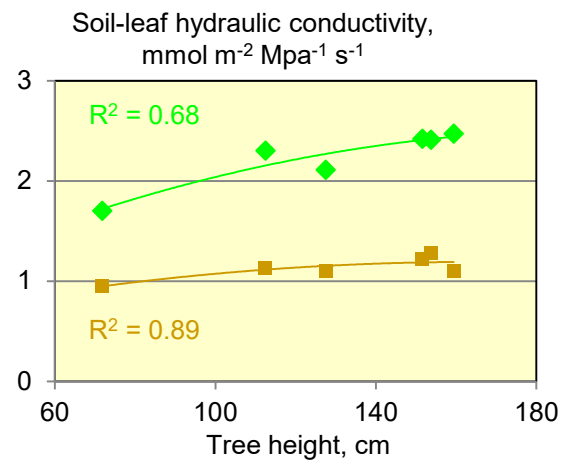
$R^2 = 0.68$

$R^2 = 0.89$

Figure 1-19. The between tree height and soil-leaf hydraaulic conductivity in early summer (green) and mid summer (brown) in six populations from a wide distribution area in Europe studied in a provenance trial at 1,340 masl in northern Spain.. Robson et al. (2012).

tree height were strong. The relationship for midsummer was erratic while the early summer relationship was disturbed by the Swedish population (Fig. 1-20). Exclusion of this population resulted in a strong relationship, $R^2 = 0.96$. One important question to raise is: why does the Swedish population grow so poorly? Considering the long distance transfer it is likely that other factors than basic physiology traits are responsible. This population originates from a region, in which night length triggers growth cessation in maany tree species and it is expected that this population would have an early growth cessation when transferred to another light climate more than 15 degrees of latitude further south. However, this was not observed (Figs 1-15). It might be speculated that the phenological response might have been uncoupled following such a long distance transfer. If this is the case the interpretation of the various relationships would be safer after exclusion of the Swedish population. Also the German population deserves a comment since its growth was exceptionally good for a population originating from a mild and humid climate (Figs 1-16). It was suggested that good growth could be attributed to a high leaf area and a good soil-leaf hydraulic conductivity in early summer. It was further pointed out that it showed comparable estimates as regards δ^{13}C and early summer light-saturated photosynthesis as the Bulgarian and Spanish populations. It is evident that the genetic constitution of the German population is well suited for the Spanish test locality plus a large phenotypic plasticity.

In conclusion, several promising results as regards the relationship between basic physiology traits and growth at population level. For utilization in breeding it is important that this type of study is followed up with estimates of genetic parameters at the within-population level.

Tree height m,

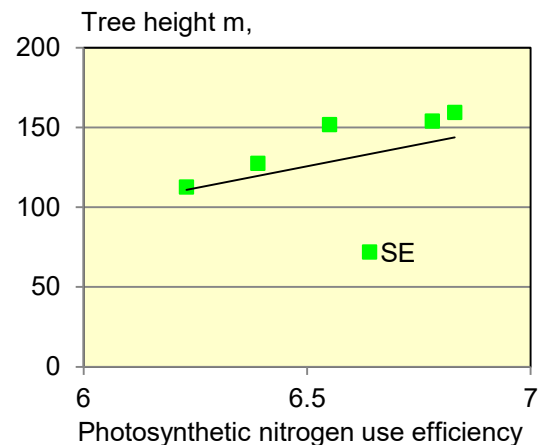SE

Photosynthetic nitrogen use efficiency

Figure 1-20. The relationship between photosynthetic nitrogen use efficiency and tree height in early summer in six populations from a wide distribution area in Europe studied in a provenance trial at 1,340 masl in northern Spain.. Robson et al. (2012).
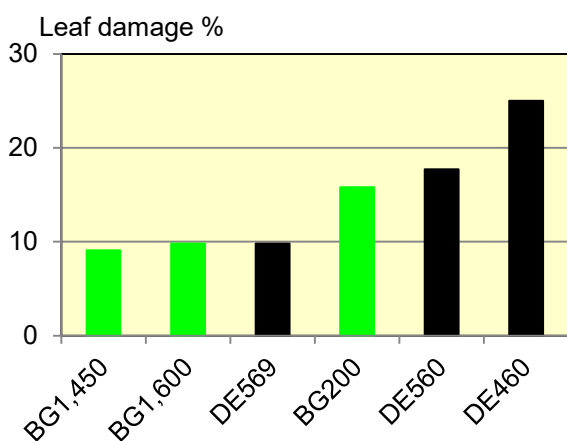
13

Figure 1-21. Leaf damage percentage following freeze testing at -5℃ of three Bulgarian (BG) and three German (DE) populations.. The elevation at population origin is indicated. Potted plants at age 2. Data from Figs.3A in
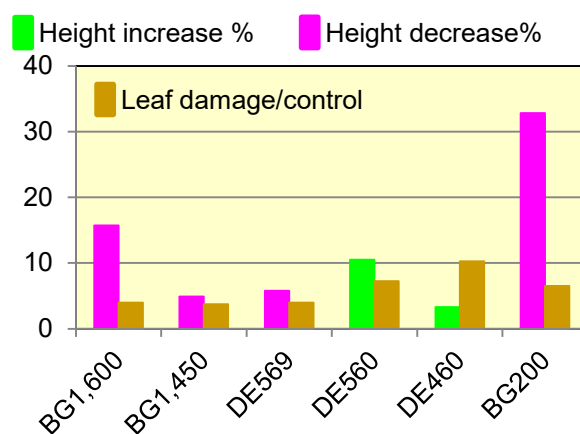
Figure 1-22. Percentage decrease or increase in plant height increment following freeze testing at -5°C in three Bulgarian (BG) and three German (DE) populations.. The ratio of leaf damage in frost treatment/control is also shown. The elevation at population origin is indicated. Potted plants at age 2. Data from Figs.3A and 3B

The effects of late frost and warming during cultivation of two-year old potted plants from three Bulgarian and three German populations were reported by Kreyling et al. (2012). The elevational range of the German populations was 460 – 569 masl. while the elevations of the Bulgarian populations had a broader range, 200, 1,450, and 1,600 masl. Each treatment had nine plants. The freeze testing for three hours at -5℃ took place during one night, May26 – May27. The warming with approximately 1.6ºC per day took place from April until October. Weekly recordings of bud flushing were recorded and a fully developed leaf was classified as leaf onset. Leaf senescence and plant heights were determined in October (dates not specified). Chlorotic and necrotic leaf area per plant was assessed five days after frost treatment with percentages 0-5, 5-25, 25-50, 50-75, and 75-100. Climatic data from meteorological adjacent weather stations were used to relate observed data to temperatures at the origin of the six populations.

On an average 14.5% of the leaf area was classified as damaged following the freeze testing while there was no frost damage in the control plants. I have summarized the main results from the ANOVA in Table 1-2, which shows that significances were noted for population effects except for leaf senescence. Both warming and freeze testing had

strongly significant effects. Fig. 1-21 reveals that the two high-elevation populations and the German DE569 had the lowest percentages of leaf damage following freeze testing at -5ºC. As regards height increment Fig. 1-22 reveals that the low-elevation Bulgarian population suffered most from the freeze testing. This population had the largest height increment (≈ 25 cm) in the control and was most affected by freeze testing with a growth reduction of 32.8%. In spite of the two largest leaf damage/control ratios, the two German populations DE460 and DE560 grew taller than the control plants. Based on results from northwards transfers of populations to Scandinavia of other tree species it is expected that such a low-elevation population as BG200 might show good growth following a northward transfer of more than six degrees of latitude. On an average senescence was delayed by 1.6 days in the freezing treatment.

A few relationships with climatic data at origin of the populations and leaf damage were determined but most of them were not strong. Since there are two groups of populations with three in each group it might be hard to trace relationships with geographic factors at the origin of the populations. I tested the relationship between mean

Table 1-2. The significances of various effects on four traits studied in three-factor experiment with three German and three Bulgarian populations exposed to late spring frost and artificial warming. Potted plants at age 2 were studied. Kreyling et al. 2012.

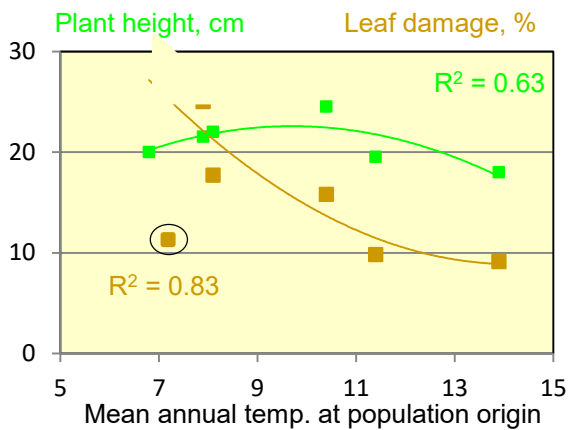| Effects	Leaf damage	Plant height	Leaf onset	Senescence
Population	*	***	**	
Spring freeze testing	***	*		***
Warming	***			*
Population x frost	*			
Population x warming				

Figure 1-23. The relationship between mean annual temperature at population origin and plant height in control material or frost damage % following freeze testing at -5°C in three Bulgarian and three German populations. The encircled square is excluded in the regression for the relationship between MATPO and frost damage. Kreyling et al. 2012.

annual temperature at population origin, MATPO. and observed leaf damage after freeze testing, which resulted in a weak relationship. Exclusion of the deviating DE569 resulted in a strong relationship, $R^2 = 0.83$ (Fig. 1-23). It is hard to explain the lower freezing tolerance of two of the German populations, which during the course of evolution were continuously exposed to the late spring frosts unless their bud flushing *in situ* takes place after the appearance of late spring frosts. Fig. 1-23 also shows a fairly strong relationship between MATPO and plant growth in the control material ($R^2 = 0.63$) but its biological meaning is unclear. It reminds of relationships from northern part of Europe, where a transfer from warmer climate to cooler results in improved growth. Also in this case population DE569 has a great impact on the shape of this relationship.

Železnik et al. (2018) studied fine root development and turnover in three populations in a Slovenian provenance trial at 545 masl. The annual precipitation of the three populations varied in the range 538 – 2,795 mm and the elevational range was 480 – 1,150 masl. In addition, fine roots in the local population were also studied. Ingrowth cores (IC) are porous containers filled with substrate. Twelve ICs per population were used. Six soil cores were collected after one year and another six soil cores were analyzed after two years. After washing the soil cores and discarding herbal roots, dead and alive fine roots of beech were measured and weighed after drying to obtain dry matter production. Minirhizotrons are transparent plastic tubes connected to a video camera, which allows estimations of root turnover. Five such tubes were installed in plots of each population. To begin with images were taken every two weeks but were prolonged owing to slow growth. It is worth mentioning that no decomposition of roots was noticed.
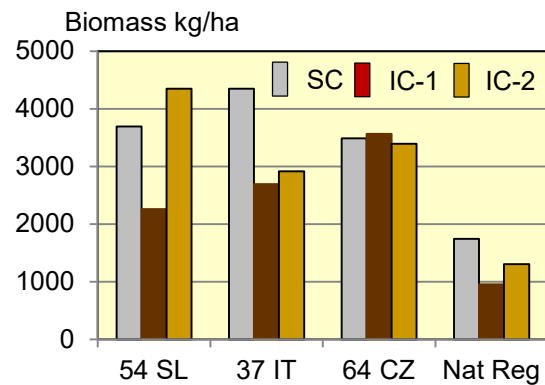

Figure 1-24. Fine root biomass, kg/ha in soil cores (SC) and in ingrowth cores (IC-1 and IC-2; 1 and 2 stand for years) in three populations and an adjacent stand in a Slovenian provenance trial. The populations originated from Slovenia (SL), Italy (IT), and Czechia (CZ). Železnik et al. 2018.

The fine root biomass was much lower in the natural stand than in the populations in the adjacent trial (Fig. 1-24), which partly might be attributed to a stronger competition in the natural stand than in the trial. Looking at the data in this figure it is expected that there were no significant differences among the three populations for biomass per hectare. The difference between the Italian population and the stand population was the only significant difference obtained. The Italian population had lower fine root turn over in all treatments (Fig. 1-25). However, differences among the populations with respect to fine root turn over were non-significant. None of the other fine root-related traits showed any significant differences. Probably the number of replications was too low to enable detection of differences. It ought to be reminded that this kind of investigation is extremely laborious which prevents testing of large materials. Finally, the differences in breast height diameter were limited.
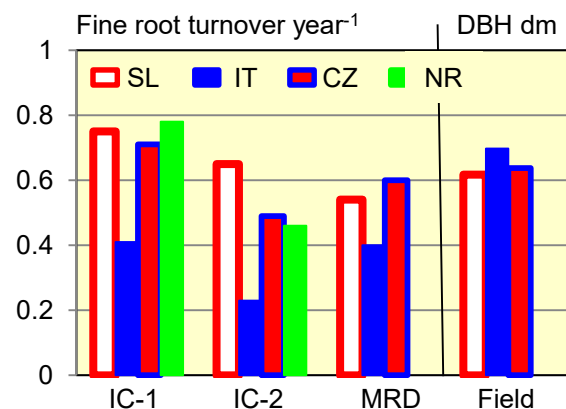

Figure 1-25. Fine root biomass, kg/ha soil cores (SC) and in ingrowth cores (IC-1 and IC-2) at two consecutive years in three populations and an adjacent stand in a Slovenian provenance trial. Železnik et al. 2018.

1.3 *In situ* studies of populations

One French study by Lander et al. (2011) on beech populations from Mount Ventoux in Provence, addressed the three points below:

1. The recent colonization from remnant populations
2. The existing pattern of genetic variation as a result of changing population sizes
3. Is there an agreement between approximate Bayesian computation of demographic reconstruction and existing genetic data

Historical maps were used to find populations suitable for this study. Three populations coined east, west, and south at Mount Ventoux in southeastern France were selected. East and west are remnant populations on the eastern and western ridges, respectively. South is a newly established and expanding area. The distance of colonization was estimated based on the present distribution minus the outer edge at 1845. At this time the contraction of beech was most pronounced. The annual rate of expansion was estimated at 27 m and 38 m in the southern and eastern populations leading to an area expansion of 65%. The number of plots and trees included in this investigation were:

East	21plots	575 trees
West	25 plots	1,228 trees
South	5 plots	129 trees

In the majority of the plots (48 of 51) 30 trees were sampled while all trees were sampled in the three remaining plots. Thirteen microsatellite markers were used for genotyping of all sampled trees.

Groups of trees belonging to clones were detected. In the analyses only one tree from such groups were included in the analyses. Number of alleles, allelic richness, expected and observed heterozygosity, and fixation index were estmated. The modified index for estimation of historical bottlenecks according to Garza and Williamson (2001) was calculated separately for each region. The structure v.2.3.3 program was used for testing of Bayesian clustering of 1825 trees and 12 loci. Approximate Bayesian Computation (ABC) analysis was used to identify if there were any bottlenecks during the history of the three populations. The DIYABC program according to Cournuet et al. (2008) was used for this purpose. Eight different demographic scenarios, with varying times of split of the populations as well as size of populations, were tested in this analysis. Huge numbers of simulations (500,000) per scenario were run.

The allelic richness and expected heterozygosities did not vary much among the three populations with ranges 4.75 – 4.89 and 0.70 – 0.71, respectively. The fixation index was estimated at 0.03 (west) and 0.064 (south). F_{ST} for among region differentiation was extremely low but significant, 0.2% while F_{ST} among plots was 2.6% and strongly significant. Thus, most of the variation was within regions. There was support for three groups based on the structure analysis.

The ABC analysis suggested that one asynchronous divergence scenario was most likely for the development unto the present situation with three populations. According to this scenario the three populations expanded from a smaller ancestral population. Further, the south population diverged from the other populations two generations (t2) ago while the east and west populations diverged one generation (t1) ago. Even if artificial regeneration had taken place in the south population it was stated that it was likely that local material was used for this regeneration. Finally, in this scenario changes in population size was allowed to occur both at one and two generations ago.

As regards the effective population size the analysis revealed that this was *never very small*. The estimated global increase of the population size from t2 to t1 was 43% but with a large imprecision of this estimate. The hypothesized bottlenecks in the history of this 4,000 hectares beech forest were not detected.

This investigation is unique since it combines historic demographic data with genetic diversity estimates in existing populations for understanding recent history of a large beech population.

Ninety-six beech trees from one northern and one southern elevation gradient in southern France were sampled for a study of nucleotide diversity and linkage disequilibrium of 56 stress response genes by Lalaüge et al. (2014). The trees grow at two elevations in each gradient; 995 (35 trees) and 1,340 masl (36) in the northern and 895 (12) and 1,517 masl (13) in the southern gradient. Phenology and abiotic stress related candidate genes were selected for this study. This investigation was limited to gene fragments with more than 800 base pairs and with ten or more segregating sites. After exclusions 45 pairwise comparisons between SNPs could be carried out.

In all, 644 polymorphic sites were detected, of them 102 were due to indels (insertion/deletion mutations). Of the 102 indels 67 had a missing heterozygous state and the remaining 35 were recoded as SNPs. With these 35 SNPs included 573 bi-allele SNPs were detected in the 58 candidate genes. The estimated nucleotide diversity ($\theta\pi$) of these 58 genes was low, 2.2 x 10^{-3}, and without any great difference between phenology and abiotic SNPs. The number of haplotypes varied in the range 2 – 20 with a median value of six. The haploid diversity was relatively high; median = 0.69. The F_{ST}s among the four populations varied in the range 0.01 – 0.04 with a global estimate of 0.021 indicating a low differentiation among the four sites.

Hill and Robertson's (1968) r^2 was used to estimate the non-random association between alleles at different loci; i.e. linkage disequilibrium (LD). Based on 4,409 pairwise comparisons the mean linkage disequilibrium within the genes was estimated at 0.26, and 49% of them were significant. The decay of linkage disequilibrium was compared for sequences >800 base pairs and with 45 pairwise comparisons for DNA fragments with two segregating sites. Sixteen of the candidate genes fulfilled this requirement. There was a large variation among the candidate genes. The half-way decay of LD was estimated at 154

base pairs. However, for two cases catalase coding genes had virtually no decay of LD. It was noted that decay of LD was higher after removal of rare alleles. Thus, at high mean allele frequency (MAF) the decay is larger than at low allele frequencies. At an increase of the allele frequency from 0.001 to 0.010 the half distance increased more than four times; 200 bp versus 900 bp. It was concluded that *The relatively low recombination rate and high levels of LD that decay relatively slowly suggest that the studied* F. sylvatica *population has a relatively small effective population size (N$_e$).*

An *in situ* investigation of the evolutionary potential of a marginal southern French beech population growing at an elevation of 987-1,048 masl. was reported by Bontemps et al. (2015). Bud flushing, leaf morphology traits, and physiological traits were assessed. The genetic relationship among the trees was traced by use of 19 microsatellites and spatial analysis. Two different methods were used for estimation of relatedness: Lynch and Ritland (1999) and Wang (2002). A relatedness coefficient (r$_{ij}$) is t*he expected fraction of alleles in the genome that two (related) individuals have identical by descent.*" Actual variance of relatedness, Vr$_{ij}$, was also estimated as well as V$_{Tot}$ r$_{ij}$ (referred as corrected), which separates the variance component due to random segregation of alleles from the total variance of the molecular-based estimate of r$_{ij}$. Relatedness was estimated for different distances from each individual. Another way of estimating the heritabilities was done by applying Bayesian animal method, which requires a pedigree-based relatedness matrix, which was not available in this *in situ* study. Therefore, a marker-based relatedness matrix was used.

Strong spatial genetic structure with a sharp decline until 25 m was found. Beyond 25 m the trees were no more related than expected by chance. In the class within 5 meters from an individual the expected relatedness was close to 0.25, which is equal to the value for half-sibs.

In Fig. 1-26 the heritabilities obtained by use of Ritland's method are visualized for the distance class up to 30 m. An extremely high heritability after correction was obtained for Phenology Score Sum, 0.84. It was still higher for the class up to 100 m, 0.92. Strangely, heritabilities for LM/LA (leaf mass over leaf area) were all negative. It was pointed out that the genetic correlations suffer from imprecision. Most of them were insignificant except for the leaf mass – leaf area correlation, around 1.00 according to Fig. 3 in the paper.

The heritabilities obtained from Bayesian method were lower but still in the range 0.20-0.41. With this method all traits had significant heritabilities including the LM/LA ratio; range 0.20-0.32. There was some agreement in the ranking of traits with repect to their heritabilities (Figs. 1-26 - 1-27). Only the genetic correlation LM – La was significant in agreement with was found for Ritland's method. Strangely, both estimation methods resulted in no correlation between LA and LM/LA although LA is a component in this ratio.
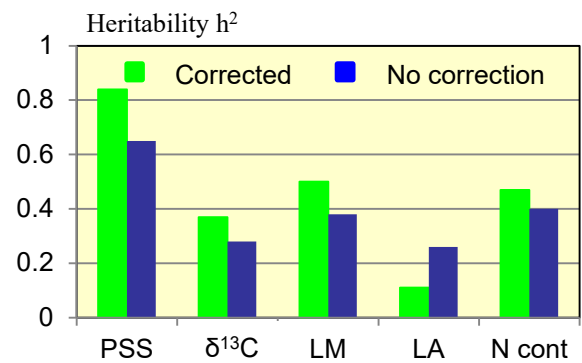

Figure 1-26. Heritabilities for five traits recorded in situ *in a southern French population at elvation 987-1,048 masl. The relationship among the trees was estimated by a method developed by Ritland (1996). The heritabilities are shown for a cut-off distance of 30 m. PSS = phenological score sum for bud flushing, δ^{13}C = carbon 13 isotope discrimination, LM = leaf mass, LA = leaf area. N cont = is nitrogen content in leaves. Bontemps et al. 2015.*

There is always a risk that estimates based on *in situ* populations and not on progenies may suffer from imprecision. However, the good statistical power obtained in this investigation speaks against an imprecision in this investigation. The good results obtained were attributed to the high proportion of highly related individuals (strong phenotypic covariance) with unrelated individuals (no phenotypic covariance) as evidenced by the strong spatial genetic structure. It was further stressed that substantial genetic diversity was revealed in this marginal population characterized by a large variation in water availability within this particular locality.

It was concluded that the studied marginal population had good possibilities to evolve thanks to the considerable additive variance observed.
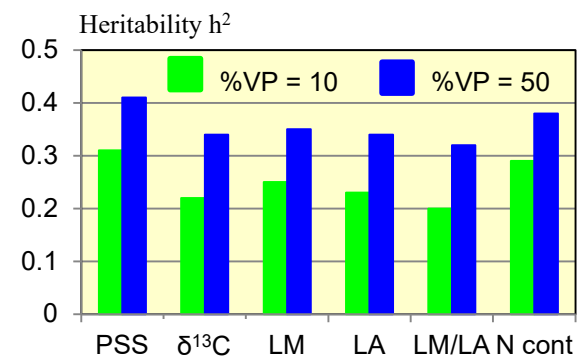
Figure 1-27. Heritabilities for six traits recorded in situ *in a southern French population at elvation 987-1,048 masl. The relationship among the trees was estimated by a method developed by Wang (2002). The heritabilities are shown for a %VP = 10and %VP 0 50. PSS = phenological score sum for bud flushing, δ^{13}C = carbon 13 isotope discrimination, LM = leaf mass, LA = leaf area. LM/LA = leaf mass per leaf area, N cont = is nitrogen content in leaves. Bontemps et al. 2015.*

17

Figure 1-28. Partitioning of the variance based on Partial Redundancy Analyses *performed on allelic frequencies in 19 mountain ranges from Jura to The Mediterranean Alps. A double-digest restriction site association DNA sequencing procedure coined ddRADseq was applied. Capblancq et al. 2019.*

Three objectives were identified for a SNP study by Capblancq et al. (2019):

1. Identification of environmental factors responsible for the existing population differentiation
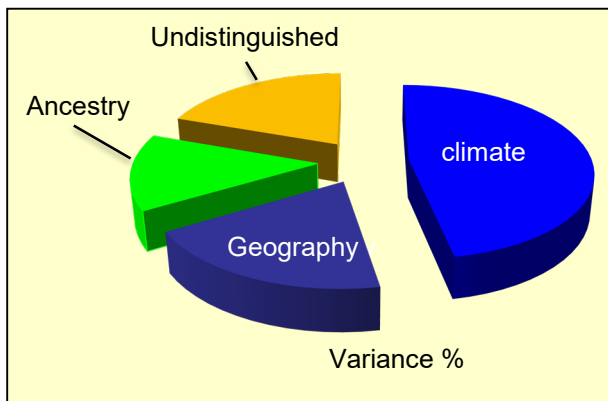2. Identification and isolation of the genetic variation associated with the existing gradients
3. Predictions of the species capacity to respond to environmental change

A double-digest restriction site association DNA sequencing procedure coined ddRADseq was applied. In all, 36 populations with 750 trees from 19 mountain ranges in Jura to the Mediterranean Alps were included in this study. A principal component analysis was used to estimate the genetic differentiation among populations. In addition, a constrained method of genetic clustering (sNMF) was used. Partial redundancy analyses (RDAs) were used to estimate the relationships between SNP data and climatic or geographic variables. RDA was also used to identify loci extremely linked to environmental variables, which might be linked to selection. Adaptive indices were looked for in a second RDA analysis limited to loci identified as outliers.

Assessment of species' capacity to respond to climate change was done by comparison of the existing RAD1 and RAD2 scores for each population with the expected scores based on a global change scenario.

The PCA and sNMF analyses gave similar results in a north – south gradient. It was suggested that this gradient originated from isolation by distance, although without a complete blocking of gene flow. However, it is hard to disentangle the effects of isolation by distance from adaptation and gene flow. One way to get some insights in this dilemma is to run partial RDAs. The partial RDA analyses on the allele frequencies allowed a partitioning of the variance attributed to climate, geography, and ancestry of the populations. Maximum and minimum temperatures, mean evapotranspiration, sum of the annual precipitation and moisture index were used as climate variables. The model including these variables in the analysis explained 35.2% of the total variation. The partitioning of this estimate revealed that almost half (47%) of this estimate was attributed to climate (Fig. 1-28) while geography and ancestry each contributed less than 20%. It was suggested that local adaptation occurred along the French Alps. Further analyses identified 65 loci as outliers with strong associations to environmental variables and thus expected to play a significant role in selection. In a new RDA on these 65 loci it was noted that the two first axes explained most of the adaptive genetic variance among the populations. The first axis explained 35% of the variance and indicated a strong contrast between high maximum temperature and high values for minimum temperatures, precipitation, and moisture index. Geographically this meant that populations from northern localities in pre-Alps, in which minimum temperatures are not very low and precipitation is important, are separated from southern mountain populations experiencing a dry climate. In the second axis there was a separation between high values of maximum and minimum temperatures on one hand and precipitation and moisture on the other hand. This axis is associated with altitudinal differences. It was observed that some of the outlier loci were specifically associated with one of the two RDA axes. This means that the two RDA axes were partly supported by different loci. This in turn means that adaptive loci were "independently" associated with the two different environmental constraints experienced by beech in The French Alps.

As anticipated the model for capacity to respond to future environmental conditions resulted in a dramatic loss of favorable regions for beech in lowlands and in valleys accompanied by a shift towards higher elevations. It was also found that high-elevation populations would need a greater change in adaptive genetic composition than was required for low-land and valley populations. Since the environmental change is very rapid in an evolutionary perspective the high-elevation populations have to rely on existing genetic variation within the populations or gene flow from already adapted populations. It was further noted that the situation for lowland populations is more problematic since they are growing in the margins of distribution and with fitness-contributing alleles already fixed. Moreover, there are limited possibilities for gene flow to compensate for such a situation.

As one take-home message, it was stressed that detection of loci involved in local adaptation is improved by RDA analyses. Such a multi-loci approach is of particular significance for genes regulating fitness-contributing quantitative traits.

Two central German populations at elevations 400 (referred to as B) and 500 (referred to as S) masl were selected for an analysis of the relationship between isozyme markers and forking of trunks (Dounavi et al 2010). Nine isozyme loci were analyzed. The distribution of forking trees within the populations was recorded. The B population with an age of 20-60 years grows on a north-eastern
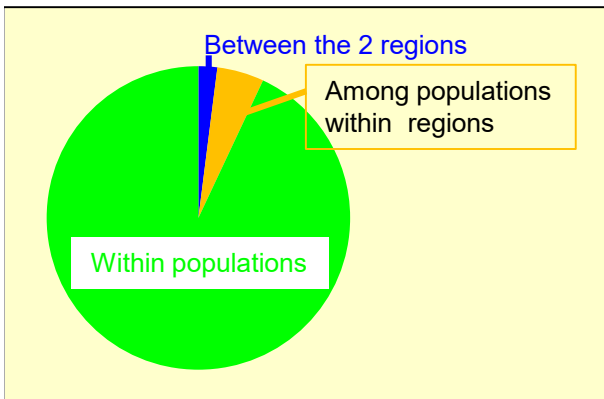
Figure 1-29. The separation of the variance components in percent between the two regions northern and southern Poland, among populations within regions, and among individuals within populations. Kempf and Konnert 2016.
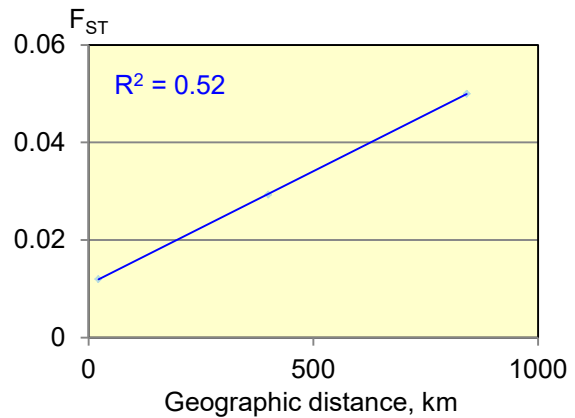

Figure 1-30. The relationship between the 66 pairwise geographic distances and estimated F_{ST}s of twelve Polish marginal populations studied with ten highly polymorphic microsatellites. Kempf and Konnert 2016.

slope with some natural regeneration occurring. The S population with an age of around 140 years grows on a northern slope without any natural regeneration. Several estimates of genetic parameters as well as spatial distribution parameters were estimated.

The allelic diversity and genetic differentiation based on the nine isozyme loci were 1.6 to 5.5% higher in the forked populations. Up to 20 meters radius in the young B population there was an aggregation of genotypes displaying kinship, which probably was caused by the limited dispersal of beech nuts. No corresponding aggregation was noted for the S population, which was explained by loss of individuals during the course of development up to the age of 140 years. Besides natural selection, thinning by man probably contributed to the loss of trees.

The forking trees grew randomly in both populations. It was suggested that *there are less distinct family structures in the group of forked trees and a more possible micro-environmental influence and random effects in the formation of forked trunks.*

The use of isozymes for this study seems as somewhat old-fashioned since microsatellites would have stronger differential power.

Bozic et al. (2013) analyzed 16 isozyme gene loci in two Slovenian populations from 275 and 657 masl, respectively. The two populations were selected owing to their different ecological conditions and anthropogenic impact. The high elevation population had limited human impact. The genetic distance according to Gregorius (1974) was estimated at 6.1%. This value must be regarded as large since the geographic distance between the two populations is only 13 km. This suggests that there was limited gene flow between these two populations. Five of the 16 loci showed a significant differentiation between the two populations with a mean genetic distance of ≈12%.

Kempf and Konnert (2016) studied the genetic diversity and fixation index by aid of ten highly polymorphic microsatellites in twelve marginal populations in Poland. Leaves were collected from around 46 trees per population in a provenance trial at latitude 49.40ºN and 20.92ºE. The geographic locations of the populations are shown in Fig. 1-31 on next page. The latitudinal range was 49.12 – 54.62ºN and with a longitudinal range of 15.90 - 22.48ºE. Population No. 42 is the only high elevation population, 700-920 masl.

The average number of alleles per locus was 13.2. The observed heterozygosity based on the ten loci was 0.698 and the expected heterozygosity was almost identical, 0.701, which indicates a high diversity in the twelve populations. Private alleles were found at low frequencies in all populations.

The F_{ST} estimates for individual loci varied in the range 0.030- 0.041 with one exception, 0.087. The analysis of molecular variance revealed that 93% of the variation was attributed to individuals within populations while the variation between southern and northern Poland had the lowest variance component, 2% (Fig. 1-29). In spite of the low variance component for region about 50% of the variation in F_{ST}s was explained by geographic distance (Fig. 1-30). As pointed out by the authors this does not mean that the relationship reflects a difference in adaptedness of the populations since microsatellites are assumed to be neutral markers. It would have been useful to have the mean F_{ST}s for individual populations to find out whether some populations could be regarded as outliers. For example the high-elevation population might be more differentiated from the other populations owing to its location in another climate zone.
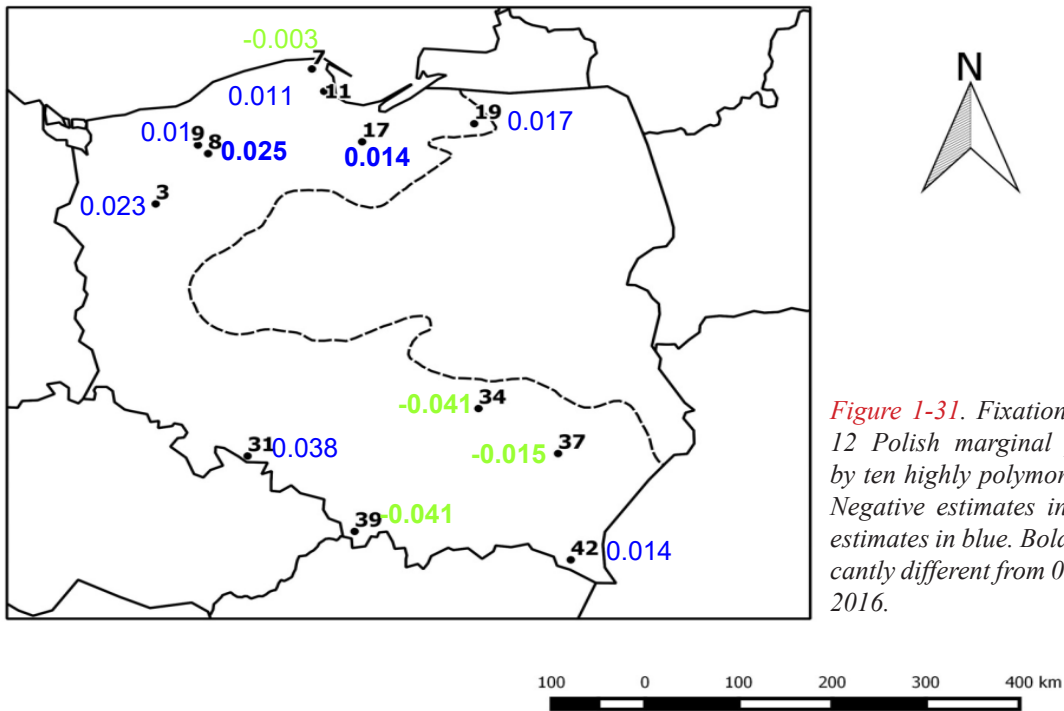
Figure 1-31. Fixation index estimates for 12 Polish marginal populations studied by ten highly polymorphic microsatellites. Negative estimates in green and positive estimates in blue. Bold = estimates significantly different from 0. Kempf and Konnert 2016.

The F_{IS} estimates are presented close to the origin of the twelve populations in the map in Fig. 1-31. In most cases the estimates were low and non-significant. In five of the populations the difference from 0 was significant (bold in Fig. 1-31), three of them with an excess of heterozygotes, all three originating from southern Poland.

The data presented suggest that there is a considerable gene flow among the twelve populations studied. This results in a substantial genetic diversity within populations.

A study of four isolated peripheral populations in Romania was published in 2017 by Ciocîrlan et al. (2017) who used seven microsatellites in their comparison with five core populations from the continuous natural range of beech in Romania. Both sides of the Carpathian Mountains were represented by the core populations. With one exception three trees per population were analyzed; five trees from population MAC in southeastern Romania. The four peripheral populations originated from an elevational range of 77-230 masl while the core populations originated from higher elevations, 315-1130 masl. Separate analyses of molecular variance (AMOVA) were carried out for all nine populations as well as for peripheral and core populations separately.

The among-population variance component for the core populations was estimated at 4.1% while this component was slightly higher, 5.4% for peripheral populations (Fig. 1-32). Considering the isolated character of the peripheral populations the differentiation seems to be rather modest. One explanation could be that there still is considerable gene flow among the isolated populations or that they are the remnants of large and continuous beech forests in Romania. Unfortunately, individual F_{ST}s were not presented but Fig. 2 in the paper indicates that the highest

F_{ST} was noted for the comparison of populations APU and TAL. APU grows at high elevation (1,130 masl.) in western Romania while TAL grows in eastern Romania at 77 masl, which is the lowest elevation of all populations. Moreover, the APU population showed high F_{ST} estimates when related to the other three peripheral populations. Similarly, the TAL population had high F_{ST} estimates also with the other four core populations. The TAL population deviated most from the other eight populations with respect to observed and expected heterozygosities (Fig. 1-33).
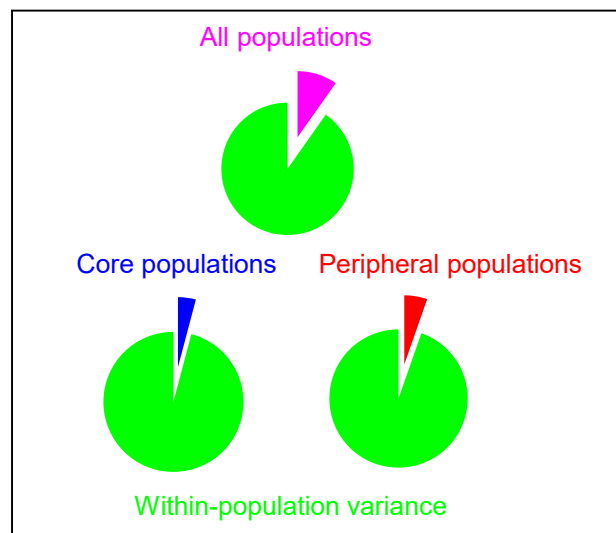

Figure 1-32. Among populations, and within-population variance components for nine populations and separately for five core and four peripheral Romanian populations. The analysis was done with seven microsatellites, one being an EST-microsatellite. Ciocirlan et al. 2017
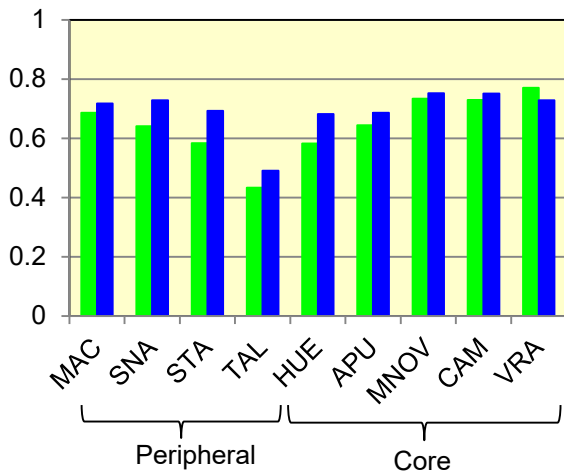
Figure 1-33. The observed (green) and expected hetero-zygosities (blue) in four isolated peripheral populations and five core populations in Romania based on analysis of seven mcrosatellites. Ciocîrlan et al. (2017).

In all, eleven rare alleles were detected, six of them in the STA peripheral populations. A low number of rare alleles was noted in this material. This might be attributed to the low number of trees tested per population
Fig. 1-34 shows that four of the populations have F_{IS} estimates above 0.10, three of them being peripheral populations. Only one of the populations, VRA, had an excess of heterozygotes. However, as far as I can understand from the paper none of the estimates differed significantly from 0.00.
In conclusion, the differences between the peripheral and core populations are less pronounced than expected from the isolated nature of the peripheral populations.
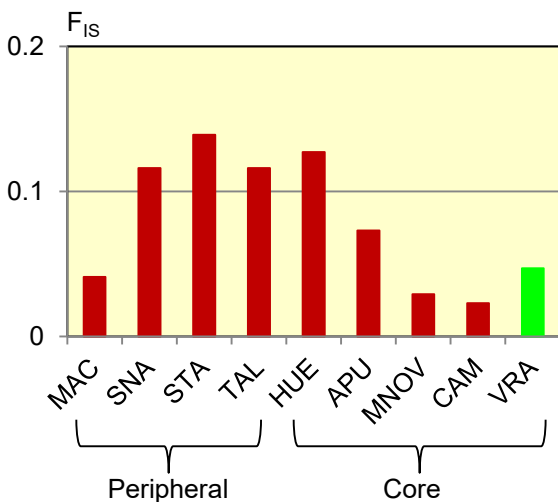

Figure 1-34. The fixation indices in four isolated periphe-ral populations and five core populations in Romania ba-sed on analysis of seven microsatellites. Ciocîrlan et al. (2017).
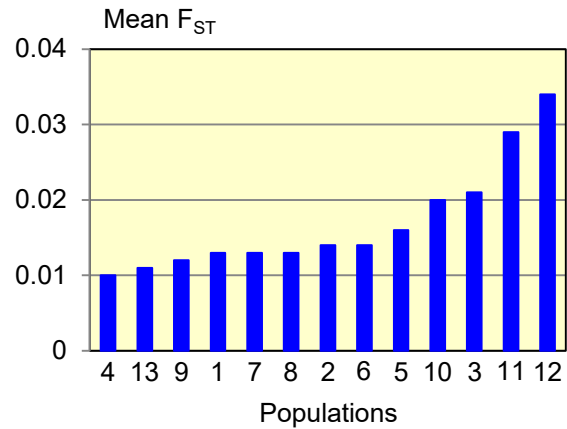

Figure 1-35. Mean F_{ST} of 13 Czech populations studied by 12 polymorhic microsatellites. Cvrčková et al. 2017.

Cvrčková et al. (2017) studied 13 Czech populations, mainly gene conservation units, using 14 polymorphic nuclear microsatellite markers. The populations had an approximate age of 120 years, and were selected to cover the natural distribution of *F. sylvatica* in Czechia. The elevational range was 270-1,135 masl. The average distance between the 30 sampled trees in each stand was 100 m.
In spite of considerable differences in allele frequencies between some of the populations most of the pairwise F_{ST} estimates were rather limited with a grand mean of 0.017. The estimates for the individual populations are illustrated in Fig. 1-35, which shows that populations 11 and 12 had the highest estimates. One *a priori* expectation was that the high elevation populations, 11 and 12, >800 masl, would differ most from the other eleven populations, which agreed with the expectation.
In contrast to a structuring of the Czech beech populations, the low F_{ST} estimates suggest a substantial gene flow among these 13 populations. Moreover, there was an extremely poor relationship between genetic and geographic distances ($R^2 = 0.0047$), which also speaks against a structuring of the populations.
There was no significant difference between observed and expected heterozygosity, which suggests that the matings are in agreement with Hardy-Weinberg equilibrium. All 13 populations showed positive estimates of fixation indices, varying in the range 0.012 and 0.113. These results were attributed to selfing and null alleles.
Private alleles were detected in some loci and the highest number was found in population No. 4. For random reasons they will remain or be lost in the populations without any impact of natural selection.
It was concluded that the results of this investigation should be utilized to select genetic resource populations. With the limited differentiation it seems as one population would be enough. However, differentiation in adaptive traits will probably give another picture.
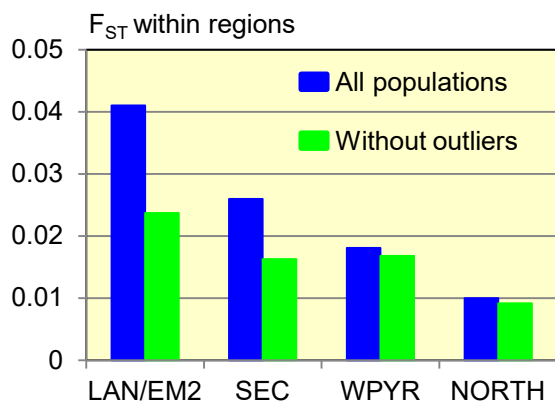
Figure 1-36. Mean F_{ST}s in four regions in France based on 16 highly polymorphic microsatellite loci including or excluding outlier populations:
LAN/EM2 = Isolated populations from Landes/Entre-deux-mers
Three regions classified as refugia:
SEC = South-eastern France/Catalonia
WPYR = Western Pyrenean
NORTH = Northern France, north of latitude 46°N.
The number of populations within regions varied between 13 and 22. De LaFontaine et al. 2013.

1.4 Spatial structure

Sixteen microsatellites were used to test the hypothesis that there would be a stronger spatial genetic structure in refugia than in recolonized areas (De Lafontaine et al. 2013). Estimates of pairwise F_{ST}s among populations within refugia and their positive relationship with geographic distances would prove spatial genetic structure. Microsatellite mapping of 2,510 trees from 65 French populations in four geographic regions in France were carried out in this study. The total numbers of alleles were 193 with an average of 12.1 per locus. The four regions and number of populations are:

NORTH: Northern France – north of 46°N, below 200 masl; recolonized 13
LAN/E2M: Landes/Entre-deux-Mers– between 44 and 45°N, below 100 masl, refugium 13
WPYR: Western Pyrenean Mountains – south of 46°N, above 600 masl, refugium 17
SEC: Southeastern France / Catalonia – south of 46°N, above 600 masl, refugium 18
There was some subsampling in LAN/E2M, in which all stands were isolated

Traditional population genetics estimates were estimated. Bayesian clustering methods (BAPS 5.4 Corander et al. 2008) and a non-spatial model implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000) were used to identify any grouping of the populations. Data from an earlier published isozyme analysis of 375 populations grouped into ten refugia and eleven recolonized regions were reanalyzed (Comps et al. 2001).

The observed and expected heterozygosities were almost identical, 0.702 and 0.694, with the lowest observed heterozygosity in the NORTH group although it had the highest allelic richness. The lowest allelic richness was noted for the LAN/E2M group. Inbreeding was low with a mean fixation index of -0.013. Only for the NORTH group a positive F_{IS} estimate was noted, 0.014. These results indicate that the diversity is higher than expected for isolated marginal populations with the assumed importance of genetic drift in such isolated populations.

The clustering analysis suggested 10 groups, six of them consisting of just one population. Five populations were assigned to a geographically distant group. Both Bayesian methods grouped 45 of the populations into the four regions, in which they were growing while 20 populations had problematic assignment and were identified as outliers. They were geographically isolated from the core area of its geographic group. Also in the NORTH region there were some isolated populations but none of them was classified as outlier population. This observation strengthens the hypothesis that the high frequency of outliers in the refugium regions must be attributed to isolation by distance and its accompanying genetic drift

The mean pairwise F_{ST}s within the four regions were highest for the LAN/E2M in analysis of all populations as well as in the analysis after exclusion of outliers (Fig. 1-36). In this region more than half of the populations were classified as outliers, which explain the high mean F_{ST} within this region. The variation of the individual F_{ST}s was several times larger for this region than the other three regions.

Also the reanalysis of isozyme data revealed a higher F_{ST} within refugia than in the colonized region, 0.024 versus 0.014.

The pairwise F_{ST} estimates in the NORTH group were significantly related to geographic distances, r = 0.36. However, this relationship explains less than 15% of the variation. In two of the ten refugia in the isozyme study by Comps et al. (2001) there was a significant relationship between geographic distance and F_{ST}; r = 0.36 for Cantabrian Mountains and r = 0.56 for western Bulgaria. Significant relationships were obtained for eight of the eleven recolonized regions but the degree of explanation of these relationships was low, never above 15%. Thus, in the majority of cases there were only weak relationships between F_{ST} and geographic distance. Even if there were a higher frequency of significant relationships between F_{ST} and geographic origin in recolonized regions than in refugia the overwhelming majority of relationships were mostly so weak that far-reaching conclusions about difference in spatial genetic structure between refugia and recolonized populations cannot be drawn.

The check for recent bottlenecks did not reveal any indications of bottlenecks in any of the populations in the four French regions.

1.5 Global warming

Four models for projection of future distribution of beech were used by Kramer et al. (2010):
1. Statistical species area modelling
2. Process-based species area modelling
3. Modelling genetic diversity at the landscape level
4. Modelling adaptive response of functional traits at stand level.

Only the genetic parts (3 and 4) of the modelling will briefly be touched upon.

For point 3 simulations of a 10 x 10 km area in mountainous Sollingen about 25 km from Düsseldorf in central Germany was used. This area was subdivided into 500 x 500 m squares and the presence of beech in each square was determined. Data from a study with eleven isozyme loci and three microsatellite loci of four populations by Maurer et al. (2008) were included. Three scenarios were run in the modelling:
A. 100 years constant means as regards temperature and precipitation for Sollingen area
B. 100 years with linear increase of temperature by 2°C and linear decrease of precipitation by 20%
C. 100 years with linear increase of temperature by 2°C and linear increase of precipitation by 20%.

For point 4 above bud flushing timing was used to trace the adaptive response to increased temperature at the leading edge of distribution. Temperatures from a time series at a Dutch meteorological station were used and two additional temperatures 3°C and 6°C were superimposed on these series. Bud flushing was simulated based on the sequential attainment of a tree's critical chilling – and subsequent forcing requirements. Minus 2°C was used as threshold temperature for severe frost damage. A quantitative genetic model with ten loci, each with two alleles, was applied. A skewed genetic model was used for the initial allele frequencies. For the development at the trailing edge of distribution the relationship between stomatal conductance and soil water availability was studied. Data on precipitation from time series at Mount Ventoux in southeastern France was used for this modelling. Annual dry, wet, and average precipitation conditions were used in the modelling of water use efficiency as well as external pollen flow fractions of 0.2.

The effect of three management regimes was studied:
1. A group conversion system
2. A shelter cut system
3. No management.

There was a limited impact of changes in temperature and precipitation on genetic diversity in the Sollingen region. The reduction of the number of reproductive trees was 1.1, 14.4, and 1.9% for scenarios A, B, and C. The reduced precipitation is responsible for the larger reduction in scenario B than in scenarios A and C. For scenarios B and C there was an increase of beech at high elevations with a slight decrease at low elevations.
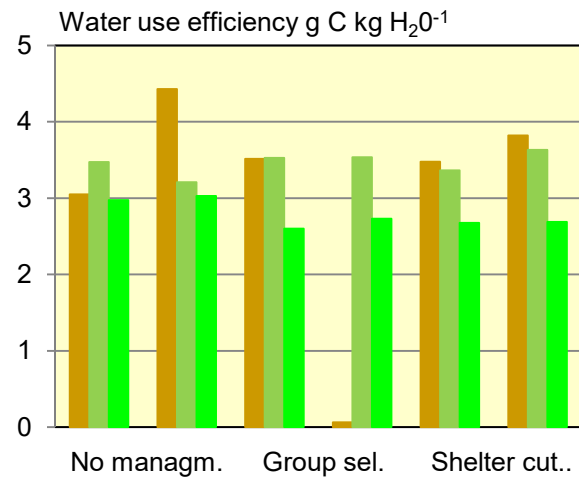

Figure 1-37. Water use efficiency for different management regimes, water availability scenarios (brown = dry, olive green = average, green = wet), and pollen flow. Column groups 1, 3, and 5 have no pollen flow while column groups 2, 4, and 6 have a fraction of pollen flow = 0.2. Kramer et al. 2010.

Bud flushing will take place earlier with the greatest impact at 6°C increase. In case of pollen flow fraction of 0.2, bud flushing will also take place earlier even in absence of a temperature increase. As regards management it was noted that the shelter cut system resulted in a faster adaptive response than the group selection cut system.

The results as regards water use efficiency are shown in Fig. 1-37. The extremely low value for the group selection dry – 0.2 pollen flow scenario was attributed to few living trees left in this case. The largest impact was noted for the no management dry – 0.2 pollen flow scenario. Fig. 1-37 reveals that the lowest water use efficiencies were noted for the high precipitation conditions. Except for this result, the effect of pollen migration was limited. Fig. 1-37.

In conclusion, there is a tendency of earlier bud flushing following increased temperatures, meaning that expansion of beech northwards in Scandinavia and The Baltic States is projected. Further, management will probably enhance the rate of adaptation. As regards the trailing part of the distribution of beech in southern Europe, there will probably be a shift of the species distribution to higher elevations owing to increased drought at lower elevations.

1.6 Miscellaneous

LeFèvre et al. (2012) pointed out that many published microsatellite studies lacked appropriate controls owing to presence or absence of null alleles, linkage disequilibrium, and error rate measurements. For these reasons development of multiplexed microsatellite markers was aimed at. Four French beech populations were used in

a study comprising 2 x 8 microsatellites. In a first kit 14 microsatellites from clone banks were scrutinized. To be included in the kit it was required that a locus should contain more than three alleles, absence of null alleles, and good amplification quality. For the second kit DNA from six trees was utilized, in which 227 microsatellites were found. To be included in the kit the motif repeat numbers should be larger than 9, which left 48 microsatellites. Following sorting of these 48 microsatellites in the same way as for kit 1 left 17 microsatellites. These loci were validated in simplex reactions, in which loci with poor amplification were discarded. After that, existence of null alleles was tested using 10 families (the female parent and 7 offspring). Error rate measurements were performed on both kits.

Of the 31 first selected microsatellites the reasons for discarding 15 of them were:

Presence of null alleles	3
Low polymorphism	3
Poor amplification quality	6
Low signal intensity	3.

It was concluded that the two kits developed should be useful in future beech population genetic studies by *providing powerful and accurate genotyping tools*. The use of these kits will certainly facilitate future population genetic studies on beech.

1.7 Summary

1.7.1 Parent – offspring relationships.
Relatively strong relationships between parental populations and offspring populations in common garden trials were reported for leaf masses or leaf sizes, R^2 0.76 and 0.57, respectively. The populations were sampled along elevational gradients in two Pyrenean valleys in France in 2009. The relationship between population elevation and leaf mass was the only strong relationship in the common garden experiment, $R^2 = 0.78$. This relationship and three other relationships between leaf functional traits and population elevation for *in situ* collected material were relatively strong, $R^2 > 0.50$.

A comparison of heterozygosities in five microsatellite loci and 16 isozyme loci in two parental German populations and their offspring populations did not reveal any difference between the two types of population. Strangely, the F_{ST}s between the South-western slope offspring population and the two north-eastern slope populations were approximately six times smaller than its F_{ST} with its parental population.

1.7.2 Field trials
Growth. In five papers results from the European beech provenance series established in year 2000 were presented. Except for two of the papers, data from just one trial were reported. In two investigations all 32 populations were studied, while in others representative samples of populations were focused on.

Generally, there were significant differences in tree height. Similarly, differences in DBH or basal area were reported for the studies in which these two traits were included. The differences in growth were expected considering the wide geographic and elevational origin of the populations. No significant relationships between tree height and geographic variables (elevation, latitude, longitude) were noted in one of the trials with 32 populations. A step-wise regression analysis would have been worthwhile to carry out. The other study did not present any correlations between geographic variables and height. According to the principle component analysis latitude, elevation, temperature at population origin, and duration of the growth period as well as precipitation during the growth period, longitude, and winter temperature at population origin influenced the tree growth.

Relatively strong relationships between leaf functional traits (carbon isotope discrimination, net photosynthesis rate, soil-leaf hydraulic conductivity) and tree height were found in one investigation; R^2 in the range 0.63 – 0.89.

The concept of ecodistance was introduced by Matyas et al. (2009) in their study of three south-eastern European provenance trials. This concept says that the phenotypic response to climate depends not only on the climatic conditions where the population is actually grown or tested, but also on the ecodistance of transfer. The mean tree height of the populations in these three trials were related to the Ellenberg quotient, $EQ = 1,000 \times T_{07} \times P_{ann}^{-1}$, in which T_{07} is July mean temperature and P_{ann} is the annual precipitation. Two of the high-elevation populations grew well in these three trials which resulted in a relationship between EQ and tree height with a minimum. One Polish and one Dutch population showed good growth at all three test localities while the opposite result was noted for one Danish population.

Phenology. Large variation in bud flushing was noted in the two studies including 32 populations. This trait seemed to be strongly correlated between years based on one of the investigations, in which the population variance component for bud flushing was estimated at 36%. High-elevation populations flushed earliest. No strong relationships between bud flushing and geographic variables were found. Contrary to this, relatively strong relationships between population elevation and leaf coloring duration were noted; $R^2 = 0.63$ (age 10) and 0.56 (age 11). In a German trial detailed studies of six populations, three each from low and high annual mean temperature, were carried out. With one exception the bud flushing of the low-temperature populations was most advanced.

In one German field trial large variation in stem quality was observed with highest frequency of straight stems in high-elevation populations. Mostly, poor quality was attributed to spring frost damage.

1.7.3 Experiments

Damage and effect on growth of potted plants from three German and three Bulgarian populations exposed to freezing tests at -5°C and warming, +1.6°C during the growth period were reported in one study. The populations differed significantly with respect to leaf frost damage. Two of the German populations had the highest percentage of leaf frost damage, 18 and 25% while the mean for all six populations was 14.5%. The drop in height growth was most pronounced in a low-elevation Bulgarian population (200 masl), 33%. Two of the German populations showed increased growth following freeze testing, 3 and 10%. As corollary of this the population effect for this trait was strongly significant.

Sophisticated equipment was used for a study of fine root development and turn-over in three populations in a Slovenian provenance trial. No significant effects among the populations were noted for the two fine-root-traits. The fine-root biomass was significantly lower in the adjacent natural stand that was attributed to a more severe competition in this stand.

1.7.4 *In situ* studies

For understanding recent history of a large beech population a unique investigation combining historic demographic data with genetic diversity in existing populations was carried out. This investigation comprised two remnant populations and one newly established population in a French mountain area. The allelic richness and expected heterozygosities did not vary much among the three populations. As a corollary of this F_{ST} among these populations was low 0.2%. No signs of recent bottle necks were found.

Phenology and abiotic stress-related candidate genes were selected for a study of four high-elevation populations on two mountain slopes. The F_{ST} including all four populations covering an elevation range of more than 600 meters was low, 0.02.

One main focus was the identification of linkage disequilibrium (LD) within the 58 stress related genes. Mean LD within genes was estimated at 0.26 and half of them were significant.

By aid of relatedness of the trees in a growing marginal stand heritabilities were estimated. No less than 19 microsatellites were used to estimate relatedness. Independent of the method used for estimation of relatedness extremely high heritabilities were noted for bud flushing, 0.30 – 0.80. Leaf area, leaf mass and N content varied in the range 0.20 – 0.40. These figures suggest that this marginal population has good prospects for future adaptation. Two methods were used in the analysis of environmental factors responsible for existing population differentiation of 19 mountain range populations in France, principle component analysis and constrained method of genetic clustering. Geographically, populations from northern localities in pre-Alps, in which minimum temperatures are not very low and precipitation is important, were separated from southern mountain populations experiencing a dry climate. A separation attributed to elevational differences was also noted.

Marker studies to compare marginal and core populations were carried out in various countries. Generally, there were limited differences between the two types of populations. In one investigation a relatively strong relationship between genetic and geographic distance was reported, $R^2 = 0.52$. A three times higher frequency of positive fixation indices than negative ones were noted in one study.

1.6.5 Spatial structure

A stronger spatial genetic structure in refugia than in recolonized areas was hypothesized and tested in 61 populations from four regions in France and Catalonia. In the recolonized northern French region there was a weak relationship between geographic distance and pairwise F_{ST} estimates, r = 0.36. Thus, there was no strong support for the above hypothesis. The pairwise F_{ST}s were much higher in the Landes region in France. This was attributed to the isolated occurrence of the 13 tested populations from this region.

1.6.6 Global warming

Simulations were run to study the impact of changed temperature and precipitation on future distribution of beech. Three management methods were included in the simulations; *group conversion system*, *shelter cut system*, and *no management*. In conclusion, increased temperatures cause an earlier bud flushing, which results in northwards expansion of beech. For the trailing part of beech distribution there will probably be a shift of the species distribution to higher elevations owing to increased drought at lower elevations. Management will probably enhance the rate of adaptation.

2. Phenology

Vitasse et al. (2009a) reported on a species trial with six species that included five beech populations from an elevational range of 131-1,604 masl. and another valley with four populations, 148-1,551 masl. A provenance trial was established in south-western France at 23 masl. and bud flushing was recorded weekly during March-May and during September-December for senescence. Leaf unfolding date was defined as the date when at least one leaf from the apical bud was unfolded. Senescence date was defined as the day when 50% of the leaves were no longer functional, i.e either coloured or fallen. Growth period duration is the difference between senescence and bud flushing. Conventional ANOVAs were run to estimate the population effects. An estimate of population differentiation (D) was done according to the following:

$$D = \sigma^2_p /(\sigma^2_p + \sigma^2_{pb} + \sigma^2_\varepsilon),$$

in which σ^2_p is population variance, σ^2_{pb}, is population x block variance component, and σ^2_ε is the residual variance component. Relationships with population altitudes were tested with Pearson correlations.

The survival varied in the range 70-100%. No significant relationships were noted for the relationship between altitude and survival but the lowest survival was noted for one of the high-elevation populations.

With one exception, significant differences among populations were noted for the three traits studied both years (Fig. 2-1). The population effect for growth period duration was about twice as strong as the population effect of the two other traits and explained 30 and 40% of the variation in growth period duration. The population originating from 131 masl. had a much longer growth period than the rest of the populations, which probably contributed much to the D estimate for this trait. The other populations did not show any trend to relationship with temperature at population origin. There was a significant effect for the rela-
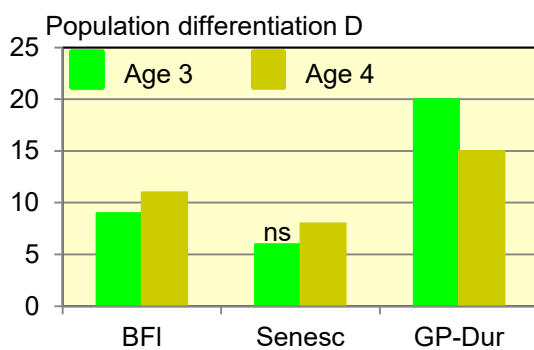
tionship between annual temperature at population origin and bud flushing, $R^2 = 0.48$. The earliest flushing was noted for high-elevation populations, which have the lowest annual temperatures. The maximum difference among the populations for flushing dates was only about five days, which means that more frequent recordings are required to possibly find a stronger relationship. The early flushing in the high-elevation populations must be attributed to a lower heat demand for bud flushing in these populations. Another relationship of intermediate strength was noted for the *temperature – senescence* relationship, $R^2 = 0.42$, which was close to significant at the 5% level. Again the earliest senescence was noted for the high-elevation populations and the observed range was almost 30 days between the earliest and latest population. It was speculated that the triggering of senescence could most likely be attributed to temperature rather than to day length since there did not exist any difference in photoperiodic conditions at the origin of the populations. However, the high-elevation populations trigger senescence earlier than the low-elevation populations and the triggering takes place at shorter night length than is the case for low-elevation populations. Even if the photoperiodic conditions are not as pronounced in southern France as in northern Europe, the 30-day difference in senescence means a reasonable difference in night length. I have preferred to refer to night length as triggering of senescence since it is well documented that an uninterrupted night length is the triggering factor in many northern tree species (Eriksson et al. 2020).

Except for growth rate, there was good agreement between the recordings during the two years when the relationships were tested at the population level (Fig. 2-2). The strength of the relationships was much less when tested at the individual plant level. The reason for the significance for growth rate with such a low correlation.

Figure 2-1. Population differentiation, D = the population variance component divided by the sum of all variance components. Bud flushing (BFl), senescence, and growth period duration (GP-Dur) based on a provenance trial at lat. 44.°74N and 23 masl. with populations from an elevational range of 131-1,604 masl. in The Pyrénées in France. Non-significance is indicated. Vitasse et al. 2009a.
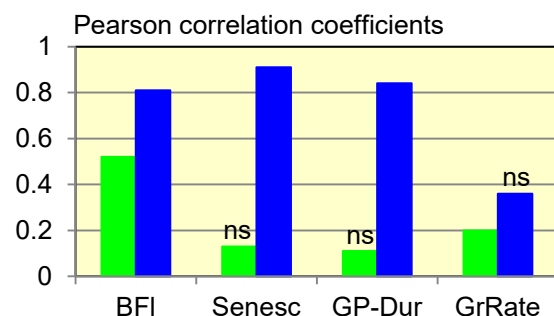

Figure 2-2. Between years Pearson correlation coefficients for four traits in a provenance trial at lat. 44.°74N and 23 masl. with populations from an elevational range of 131-1,604 masl. in The Pyrénées in France, Green columns refer to correlations based on individual plants and blue columns refer to correlations at the population level. Non-significant correlations are shown. Vitasse et al. 2009b. .

Table 2-1. *The impact of a shift in elevation of 100 meters or one degree centigrade on bud flushing, senescence, and duration of the growth period based on recordings* in situ *of these traits along two slopes, 131 to 1,604 masl. in The French Pyrénès. Vitasse et al. 2009c and 2009b.*

	Relationships. Traits at days - elevation Elevation range: 131-1,604 masl.		Relationsips: Temperature:- days bud flushing March – May Senescence August – November Growth period duration entire year		Increase or decrease of GP-Dur per one ºC increase
Bud flushing	*Positive*, high-elevation populations flushes late	Weak; 1.1 days per 100m not significant	*Negative*, High-elevation populations flushes late	$R^2 = 0.63$ $R^2 = 0.15$ for the long-time series	-1.9
Senescence	*Negative*, earliest cessation in high-elevation populations	Intermediate; not significant	*Positive*, earliest cessation in high-elevation populations	$R^2 = 0.75$	+ 5.6
Duration of the growth period	*Negative*, longest duration of the growth period in low-elevation populations	intermediate; not significant	Positive, longest duration of the growth period in low-elevation populations	$R^2 = 0.89$	+ 8.0

coefficient as 0.20 must be attributed to the large number of plants recorded.

Four significant relationships between traits were observed (Fig. 2-3). However, only one relationship, senescence – growth period duration, explained a substantial part of the variation. The three others explained less than 40% of the variation.

The bud flushing and senescence of the above studied populations were followed in situ during 2005 - 2007 by Vitasse et al. (2009c). Beech was one of four species in this investigation. The other three were *Acer pseudoplatanus,*
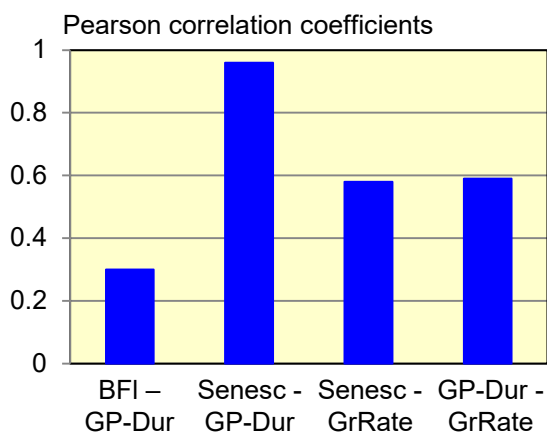

Figure 2-3. Significant Pearson correlation coefficients between four traits in a provenance trial at lat. 44.º74N and 31 masl. with populations from an elevational range of 131-1,604 masl. in The Pyrénées in France, Bud flushing (BFl), senescence (Senesc), growth period duration (GP-Dur) and growth rate (GrRate). Vitasse et al. 2009b..

Fraxinus excelsior, and *Quercus petraea*. The objectives of this study were phrased as follows: *to quantify the relative gain in canopy duration (duration of the growth period) per one degree centigrade increase and the relative contribution of spring autumn phenology to this relative gain*. Ten trees per population were recorded for bud flushing and senescence every ten day during March-May (flushing) and September-November (senescence). Observations were used by binoculars at a distance of approximately 15 meters from the tree. Bud flushing was defined as the date when there was one fully developed leaf in 50% of the buds. Senescence was defined as the date when 50% of the leaves were either colored or had fallen. Data loggers were used to measure the temperature at each locality. Solar radiation was also recorded.

Good agreement between the phenological observations over years was reported. I have tried to summarize the phenology recording results in Table 2-1. The relationships of the three phenology traits and elevation were linear but the slope for bud flushing and elevation was not pronounced. Unfortunately no estimates of R^2 were presented. As expected the latest bud flushing and earliest senescence were noted for the high-elevation populations. The relationship between these two traits and elevation were positive, which indicates that senescence influences growth period duration more than bud flushing. The three relationships between temperature at the different elevations and the phenology traits showed the same pattern as the ones for elevation and they were stronger according to the graphic illustrations in Figs. 1-2 in the paper. It was pointed out that temperature affected senescence more than bud flushing, which differed from the other three species studied.
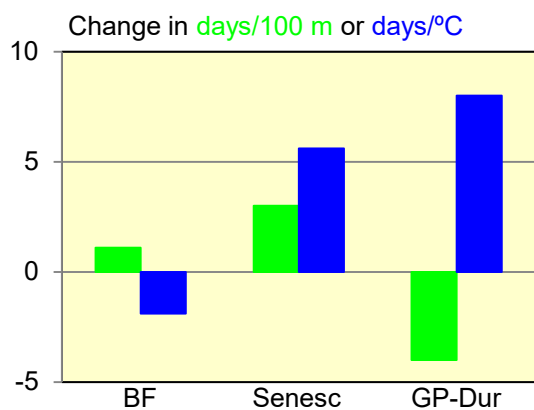
Figure 2-4. The increase or decrease of the duration of the growth period in number of days for each 100 m increase of elevation or increase per degree centigrade for the three phenology traits studied in this investigation. Bud flushing (BFl), senescence, and growth period duration (GP-Dur) recorded in situ of populations from an elevational range of 131-1,604 masl. in The Pyrénées in France, Vitasse et al. 2009c.

In Fig. 2-4 I have summarized the figures for change of the growth period duration per 100 meters difference in elevation or for the difference in temperature by one degree centigrade. This figure reveals that senescence is considerably postponed with increasing temperature, which in turn leads to a substantial prolongation of the growth period.

The contribution of bud flushing was less than half of the contribution by senescence to the extension of the growth period by temperature increase (Fig. 2-5). Beech differed from the other three species in this investigation. Two of them *Acer pseudoplatanus,* and *Fraxinus excelsior* did not show any impact of senescence on growth period prolongation. My interpretation of these results is that senescence in *A. pseudoplatanus* and *F. excelsior* is strongly regulated by night length while this is not the case with *F. sylvatica*. This interpretation is supported by the stronger impact of temperature than elevation for senescence in beech.

It was stated that understanding of environmental signals triggering senescence are poorly understood, which is surprising with the wealth of data supporting the crucial role of night length for triggering of growth cessation in northern tree species (Eriksson et al. 2013 for a summary). It should be remembered that differences in photoperiod increase with latitude. Four hypothesis for triggering were presented:

1. Increased temperature would delay growth cessation
2. Increased temperature would speed up growth cessation
3. Night length triggers growth cessation
4. Low temperatures in combination with prolongation of night length triggers growth cessation

The authors stated that the first hypothesis was supported by their results while I prefer to interpret their results as a support for the fourth hypothesis. In bottom there is a
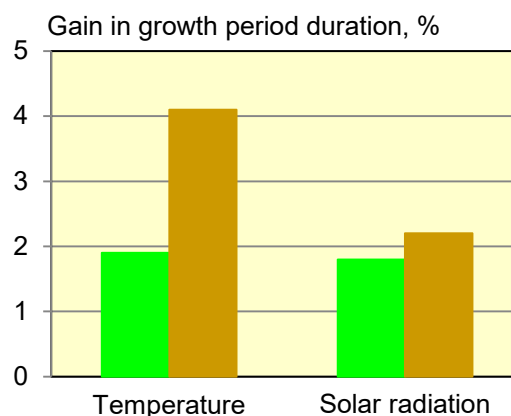

Figure 2-5. Relative gain in growth period duration, %, attributed to bud flushing or senescence, on a per day basis for 1℃ increase in temperature or on a solar radiation basis. The populations in this study originated from an elevational range of 131-1,604 masl. in The Pyrénées in France, Green columns refer to the contribution from bud flushing and brown columns contribution from senescence Vitasse et al. 2009c.

night length triggering of growth cessation that can be modified by temperature and water availability. I argued above that the neglection of differences in night length at growth cessation was misleading and this comment is still valid for this investigation.

Finally, the increased growth following a prolongation of the growth period was pointed out and that a prolongation thanks to early bud flushing will contribute more to such an increased growth thanks to higher solar radiation during flushing than during senescence.

The data from Vitasse et al. (2009c) were once more treated in paper by Vitasse et al. (2009b), in which a longtime series, >22 years, of phenological recordings in two lowland stands from Fontainebleau at lat. 48.41ºN and 120 masl. were followed. Two more species, *Abies alba* and *Ilex aquifolium* were included in the observations from the two Pyrénéan Valleys in this paper.

Most of the results from the elevation transects were presented before but the amplitude for flushing in 2005 and 2006 were given. The first and somewhat cooler spring had larger amplitude, 20 days, than the warmer 2006, 15 days. These figures were much lower than for the five other species.

There was a weak and non-significant relationship between temperature and bud flushing in the Fontainebleau populations in contrast to *Quercus petraea and Fraxinus excelsior*. However, the R^2s for these species were both lower than 0.40. It was stated that phenology appears to be a very heritable trait. This is certainly true for species from the boreal and temperate zones of the world, in which late spring frosts and early autumn frosts may be detrimental for the future development of seedlings and trees. It was further pointed out that the obtained results suggest that population sensitivity to temperature is stable for a given species, in spite of its possible
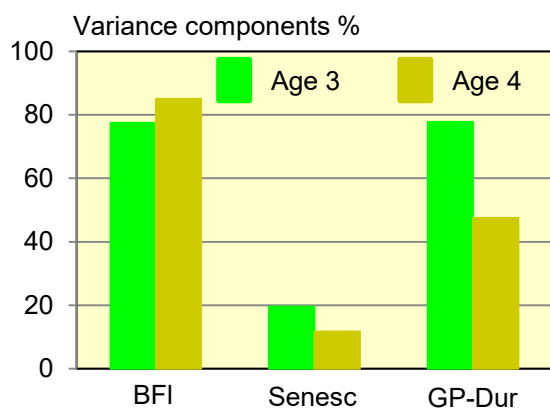
Figure 2-6. Percentage variance components for impact of trial elevation on bud flushing (BFl), senescence, and growth period duration (GP-Dur) based on five trials at an elevational range of 133-1,180 masl. in Gave Valley in The Pyrénées in France, Vitasse et al. 2010.

Figure 2-7. Mean difference in days between shortest and longest duration of bud flushing (BFl), senescence, and growth period duration (GP-Dur) based on five trials at an elevational range of 133-1,180 masl. in Gave Valley in The Pyrénées in France, The differences are shown for observations at age 3. The three colors refer to populations originating from different elevations. Vitasse et al. 2010.

local adaptation. As stated by the authors the results from this investigation with beech populations from a limited part of the distribution of species suggest that population sensitivity to global warming might be stable for a given species in spite of its possible local adaptation. Although this investigation comprised populations from a limited part of distribution of beech but it covered several climate zones, which strengthen its value.

With the purpose of studying the phenotypic plasticity in phenological traits, three beech populations from 488, 773, and 1,190 masl. in the Gave valley in French Pyrénées were selected for establishment of trials at 131, 488, 833, 1,190, and 1,533 masl in this valley by Vitasse et al. (2010). Bud flushing was recorded every ten days during March – May. Leaf unfolding date was defined as the date when at least one leaf from the apical bud was unfolded. Leaf fall and leaf coloring was monitored every ten days during September – December. Senescence date was defined as the day when 50% of the leaves were no longer functional, i.e either coloured or fallen. The time between senescence and the bud flushing was regarded as the duration of the growth period. In the ANOVAs, trial elevation and population were regarded as random effects.

In Fig. 2-6 I have illustrated the percentage variance components for the three phenology traits studied. This figure reveals that test locality elevation is the dominating effect as regards bud flushing and duration of the growth period. With such a strong effect of test locality it is expected that other random effects will have a low share in total variance. In spite of the small percentage variance components, 2.4 and 3.5%, the population effect was significant for bud flushing. For the other two traits no significant population effect was noted, which is somewhat strange regarding the large difference in elevation at the origin of the three studied populations. The high-elevation popula-
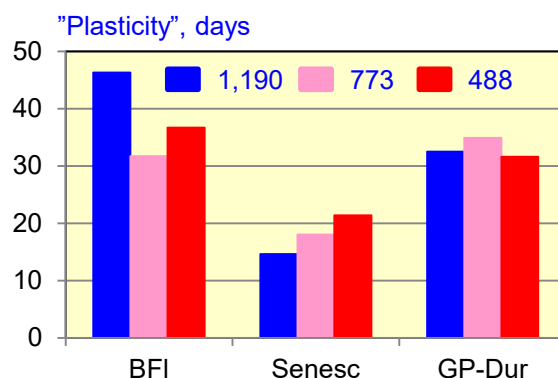
tion had the earliest bud flushing in all trials, which probably can be attributed to its lower heat sum requirement for flushing than in the other populations.

The relationships between temperature at the test localities and bud flushing of the three populations were close to straight lines at both ages. For the high elevation population the earliest flushing at the lowest test locality occurred around April 10. The two other populations flushed a few days later. Each degree of increase meant more than five days earlier flushing. For senescence hyperbolic curves were noted with earliest senescence at the high and the low elevation test localities. The early senescence at the low elevation test locality was attributed to drought preventing further growth. No population difference for senescence was noted, nor was there any population x test locality interaction. The duration of the growth period increased with increasing temperature at the test locality and with some flattening at the two lowest test localities. No population or population x test locality interaction effects were proven.

Since the main objective of this paper was to study phenotypic plasticity at the population level I have tried to illustrate this based on data in Fig. 2 of the paper (Fig. 2-7). It is obvious that phenotypic plasticity is most pronounced for bud flushing and least for senescence. The latter is due to the hyperbolic character of the relationship for this trait. The phenotypic plasticity for growth period duration seems to be more dependent on flushing date than on senescence.

The more or less absence of population differences in spite of their origin from different climatic zones in this valley is the most striking result in this investigation. This lead to the conclusion that phenotypic plasticity was mainly caused by environmental conditions and not by genetic differences.

In conclusion, the above series of papers constitute an important contribution to the understanding of evolution of adaptive traits in a geographic region with large difference in temperature climate. The results are of importance for sampling for genetic conservation and for selection of material for reforestation.

Bud flushing in *Fagus sylvatica* populations along two slopes, one northern and one southern aspect in The French Alps was followed in 2006 and 2007 (Davi et al. 2011). In the northern slope, populations from 961 masl. to 1,528 masl. were followed. The corresponding elevations for the southern slope were 1,115 masl. and 1,530 masl. This study gives valuable information on an important trait in beech.

The major results of this investigation were:

A rapid sigmoidal response curve for bud flushing was noticed.

Mean bud flushing was about seven days earlier in spring 2007 than in 2006, which was attributed to warmer 2007. Delay of bud flushing increased with 1.8 days per 100 meters based on the extremely strong relationship between bud flushing and elevation, r > 0.98.

Local adaptation is one possible explanation for the results observed.

Vitasse et al. (2013) who studied bud flushing in populations from the Swiss Alps in eight common gardens trials presented two hypotheses for their study:

1. There is a genetic differentiation for bud flushing between low and high-elevation populations caused by a strong directional selection for late bud flushing at high elevation
2. There is a lower phenotypic plasticity in high-elevation populations owing to this strong directional selection

One eastern and one western slope were selected with elevational ranges of the four populations from each slope, 610 – 1,280 and 540 – 1,240 masl, respectively. Four common garden experiments with potted plants were established along transects in each of one eastern and one western slope. Three blocks were used with five pairs of individuals from each population included in a fully randomized experiment. Temperature was recorded by data loggers at each test locality. A late frost occurred in mid-April at the highest experimental sites in both transects. For bud flushing weekly observations took place at age 2 in a five-degree scale; score 0 = no bud activity, score 4 = one leaf fully unfolded. The apical budset was recorded in late July, 20-21 in western transect and July 26-27 in the eastern transect,.
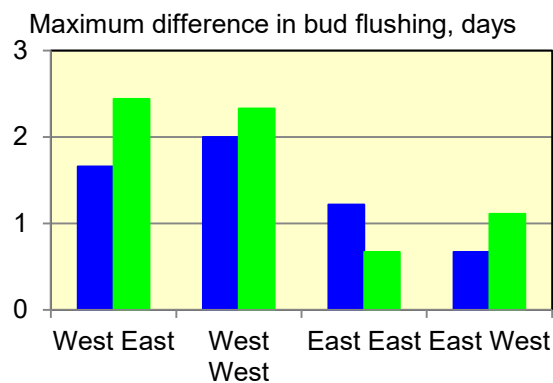

Figure 2-8. The maximum difference in bud flushing between high-elevation populations (blue) and low-elevation populations (green) tested at eight test localities along two elevation transects. West East stands for eastern populations tested in the western transect, and so on. Vitasse et al. 2013.

There were strongly significant population differences for:

Transect, east versus west
Elevation within transect

It was remarked that the difference in elevation between the highest test localities in the two transects, 1,400 versus 1,708 masl, contributed to the strong significance between the two transects.

Increasing temperature contributed to early bud flushing with a good fit to a straight line between mean 30-day temperature in °C before bud flushing at test locality and date for bud flushing. The slope of this regression was used as an estimate of phenotypic plasticity. Before we turn to such estimates of phenotypic plasticity it might be useful to examine some observed data. Therefore, Fig. 2-8 was drawn to illustrate observed maximum differences between populations originating from high (blue) and low (green) elevations and tested in common garden experiments in the eastern and western transects. It should be noted that the maximum differences were all between highest test locality and the lowest test locality flushing dates. In three of the four combinations the difference between high and low elevation origin suggested that the phenotypic plasticity is larger in low-elevation populations than in high-elevation populations supporting hypothesis 2 above.
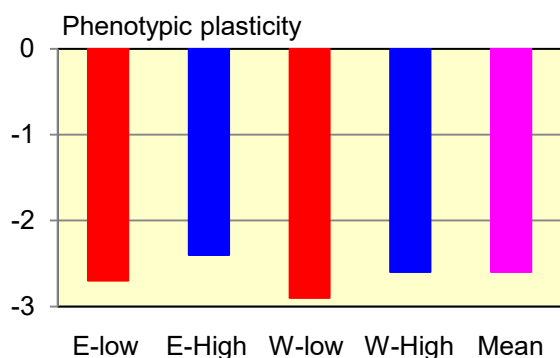
Figure 2-9. Phenotypic plasticity estimated as the slope of the rgeression line between temperature at test locality and date for bud flushing in populations from two elevational gradients, one eastern and one western in Swiss Alps. Separate estimates are shown for low elevation and high elevation populations. Vitasse et al. 2013.

This is further supported by analysis of all data from intermediate elevation test localities as well (Fig. 2-9) and amounted to -2.6 days per K (= temperature lapse rate for March – April; 0.49 K per 100 m difference in elevation in the western transect and 0.37 K for the eastern transect; K = Kelvin) for the pooled material. The differences between high- and low-elevation origins are not substantial but they point in the direction hypothesized.

Independent of populations it was noted that budset occurred latest in the two high-elevation sites. There was a tendency that low-elevation populations reached budset later than high-elevation populations. In agreement with Fig. 2-8 I have illustrated the maximum difference in percentage units for budset between the earliest and latest budset in Fig. 2-10. A similar picture can be seen in the two figures with larger difference in the same three combinations. Thus, there is support for hypothesis 2 also for budset. Since recordings of budset took place just once no estimates corresponding to those for bud flushing could be obtained
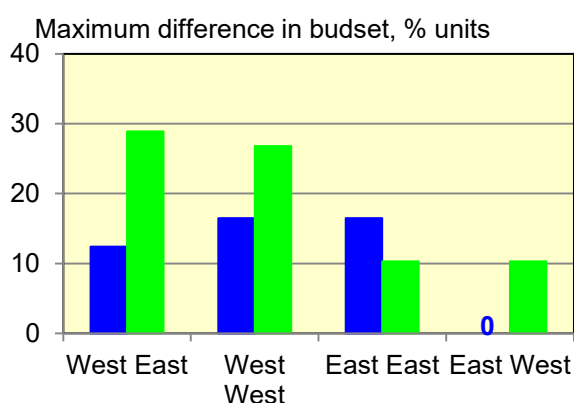

Figure 2-10. The maximum difference in budset between high-elevation populations (blue) and low-elevation populations (green) tested at eight test localities along two elevation transects. West East stands for eastern populations tested in the western transect, and so on. Vitasse et al. 2013.
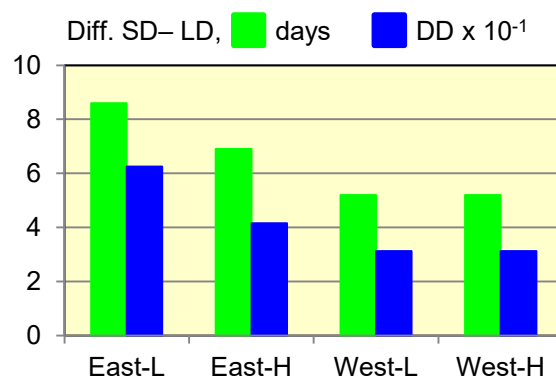

Figure 2-11. The difference in number of days and DD degree days x 10^{-1} for bud flushing between short day (SD) and long day (LD) treatments in one high- and one low-elevation populationi in each of one eastern and one western transect in Swiss Alps. The difference between high- and low-elevation populations was around 500 m. Basler and Körner 2012..

The role of photoperiod on bud flushing in 14 tree species, including, beech, was studied under controlled conditions in growth chambers by Basler and Körner (2012). They collected scions at two elevations in one eastern and one western slope in Swiss Alps. The elevation for the low collection site was 500 masl. from the western slope and 700 masl. for the eastern slope; the corresponding high elevations collection sites were 1,000 1,100 masl. The scions were collected on March 2-3 and placed in vessels with water and exposed to simulated day lengths corresponding to February – April (short days) and March – May (long days) with continuous increase of temperature during the course of the experiment. Each treatment comprised five scions. Bud flushing was recorded in a four-degree scale with 1 = dormant bud and 4 = leaf unfolding and stalk visible.

The flushing occurred significantly later in the short-day treatment than in the long-day treatment. The differences in number of days and temperature sum in degree days are illustrated in Fig. 2-11. This figure reveals that that bud flushing took place later in the short-day treatment than in the long-day treatment for all combinations. Similarly, the heat demand for flushing was largest in the short-day treatment. The most pronounced differences were noted for the two eastern populations. When temperatures from January 1 until sampling were included in the calculation of degree days (threshold temperature = 0°C), the bud flushing took place at lower degree days in the high-elevation populations than in the low-elevation populations. It was concluded that bud flushing is not only dependent on a certain heat sum but also dependent on photoperiodic conditions. It was hypothesized that the photoperiodic regulation of bud flushing would prevent too early bud flushing with its increased risk for frost exposure.

A review of the current knowledge on temperature and photoperiodic conditions for breaking of dormancy and bud flushing was published by Vitasse and Basler (2013).

1. It was noted that the variation in date for bud flushing between years did not vary much, and less than many other species
2. Only a 2-day shift of bud flushing in response to increased temperature during recent years
3. In comparison with other tree species, its flushing took place earlier than other tree species at high elevation but later than those species at low elevation

In summary, *beech can be considered as a late-flushing species under warm or mild climates, but not necessarily under colder climates within its range*. It was stated that the results as regards dormancy release are contradictory. The linear relationship between number of chill days from November 1 to date of bud flushing and degree days from January 1 to bud flushing suggests that the full requirement for chilling is not reached by most beech populations.

One study by Faluci and Calamassi (1996) reported that long-day conditions might at least partly substitute for chilling to achieve dormancy release. This result was later on confirmed by Caffarra and Donelly (2011).

Two models for the interaction between chilling and photoperiod for bud flushing were presented:

According to the first model there is a threshold for chilling before the onset of photoperiodic influence on bud flushing.

In the second model it is predicted that the heat demand for bud flushing decreases with increased day length

There are results that point at model 2 as relevant for beech with its strong demand for chilling to release dormancy.

There are reasons to believe that the interaction between chilling and forcing varies dependent on the climatic conditions in the range of distribution. The suggestions by Vitasse and Basler are summarized in Table 2-2. There is support for the predictions in this table from recordings of bud flushing in two low-elevation and two high-elevation populations in Switzerland followed during 27 years. The maximum difference in bud flushing dates was 27 and 33 days for the high-elevation populations versus 18 and 25 days for the low-elevation populations (Fig. 2-12).

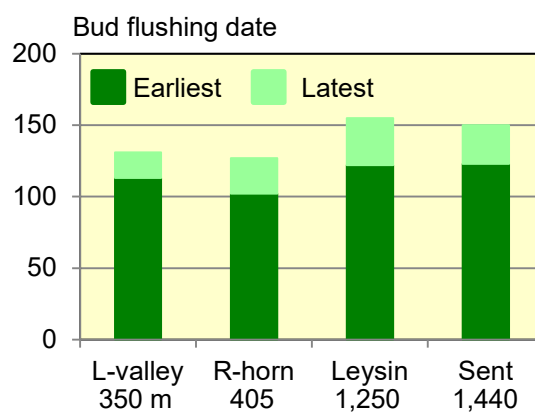It seems as demand for chilling to break dormancy is not

Figure 2-12. Earliest and latest bud flushing dates in two low-elevation and two high-elevation Swiss populations followed over 27 years. The elevations of the population origin are indicated. Vitasse and Basler 2013..

always fulfilled. Bud flushing might still occur and is promoted by long days. As regards initiation of bud flushing there seems to be a complex interaction between chilling and photoperiod, which varies among populations.

The different responses among populations to environmental factors complicate the predictions for future responses of beech populations to global warming, which were thoroughly discussed by the authors. A most interesting paper.

The impact of temperature and photoperiod on bud flushing in two pioneer tree species and two climax tree species was compared following treatment under controlled conditions (Caffarra and Donnelly 2011). Grafts of clones from an International Phenological Garden in Germany were included in this investigation. Three experiments were conducted:

1. Effects of light intensity. One-node cuttings were exposed to ambient conditions for chilling and then transferred to two light conditions, 75 and 250 μmol m^{-2} s^{-1} in growth chambers at 24°C and 16 h day
2. Effects of forcing temperatures. Dormant twigs were kept at 3°C for 16 days. One-node cuttings were then placed in growth cabinets at temperatures of 6, 12, 18, 24 and 32°C and an incubator at -3°C wuith a day length of 16 h.
3. Effect of chilling duration and forcing photoperiod.

Table 2-2. Hypotheses as regards bud flushing in high- and low-elevation populations and observed responses. Vitasse and Basler 2013.

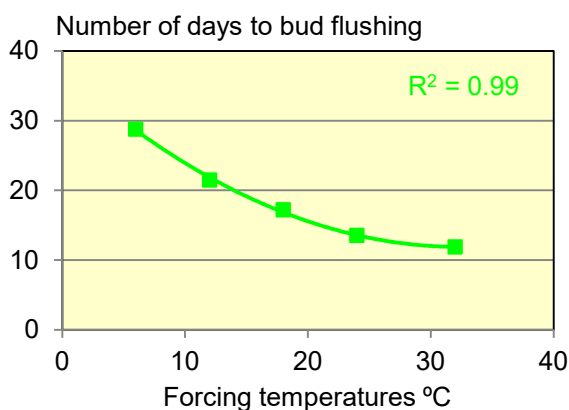| Population origin	Hypothesis	Response
Mild climate, low elevation southern regions	Under mild winter the chilling requirements for bud flushing is not reached, which leads to a higher amount of heat for bud flushing and thus a delay of it.	The increasing day length during spring probably compensates for such a delay leading to limited year to year variation in date for bud flushing
Harsh climate, high elevation northern regions	The chilling requirements for breaking of bud dormancy are mostly fulfilled, which removes the interaction between chilling and day length in regulation of bud flushing	Bud flushing becomes dependent on temperature, which leads to a relationship between temperature and bud flushing. With the interannual variation in spring temperature bud flushing dates will vary.

Figure 2-13. Experiment 2. The relationship between forcing temperatures and days to bud flushing. The photoperiod was16 h daylight. Caffarra and Donnelly 2012.
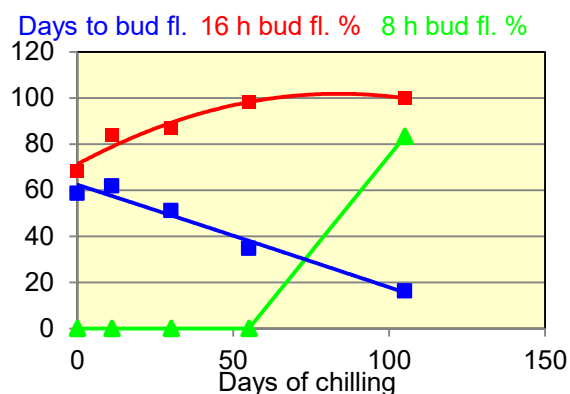

Figure 2-14. Experiment 3. Number of days to bud flushing and percentage of bud flushing in two photoperiodic treatments; 16 h daylight and 8 h daylight. Caffarra and Donnelly 2012..

The plants were kept under temperature control until November and then exposed to temperatures below 12ºC for less than two weeks. After that the plants were kept at 3ºC for 0, 11, 30, 55, and 105 days. At the end of each treatment eight plants were placed in growth cabinets with 22/14ºC day/night temperatures and 16 h or 8 h day length. Four plants per treatment were exposed to the different regimes.

This investigation is not genetic but it gives useful information on responses to chilling and photoperiodic treatment on bud flushing.

Bud flushing under high light intensity in experiment 1 took place around four days earlier in high light intensity than in in low light intensity. Bud flushing started earlier with increasing forcing temperatures with an extremely good fit to a polynomial relationship between forcing temperatures and days to bud flushing (Fig. 2-13). There was an extremely strong relationship ($R^2 = 0.93$) between days of chilling and bud flushing in experiment 3 (Fig. 2-14). The impact of photoperiod was also strong with no bud flushing until 105 days of chilling was applied. Days to bud flushing was linearly related to days of chilling, varying between 16 days for 105 days of chilling to 62 days for 11 days of chilling.

Bud flushing of six populations covering the latitudinal range 42.25 – 53.00ºN was followed during two years in one Austrian and one German field trial by Schueler and Liesebach (2015). The trials were located at 48.25 ºN, 350 masl. and 54.30 ºN, 40 masl. Recordings took place at ages 12 – 13 in the German trial and at ages 13 – 14 in the Austrian trial. Only two recordings were carried out in the German trial at age 12. Bud flushing in the upper part of the crowns were used for recording of flushing in seven classes, 0 = dormant bud to unfolded leaves in class 7. The shift from stage 4 folded and hairy leaves begin to appear to stage 5 leaves are spread out, was used as the best stage for discrimination of bud flushing among the six populations. Temperature sums for bud flushing was

calculated with +5 ºN as threshold temperature. Pearson correlations based on mean values were calculated to estimate the strength of the relationships between years and trials. For relationships within the two trials individual tree data were used.

In the Austrian trial bud flushing took place on April 25 one year and on May 2 another year suggesting that temperature sum has an impact on bud flushing. There were strongly significant population effects (p<0.001) for Julian days, temperature sum, and daylength at bud flushing for each year at the Austrian test locality and for age 13 at the German test locality. There was a very strong stability across years and trials for Julian days at bud flushing, temperature sum at bud flushing, and daylength at bud flushing (Fig. 2-15).

It is somewhat surprising that relationships between bud flushing and geographic variables were not presented. In Fig. 2-16 I have plotted the relationship between popula-
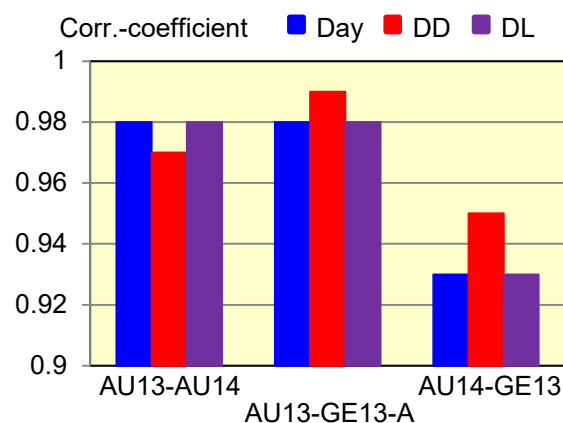

Figure 2-15. Pearson correlation coefficients for the relationships of Julian days to flushing (Day), temperature sum at bud flushing (DD), and daylength at bud flushing (DL) between years in the Austrian trial and between Austrian trial and the German trial. The numbers refer to age of assessment. Schueler and Liesebach 2015.
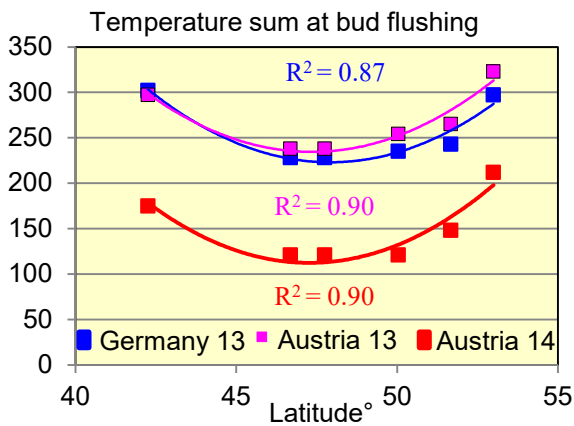
Figure 2-16. The relationship between population original latitude and the temperature sum for bud flushing in degree days with +5°C as treshold temperature for six beech populations studied during two years in one trial in Austria and one year in one German trial. Schueler and Liesebach 2015.

tion latitudinal origin and temperature sum at bud flushing. There were strong 2nd degree polynomial relationships for the three test occasions. The biological interpretation of the observed relationships is not straightforward. The Spanish population from latitude 42.25ºN exerts a strong impact on this relationship. A first interpretation might be that this pattern should be attributed to the high elevation of the Spanish population; 950 masl. However, the Austrian population from latitude 42.25ºN at 1,050 masl did not perform unexpectedly. Similar relationships were no-
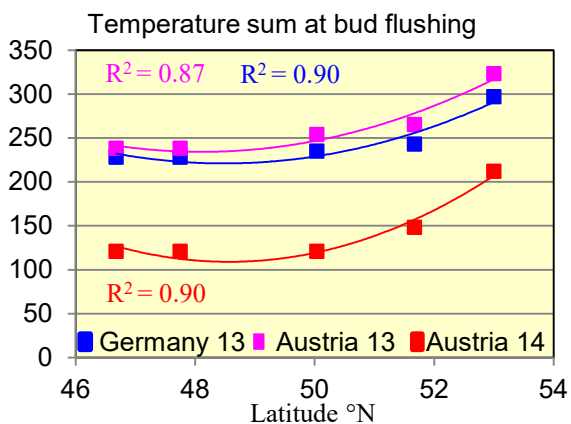

Figure 2-17. The relationship between population original latitude and temperature sum at bud flushing date for five beech populations studied during two years in one trial in Austria and one year in one Germany trial. Schueler and Liesebach 2015.
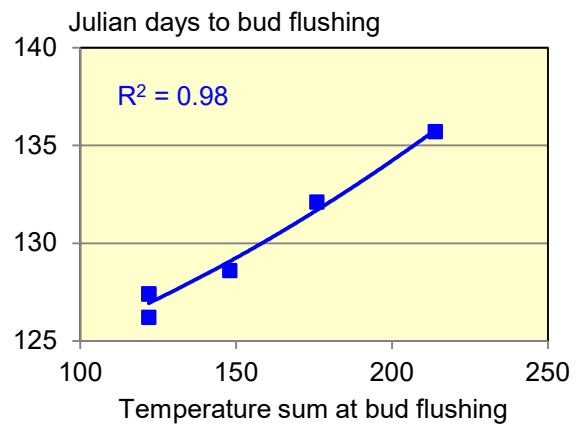

Figure 2-18. The relationship between temperature sum at bud flushing date and Julian days for flushing in six beech populations studied during two years in one trial in Austria and one year in one German trial. Two populations at the temperature sum of 122 degree days had identical flushing date.Schueler and Liesebach 2015.

ted for the relationship between latitudinal origin and Julian day for bud flushing. In Fig. 2-17 this relationship is plotted after exclusion of the Spanish population, which results in relationships that are expected, i.e. increase or decrease with increasing latitude.

Of greatest interest for the question of the impact of temperature on bud flushing is to test the relationship between temperature sum at bud flushing and bud flushing date. This relationship is illustrated for the German trial in Fig. 2-18, which shows that there is an extremely strong relationship, $R^2 = 0.98$. An equally strong relationship was also noted for the recordings in 2007 in the Austrian trial while the Spanish population again deviated strongly from the performance of the other populations. It would have been interesting to speculate around the adaptation of this Spanish population.

The authors stressed the importance of daylength for bud flushing partly based on the observation that a lower temperature sum was required following transfer to more northern localities. In contrast to this, two of the three relationships between temperature sum at bud flushing and Julian day at bud flushing suggest that temperature is the most important factor for bud flushing. Experiments under controlled photoperiodic and temperature conditions are probably required to distinguish effects of photoperiod and temperature sums on bud flushing. Usually, linear relationships between temperature sum and biological phenomena are assumed. This might be an oversimplification.
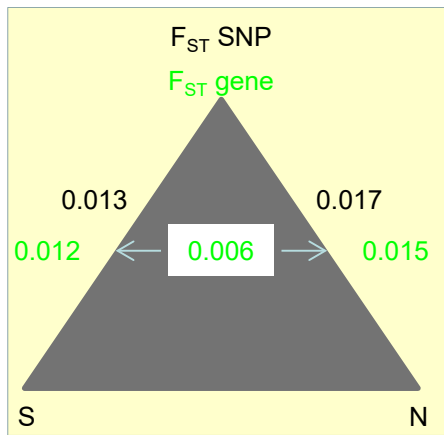
Figure 2-19. F_{ST}s for SNPs and genes between and within slopes. The analysis comprised 53 candidate genes and 546 SNPs related to bud flushing. Csilléry et al. 2014..

A long series of observations (20 years) of bud flushing and leaf coloring at 47 localities (55 – 1,050 masl.) in Slovenia were utilized to estimate temperature impact on these two traits (Cufar et al. 2012). Temperature recordings from ordinary meteorological stations were used in the estimations.

They reported fairly strong relationships between elevation and mean dates for bud flushing and leaf coloring, R^2 = 0.73 and 0.66, respectively. Bud flushing was delayed by 2.6 days per 100 m in elevation while leaf coloring was negatively correlated with elevation and a change of 1.9 days per 100 meter.

As regards bud flushing March temperatures had a greater impact on low-elevation populations than on high-elevation populations. April temperatures were more related to bud flushing of high-elevation populations. Leaf coloring was related to temperatures in August and September.

It was also noted that bud flushing occurred 1.52 days earlier per decade at 1,000 masl while no shift was noted for low-elevation populations. No clear trend was noted for leaf coloring. I suspect that leaf coloring is strongly regulated by night length and that temperature has a modifying effect on leaf coloring.

To detect SNPs and genes regulating bud flushing Csilléry et al. (2014) studied two populations from a northern slope (995 and 1,340 masl, 35 and 36 trees) and two from a southern slope (895 and 1,517 masl, 12 and 13 trees) on Mount Ventoux in southeastern France. Based on the report by Lalagüe et al. (2014) 53 candidate genes and 546 SNPs were selected for this investigation. F_{ST} estimations both at gene and SNP levels were calculated for the comparison of northern and southern populations as well as within northern and southern slopes. To detect selection caused by climatic stress F_{ST} outlier methods were used according to the following:

Elevation-related stress, the two high-elevation populations versus the two low-elevation populations
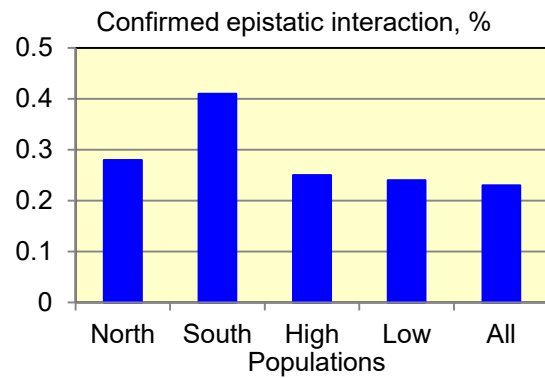Hierarchical with the two populations from each slope nested within each slope.

Figure 2-20. The percentage of confirmed epistatic interactions according to Ohta (1982) between SNPs in the two slopes, north and south, between SNPs at different elevations, and between SNPs for all four populations. Scilléry et al. 2014..

For SNPs F_{ST} loci with allele frequces <0.05 were not included in the analysis.

To detect epistatic selection Ohta's test (Ohta 1962), which separates the variance of linkage disequilibrium within (D^2_{IS}) and between populations (D^2_{ST}). If the ratio D^2_{IS} / D^2_{ST} is large epistatic selection has occurred. Ohta's test was performed for all four populations, for the two northern and two southern populations separately and separately for the two high-elevation and two low-elevation populations.

Fig. 2-19 reveals that the F_{ST}s were relatively low and no difference between the two slopes was noted for the SNPs. or genes (negative F_{ST}) The differences observed suggest that selection outweighs gene flow among these four geographically adjacent populations.

The confirmed cases of epistatic interaction illustrated in Fig 2-20 were low (0.23 – 0.41%) for all five cases tested; within slopes, between high elevations, between low elevations, and between all four populations. It should be remembered that number of pairs tested varied between 72,998 and 87,834, which means that there is a high probability to observe disequilibrium for random reasons.

Most outliers were noted for the northern high-elevation population and fewest epistatic interactions in contrast to the southern low-elevation population with its opposite pattern. This suggests that directional selection had taken place in the northern high-elevation population. Owing to the low number of trees sampled in the two southern populations, altogether 25 trees, far-reaching conclusions based on these two populations cannot de drawn. The hierarchical model revealed two outlier SNPs in genes 23_1 and 88_1. In the analysis accounting for more realistic demographic scenarios, an additional outliner was detected, gene 142. Evidence for divergent selection was found for gene 23_1 and for gene 142 in the northern population. Weak signals for selection were noted for SNPs 23-1 and 142. At the gene level genes 58 and 134_2_2 showed weak signals of selection. Low F_{ST}s were noted for several genes suggesting that these genes were exposed to sta-
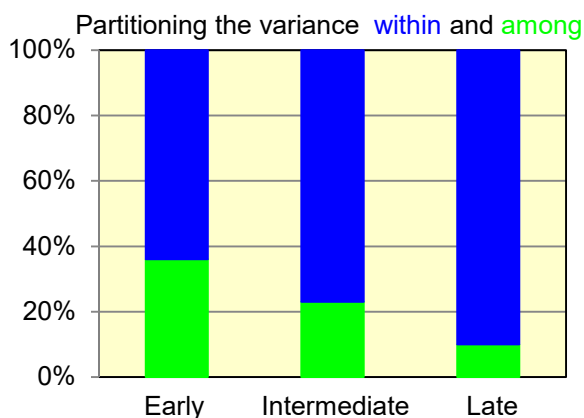
Figure 2-21. Partitioning the variance within and among

Figure 2-21. Partitioning of the variance among and within populations according to AMOVA in three Polish populatons, in each of them 30 trees were classified as early, intermediate, or late bud flushing. Five polymorphic microsatellites were analyzed. Kraj and Sztorc 2009.

E_{ST} R_{ST}

Figure 2-22. F_{ST} and R_{ST} across three Polish populations, in each of them 30 trees were classified as early, intermediate, or late bud flushing. Five polymorphic microsatellites were analyzed. Kraj and Sztorc 2009.

bilizing selection. Contrary to this the outlier SNPs were interpreted as caused by directional selection.

The varying bud flushing times in three southern Polish populations were related to genetic setup in five polymorphic microsatellite loci by Kraj and Sztorc (2009). The populations grow in the latitudinal range 49.41-50.12 ºN and longitudinal range 19.96-19.94ºE. Seven classes were used to assess the flushing and for assignment them into three phenology groups, early (classes 6-7), intermediate (4-5), and late (1-3). In each population 30 trees belonging to each of the three phenology groups were sampled for microsatellite identification. The effective number of alleles (N_e) and the expected Hardy-Weinberg heterozygosity (H_e) were estimated. F_{ST}s according to Weir and Cockerham (1984) and R_{ST}s according to Slatkin (1985) between individuals and groups of phenology were calculated. AMOVA was run to estimate the partitioning of the variance to the different hierarchical levels. Finally, a principle component analysis (PCA) was run.

N_e of the five microsatellites did not vary much, 7.9-8.6 and the corresponding variation of expected heterozygosity was 0.851-0.883. No significance was noted for these parameters. The among-population variance component was much higher in the early flushing group and was lowest in the late-flushing group (Fig. 2-21). In the principle component analysis (PCA) three separate groups were noted in the early-flushing group, which was related to geographic origin. This result explains the large among-population variance in the early-flushing group. Such a clear separation was not noted for the intermediate-flushing group and still less so for the late flushing group. In agreement with the PCA analysis the F_{ST} and R_{ST} for the early-flushing group were largest while it was smallest for the late-flushing group (Fig. 2-22).

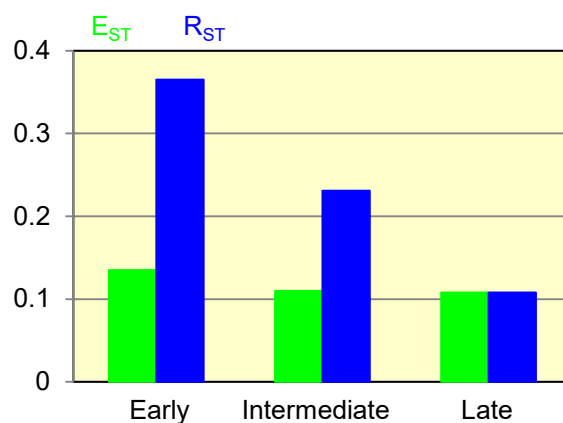The improvement of fitness of an individual by late flushing was pointed out since late spring frosts occur frequently. There is a trade-off between late flushing and growth. A too late flushing leads to a short growth period and thereby less growth, which in turn means that such a seedling or tree may be outcompeted by other plants or trees. It was clearly pointed out that the genetic differences obtained in this investigation do not mean that they are related to differences in fitness. The results call for progeny testing of trees belonging to differences in timing of flushing.

The effect of urbanization and elevation on *in situ* bud flushing based on a long series of observations (1980 – 2009) in stands around three German large cities, Cologne, Frankfurt, and Munich, was reported by Jochner et al. (2012). The number of points for observation around the three cities varied in the range 27 – 45.

Of the 78 temporal trends estimated 47 were negative and significant while one significant positive trend was found. In all 73 trends were negative. The mean was estimated at 0.23 days earlier bud flushing per year. Generally the multiple regression analysis revealed an earlier onset of bud flushing at lower elevation and higher urbanization. However, only the difference in elevation was significant. The delay of flushing per 100 meter of elevation was estimated at 1.34 days.

Fu et al. (2013) studied the effect of warming on bud flushing following exposure of the plants to warming with 1 – 6ºC above ambient temperature in growth chambers. One Belgian population was used in this study. Since only one population was used I limit this presentation to the two main conclusions in this paper. Leaf unfolding was advanced by warming but was not linearly related to temperature with less effect at temperatures 5 – 6ºC above ambient temperature. The lower the chilling temperatures to break dormancy during winter the more heat was required for bud flushing.

Table 2-3. The results of simulations with respect to change in temperature sum for bud flushing in degrees in nine different scenarios. The end results after six generations are shown. The changes in average elevation are also illustrated. Oddou-Muratorio and Davi 2014..

Scenario	1,000	1,200	1,400	1,600
A. Without adaptive evolution, Thus $h^2 = 0$	-0.42	+0.48	- 0.08	0.00
	Average elevation 202 m			
B. Adaptive evolution via selection, $h^2 = 1.0$	+1.18	-4.72	-2.85	-1.06
C. As B, but evolution without mortality .	0.00	-0.24	-0.52	-0.31
	Average elevation + 37 m			
D. As B, but selection via differential mortality and evolution without differential reproduction;	+1.33	-4.76	-2.89	-0.88
	Average elevation +168 m			
E. As B, but mortality occurred only at the end of the growing season when a critical value of carbon was exceeded	-0.23	-4.93	-2.63	-0.94
	Average elevation +16 m			
F. As B, mortality caused by a critical low level of carbon at bud flushing	+0.55	-0.03	+0.03	+0.06
G. As B, but $h^2 = 0.6$	+0.60	-3.79	+2.07	-0.90
Ha. Evolution with frost effect on leaf area index (LAI), each late frost reduced LAI by 10% per degree below the critical temperature	-1.21	-0.41	-3.71	-0.93
	Average elevation + 75 m			
Hb. Evolution with frost effect on leaf area index (LAI), each late frost reduced LAI by 20% per degree below the critical temperature	-1.15	-0.21	-4.01	-1.17
	Average elevation + 27 m			

2.2 Modelling

Simulations of local adaptation based on a Physio-Demo-Genetic (PDG) model was presented by Oddou-Muratorio et al. (2014), in which bud flushing was selected as the genetic trait. A series of physiology related traits with published data was used in this investigation.

The physiology related module at individual tree level included biomass, leaf area index, leaf mass per area, crown projection, canopy clumping, water stress index, maximum carboxylation rate, and onset date of rest. Critical value of the state of forcing (temperature sum for bud flushing) was the only parameter that was allowed to vary among the trees. Bud flushing surveys in two years at two elevations, 1,117 and 1,340 masl., were used to obtain estimates for average critical value for the state of bud flushing forcing.

The demographic module included carbon reserve at the end of the year, carbon required for the complete development of leaf, biomass allocated to wood, sufficient biomass for seed production, female fecundity, pollen dispersal, mating, seed dispersal and recruitment.

The quantitative genetic module for bud flushing relied on the variation in bud flushing and the variation in environmental conditions among localities. It was assumed that bud flushing was triggered by temperature sum only. Simulations were carried out in such a way that bud flushing along an elevational gradient from 700 to 1,700 masl should be mimicked. A subdivision in discrete groups with an elevational distance of 200 m was done for the elevation range 800-1,600 masl. It was assumed that bud flushing was regulated by ten independent biallelic loci with complete additive effects.

The initial population in the simulation included 500 trees all of an age of 40 years and showing a normal distribution. The simulations were based on six non-overlapping generations. The scenarios tested are briefly presented in Table 2-3.:

The simulations resulted in extinction of the low-elevation population and the change in temperature sums for bud flushing at the other levels are summarized in Table Oddou-2-3. The elevational range of the populations was also affected by the mortality, which is indicated in this table.

In scenario A there was only random effects on temperature sum for bud flushing since the heritability was 0.00. The maximum difference in bud flushing was estimated at 12.2 days and 35.2 days between lowest and highest
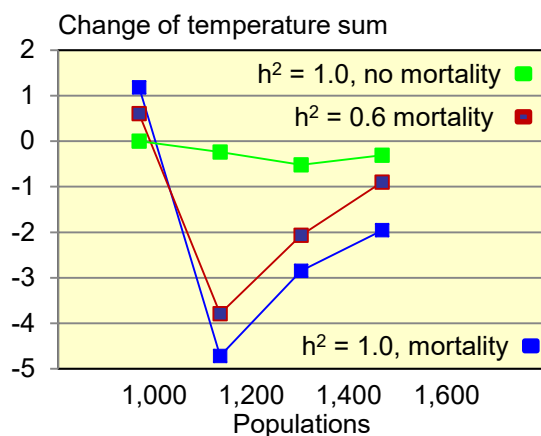
Figure 2-23. The results from simulations of scenarios B (blue), C (green) and G (brown). See Table 2-3 for explantion of the three scenarios. Oddou-Muratorio and Davi 2014..

elevation. A reduction of the growing season from 210 to 160 days was noted and the water stress index decreased with 33%. The highest photosynthetic level was noted for 1,078 masl. with a flat maximum between 1,050 and 1,300 masl. The highest ring increment and seed production was found at 1,258 masl with steeper ring width decrease than the decrease in seed production. The lowest values for carbon reserves were found at 1,160 – 1,400 masl. At bud flushing, the demand for carbon resources was below the threshold from ≈1,000 masl and at higher elevations. Finally, mortality was higher at low and intermediate levels.

Scenario B. Significant differences in change of temperature sum for bud flushing were found between populations (Fig. 2-23) with an increase for elevation 1,000 masl and decrease for the other three elevations, which means an earlier bud flushing in the latter three populations. The increase of the temperature sum in population 1,000 masl. was attributed to mortality caused by lack of sufficient carbon resources before bud flushing. The difference between scenarios A and B was strongly significant. The selection was twice as intensive in population 1,200 masl as in elevations 1,400 and 1,600 masl. The temperature sum difference of 5.9° between the lowest elevations corresponds to a two-day difference in bud flushing. The strong response in just five generations must be attributed to the heritability of 1.00 in this scenario.

Scenario C. Temperature sum requirement for bud flushing was not much influenced without mortality.

Scenario D. The results are similar to the results in Scenario B, which suggests that the difference in reproduction had a limited effect on adaptation.

Scenario E. Low level of carbon reserves had strong effects on mortality and as corollary of this, also strong effects on temperature sum, again with the greatest effect at 1,200 masl.

Scenario F. The low level of carbon reserves at bud flushing did not influence temperature sum much. The absence of mortality in populations 1,200 masl. and 1,400 masl explained the limited effects on temperature sum in these two populations.

Scenario G. The heritability of 0.60 in this scenario resulted in smaller changes than in scenario B with its heritability of 1.00. It was stressed that the effects on populations 1,200 masl.and 1,400 masl. were still pronounced.

Scenario Ha. There was another pattern of response compared to Scenario B. A reduction of TS was noted for population 1,000 in this scenario instead of an increase in Scenario B. Population 1,400 masl.was most affected while population 1,200 masl. was affected to a lmited extent.

Scenario Hb. The pattern was quite similar to Scenario Ha.

For the Ha and Hb scenarios it was remarked that only trees that had initiated bud flushing could suffer from frost damage. This means that the late flushing populations at the two highest elevations avoided frost damage. The results from simulations of the nine scenarios showed that differential mortality was the strongest factor influencing evolution and thus stronger than differential reproduction (Fig 2-23). Further, the influence varied among the elevational populations resulting in a non-monotonic evolution with elevation. Finally, the impact of the heritability of the trait on the evolution was strong (Fig 2-23).

The authors concluded that the PDG-model is a useful tool *for predicting the evolution of nonequilibrium forest populations under CC (CC = climate change*). However, a cautious interpretation of the data was recommended owing to the large amount of data involved in the simulations.

This study showed a non-linear reationship between elevation and bud flushing. It also showed that phenotypic plasticity variation among elevations and years was more pronounced than genetic variation.
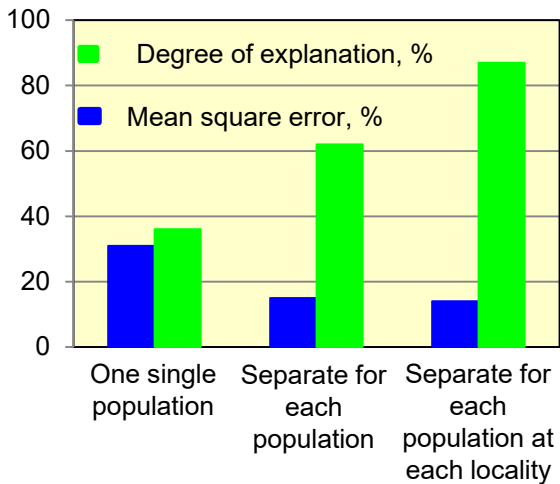
Figure 2-24. Mean square error % and degree of explanation, %, for modelling of bud flushing of beech populations in series of provenance trials in Europé including populations from the entire distribution area of beech in Europé. In the first model it was assumed that the material constituted one single population without any populaion dfferentiation. In the second model the environmental requirements for each population were considered. In the third model there was a consideration of the environmental requirements for each population at each site. Kramer et al 2017.

Kramer et al. (2017) focused on modelling of bud flushing in beech and outlined two hypotheses regarding bud flushing in *F. sylvatica*:

1. The critical chilling- and forcing requirements for foliar bud-burst of trees are adapted to local climatic conditions, thereby differing among provenances.
2. The critical chilling-and forcing requirements themselves are phenotypically plastic, i.e. varying among trial sites for the same provenances.

It is assumed that breaking of dormancy requires a certain chilling. It is further assumed that once dormancy is broken a certain temperature sum is required for bud flushing, which is coined as forcing. Kramer et al. (2017) utilized data on bud flushing from different series of provenance trials in Europe. The scales for phenology scoring varied, which made a harmonization necessary. No less than 16 models were used to test the prediction of flushing dates for the different populations. The chilling requirements for breaking dormancy as well as the heat demanded for bud flushing were considered in the 16 models tested. Three models were used to assess the hypotheses presented above:

1. No population differentiation, thus one single population composed of all tested populations
2. The model was adjusted for the chilling and forcing requirements for each population separately
3. In the third model there was a consideration of the environmental requirements for each population at each site separately.

Fig. 2-24 shows the mean square errors (MSE) of the three models analyzed as well as the degree of explanation of the models. Model 1 had a large MSE, 31% of total MSE, and the model explained only 36% of the variation in bud flushing. In model 2 the MSE was reduced to 15% and the degree of explanation increased considerably, 62%. The third model resulted in an extremely high degree of explanation, 87% with a MSE of 14%. It must be regarded as amazing that a mathematic model of a biological process reaches such a high degree of explanation. Particularly, since the temperature recordings did not take place at the trial sites but at some distance from them as well as the need for harmonization of the different scorings in the different trials.

The different performance of the populations across test localities means that phenotypic plasticity at the population level occurs. It was stressed that phenotypic plasticity itself is also an adaptive trait and has to be considered in predictions of the effect of global warming on survival and distribution of tree species.

2.3 Summary

2.3,1 Observed results

Most papers treat bud flushing while less than half of them reported on senescence. Populations from elevation origins varying in the range 100 – 1,600 masl were studied. With such a large variation in origin large population differentiation for bud flushing, growth cessation, and growth period duration was expected and observed. Mostly, earliest bud flushing and latest growth cessation was observed for low-elevation populations. One deviating result of the relationship between population latitude and bud flushing date with a minimum was noted. This was attributed to one Spanish high-elevation population. Exclusion of this population changed this relationship with a minimum to an increase with increasing latitude.

Three papers reported results from long-time observations. The delay in bud flushing varied from limited impact to ≈ 2.5 days per 100 m elevation while the corresponding for growth cessation was estimated at 1.9 days. Both elevation and temperature had strong impacts on growth cessation. In one study the corresponding changes in number of days per degree centigrade were estimated at 1.9 for bud flushing and 5.6 days for senescence.

Phenotypic plasticity for bud flushing was in focus in several papers. Larger phenotypic plasticity in low-elevation populations than in high-elevation populations was reported in one paper while the opposite was noted in another investigation. Only in one paper plasticity of senescence was reported. In this case a strong phenotypic plasticity was noted.

2.3.2 Modelling

A new Physiology-Demography-Genetic modelling was used to predict future distribution of beech in Europe. The

physiology part contained several functional traits as well as morphology traits. The demographic model contained carbon required for development of different organs including fecundity and pollen and seed dispersal. The quantitative genetics part relied on bud flushing, which was assumed to be triggered by temperature. Nine scenarios with varying heritabilities, mortality, carbon levels, and frost occurrence were modelled. The results from simulations of the nine scenarios showed that differential mortality was the strongest factor influencing evolution and stronger than differential reproduction.

Another modelling study focused on bud flushing, which was assumed to depend on chilling and forcing temperatures.

Three models were used to assess the hypotheses presented above:

1. No population differentiation, thus one single population composed of all tested populations
2. The model was adjusted for the chilling and forcing requirements for each population separately
3. In the third model there was a consideration of the environmental requirements for each population at each site separately.

Data from several provenance trials were used in this modelling. The degrees of explanation of the models 1 to 3 were 36, 62, and 87%, respectively. Thus, model 3 was the most useful for predictions of future evolution of bud flushing in beech. It should be noted that phenotypic plasticity also is genetically regulated.

3.Drought

3.1 Ex situ studies

The performance of several growth and physiology traits in one German population (lat. 51.75ºC, long. 9.52ºE 440 masl.) and one Polish marginal population (lat. 49.25ºC, long. 22.78ºE 800 masl.) was studied by Rose et al. (2009). It was hypothesized that the marginal population would be less sensitive to drought and that the root system is critical for coping with drought. Germinated seedlings were transplanted into pots on April 19 and were growing for 14 weeks before drought treatment was initiated on July 25. Three levels of water in the substrate were applied:

Control	40% water
Moderate stress	20% water
Severe stress	10% water

The treatments were terminated on September 21 when several leaf and growth traits were assessed. The day before termination of the experiment predawn water potential was measured. Carbon isotope discrimination (δ^{13}C), and content of calcium, magnesium, nitrogen, and potassium were determined in the dried material.

It was noted that the Polish beechnuts were heavier than the German beechnuts. I have summarized the results as regards population effects and population x treatment effects in Table 3-1. Five traits without significant effects
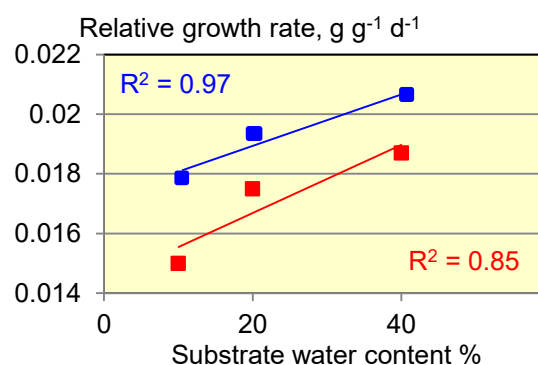

Figure 3-1. The relationships between water percentage in substrate and relative growth rate in one German population (blue) and one Polish population (red). The drought treatment lasted for 14 weeks, g = gram and d = days. Rose et al. 2009.

for any of these two parameters are not listed in this table. Six of the 17 traits showed significant population effects while ten showed significant population x treatment interaction effects. I have illustrated the relationships between drought treatment and relative growth rate in Fig. 3-1 as an example of a strongly significant population effect. There was a higher relative growth rate in the German than in the Polish population and the development is fairly parallel in the two populations. As corollary of this, the population x treatment interaction was non-significant. Contrary to this, a significant interaction was noted for shoot length but no significance for the population effect was noted for shoot length. The interaction is reflected in Fig. 3-2. ,which shows population rank changes.

It was concluded that the hypothesis of greater tolerance of drought of the marginal population was confirmed. This was mainly based on higher root/shoot ratio and

Table 3-1. Significant population and population x drought treatment effects in an experiment with artificial drought treatment of one German and one Polish beech population. Empty boxes mean non-significance. Rose et al. 2009.

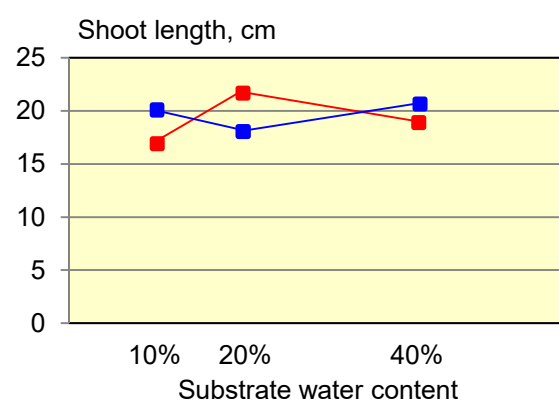| Trait	Population	Pop. x treatment
Relative growth rate, RGR g g^{-1} day^{-1}	***	***
Shoot length		*
Leaf K content	*	
Root/shoot ratio	*	
Specific leaf area	**	
δ^{13}C	*	***
Fine Root/Leaf Area ratio, FR/LA		***
Leaf Mg content		*
Leaf water content		***
Predawn water potential		***
Rot collar diameter		***
Specific root area SRA	**	*
Stem biomass		*

Figure 3-2. Shoot length following drought treatment to different levels of water percentage in substrate in one German (blue) and one Polish population (red) following 14 weeks of drought treatment. Rose et al. 2009.
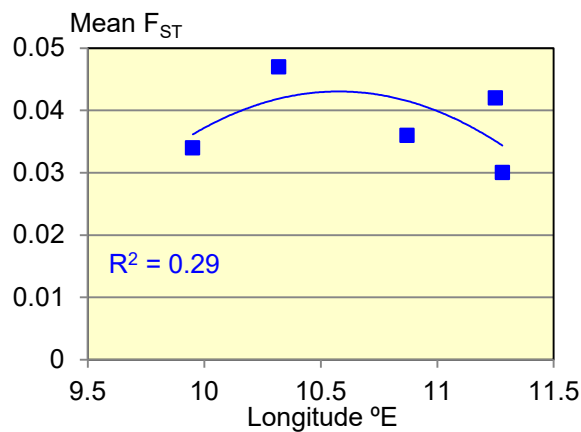
Figure 3-3. Mean population F_{ST} plotted against longitudinal origin of five German beech populations growing along a precipitation gradient. Carsjens et al. 2014.
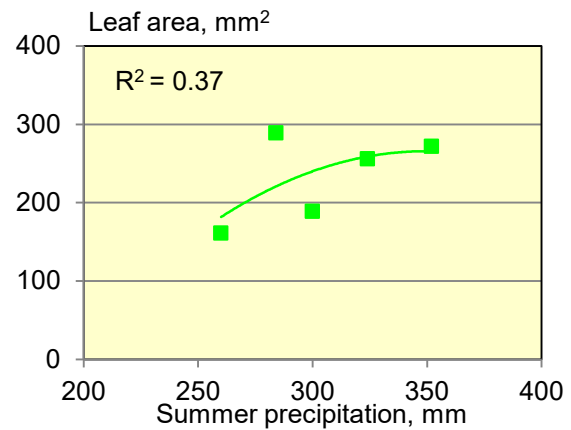

Figure 3-4. The relationship between summer precipitation at population origin and leaf area in five German populations of beech in the control material in an experiment with artificial drought treatment. Carsjens et al. 2014..

root surface area/leaf area in the marginal population. It was also pointed out that the results are valid for the first growth period. Before far-reaching conclusions can be drawn long-time resistance to drought of marginal populations must be evaluated.

The hypothesis that populations from dry sites respond less to drought than populations from mesic sites was tested in five German populations by Carsjens et al. (2014). The populations had a summer precipitation range of 260 – 350 mm. The plants were cultivated in 5-L pots filled with coarse sand. Besides growth, abscisic acid (ABA) and stress-related genes were analyzed, [9-cis-epoxy-dioxygenase (NCED), protein phosphatase 2C (PP2C), early responsive to dehydration (ERD)] and stress protection [ascorbate peroxidase (APX), superoxide dismutase (SOD), aldehyde dehydrogenase (ALDH), glutamine amidotransferase (GAT) that are involved in drought acclimation]. Two drought treatments aiming at substrate water contents of 2% and 10% started on May 25 and lasted until September 17. Five entire plants from each population and moisture regime were harvested after 4, 9, and 16 weeks of drought treatment. One fully expanded leaf per tree was used for molecular analysis. Membrane analysis was performed on leaf discs from fresh leaves. Weights and size of different parts of the plants were assessed. Twenty leaf discs were used for the study of conductivity in which maximum and relative electrolyte leakage were determined. Relative conductivity was also studied after treatment of the leaf discs with the herbicide paraquat, which is known to cause massive oxidative stress

Nine highly polymorphic microsatellites were used for genotyping of the parental populations.
For microsatellite markers the mean pairwise genetic distance among the parental stands was fairly low, 0.037. Almost all variation of the microsatellites, 97%, was found within populations. The limited among-population variation was attributed to neutrality of the microsatellites. The dendrogram based on the nine microsatellites showed that the 260 and 300 populations were related while all others did not show any clear connection with each other. This is somewhat surprising since the geographic distances among the populations is not especially large. The maximum distance being 130 km, which means that wind pollination would be a constraint to differentiation. There was no relationship between F_{ST} and longitude of the populations (Fig. 3-3). Nor was there any strong relationship with summer precipitation of the populations. In both cases population 284 disturbed the relationship. Without this population there was a strong 2nd degree polynomial relationship, $R^2 = 0.98$.
The leaf area in the control material is visualized in Fig. 3-4, which shows that population 284 again disturbs a trend. Similarly, it deviates from a good fit to a relationship between summer precipitation and percentage reduction of leaf area compared to the control treatment (Fig. 3-5). Most of the reduction of leaf area in population 354 from the most mesic site occurred between weeks 9 and 16 weeks of drought; 15 and 49% reduction, respectively. Population 260 from the driest locality had the lowest reductions in both treatments, 9 and 13%, respectively,
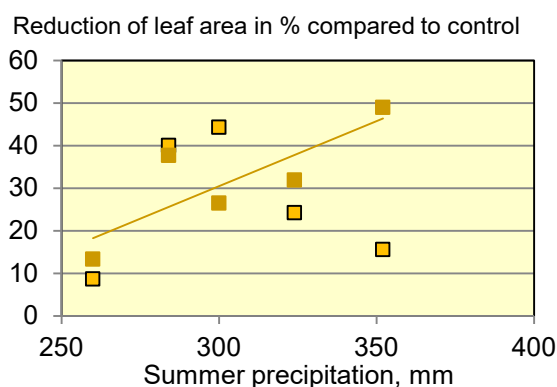
44

Figure 3-5. The relationship between summer precipitation at origin of five German beech populations and percentage reduction in leaf area following 16 (brown) or 9 weeks (light brown) of drought treatment. Carsjens et al. 2014..
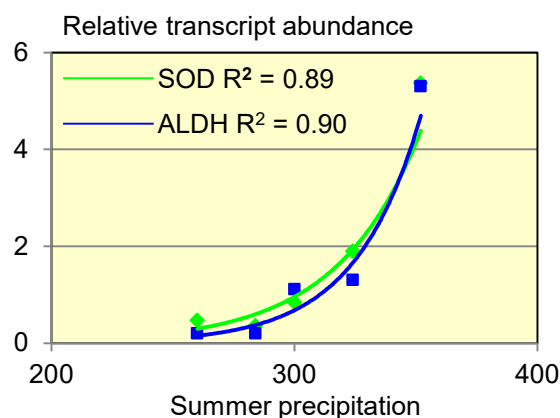
Figure 3-6. The relationships between summer precipitation at population origin and relative transcript abundance for two genes; superoxide dismutase, SOD and aldehyde dehydrogenase, ALDH, estimated after 16-week drought treatment. Carsjens et al. 2014.

which suggests that this populations has a fairly good adaptedness to dry conditions.

The relative electrolyte leakage (REL) in H_2O increased in four of the populations from the 4-week treatment to the 16-week treatment. There was a weak trend that REL decreased with decreasing precipitation at population origin. The REL after paraquat treatment was significantly lower in the most mesic population (354), which means that it can withstand oxidative stress better than the other four populations.

I have tried to summarize the most important information as regards the physiology-oriented part of this investigation in Table 3-2. For several traits there was an increase with increasing time of drought treatment. PP2C that is known to be induced by high ABA levels and its involve-

ment in ABA signal transduction constitutes an exception with an opposite trend. Also for this gene population 284 deviated from the trend shown by the other populations.

It was stated that the relative transcript levels of SOD, ALDH, and GAT ordered the populations according to their precipitation levels. This is illustrated in Fig. 3-6 for SOD and ALDH, which shows extremely god fit to the two exponential curves. These results were against expectation of higher levels of transcripts from these genes in populations from drier sites. A consequence of higher transcript levels at the end of the season in mesic populations than in those from dry sites means that there is an increased need to detoxify products of oxidative stress.

Table 3-2. A synthesis of the results from gene expression studies in one drought experiment with five German beech populations during 4, 9, and 16 weeks. The populations were selected along a precipitation gradient. The figures related to the populations correspond to the range of summer precipitation 260 – 352 mm. Carsjens et al. 2014.

| Traits studied	Difference among populations	Trend with respect to duration of drought treatment	Remarks
NCED 9-cis-epoxy-dioxy-genase	Increase with precipitation	increase	
PP2C protein phosphatase 2C	No clinal variation	decrease	Highest in pops 4-284 and 1-352
ERD early responsive to dehydration	Increase with precipitation	Mostly increase	
SOD superoxide dismutase		Increase in population 352	Strong response only for pop 1-352
APX ascorbate peroxidase	Weak trend for increase with precipitation	Peak at 9 weeks	
ALDH aldehyde dehydro-genase	SOD superoxide dismutase	Dramatic increase between 9 and 16 weeks	Strong response and only for pop 1-352
GAT glutamine amidotrans-ferase		Dramatic increase between 9 and 16 weeks	Strong response and only for pop 1-352
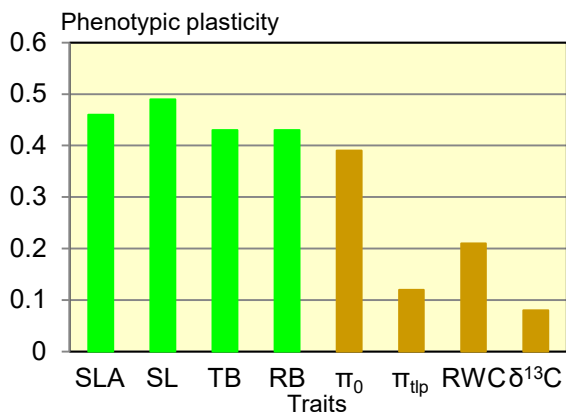
Figure 3-7. Phenotypic plasticity for traits with estimates < 0.50. SLA = specific leaf area, SL = shoot length, TB = total biomass, RB = root biomass, π_0 =osmotic potential at full hydration, π_{tip} = , πtlp= osmotic adjustment (early – late), RWC = relative symplastic water content at RWC_{tp}, $\delta^{13}C$ = carbon isotope discrimination. Knutzen et al. 2015.

The five populations in the above study by Carsjens et al. (2014) were used in another artificial drought experiment by Knutzen et al. (2015). Seedlings of a maximum height of 20 cm were excavated and the soil was washed off the roots before they were planted in 5-L pots with coarse fluviatile sand. There was no difference in mean annual temperatures, tree species composition, above ground structure, tree age, or soil chemical conditions in the five localities. Four drought treatments started on May 25 the following year aiming at 2, 4, 6, and 10% of water content in the soil. Ten replications were used for each treatment with one seedling per block and treatment. Three harvests were carried out on June 25 (early summer), July 30 (midsummer), and September 17 (late summer). Different parts of the plants were separated and dry weights were recorded as well as root specific area (SRA; m^2 kg^{-1}). One week before harvest the following traits were analyzed:

   Leaf stomatal conductance, g_s
   Leaf water potential [xylem pressure potential Ψ_{leaf} (max) at relative water content (RWC) = 100]
Osmotic potential at full turgor π_0
Osmotic potential at the turgor loss point (RWC_{tlp})
The turgor potential at full hydration Ψ_p
Tissue elasticity near full hydration ε in MPa
Carbon isotope discrimination $\delta^{13}C$
   Total N content
   Total C content.
In addition, several derived traits were calculated. The *phenotypic plasticity index* (PI) was calculated as PI = (maximum median − minimum median)/maximum median.

There was a strong focus on physiology aspects in this paper.

Significant treatment effects were noted for ten of the 16 morphology associated traits. The corresponding for physiology traits was two out of eleven traits. Significant po-
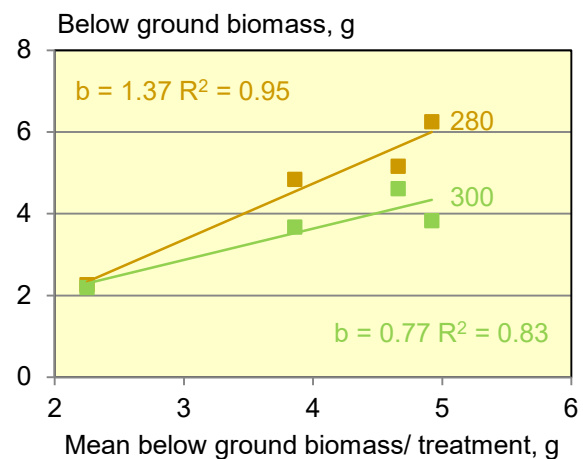

Figure 3-8. The relationships between below ground biomass and treatment mean biomass for below ground biomass for populations 300 (above) and 280 below. The slope (b) is given. Knutzen et al. 2015.

pulation effects were obtained for the below parameters and their phenotypic plasticity is given, for which estimates could be obtained:

Number of leaves	**	0.68
Total leaf area	*	0.82
Leaf size	**	0.62
Shoot length	**	0.49
Fine root mass	**	0.88
Modulus of elasticity ε	**	0.91
Leaf water potential at		
full hydration $\Psi_{leaf(max)}$,	**	0.98
Elastic adjustment (moist – dry)	**	-
Elastic adjustment (early – late)	**	1.00

In Fig. 3-7 the estimated phenotypic plasticities for traits <0.50 are illustrated, i.e. traits that are strongly genetically regulated. Three of the physiology traits had low estimates, < 0.25, of phenotypic plasticity. One way for understanding the characteristic of the individual populations is to analyze Finlay-Wilkinson diagrams in which the performance of the individual populations are plotted against treatment mean values as illustrated for the two extreme populations in Fig. 3-8. Population 280 had the highest below ground biomass production of the five populations in the control treatment. It responded strongly upon increased water availability while population 300 did not. In Fig. 3-9 I have illustrated the regression coefficients for all relationships between the two biomass traits and treatment mean biomass. This figure illustrates that populations 280 and 320 responded strongly to increased water availability with respect to above ground biomass. It was expected that population 350 would have a high slope estimate if it was adapted to the mesic conditions at its site of origin. It was also expected that population 260 would have lowest slope estimates assuming it was well adapted to the dry conditions at its site of origin. This analysis reveals that the assumed adaptedness to water availability conditions of these five populations must be rejected, which was supported in the paper. However,
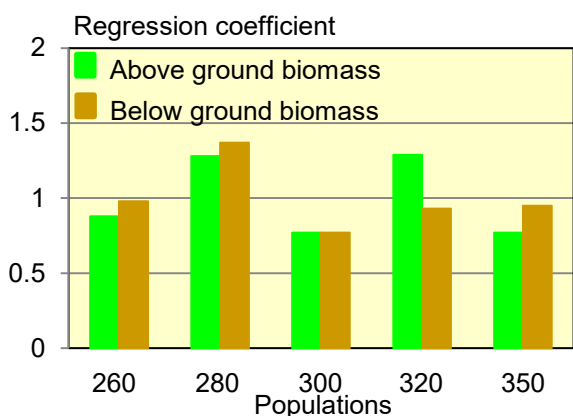
Figure 3-9. The mean regression coefficients for so called Finlay-Wilkinson diagrams for the relationships between above ground or below ground biomass in four drought treatments for five populations. The summer precipitation varied in the range 260 – 350 mm. Knutzen et al. 2015.

three significant effects for each of the relationships between precipitation at population origin and above ground biomass or below ground biomass were obtained. All six had R^2 estimates >0.85 suggesting that adaptedness varied. Too far-reaching conclusions should not be drawn owing to the low number of populations studied. There are at least two conditions, which can explain the absence of difference in adaptedness to drought conditions. 1. A strong gene flow among populations as indicated by the limited among-population differentiation in the study of molecular markers . 2. It is likely that the differences in precipitation at the sites of origin are too small to cause disruptive selection for water availability in presence of substantial gene flow.

It was expected that plants from localities with high water availability would reduce their leaf conductance more under water shortage than plants from dry origin. However, this was not observed. It was noted that beech compared to other species had a low capability to osmotic adjustment but the two populations from the highest precipitation origin were characterized by pronounced elastic adjustment. However, these two populations have a higher risk for hydraulic failure owing to more rigid cell walls in case of low water availability.

Several significant relationships between treatment or precipitation at population origin and the various traits studied were illustrated graphically, and R^2 estimates were presented in the supplementary material.

The above two investigations constitute important steps for understanding of mechanisms regulating drought tolerance in beech in relation to possible variation in drought adaptedness in natural beech populations. As stated by Carsjens et al. (2014): *It is clear that more work is required in future to identify adaptive traits in beech and unravel their molecular basis.*

The effect of drought treatment and type of soil on central and marginal populations of beech was studied by Thiel et al. (2014). Three German populations and one popula-
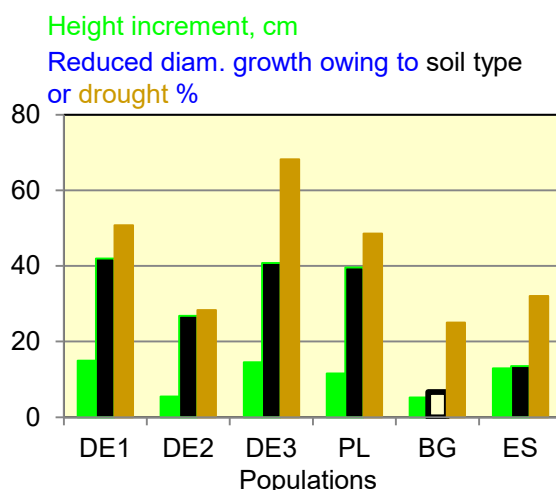

Figure 3-10. Height increment, percentage reduction of diameter increment in sandy loam compared to loamy sand (soil), and percentage reduction of diameter increment in drought treatment in three central (DE1 – DE3) and three marginal beech populations (PL, ES, BG).. DE = German, PL = Polish, ES = Spanish, and BG = Bulgarian population. The open column for soil indicates that the control plants grew less than the treated plants. Thiel et al. 2014.

tion each from Poland, Spain and Bulgaria were included. The latter three were regarded as marginal populations. Two types of soil were used sandy loam and loamy sand. The latter is a drier substrate than sandy loam. Nine potted plants per treatment were exposed to the different treatments. The 36-day drought stress treatment started on May 9 and was terminated on June 13, at this date 22% of the plants showed severe drought damage. After June 13 all plants got the same treatment. Drought damage in six classes (0, 1-5, 6-25, 26-50, 51-75, 76-100%) was visually determined at the last day of drought treatment. As soon as necrotic tissue appeared a leaf was regarded as damaged. Height and diameter of the plants were recorded shortly after transplantation in April and at the end of September.

The percentage of damaged leaves in the combined loamy sand and drought treatment was 39%, which was conspicuously higher than the 5.4% in the control treatment or in the sandy loam drought treatment, 12.8%. The Bulgarian population had significantly less leaf damage, 6.5%, than the DE3 population (23%). No other significant population differences were noted.

There was a strongly significant population difference in height increment, which is reflected in Fig. 3-10. The DE2 and BG populations had much smaller increments than the other four populations. The Bulgarian population was unique having a larger diameter and height increment in loamy sand than in the control. Nor did it show any diameter increment reduction in the loamy sand (Fig. 3-10). The type of soil did not result in any significant population effect but a significant population x soil interaction was found. As seen from Fig. 3-10 the soil treatment caused strong reductions in diameter increment in the Polish and the German populations in contrast to the southern
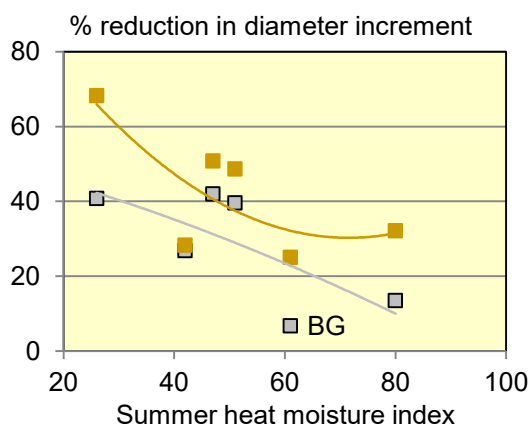
Figure 3-11. The relationships between summer heat moisture index and percentage reduction of diameter increment in sandy loam compared to loamy sand (grey), and percentage reduction of diameter increment in drought treatment (brown) in three central and three marginal beech populations . Thiel et al. (2014).

European populations, BG and ES. Together with the DE2 population they did not suffer as much from drought as the three other populations. The expectation that the Bulgarian and Spanish populations originating from regions with summer drought would have achieved adaptedness for prolonged dry summer conditions was thus fulfilled.

Several relationships between summer heat moisture index (SHMI) and traits were tested. Only the relationship with survival gave a strong relationship, $R^2 = 0.69$ while height increment, basal diameter increment, and mean leaf damage had R^2s in the range $0.01 – 0.31$. It was pointed out that the low number of populations might contribute to absence of strong relationships with SHMI. I tested the relationships between SHMI and percentage reduction in diameter increment (Fig. 3-11). None of them were particularly strong, $R^2 = 0.52 – 0.59$. Especially, the Bulgarian population is far below the fitted curve for the soil treatment.

Several results single out the Bulgarian population as different from the other populations and it was speculated that it might originate from another refugium during the latest glaciation than the other populations. The poor growth performance of the German DE2 population is noteworthy since it most likely originates from the same refugium as the two other German populations. The microsite conditions might have caused another genetic setup during the course of evolution of this deviating population. It was pointed out that a single drought treatment during the juvenile phase might not be enough to recommend populations for future regeneration of beech under

global warming. However, the speed of change seems to be so fast that postponing recommendations until results from long-time trials are available is not satisfactory.

One thought-worthy conclusion based on the published results was: *Therefore, the concept of marginality should be extended from a geographic to a more site-related concept.*

In another study by the same group of scientists (Kreyling et al. 2014) four hypotheses were addressed:

1. Frost tolerance depends on the climatic conditions at the origin of the populations
2. Warming reduces frost tolerance
3. Differences in winter frost hardiness are less than in resistance to late spring frosts
4. Preceding water stress increases frost resistance independently from phenological differences

Rather complex experiments were carried out at two localities; one designated as cold and the other as warm. No joint analysis could be carried out since the experiments started two different years. Four German populations from the central distribution of beech in Europe and three marginal populations, one from each of Bulgaria, Poland, and Spain were included in this study. All seven populations were autochthonous.

I have tried to summarize the treatments in the two experiments and a synthesis of results in Table 3-3.

The expectation in the first hypothesis was largely confirmed. It was suggested that there was stronger selection in combination with limited gene flow in the marginal populations than in the central populations. This would give rise to population differences.

The warming increased the survival contrary to hypothesis 2. Contrary to the central populations, there was a tendency to decreasing frost tolerance in the marginal populations, which evidently puzzled the authors.

The third hypothesis was confirmed. There were limited differences in winter frost damage among populations but large variation in spring frost damage.

Drought treatment caused reduced frost susceptibility.

The large mortality had deserved a discussion, especially the significant population effect with such high mortality as 99.2% in the control.

The large standard errors of most estimates were interpreted as large genetic variation within populations which might be true but there were no replications of individual seedlings. Therefore, purely environmental factors cannot be ruled out as cause of the large standard errors. However, the uniform environmental conditions speak in favor of the interpretation by the authors. If the interpretation by the authorsis correct, the large production of beechnuts by individual trees offers good prospects for future adaptation.

Cold locality	Warm locality
Mean plant height at the start of the experiment was measured. Watering with rainwater took place twice a week. Two warming treatments were applied with wind shelter reducing wind speed by 70% and by IR radiation, which increased the temperature in the soil at 2 cm below ground (-2 cm) with 1.5°C. The minimum temperature at -2 cm in the control was -19.1°C and in warming it was -15.8°C. The corresponding for air temperatures at 50 cm above ground were -18.6°C and -17.1°C. Twelve plants per population and treatment were included. Survival in the warming treatment was 15.5% and 0.8% in control. Strongly significant differences among populations, with higher survival in populations from cold sites. On January 11, 15 lateral buds from 12 plants per population and warming treatment were sampled. Subsamples were used in freezing treatments. Relative electrolyte leakage (REL) after freeze testing was determined at six levels from -10°C – -196°C. Frost tolerance was estimated as LT_{50}. No significant effect of warming or population and no relationship with origin mean temperatures and frost tolerance. Mean LT_{50} differed between central and marginal populations. Improved frost tolerance in central populations and reduced frost tolerance in marginal populations. Fine root frost tolerance was quantified by REL in three populations, one population from each of Bulgaria, Germany and Spain. The German population had the highest frost tolerance of these populations, LT_{50} = -23.1°C versus -14.1°C (ES) and -10.1°C (BG). Winter survival was assessed in May. Temperature data for the population original sites were obtained from WorldClim and used for relationships with observed data. Strong relationship for marginal populations, R^2 = 1.00. Less strong for central populations, R^2 = 0.50.	Mean plant height at the start of the experiment was measured. Watering with groundwater took place twice a week. One drought event started on May 9 and lasted for 36 days. After the drought treatment the seedlings were supplied with the amount water they had missed during the treatment. The drought treatment increased the frost tolerance of the seedlings during winter and spring the following year. Bud flushing was delayed in the drought treated seedlings. On February 6 and April 16, 15 lateral buds from four seedlings per population and drought treatment (control and 36-day no watering) were sampled. Subsamples were used in freezing treatments. Relative electrolyte leakage (REL) after freeze testing was determined at six levels from -10°C – -196°C. Frost tolerance was estimated as LT_{50}. Mean LT_{50} in February second winter was -39.3°C without significant population differences. On April 16 there was significant and positive relationship between LT_{50} and mean minimum April temperatures at population origin. R^2 marginal = 0.96, R^2 central = 0.51. Bud flushing was recorded in four classes on April 16 two consecutive years. Significances were noted for population x year interaction and year but not for population in spite of a wide range of percentages in bud dormancy on this date, 15 – 50%. Large standard errors explain the absence of significances for populations (Data were not shown for all populations every year).

Four hypotheses were presented in an investigation comprising three German populations studied in the three regions with different levels of water availability by Baudis et al. (2014):

1. Reduction of stomatal conductance and biomass production are reduced following drought exposure
2. Stomatal conductance and biomass growth are reduced in response to competition from other plants
3. Populations from dry sites are better adapted to drought conditions; moreover populations are better adapted to their original site condition
4. There is a close relationship between physiological traits and growth traits

Experiments with three populations from northern Germany (NG), central Germany (CG), and alpine Germany (AG) were established in each region. In some plots the seedlings were planted with the understory plants left, in others all competing plants within a radius of 20 cm were removed. Roofs were used to regulate precipitation. The reduction of precipitation varied in the range 23.2 – 35.6% at the three test localities. Stomatal conductance (g_s) was measured at some dates and transpiration rate was calculated. Photosynthetic active radiation (PAR) was measured once in July on plant apexes. Plant height, basal stem diameter, and leaf growth rates were determined.

The southern German population had the highest survival of the three populations 91.8% and the central German population had the lowest survival, 85.9%. For most of the traits assessed there were no significant population differences. Some treatment effects were noted; drought and competition affected stomatal conductance significantly at the measurement in July but not in September. For the July assessments there was a reduction in stoma-
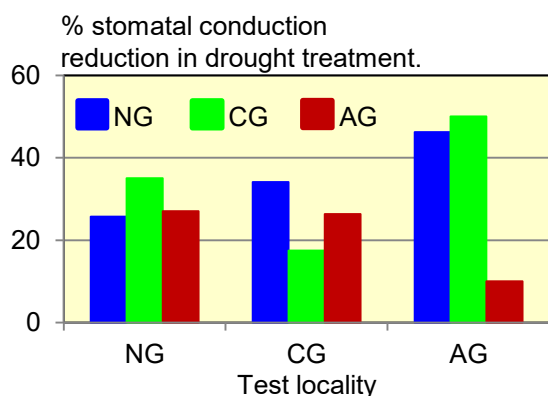
Figure 3-12 *Difference in stomatal conductance between control and drought treatment in % of three German populations from northern (NG), central (CG) , and alpine Germany (AG) grown in these three regions. Data obtained from Fig. 1 in the paper. Baudis et al. 2014.*

tal conductance under drought conditions in the three populations at all three test localities (Fig. 3-12). It seems as the population from southern Germany (AG) suffered less from drought than the central population. However, the measured conductivities were low, which means that large percentage differences might be observed even with small differences.

There was a significant population effect on plant height growth rate (Fig. 3-13) and crown projection area. The poorest height growth was noted for the NG site and the highest for the CG locality. It is somewhat surprising that the high-elevation population, AG, performs so well under low-land conditions. Relative growth rate is dependent on the starting plant height, which complicates the interpretation of growth in absence of values of initial height. It would have been useful to have plant heights before the onset of treatment as well as after finishing the experiments. It was concluded that the growth results did not support the third hypothesis.

Except for crown projection area, vegetation cover did not show any significant effect on any other traits. Crown projection area also showed significance for the interaction effect, vegetation cover x population. The second
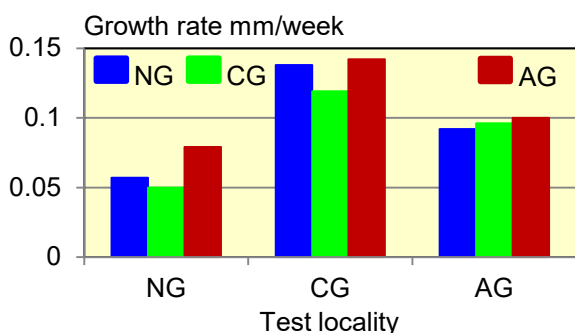

Figure 3-13 *The relative growth rate for plant height in mm/week of three German populations from northern (NG), central (CG) , and alpine Germany (AG) grown in these three regions. Data obtained from Fig. A8 in supplementary information. Baudis et al. 2014.*
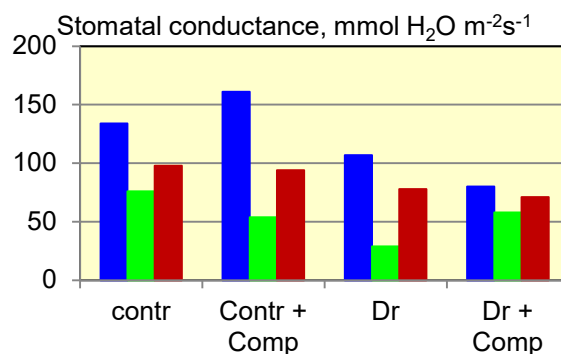

Figure 3-14 *Stomatal conductance of three German populations included in an experiment at three localities in Germany with increasing water availability northern trial (blue), central trial(green), and alpine Germany trial (brown) with four treatments: Control, Control + competition from surounding vegetaion, Drought, and Drought + competition from surounding vegetaion. Data from Fig. 2 in the paper. Baudis et al. 2014.*

hypothesis presented above (Reduction of stomatal conductance and biomass growth in response to competition from other plants) was rejected since there were no consistent effects of drought and competition. Fig. 3-14, which does not contain any genetic information, presents results concerning the effect of drought and competition on stomatal conductance. The lowest conductivity in all treatments was noted for the central German trial, while the conductivity was highest in the northern trial.

It might be speculated that the contrasts in the treatments were too limited. However, the range for precipitation during May – August was approximately 250 – 340 mm, which might have caused some differentiation between the AG population and the two other populations, which both had approximately 250 mm precipitation during May – August.

A study was carried out by Harter et al. (2015) with the objective of studying the relationship between population genetic diversity and phenotypic plasticity (for estimation of this trait see p. 46) for growth or phenology traits. Three German and three Bulgarian populations were included in this study. Unfortunately, the elevation at population origins was not given, which means that the impact of population elevation cannot be evaluated. Besides control, three treatments were applied; drought, warming, and combined drought and warming. The experiment took place outdoors with plants in 12-L pots. In the drought treatment the plants did not receive any water for 60 days starting on May 17. In the warming treatment infrared radiation was applied resulting in an increase of temperature by 1.6°C. The date when the first green leaf of a bud was visible was used for determination of bud flushing. Weekly recordings of bud flushing were carried out. Senescence was reached when all leaves had turned brown. How senescence was followed was not informed. There were three plants in three replications in each treatment. Ten isozyme loci were studied to get population genetic diversity estimates of the populations. These data
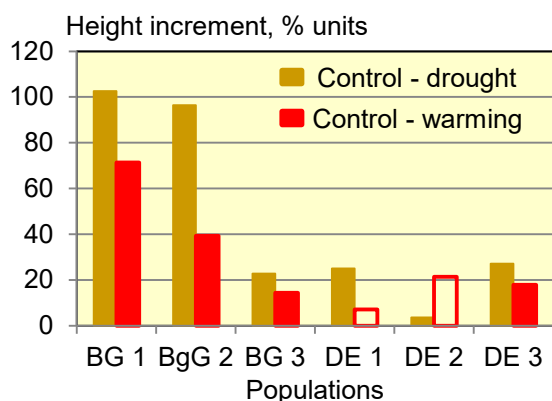
Figure 3-15 The difference between control and drought treatment and control and warming treatment in percentage units for median height increment in an experiment with artificial drought and heat treatment with three populations from each of Bulgaria and Germany. The height increment refers to the second growth period. Empty column = larger increment in treatment than in control.. Harter et al. 2015.

were related to the phenotypic plasticity of growth and phenology data.

There were significant effects for population as regards height increment and bud flushing. Warming influenced the bud flushing in a significant way but not height increment. No significant effects were noted for leaf senescence. Since there was no information how the recording for this trait was done, it is hard to come to a solid interpretation for the cause of this. At the regional level (Bulgaria versus Germany), bud flushing was the only trait that had a significant effect.

Based on Fig. 3 in the paper I have illustrated the difference in percentage units between median values for control on one hand and drought and warming treatments on the other hand (Fig. 3-15). Unfortunately, the quality of figure 3 in the paper was not good enough to read the median values for the combined drought and warming treatment, which would have been useful for the interpretation of the results. Fig. 3-15 gives an idea about the phe-
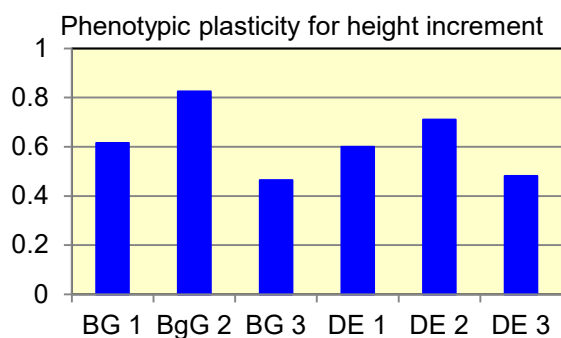

Figure 3-16. Phenotypic plasticity for height increment during the second growth period in three Bulgarian and three German populations in an experiment with artificial drought and heat treatment in an investigation, which was carried out in Germany. Phenotypic plasticity estimated according to Valladares et al. (2006). Harter et al 2015.
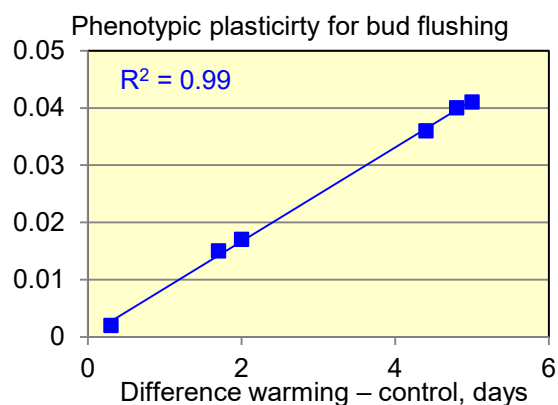

Figure 3-17. The relationship between bud flushing in warming treatment and control in days in three Bulgarian and three German populations in an experiment with artificial drought and heat treatment and estimated phenotypic plasticity. Bud flushing refers to the second growth period. Harter et al 2015.

notypic plasticity for height increment. The largest differences in both treatments were noted for two of the BG1 and BG2 populations, which to a large extent can be attributed to their good growth in the control treatment. The DE2 population showed the poorest growth of all populations with a height increment of 25 % compared to the height increments of more than 100 % units in the BG1 and BG2 populations. Warming caused reduced growth in four populations while warming caused an increased growth of approximately 20 percentage units in the DE2 population. The calculated values for height increment phenotypic plasticity varied in the range $0.46 – 0.82$ (Fig. 3-16). I would expect some relationship between the results in Figs 3-15 and 3-16. Such a relationship is disturbed by the phenotypic plasticity of DE2, which was the second largest estimate, 0.71. Moreover, there is no support for a large estimate from the median values of Fig. 2B in the paper. Could it be a miscalculation of the phenotypic plasticity estimate for population DE2? I missed a discussion of the performance of the DE2 population in this paper.

The difference between flushing dates in the warming treatment and the flushing dates in the control material was read in Fig. 4A in the paper. Thus obtained number of days was plotted against the published phenotypic plasticity estimates in Fig. 3-17, which shows a perfect relationship. This suggests that just measuring differences is as good for estimation of phenotypic plasticity as calculation of phenotypic plasticity. However, when data from several treatments are used for estimation of phenotypic plasticity simple measurements cannot be used for a combined estimate of phenotypic plasticity. A comparison of Figs. 3-16 and 3-17 reveals that the phenotypic plasticity for bud flushing was up to 40 times smaller than for height increment. Since there was a population difference in flushing date between control and warming treatment, certainly small but significant, the statement that photoperiod was responsible for this difference is not supported by the obtained data.

The isoenzyme analysis revealed that 90% of the variation was within populations. The differentiation among the Bulgarian populations was larger than among the German populations (F_{ST} 0.09 versus 0.03). It should be noted that the elevational range of the Bulgarian populations was large, 200 – 1,600 masl, which probably explain the relatively large F_{ST} for the Bulgarian populations. A significant relationship between allelic diversity and height increment plasticity was noted. However, the degree of explanation for this relationship was less than 45%. As seen from Table 2 in the paper population DE2 deviated strongly from the regression line (Fig. 5 A in the paper) supporting my suspicion of an unexpected high phenotypic plasticity estimate for plant height increment in this population. It was admitted that diversity estimated by isozymes might not be the best method for such estimates. It was suggested that molecular markers would give more informative results. One might question why molecular markers were not used in this study?

No significant relationships were noted for phenotypic plasticity and climate parameters at the origin of the populations.

A larger number of populations from an ecological gradient studied with nuclear microsatellites and growth and phenology in different treatments might give a stronger support to the hypotheses presented in the paper, or alternatively a rejection of these hypotheses.

Sánchez-Gómez et al. (2013) studied population variability in leaf functional traits under reduced water availability in the six populations listed below:

Spain	42.02ºN	1,325 masl
Italy	46.04ºN	1,130 masl
Germany 1	48.94ºN	525 masl
Germany 2	47.74ºN	880 masl
Germany 3	48.18ºN	560 masl
Sweden	58.86 N	150 masl

Seedlings from the six populations were exposed to two treatments in green house; control and water deficit. Control treatment was watered to field capacity during the entire experiment, which ended at Julian day 248. The water deficit treatment was designed according to the following:

Julian days 112-150 watering to field capacity (T1)
Julian days 151-178 water in the pots was depleted to 15% (T2)
Julian days 179-200 water in the pots was depleted to 13% (T3)
Julian days 201-248 watering to field capacity (T4)

The traits studied are listed in Table 3-4. The main focus of this report was on plant physiology but population differences of the studied populations were also discussed.
I have tried to summarize the main results In Table 3-4 and illustrated the development of area-based maximum photosynthetic rate during the course of this experiment in Fig. 3-18. The drought treatment caused a considerable drop in the maximum photosynthetic rate, especially in
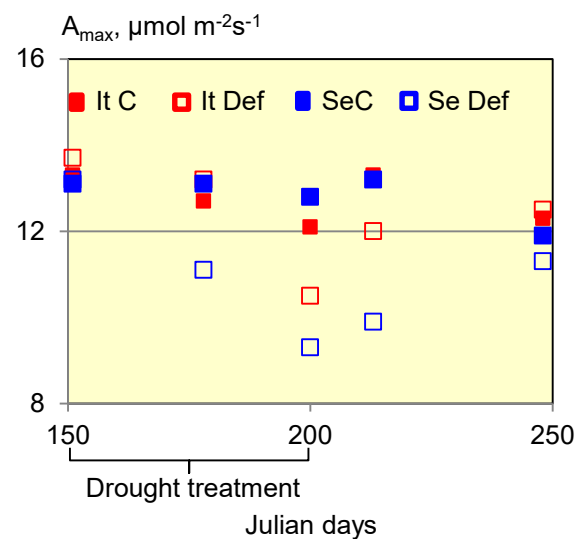

Figure 3-18 The development of A_{max} (area-based maximum photosynthetic rate) for one Italian (red) and one Swedish (blue) population exposed for water deficit between Julian days 151-200 (open squares and in control material filled squares) with full field capacity of water. Sánchez-Gómez et al. (2013).

the Swedish population. As seen from Table 3-4 population effects were significant for nine of the ten traits studied. It was noted that the principle component analysis resulted in different patterns for the two treatments. Under no water limitation the Spanish and Italian populations grouped together and differed from the German group of populations as well as from the marginal population from Sweden. This grouping did not remain in the water deficit treatment.
It was stated that tolerance to water deficit was related to photosynthesis nitrogen use efficiency (PNUE). Thus, the Spanish population had the highest PNUE in the drought treatment in this investigation and one of the smallest differences between the two treatments (Fig. 3-19).
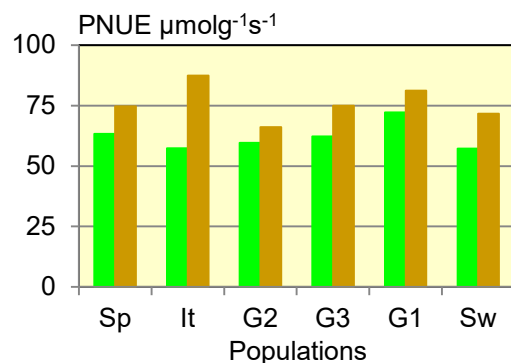

Figure 3-19. Least square means of photosynthetic nitrogen use efficiency (PNUE) in a water deficit (brown) treatment and control (green) at Julian day 200 in six populations arranged according to increasing original latitude. Es = Spanish, G = German, It = Italian, and Sw = Swedish. Sánchez-Gómez et al. (2013).

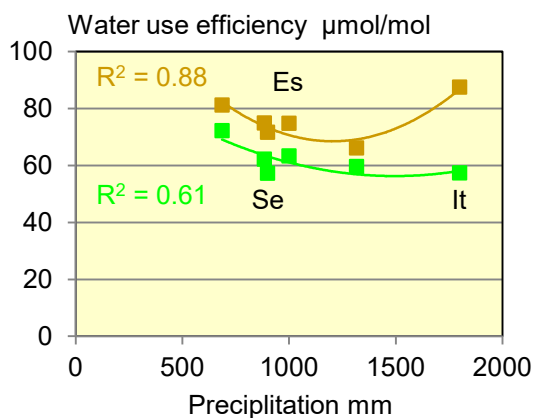| Trait	population	Treatment	Population x Treatment
A_{max}, area-based maximum photosynthetic rate	***	***	***
g_i stomatal conductance	**	***	n
Φ_{PSI}, effective quantum efficiency of PSII	***	ns	ns
SLA specific leaf area	*	***	ns
N_m, mass-based nitrogen content	***	**	ns
N_a, area-based nitrogen	***	***	ns
$\delta^{13}C$, carbon isotope discrimination	*	***	*
PNUE, photosynthetic nitrogen use efficiency	**	***	*
A_{mmax}, mass-based maximum photosynthetic rate	ns	***	*
WUE_i, instantaneous water use efficiency	**	***	*

Figure 3-20. The relationships between annual precipitation at population origin and least square means of water use efficiency in a water deficit (brown) treatment and control material (green) at Julian day 200. Sánchez-Gómez et al. (2013).
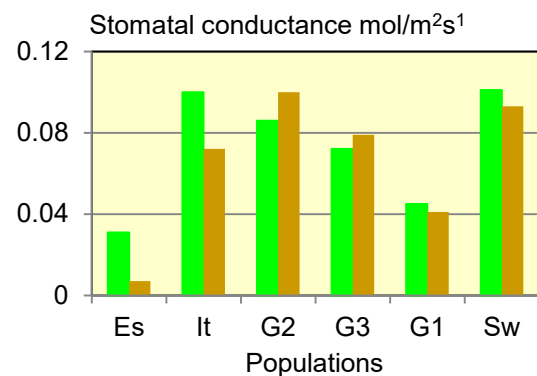

Figure 3-21. The stomatal conductance in control (green) and a water deficit (brown) treatment at Julian days 200 and 213 in six populations arranged according to increasing original latitude. Es = Spanish, G = German, It = Italian, and Sw = Swedish. Sánchez-Gómez et al. (2013).

There was a fairly strong relationship between annual precipitation at population origin and water use efficiency in the control material (Fig. 3-20). The strong relationship between the two variables in the drought treatment (R^2 = 0.88) is hard to explain. The high elevation Italian population (1,130 masl) was characterized by a large difference between the two treatments (Fig. 3-20) while the high-elevation Spanish population (1,325 masl) had a smaller difference between the two treatments. The low impact on drought of the Spanish population (1,000 mm) might be attributed to an adaptation to summer drought. The relatively strong response of the Swedish population (900 mm precipitation, Fig. 3-20) was expected since it originates from the maritime climate of south-western

Sweden. The German G1 population deviated significantly from the other five populations in the control treatment while the inter-population differences showed a complex pattern in the water deficit treatments. I could not trace any clear relationship between population latitude and water use efficiency.

I have illustrated the difference between the two treatments (plasticity) of the populations for stomatal conductance. This trait differed considerably between the two treatments (Fig. 3-21). The Spanish population had much lower stomatal conductance in both treatments than the other five populations. For stomatal conductance the G1 population differed from the rest of the populations suggesting a different adaptation.

Table 3-5. Significances for effects of population and treatment on different gas exchange traits in an experiment with drought treatment of six populations from a wide latitudinal range 41.07-56.52. Aranda et al. 2014.

Trait	Peak of drought		Before drought
	Population	Treatment	Population
A_{net}	***	***	ns
G_{sw}	***	***	ns
Φ_{PSII}	*	***	ns
Ψ_{pd}	ns	***	ns
Ψ_{md}	**	**	ns

Table 3-6. Spearman correlation coefficients, r, for trait relationships in the two treatments; well-watered and water-stressed. Only significant relationships are presented. Ψ_{12} means 12% reduction of the water potential. The treatment involved in the significant relationship is indicated. Aranda et al. 2014.

Trait	Wood density, g cm^{-3}	Water potential Ψ_{12} MPa
Xylem specific conductivity Ks, mol m^{-1} s^{-1} MPa^{-1}	Water-stressed, negative r = 0.88**	Water-stressed, positive r = 0.83**
Effective quantum efficiency of photosystem II, Φ_{PSII}	Water-stressed, negative r = 0.77*	Well-watered, negative r = 0.83**
Net assimilation rate, A_{net}, mol m^{-2} s^{-1}	Water-stressed, negative R = 0.77*	Well-watered, negative R = 0.83**

It is valuable that links between genetics and plant physiology are created to get a basic understanding of causes behind observed genetic differences in tree growth.

The plants studied in the above paper were followed for a second growth period with an additional water deficit treatment (Aranda et al. 2014). The objective of this investigation was to identify genetic differences among populations as regards physiological and hydraulic–related traits and their phenotypic plasticity. The same treatment as in the above paper was applied starting at May 20. After 50 days there was a peak in drought with 8% of water volume in the substrate. Gas exchange was measured at the start of the drought treatment and at the peak of drought. As in the previous study the following traits were measured:

   Net assimilation rate, A_{net}
   Stomatal conductance G_{wv}
   Effective quantum efficiency of photosystem II, Φ_{PSII}

In addition, water potentials at predawn, Ψ_{pd}, and midday, Ψ_{md}, were measured at the peak of drought.

After the drought treatment the plants were watered to field capacity and xylem cavitation was measured and the hydraulic conductance, k_i, of each sample was measured. The percentage loss of hydraulic conductance was calculated, PLC. The xylem-specific conductivity was also calculated, K_s. Wood density was determined. Finally, phenotypic plasticity was estimated as a log-response ratio of the traits, ln in well-watered treatment – ln in drought treatment. Ten to twelve plants per population were included in this experiment.

Table 3-5 summarizes the main results from the gas exchange measurements. Before drought was applied no significant effects were noted for populations for any of the five traits listed in this table. In contrast to the other populations the German G2 population did not show any decrease in Φ_{PSII}. Its phenotypic plasticity was also non-significant. The treatment effect for Ψ_{50} (loss of 50% of hydraulic conductance) of this population was significant. It seems as G2 is an outlier population. The Spanish and Italian populations had the highest estimates of A_{net} and G_{wv} in both treatments while G1 had low estimates in both treatments.

Except for the Swedish population, no significant treatment effects on wood density were noted. As regards hydraulic conductance no plasticity was noted. In spite of a large variation in hydraulic conductivity among the
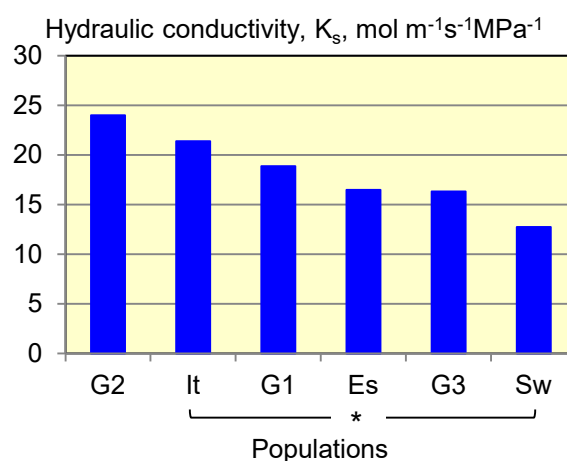

Figure 3-22. Hydraulic conductivity in six populations arranged according to decreasing K_s at peak of drought in a treatment with reduced water availability at age 2. The only significant difference Italy – Sweden is illustrated.. Es = Spanish, G = German, It = Italian, and Sw = Swedish. Aranda et al. (2014).

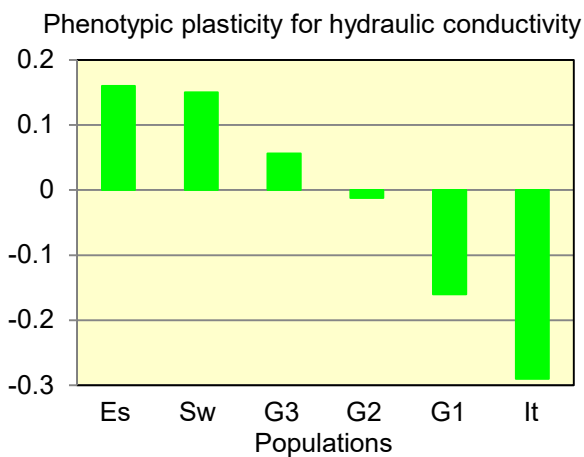## Phenotypic plasticity for hydraulic conductivity

Figure 3-23. Phenotypic plasticity in hydraulic conductivity in six populations at age 2. Two treatments were applied, control and reduction of water potential to 8% in a drought treatment. Positive estimates indicate higher value in the well-watered treatment while negative estimates mean higher estimates in drought treatment. Es = Spanish, G = German, It = Italian, and Sw = Swedish. Aranda et al. 2014.

populations only once there was a significant difference between two populations. The Swedish population had a significantly lower hydraulic conductivity than the Italian population in the water stress treatment (Fig. 3-22). The variation among populations in Ψ_{50} in the drought treatment was limited, -3.34 - -3.56 MPa. Significant effects on Ψ_{md} were noted for populations and treatment while the interaction population x treatment was insignificant. The three German populations had lower values of Ψ_{md} than the other populations.

There were differences in phenotypic plasticity of hydraulic conductance among the populations (Fig. 3-23) but standard errors of these estimates were large, which means that no significance was noted for this trait.

Significant trait relationships are presented in Table 3-6. They originate from Fig. 4 in the paper, in which only strong relationships are shown. As seen from Table 3-6 some of the relationships are strong, r> 0.80. However, the graphic presentations of them do not indicate such strong correlation coefficients. It is hard to draw any far-reaching conclusions based on these relationships. The negative relationship between wood density and K_s in the water-stressed material was expected since a relatively large wall volume reduces the risk for cavitation via high K_s.

It was concluded that there were small but significant differences in the drought response traits of trees of the provenances tested. A comment to this is that genetic studies mostly need larger numbers of tested plants than are feasible in sophisticated plant physiology studies. Without large materials it is hard to detect genetic differences. This can explain absence of genetic differences in some cases. The role of the traits studied in adaptation to drought conditions was discussed. It is evident from the results that the two southern populations have a higher

adaptedness to drought conditions than some of the other populations. However, for future evolution it is critical that there is enough additive variance for traits contributing to fitness. Unfortunately, such studies require large number of plants/trees per family as well as many families.

Another study of the same group focused on population differences in leaf metabolic profiles (Aranda et al. 2017). Two-year old seedlings of one population from each of Spain Germany, and Sweden were studied in this case. In all 63 compounds including organic and fatty acids, saccharides and poly-alcohols, amino acids, and polyphenolic and terpenoid compounds were studied. In addition, physiology traits, most of them common with the previous study, were also analyzed.

The impact of water deficit on the production of metabolic compounds was limited. The Spanish population differed from the German and Swedish populations by its larger concentration of fumaric acid, succinic acid, threonine, and valine, as well as the secondary metabolite kaempherol. As seen from the graphic illustrations in the paper there was a large variation among the six or seven plants analyzed, which reduced the precision of the estimated amounts of the different metabolic compounds.

There were significant differences among populations in the drought treatment for carbon/nitrogen ratio, net photosynthesis on leaf area basis, ^{15}N isotope fractioning, nitrogen use efficiency, and water use efficiency.

It was concluded that population differences in metabolic compounds were larger than differences in physiological traits, which calls for more focus on metabolomics.

One investigation with the purpose of studying the adaptedness to soil conditions was carried out by Manzanedo et al. (2018). Seedlings 2 – 3 year old were collected in three localities at different elevations in Germany with differing soil moisture content and were transplanted to pots (13 x 13 x 13 cm) and placed in greenhouse for a factorial experiment with 36 (3 x 3 x 2 x 2) different treatment combinations:

  3 populations
  3 soil types from the population origins
    Inoculation with soil from these localities or no inoculation
  2 drought treatments

This investigation was carried out in The Botanical Garden, Bern, Switzerland. In all, 25 blocks were used, of which 12 were randomly selected for a 6-week drought treatment. A 50% reduction of soil water was aimed at. The objective of this mild drought treatment was to study the effect of temporary drought likely to occur owing to global warming. Above ground biomass was determined after defoliation in November - December. The occurrence of mycorrhiza on all root fragments was recorded in 18 plants from each of six blocks. Initial size of the seedlings was measured to use as covariates in the statistical analyses. In the following the populations will be referred
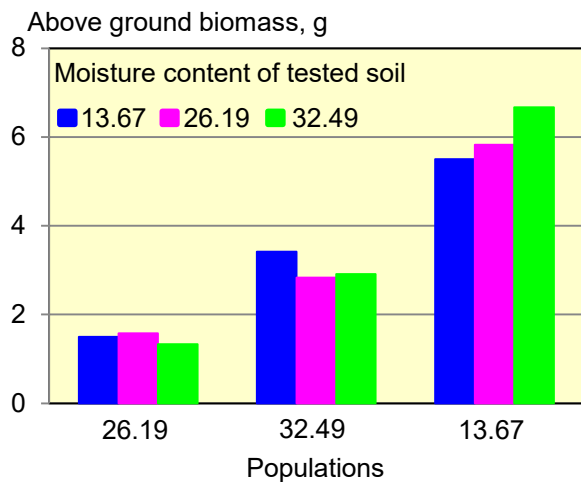
Figure 3-24. Above ground biomass increment in three German populations originating from sites with soil moisture content at 10 cm of 13.67, 26.19, and 32.49% and cultivated on soil from these three localities. Manzanedo et al. 2018.).

to as 13.67, 26.19, and 32.49, which are the soil water content at 10 cm in the soil. Similarly, the soil from the three localities will also use this identification. No exact geographic information on the localities was given. The 13.67-population originated from an elevation span of 3 – 140 masl. The corresponding spans for the two other populations were 285 – 550 and 460-880 masl.

The ANOVA revealed that the population effect on above ground biomass increment was strongly significant (Fig. 3-24) with the 13.67 low-elevation population showing the largest increment and population 26.19 had the poorest growth increment. The growth substrate did not influence the growth in a significant way. Nor was there any significant effect of drought treatment. The interactions population x soil origin and population x drought treatment were significant at 0.01 and 0.05% levels. Only
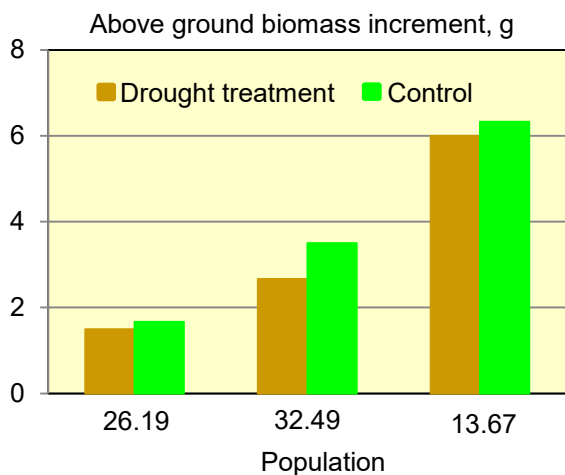

Figure 3-25. Above ground biomass increment in three German populatins originating from sites with soil moisture at 10 cm of 13.67, 26.19, and 32.49% and exposed to a mild drought treatment; 50% reduction of soil water for six weeks. Manzanedo et al. 2018.
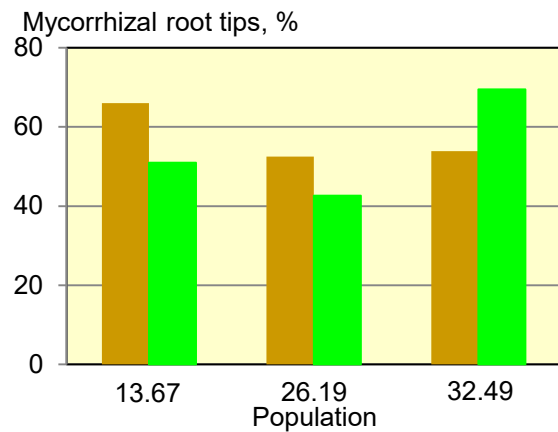

Figure 3-26. The percentage of root tips with mycorrhize in drought and control treatments in three German populations originating from different soil water content at their site of origin. Manzanedo et al. 2018..

the 26.19 population had the highest increment in the soil from its own original site. The lack of best performance of the two other populations in their own soil was attributed to local maladaptation to fungi existing in their "own" soil or constraints caused by local pathogens. These results were interpreted as evidence for no adaptation to soil conditions during the course of evolution of these populations.

The largest drop in growth following drought treatment was noted for the 32.49-population amounting almost to 25% (Fig. 3-25). The significant population x drought treatment interaction must be attributed to the difference in growth reduction among the populations. It was suggested that 32.49-population in contrast to the other two populations had evolved under conditions without pronounced drought conditions. Fig. 3-26 reveals that the percentage of root tips with mycorrhiza in two populations increased in the drought treatment while it dropped in the 32.49-population. It was suggested that mycorrhiza support the host seedlings under limited water availability in the 13.67- and 26.19-populations. It was speculated that mycorrhizal communities do not differ as much as had been expected. As a corollary of this the limited impact of mycorrhiza could be explained. There was no increase in mycorrhiza presence when the cultivation took place in the "own" soil. It was stated that mycorrhizal communities were unresponsive to plant origin, soil origin, and stressful conditions.

The authors did not expect such a strong population effect on biomass increment as was observed considering the substantial gene flow among populations. It was speculated that the growth conditions in Bern were most related to the conditions of the 32.19-population and might have contributed to the observed population differences. The impact of elevation on the results cannot be evaluated since only ranges of elevation were given for the populations. it is likely that the differences in elevation probably paid a role in the differentiation among populations.
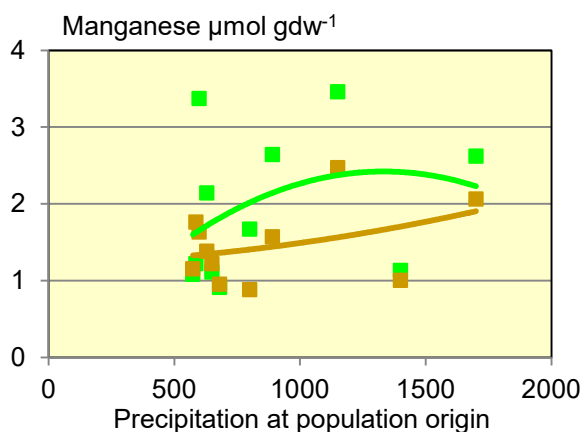
Figure 3-27 *Manganese content in roots of eleven German populations in control (green) and drought (brown) treatment during the first growth period. Peuke and Rennenberg 2011.*

A German study with the objective to estimate the effect of summer drought treatment on macro and micro nutrient elements in eleven German populations from regions with varying precipitation (574 – 1,700 mm) was conducted by Peuke and Rennenberg (2011). Twelve weeks after sowing, plants were separated into two groups, one with continued watering every second day and the other group with a watering regime aiming at 20% of the maximum water-holding capacity in the substrate. The duration of the drought treatment was not presented. At harvest leaves, stems, and roots were separated. Ca, K, Mg, Mn, B, Fe, Al, and Zn were determined by an inductively coupled plasma atomic emission spectrometer. Chloride and other anions were measured in solutions in a
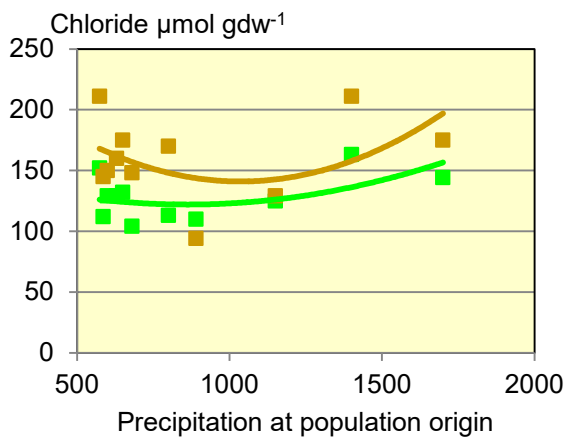

Figure 3-28 *Chloride ion content in leaves of eleven German populations in control (green) and drought (brown) treatment during the first growth period. Peuke and Rennenberg 2011.*
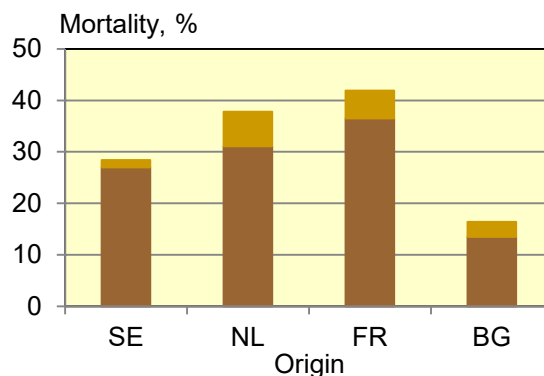

Figure 3-29 *The mortality in one population from each of Sweden, The Netherlands , France, and Bulgaria in 2001 (dark brown) and 2012 (light brown) in a provenance trial in The Netherlands established in 1998. Eilmann et al. 2014.*

high-performance liquid chromatography system and separated in an ion exchange column. Previously published data on water-soluble compounds were also discussed in this report.

Except for boron, there were significant differences among populations for all elements. Generally, there was a reduction of the metal ions following drought treatment with significant effects for most elements. No significant effects were noted for K and Mg.

Although the objective of this investigation was to trace any relationship between precipitation of the population origins and different elements no such relationships were illustrated in the paper. I have selected two relationships, which had large difference between control and drought treatment (Fig. 3-27 and 3-28). All relationships were weak. It was concluded that:.

The effects of drought on mineral concentration in roots, shoots and leaves seem to vary randomly across different provenances of beech A conclusion easy to agree with.

The possibility of introducing southern populations from areas with pronounced summer drought to more northern areas was studied by Eilmann et al. (2014). It was expected that such populations would match future drought owing to global warming. One population from each of Bulgaria (lat. 41.57°N, 1,450 masl), France (lat. 42.92°N, 670 masl), Sweden (lat. 56.12°N, 40 masl), and The Netherlands (lat. 51.94°N, 45 masl) covering much of beech distribution in Europe were selected for this investigation.

Besides growth and phenology, detailed studies of wood-anatomical traits were carried out (Table 3-7). Bud flushing comprised five classes with 1 = dormant bud and 5 = leaves completely unfolded.

Fig. 3-29 reveals that the mortality was highest during the first three years after establishment of the trial and that the lowest mortality was noted for the Bulgarian population
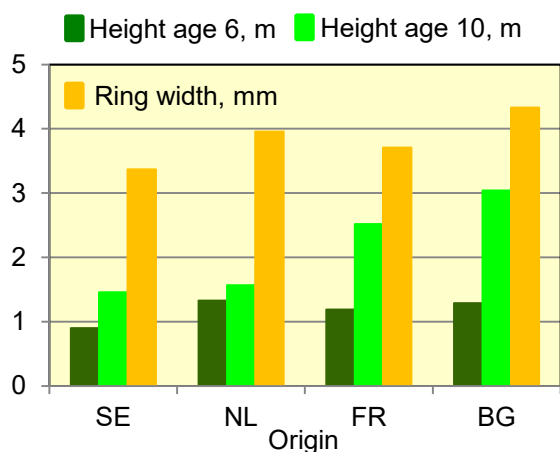
Figure 3-30. Tree height at ages 6 and 10 and tree-ring width at age 12 of one population from each of Sweden, The Netherlands, France, and Bulgaria in a provenance trial established in 1998 with 2-years old seedlings in The Netherlands. Eilmann et al. 2014..

This population showed the best individual tree growth and the largest tree-ring width (Fig. 3-30). Most of the superiority was achieved between ages 6 and 10. One of the reasons for this superiority was attributed to early start of the growth period, which differed considerably from the other three populations (Fig. 3-31, the higher the value the earlier the bud flushing). During the early part of the growth period the growth conditions are more favorable (long days and high photosynthetic capacity) than at the end of the growth period. Increased competition among populations might also contribute to larger differentiation among the populations.

Vessel density was the only wood-anatomical trait that did not show a significant population effect (Table 3-7). There was a similar development over time with respect to ring-width growth. There was a drop in tree-ring width in 2003, which was a dry year, while it peaked in 2007 with its favorable growth conditions. The most pronounced drop in 2003 was observed for the French population from The Pyrénées while the Bulgarian population was least affected by this drought. The poor performance of the Swedish population follows a general trend that

Table 3-7. The significances of population and population x time for wood traits studied in a Dutch provenance trial with one population from each of Bulgaria, France, Sweden, and The Netherlands. For the wood-anatomical studies thin sections from five trees per population were sampled. Eilmann et al. 2014.

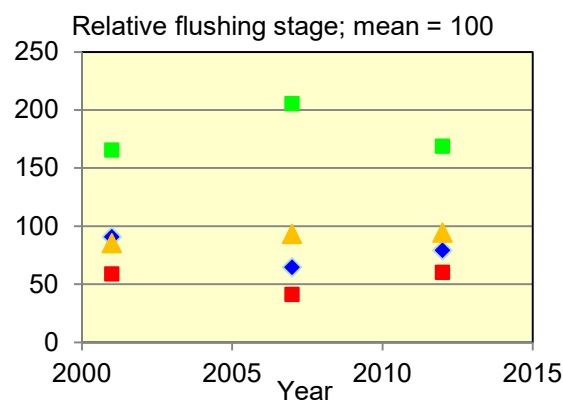| Trait	Population	Pop. x treatm.
Tree ring width	***	**
Mean vessel area	**	ns
Vessel density	ns	ns
Cumulative vessel area	***	**
Conductive area	***	ns

Figure 3-31. The relative flushing stage in one French (red) one Dutch (orange), one Swedish (blue), and one Bulgarian (green) population growing in a provenance trial in The Netherlands, which was established in 1998. Eilmann et al. 2014.

southward transfers of northern populations result in poor growth, which at least partly must be attributed to an early growth cessation. The ring-width growth was correlated with temperature before and during the growth period. Except for conductive area, the other wood-anatomical traits were significantly correlated with ring width.

The relatively poor performance of the French population was a surprise. It was expected that this population originating from a locality with dry summer climate would perform better. It was noted that *the water transport capacity of the Pyrénée Orientales provenance is much lower than that of the three other provenances*.

As regards the wood-anatomical traits, the most pronounced difference among the populations was noted for cumulative vessel area (Fig. 3-32). It was remarked that the Bulgarian population had the most efficient water-conducting system with high frequency of large vessels, largest cumulative vessel area, and conductive area. This means that this population has a more efficient water conductance than the other three populations. These characteristics of the Bulgarian population are most likely responsible for its good growth performance.

In conclusion, the performance of the Bulgarian population suggests that northward transfer of drought adapted populations might be used to mitigate drought conditions caused by global warming at more northerly localities. However, the performance of the French population contradicts this suggestion. Studies of a larger number of populations are required to reach a final conclusion as regards such northward transfers to meet future climatic conditions.

The impact of a short drought period on physiology-related traits such as photosynthesis, gas exchange, rapid light curves of chlorophyll a, and water use efficiency were studied in three Slovak beech populations from varying elevations, 530, 625, and 1,250 masl. by Pšidová et al. (2015). The annual precipitation of the populations were 596, 693, and 885 mm. The drought treatment consisted of a period of seven days without any water supply. This was followed by a recovery period of three days
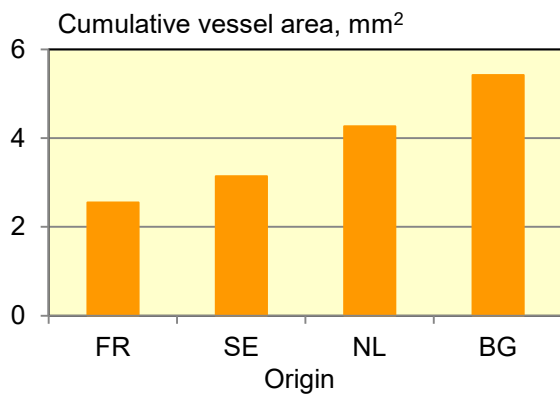
Figure 3-32. Cumulative vessel area at age 11 in one French, one Swedish, one Dutch, and one Bulgarian population growing in a provenance trial in The Netherlands,. Eilmann et al. 2014.

with watering and the recovery of the seedlings was recorded. The experiment was carried out under controlled conditions. Ten seedlings per population and treatment were analyzed.

Below, I have tried to summarize the main findings in this experiment. The populations are identified by their elevations at their sites of origin. Drought means estimates obtained after termination of the seven-day no-watering period; recovery means estimates after three days of recovery:

P_N = net photosynthesis effect:
the 625masl-population with an annual precipitation of 693 mm had a significantly lower estimate in drought treatment than all populations in the control; after recovery the 1,250 population had a significantly lower estimate than the control,

g_s = stomatal conductance:
the 625- and 1,250-populations had significantly lower estimates in drought treatment than all populations in the control; After recovery the 1,250-population had a significantly lower estimate than all populations in the control,

Ψ_{pd} = predawn water potential:
strong treatment effect, no population difference in control, drought, or after recovery within treatments; much of the strong drought treatment effect disappeared after three days of recovery,

$P_{N/gs}$ = WUE water use efficiency:
After recovery from drought treatment the 1,250-population had significantly higher WUE than in the two other populations (Fig. 3-33),
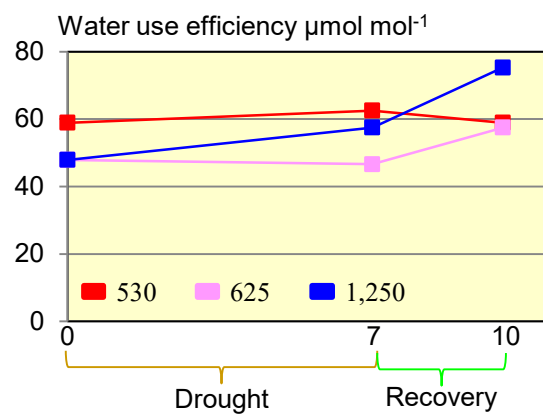

Figure 3-33. Water use efficiency in three Slovak populations with different annual precipitation in mm after 7 days without watering followed by 3 days of rccovery from the drought treatment. The elvation at population origin is indicated. Pšidová et al. (2015).

Φ_{PSII} = effective quantum yield of PSII;
The 625-population had the highest ability to utilize absorbed light in drought treatment.

It was concluded that the 1,250-population originating from the locality with highest precipitation exhibited the lowest resistance to water deficit at the level of leaf gas exchange, as well as the poorest recovery ability after re-watering.

The vulnerability to xylem cavitation was studied in 17 populations in a French trial belonging to an international series of beech provenance trials (Wortemann et al. (2011). A representative sample of six populations was also studied in a Slovak and a Spanish trial. Genotyping with six chloroplast microsatellites was carried out. Sunlit second order branches from the upper part of the crown were used for the vulnerability analysis. One specimen 28 cm long was tested for loss of conductance. Three levels of percentage loss on conductivity were estimated, P12, P50, and P88. At P12 an estimate of the xylem pressure at which embolism appears is determined while P88 is an estimate of xylem pressure at critical embolism.

The range of population means for P50 amounted to approximately 0.4 MPa. No significant effect of population for any of P12, P50, or P88 was noted. This absence of significance was attributed to the large variation within the populations with ranges up to 0.6 MPa. The estimates of populations on P50 varied among the three trials with an approximate range 0.4 – 0.6 MPa.
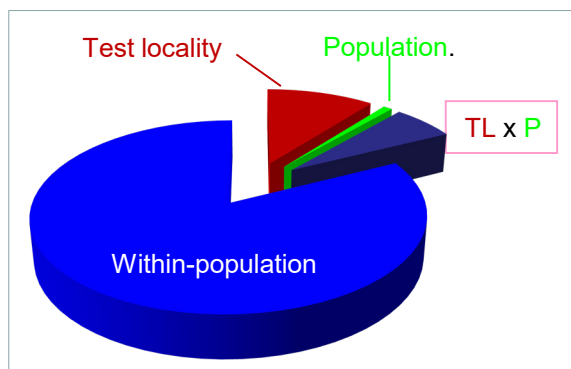
Figure 3-34. The percentage variance components of different effects on P50 conductivity in six beech populations studied in one field trial at age 15 in each of France, Slovakia, and Spain. Wortemann et al 2011.

In spite of absence of replication of individual trees (genotypes) the variance component for within-population effects was estimated (Fig. 3-34). It was justified by results from other published studies indicating strongly conserved P50 estimates among copies of single genotypes. This figure reveals that the effect of test locality is somewhat larger than the test locality x population interaction. The interaction indicates that the environmental conditions exert great impact on P50. The absence of significance for the population effect is reflected in its small variance component, 0.9%. Larger number of trees analyzed per population would be required to reveal population effects. This is hard to accomplish since measurements of conductivity is a laborious and thus demanding task.

In a Serbian provenance trial tree ring width, mean vessel area, vessel density, and water conductive area were studied during a seven-year period in three populations from mesic conditions (two German and one Croatian) and one Serbian population from dry conditions (Stojnic et al. 2013).

There was a fairly similar development of all four traits in the four populations without any significant population effects for any of the traits. Fig. 3-35 reveals that the ranking of the populations more or less remained from 2006 to 2012. Tree ring width and mean vessel area was largest in the Croatian population in 2012 while it had the lowest water conductive area. However, none of the differences were significant. The most important result was the similarity between the dry Serbian population and one of the German populations originating from a locality with approximately the double precipitation as the Serbian locality. This means that adaptation to water availability could not be proven

3.2 *In situ* observations

Xylem cavitation resistance is believed to be correlated with tolerance to extreme drought stress (Wortemann et al. 2011). Therefore, studies were carried out by Herbette et al. (2010) to estimate vulnerability to cavitation
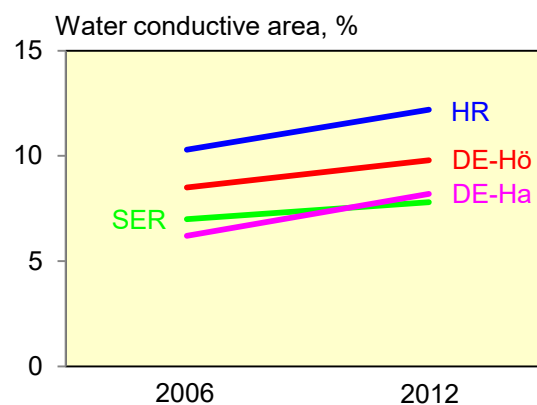

Figure 3-35. Water cunductive area in percent in four populations from Croatia (HR), Germany (DE-Hö and DE-Ha), and Serbia (SER) growing in a provenance trial in Serbia (Lat. 45.18°N, 1ong. 19.79°E, 370 masl) in year 2006 (prior to the establishment of the trial) and in 2012. Stojnic et al. 2013.

in French beech populations along a latitudinal gradient and two elevational gradients. The latitudinal range was 44.16 – 49.06ºN. One north facing and one south facing elevation gradient at latitude 44.06ºN were included with the elevational ranges, north 950-1520 masl. and south 930-1565 masl. The analysis of conductivity was carried out on branches with a diameter of 0.7 – 0.8 cm and 28 cm long collected *in situ*. Analyses were performed on specimens from the same side of the trees to avoid bias. The xylem water pressure resulting in 50% loss of hydraulic conductivity (P_{50}) was used as an estimate of vulnerability to cavitation.

There was a significant difference among the populations from the latitudinal range and a good fit to a straight line of the relationship between latitude and P50 with the lowest vulnerability of the northernmost population (Fig. 3-36). This finding was unexpected since the southern and most vulnerable population for cavitation grows under conditions with extended droughts, which might have provoked adaptation both to drought and vulnerability to cavitation. It was speculated that growth processes may have been favored for drought resistance instead of vulnerability to cavitation. Furthermore, it was stated that vulnerability to cavitation most likely is dependent on the environmental conditions during xylem vessel formation and thus differences in vulnerability among populations is dependent on phenology as well as ambient conditions during building up of xylem. Fig. 3-37 reveals that populations growing on the north-facing slope are less vulnerable than the populations on the southern slopes. At the southern slope there is a tendency to a clinal variation in vulnerability. However, these three populations did not differ significantly. The high-elevation population from the north-facing slope differed significantly from the other two north-facing populations. It was stated that the side of the mountain slope was more important for vulnerability to cavitation than elevation.
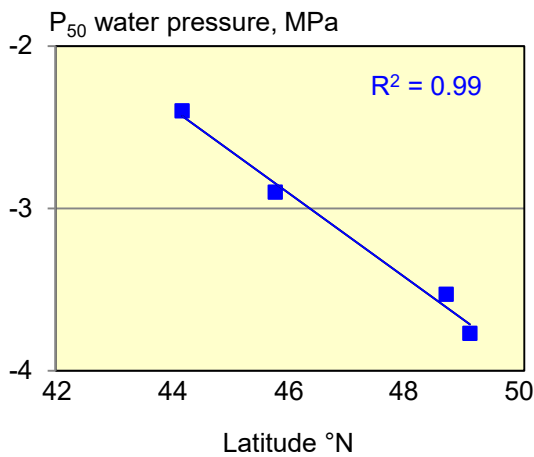
Figure 3-36. *The relationship between latitude at population origin of four French beech populations and water pressure at 50% reduction of hydraulic conductivity. Herbette et al. 2010.*

AFLP markers were used to study genetic differentiation between "close-to-natural" beech stands from three regions in Switzerland (Pluess and Weber 2012). In each region one mesic and one dry site with 39 – 41 trees were represented with an average distance of 540 meters between the pairs. The distances between the regions were 87, 128, and 186 km with mean tree ages varying in the range 111 – 135 years. Tree height and DBH were assessed. Two restriction enzymes, EcoR1 and Mse1 and ten primer-pairs were used for AFLP genotyping. Various F_{ST}s and clustering were estimated. Genetic diversity, differentiation, and clustering were estimated. The hierar-
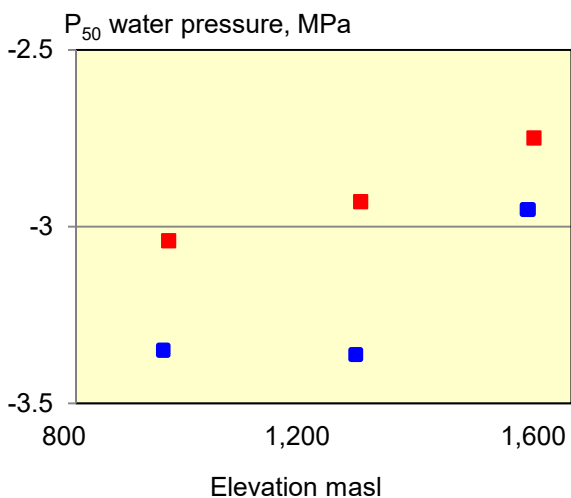

Figure 3-37. *The relationship between elevation at population origin in two elevational gradients in southern France and water pressure at 50% reduction of hydraulic conductivity, P50. Blue squares refer to the north-facing slope and red squares to the south-facing slope. Herbette et al. 2010.*
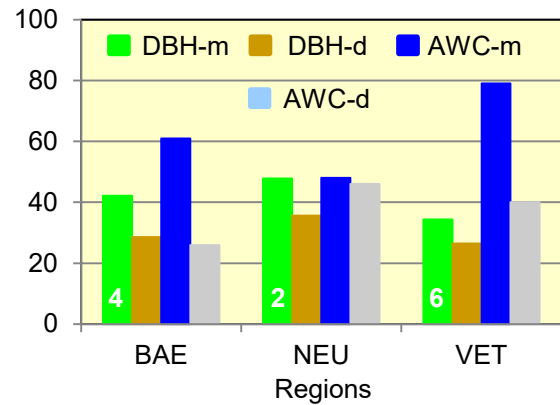

Figure 3-38. *In situ* asessments of breast height diameter in cm in mesic (DBH-m) and dry (DBH-d) sites and their corresponding available water capacity (AWC) in three regions in Switzerland. The number of AFLP outlier markers for separation of mesic and dry sites within these three regions are shown. Pluess and Weber 2012.

chical Bayesian method BayScan according to Foll and Gaggiotti (2008) was used to detect markers under selection. Drought indices (DRIs), available water capacities (AWCs), and field capacities (FCs) for the six stands were calculated. There were only minor differences between mesic and dry sites. DRI, AWC, and FC were related to AFLP markers with varying frequencies along the humidity gradient.

Fig. 3-38 reveals that there are substantial differences in growth between the mesic sites and their corresponding dry sites. Similarly, the available water capacity differs between the pairs in each region. This difference cannot be explained by difference in age since the trees in the dry sites were somewhat older than their counterparts in the mesic sites.

The percentages of polymorphic loci were somewhat higher in the mesic stands than.in the dry stands. Similarly, the expected heterozygosities were also slightly higher in mesic sites than in dry sites. However, there were no relationships between growth traits and genetic diversity estimates. One plausible explanation is that growth traits most likely are polygenically inherited and for this reason one cannot expect strong relationship with individual markers. A weak relationship between all pairwise F_{ST}s and geographic distance was noted, $R^2 = 0.16$. No grouping of the individual trees was found in the Bayesian cluster analysis.The inbreeding coefficient was low and no significant deviation from zero in any of the stands was noted. The global F_{ST} was low, 0.028, suggesting substantial gene flow among the six populations.

The Bayesian outlier analysis resulted in 13 markers that had a higher differentiation than expected under the null hypothesis of no differentiation between dry and mesic
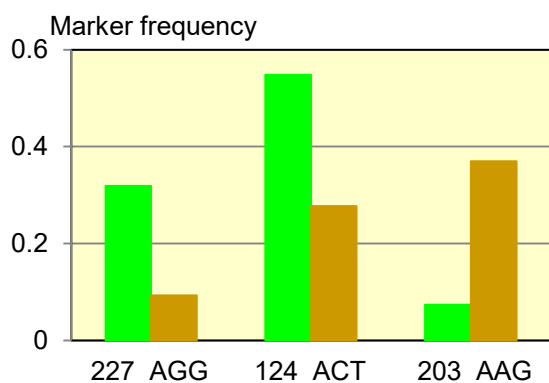
Figure 3-39. *Frequencies of three markers in mesic (green) and dry (brown) sites in each of three regions in Switzerland with contrasting frequencies in the two types of site conditions. The total number of trees analyzed was 241. Pluess and Weber 2012.*

sites. In the comparison of all mesic sites with dry sites, three markers were detected that might be linked to genes under selection or possibly selected for drought tolerance (Fig. 3-39). Seven of the 13 markers had similar changes in marker frequencies in more than one region, which supports the notion that they reflect adaptedness or are linked to fitness-contributing genes. A larger number of markers (41) were related to the three drought variables, AWC (16), FC (4), and DRI (29).

The main conclusion by the authors of this publication was that microevolutionary processes occur within short geographic distances even in presence of strong gene flow. The differentiation observed did not show any strong relationship to growth traits. Therefore, the cause of the difference in genetic diversity between mesic and dry site populations remains unresolved.

The same six stands in three regions were included in another investigation by Weber et al (2013) with the aim:
1. To evaluate the drought response of beech near its dry distribution limit
2. To test if drought response is more pronounced at dry than at mesic sites
3. To test if changes in climate have an impact on the pattern of growth responses for dry and mesic sites.

Ten dominant trees in each of the six stands were selected for this study. Ring width was recorded on two cores taken at 120° on each trunk.

Several measures of sensitivity of growth response to annual variation in weather condition were estimated. The mean sensitivity (MSs) at a site was estimated by analyzing the percentage variability in annual growth from one year to the next. Years with strongly deviating growth during five-year periods were coined pointer years. Pointer years thus identify the temporal distribution of extreme years.
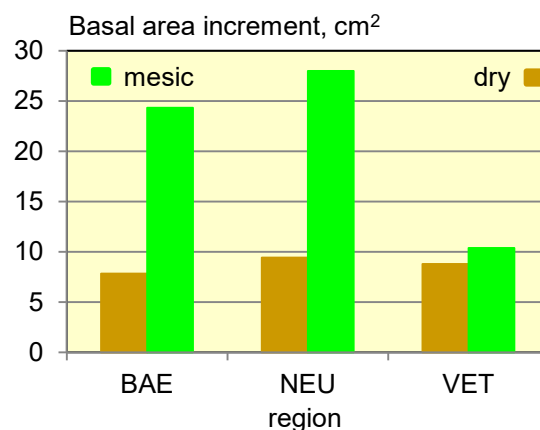

Figure 3-40. *Basal area increment in mesic (DBH-m) and dry (DBH-d) sites in three regions in Switzerland. Each region had one dry and one mesic site. Weber et al. 2013.*

Fig. 3-40 gives information on the growth at the six sites. There was a conspicuous difference in basal area increment between the dry and mesic sites in the regions BAE and NEU while the difference in the third region was much less and non-significant.

For the first period, 1930 – 1954 the sites with the lowest AWC (available water capacity) were the most sensitive. This was particularly observed for the BAE dry site, which MSs dropped and during the last period, 1980 – 2004 had one of the lowest estimates of MSs.

The number of positive pointer years increased over time for the pooled data from the mesic sites while it declined in the dry sites (Fig. 3.41). Only one negative pointer year was noted for the mesic sites for the period 1930-1954.

The results as regards mean sensitivity and pointer years showed that responses to drought were more pronounced
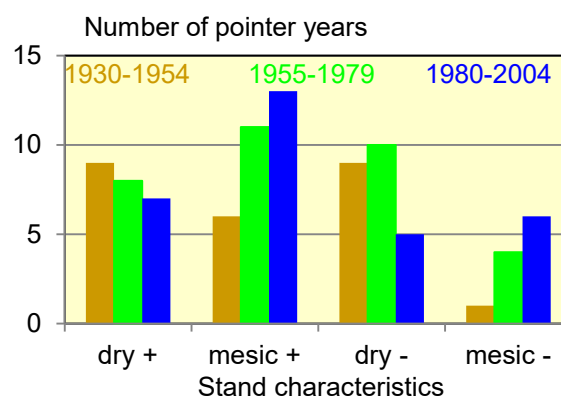

Figure 3-41. *Number of positive and negative so called pointer years in three pairs of stands classified as being dry or mesic in Switzerland. Years with growth deviating strongly positively or negatively during five-year periods were coined pointer years. Weber et al. 2013..*

at mesic sites than at dry sites, which is contrary to the second hypothesis above. The water availability is obviously very critical for growth at mesic sites. The populations at the mesic sites probably have a specific adaptedness to high available water in soil but with a narrow window as regards this availability.

Finally, it was assumed that the phenotypic results observed reflect genetically conditioned differences.

3.3 Summary

Eilmann et al. (2014) stated that *Planting provenances originating from southern to northern locations has been discussed as a strategy to speed up species migration and mitigate negative effects of climate change on forest trees.* Several of the papers in this chapter treated this strategy.

The hypothesis that populations from dry localities are better adapted to drought than populations from less dry conditions was tested in most papers. Drought treatments of young plants as well as *in situ* studies of drought tolerance were conducted. About half of the reports supported this hypothesis that deoughtadaptation had taken place. One reason for the limited support of the hypothesis might be attributed to too small difference at the origins of the populations studied.

The traits studied varied considerably from growth traits to basic physiology traits such as water use efficiency. Different traits might be differently affected by drought. Moreover, the limited number of investigated populations can at least partly explain the lack of significant population differences. In one investigation there were strong and positive relationships between summer precipitations at population origin and relative transcript abundance for two genes related to drought resistance, which was contrary to expectation.

In conclusion, the hypothesis that populations from dry origins are better adapted to drought than populations from wet origins is only partly supported by the reports in this chapter.

Relationships between performance and geographic or climatic variables were sometimes observed even if individual populations deviated much reducing the degree of explanation of the relationship considerably. The deviating populations might be adapted to specific environmental conditions, which did not follow general climatic edaphic, or geographic trends. Generally, larger number of populations well distributed over the latitudes, longitudes, or altitudes is required to detect and prove any relationships.

Large number of physiological traits were studied. The absence of population differences in spite of widely varying climatic conditions at population origin can be attriibuted to strong stabilizing selection for a particular genetic set-up. Alternatively, larger number of individuals are required to prove population differences.. If possible a more representative sample of populations should be studied.

The present knowledge should be used to select a few of the physiological traits with significant population differences for future studies of these traits with a large and representative number of populations. From a breeding as well as evolutionary point of view it is desired to obtain estimates of additive variance within populations for key physiological traits. Such studies should be a next step in the search for drought tolerance.

4. Pollen and nut dispersal

4.1 *In situ*

The objective of a Polish study by Chybicki et al. (2009) was to test whether the effect of isolation-by-distance is valid within two beech populations. One population (coined B) within a nature reserve and with the size of almost ten hectares was selected for this study. It was assumed that beech has existed in this area for a long time. There was no management in this population since 1984 when it was declared as nature reserve. The second selected population (coined L) grows in a highly fragmented forest island in an agricultural landscape. The presence of beech was only 6% in this forest, which is dominated by *Pinus sylvestris.* Beechnut collection takes place in this population and management occurs. In the center of each stand a circle with a radius of 100 meters was GPS mapped. In total, 241 and 276 trees were analyzed with respect to nine microsatellites in the two populations. A spatial autocorrelation analysis was used to identify any spatial genetic structure. The kinship coefficient according to Loiselle et al. (1995) was used for this estimation.

The expected heterozygosity for individual loci varied in the ranges 0.385 – 0.870 in the B population and 0.168 – 0.859 in the L population. Two loci showed significant deviations from Hardy-Weinberg expectations. The locus with strongly significant deviation from Hardy-Weinberg expectation was excluded from the spatial analysis. The proportions of individuals showing inbreeding with significant differences from zero were 12.9% (B) and 7.2% (L). The inbreeding coefficients for the two populations were estimated at 0.038 (B) and 0.022 (L) with a significant difference between the two populations. The number of selfed trees was low in both populations 1.7% (B) and 0.4% (L). Thus, most of the inbreeding occurred among distant relatives. Probably, selfed individuals were outcompeted during the course of development of the stands. In agreement with the isolation-by-distance model the highest kinship was noted for nearest neighbors in both populations. Significant kinships were observed to a distance of 26 meters (B population) and 39.6 m for the L population. For both populations the observed correlograms fitted well to the expected logarithmic curve according to the isolation-by-distance model; 47% (B) and 60% (L). Wright´s neighborhood estimates did not differ much between the two populations; 139.8 m (B) and 148.4 m (L). An attempt to utilize the observed data to estimate the relation between beechnut and pollen dispersal was presented with a wide range 1:10 to 1:100. Unless there is a mass transportation of beechnuts via animals such ratios are expected based on the limited dispersal of the heavy beechnuts.

The effect of habitat fragmentation on genetic drift and gene flow in central Italian marginal populations of beech was studied by Leonardi et al. (2012). The 27 populations studied were grouped in three classes based on their geographic location with respect to their distance from the main continuous distribution of beech; *near-marginal*, *remote-summit,* and *remote-abyssal*. Near-marginal populations are pure beech stands with large population sizes >1,000 adults and they grow at "normal" altitudes for these latitudes. Remote-summit populations are growing at the upper ridges of mountains and faraway from the main distribution of beech and have smaller size than near-normal populations. Remote-abyssal populations grow at elevations <600 masl in mixed stands far away from the main populations of beech. These populations usually grow in small patches under sub-optimal growth conditions for beech. Eleven isozyme loci were used in this study. Allelic richness, polymorphic loci percentage, and observed and expected heterozygosities were estimated. Principal component analyses, bottleneck, and fixation indices were also estimated. F_{ST}s for pairwise genetic distances were related to geographic distances.

There was a large difference among loci with respect to variability parameters. Therefore, the locus effect on the variability was removed by entering the locus effect in ANOVAs and GLMs. The remote-abyssal populations showed slightly but significantly less allelic richness than the other two groups of populations. Similarly, the expected and observed heterozygosities were significantly different among the groups while no difference was noted for percentage of polymorphic loci.

As regards among-population differentiation, significant allele frequency heterogeneity was observed for all loci except one. The among-group F_{ST} was low and estimated at 1.4% while the total F_{ST} among all populations was 4.6%. This shows that most of the variation remained within populations. The F_{ST} within the remote-abyssal group was ≈5%, which was significantly different from the estimate for the near-marginal group, ≈2.3%. The larger F_{ST} within the remote-abyssal group was attributed to a stronger genetic drift in this group than in the other two groups. The correlation between geographic and genetic distances for all pairwise population comparisons was significant but the degree of explanation was very low ≈5%. After exclusion of the remote-abyssal group the correlation became much stronger, ≈21%. The significant relationships were interpreted as a significant role of gene flow for the existing genetic variability.

The principal component analysis did not distinguish three clear groups with a wide spread of the remote-abyssal group.

The fixation index was positive in 21 of the 27 populations with a mean estimate of 0.07, which suggests that genetic drift was of some importance during the course of development of these populations.

It was concluded that the size of populations influenced the existing genetic variability and this was more important than distance from the main beech population. This was especially pronounced for the remote-abyssal population.
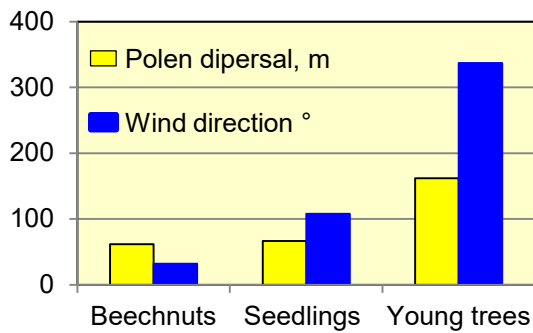
Figure 4-1. Pollen dispersal estimated for beechnuts, seedlings, and young trees in a former agroforestry population approximately 30 years after agroforestry was abandoned and regeneration started. The angle of the dominating wind direction is shown with south to north =360°. Milléron et al. 2012.
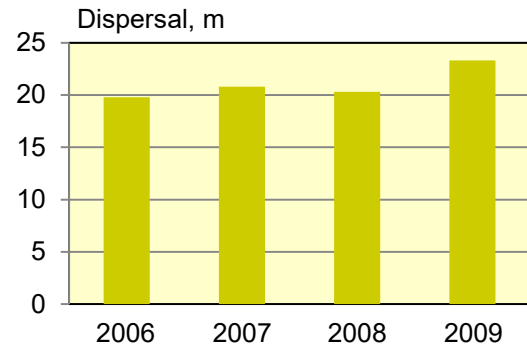

Figure 4-2. Median estimate of beechnut dispersal during four years in a former agroforestry population approximately 30 years after agroforestry was abandoned and regeneration started. The classical ecological inverse modelling method (SSS) was used for these estimates. Millerón et al. 2013.

The impact on pollen distribution in an abandoned agroforestry area in Spain following canopy closure was studied by Milléron et al. (2012). Reforestation has taken place in the study area since 1974 resulting in 1,428 young trees per hectare. A so called intensive sampling plot (ISP) with a 25 meter radius in the center of a 50 x 50 m square containing 396 trees was included in this study. Two groups of the regenerated material were identified; seedlings with DBH < 2.5 cm and height < 50 cm and young trees with DBH > 2.5 cm and height > 50 cm. Besides, 49 beechnut traps one m^2 each were placed in the ISP. Embryos and mesocarps of the beechnuts were genotyped, which allowed an unambiguous identification of each female tree. Six highly polymorphic satellites were used for genotyping. The neighborhood model according to Burzyk et al. (2006) was used for analyses of beechnuts, seedlings, and young trees. The impact of wind direction and leaf phenology were considered in the interpretation of the results.

Fig. 4-1 reveals that the pollen dispersal was more than twice as large for the young trees as for seedlings and beechnuts. It is evident that at the time of establishment of the young trees there was no or limited interception of pollen in the agroforestry population with its low tree density of approximately 15 trees per hectare. The difference in pollen dispersal between seedlings and beechnuts was not substantial 66.4 versus 61.9 meters. Leafing out took place during the entire period of pollen release, which means that both adults and young trees contributed to interception of pollen.

A large part of the pollinations took place with pollen from outside sources, 67%.

Only the southeast – northwest wind direction (337°) for pollen dispersal in the establishment of the young trees seemed to be consistent, contrary to the seedlings and beechnuts, for which random pollen dispersal could not be ruled out.

Selfing was highest in beechnuts, 5.3%, lowest in the young trees and slightly higher in seedlings, 1.2% and

1.6%, respectively. It is evident that culling of selfed individuals takes place during the course of development up to young trees.

It was concluded that the pollen dispersal ability of beech is influenced by the density and structure of the sub-canopy individuals.

The dispersal of beechnuts in the same former agroforestry population was reported by the same group (Millerón et al. 2013). During the mast year of 2008 31.2% of all viable beechnuts were genotyped separately for mesocarp and embryo. Beechnuts were collected in 49 traps, 1m x 1m, at regular intervals during the 4-year period 2006 – 2009. Leaves from 185 young trees within the so called isolated sampling plots were also genotyped. Six highly polymorphic microsatellites were used. Five methods were used for estimation of primary and effective dispersal models:

SSS = Summed seed shadow model (ecological, Ribbens et al. 1994)

OBS = direct fitting of spatial distances obtained with the parentage analysis (genetic, Jones et al. 2005)

CSM = competing sources model, (genetic, Robledo-Arnuncio and Garcia 2007)

MCM = Moran and Clark model, (genetic Moran and Clark 2011)

GSM = gene shadow model a combined ecological and genetic model (Jones and Muller-Landau 2008).

The number of assigned parent pairs was 110 and assignments for single parents was 129. The variation in estimated beechnut dispersal distances by the SSS model for the individual years is shown in Fig. 4-2. As seen from this figure the variation was limited and non-significant in spite of large variation in beechnut production. Years 2006 and 2009 were mast years.

It was noted that most beechnuts fell under the canopy of the trees but the percentage of viable beechnuts was low, 14.7%. Part of the loss of vital beechnuts was attributed to insect larvae. There was a fairly good agreement in beechnut dispersal distances independent of model used
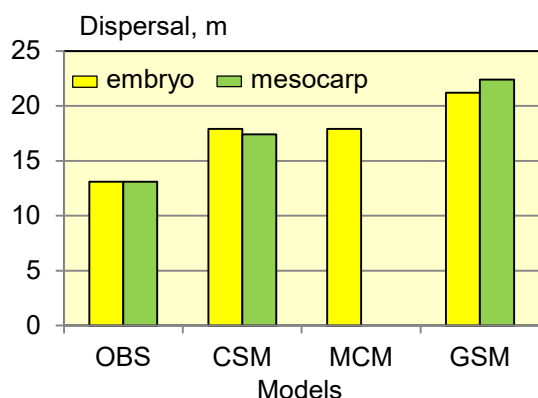
Figure 4-3. Median estimate of beechnut dispersal with four different models. The study took place approximately 30 years after agroforestry was abandoned and regeneration started. Three genetic models were used OBS, CSM, and MCM, OBS = fitting of spatial distance by parentage analysis, CSM = competing sources model, MCM = Moran and Clark model. GSM = gene shadow model a combined ecological and genetic model. Millerón et al. 2013..

to estimate the distance (Fig. 4-3). There was a good agreement between embryo and mesocarp analyses.

The dispersal distances via genotyping of the young trees varied considerably among the methods (Fig. 4-4). Much larger estimates were noted for young trees than for the analysis of beechnuts, 140 – 361 m. The great difference in dispersal between estimates from beechnuts and young trees was attributed to spreading of beechnuts by birds, which are responsible for long-range transfers while small mice contribute to short-range transfers. Survival under the canopy might be hampered by pathogens, seed predators, or herbivores in this way contributing to difference in dispersal between beechnut and young trees.
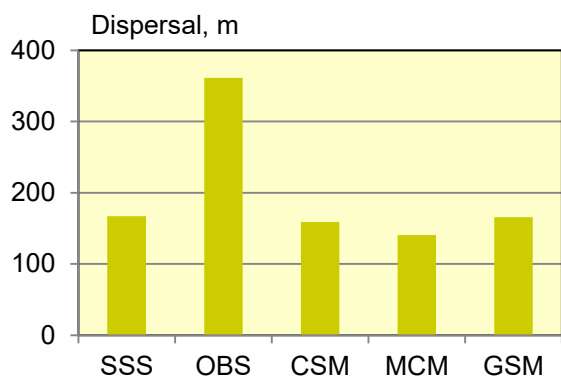

Figure 4-4. Median estimate of beechnut dispersal based on young trees with five different models in a population approximately 30 years after agroforestry was abandoned and regeneration started. Three genetic models based on parentage analysis were used OBS, CSM, and MCM. OBS = observed dispersal distance, CSM = competing sources model, MCM = Moran and Clark model. The ecological SSS = Summed seed shadow model ,and the combined ecological and genetic model GSM = gene shadow model. Millerón et al. 2013..
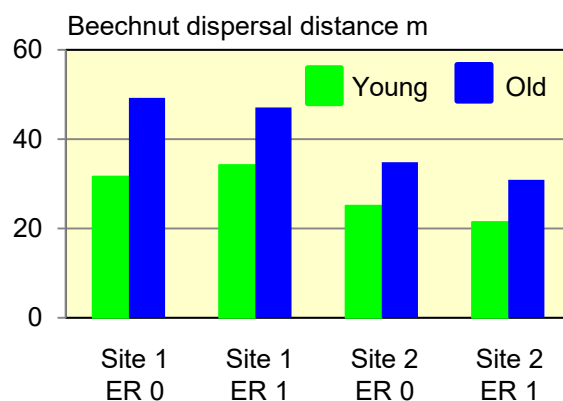

Figure 4-5. Beechnut dispersal distances from categorical parentage analysis for young seedlings, one year old, and old seedlings, >4 years old in two populations in Mont Ventoux in south-eastern France estimated by ten polymorphic microsatellites. ER 0 stands for no error in genotyping, and errors in genotyping, ER 1. Bontemps et al. 2013

Further, light conditions under the canopy might be unfavorable for germinating seedlings reducing their chance for survival.

In conclusion, the difference between primary dispersal of beechnuts and the effective dispersal as seen from the young trees is the main take home message from this investigation.

Two sites with recolonized beech with trees less than 150 years old were selected for a study of nut and pollen dispersal. The first site comprised an area of 1.93 hectares and the other 0.84 ha (Bontemps et al. 2013). To be included in this investigation a tree had to have a DBH of >10 cm or carry nuts. Trees were often found in clusters, which might be attributed to previous coppice forestry. Only one of the trunks in such a cluster was mapped. Two classes of the regenerating seedling were identified; less than one-year old and the second with +4 years-old. Lack of fruiting between these ages permitted such a classification. At locality 1 256 seedlings and at locality 2 355 seedlings were mapped with markers from ten microsatellite loci. At locality 2 only 22 seedlings less than one year old were available. The adult trees were also mapped to trace parentage.

Several calculations were carried out to estimate the dispersal. The impact of tree DBH, seedling mortality, and difference in parent offspring elevation were considered in various calculations.

Several figures were presented for the dispersal dependent on the calculation method used. One result is presented in Fig. 4-5. This figure reveals that dispersal is farther in the old population than in the young population and that dispersal is wider at site 1 than at site 2. Mortality of seedlings close to a parental tree up to age four owing to competition; so called density dependent mortality, is one possible explanation for the difference between young and old seedlings.
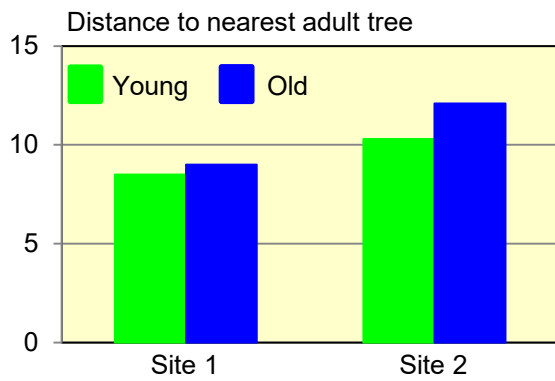
Figure 4-6. *Distance to nearest adult tree for young seedlings < 1 year and old seedlings >4 years old at two sites in Mount Ventoux in south-eastern France estimated by ten polymorphic microsatellites. Bontemps et al. 2013.*

The estimation of the average distance of seedlings to the nearest adult tree lends further support for density dependent mortality, DDM, as seen in Fig. 4-6. Suggestions for the difference between the two sites were presented:

Between-year variability in ecological factors; wind conditions, date for seed maturity,

Total nut production

Animal impact on nut dispersal

Curiously, the spreading was up-slope at site 1 while the opposite was the case at site 2. It should be noted that spreading may be influenced by animals without possibility to record. This may partly explain the difference in spreading along the slopes of the two test sites. The below figures for beechnut and pollen dispersal were reported:

Beechnut Young   11.87 m
Beechnut Old   21.63 m
Pollen dispersal   48.18 m

Seed migration rates %:
Site 1 young =   12
Site 2 young =   20
Site 1 old =   33
Site 2 old=   72

Mean DBH at site 1 was best proxy for female and male fecundity while total DBH was the best proxy at site 2.

At site 1 the difference in elevation (parent - offspring) suggested upslope dispersal, v = -0.03; v is a parameter related to the effect of diameter and elevation on female fecundity.

At site 2 the difference in elevation (parent - offspring) suggested downslope dispersal, v = 0.05

Selfing or inbreeding did not exist to any large extent since the fixation index did not differ significantly from 0. One important result in this investigation is the congruence among the trends as regards dispersal among young versus old seedlings, even if absolute figures differ. The limited beechnut dispersal from mother trees is another striking observation in this investigation. To mitigate the impact of global warming the authors recommended forest managers to increase effective dispersal and favor regeneration in plots with the highest probability for seedling survival.
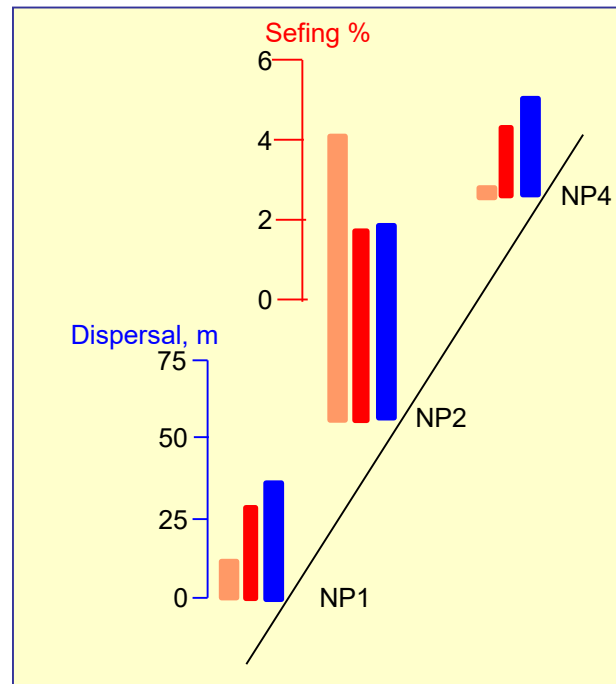

Figure 4-7. *The estimated pollen dispersal (blue) and selfing percentage in three French high-elevation populations (1,020 – 1,340 masl.). The orange and red column columns refer to selfing estimated with two different methods. The orange columns refer to a complex analysis considering the local configuration in the stands. The basal area, m²/ha was 13.4, 8.5, and 14.3 for NP1, NP2, and NP4, respectively. Gauzere et al. 2013.*

Gauzere et al. (2013) studied the effects of phenological synchronization with neighbor trees, local density, and diameter of female trees on pollen dispersal, selfing, migration, and effective number of males per matings of female trees in three French high-elevation populations, 1,020 – 1,340 masl. Bud flushing was observed at several occasions in populations NP1 and NP4 while only one observation was done in NP2. Different methods for estimates of effects were used. The calculations were described in the following way: *First, we measured the mean phenological lag, PL_{mean}, which describes whether a mother tree flowers early ($PL_{mean} > 0$) or late ($PL_{mean} < 0$) compared to its neighbours. Second, we computed the sum of the absolute phenological lag.* The effective number of fathers pollinating each mother, N_{epi}, was also estimated. The position of each tree in the three stands was determined. Genotyping took place with 13 polymorphic microsatellites.

The pollen dispersal distances varied in the range 35 – 63 meters with the highest estimate in NP2. However the differences among the populations were not significant (Fig. 4-7). The migration rate into NP1 was lowest with a mean percentage of 43%. The highest mean was noted for NP4 (64%). The lowest percentage in one NP1 tree, 12%, and the highest was found in one NP4 tree, 86%. Fig 4-8 gives information on the variation in migration in the three stands. A high canopy density probably blocks
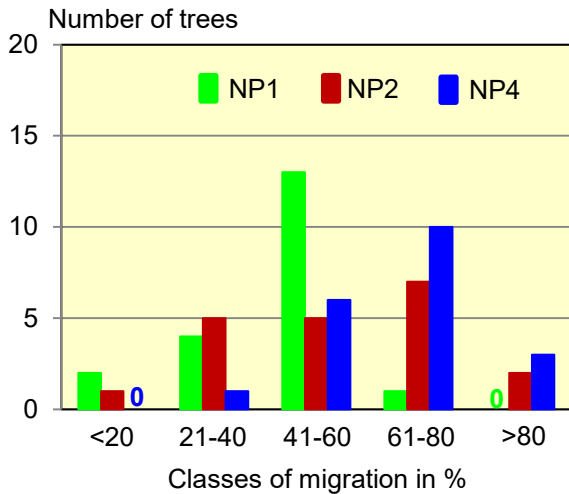
Figure 4-8. The number of trees in different classes of migration in three French high-elevation populations (1,020 – 1,340 masl.). The basal area, m²/ha was 13.4, 8.5, and 14.3 for NP1, NP2, and NP4, respectively. Gauzere et al. 2013.
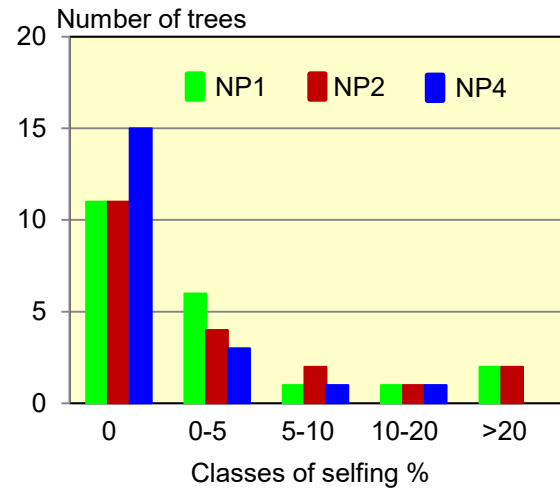

Figure 4-9. The number of trees in different classes of selfing in three French high-elevation populations (1,020 – 1,340 masl.). The basal area, m²/ha was 13.4, 8.5, and 14.3 for NP1, NP2, and NP4, respectively. Gauzere et al. 2013.

pollen flow from distant trees. Large trees are probably strong competitors and in this way reduce the blocking effects of adjacent trees, which results in low selfing and high migration rates.

The estimated phenological lags differed between the populations in the following way:

NP1 – NP2 = 4 days
NP1 – NP4 = 13 days
NP2 – NP4 = 9 days

The within-population phenological lag varied significantly among the populations with the highest estimate for NP2 and the lowest in NP4. The effects of population, the female tree DBH, the competitor density within a radius of 10m, and the phenological lag explained the variability in migration. As expected, trees with large DBH and growing under low density showed the highest migration rates while small trees with synchronous phenology showed the lowest migration rate. Owing to protogyny in beech no pollen from the own stand will be available when the early female flowering occurs, which increases the probability for matings with migrant pollen. In agreement with this expectation the early female flowering trees in NP2 showed highest migration rates. Since no pollen donor populations occur below NP1, early flowering trees in this population cannot rely on migrant pollen. The rapid and synchronous development of female and male flowering in NP4 would explain the absence of a difference in migration between early and late flowering trees in NP4.

The percentage of selfings varied considerably among the trees but the majority of trees had selfing percentages below 5% (Fig. 4-9). One tree in population NP2 had as high percentage as 48%. The difference between NP2 and NP4 was significant. A low number of clonal copies and low competition from other trees resulted in low selfing in agreement with expectation. The phenological lag had

a significant impact on selfing, which was attributed to one outlier female in NP4 with almost 20% selfings. The significance disappeared after exclusion of this family. In the analysis including the configuration of the stands the selfing percentages per population varied more and differed significantly among the populations (orange columns in Fig. 4-7).

The variation in the effective numbers of males on individual females, N_{epi}, was large among trees but the difference among the populations was non-significant.

This study is unique since it concerned the impact of ecological factors on the mating pattern among and within three high-elevation populations from one mountain slope. Finally, large within-population variability was noted for the traits studied.

One French study by Lander et al. (2011) on beech populations from Mount Ventoux in Provence, addressed the three points below:

1. The recent colonization from remnant populations
2. The existing pattern of genetic variation as a result of changing population sizes
3. Is there an agreement between approximate Bayesian Computation of demographic reconstruction and existing genetic data?

Historical maps were used to find populations suitable for this study. Three populations coined east, west, and south at Mount Ventoux in southeastern France were selected. East and west are remnant populations on the eastern and western ridges, respectively. South is a newly established and expanding area. The distance of colonization was estimated based on the present distribution minus the outer edge at 1845. At this time the contraction of beech was most pronounced. The annual rate of expansion was estimated at 27 m and 38 m in the southern and eastern populations leading to an area expansion of 65%.

The number of plots and trees included in this investigation were:

East   21 plots   575 trees
West   25 plots   1,228 trees
South   5 plots   129 trees

In the majority of the plots (48 of 51) 30 trees were sampled; in the three remaining plots all trees were sampled. Thirteen microsatellite markers were used for genotyping of all sampled trees.

Groups of trees belonging to clones were detected. In the analyses only one tree from such groups were included in the analyses. Number of alleles, allelic richness, expected and observed heterozygosity, and fixation index were estimated. The modified index for estimation of historical bottlenecks according to Garza and Williamson (2001) was calculated separately for each region. The structure v.2.3.3 program was used for testing of Bayesian clustering of 1,825 trees and 12 loci. Approximate Bayesian Computation analysis was used to identify if there were any bottlenecks during the history of the three populations. The DIYABC program according to Cournuet et al. (2008) was used for this purpose. Eight different demographic scenarios, with varying times of split of the populations as well as size of populations, were tested in this analysis. Huge numbers of simulations (500,000) per scenario were run.

The allelic richness and expected heterozygosities did not vary much among the three populations with ranges 4.75 – 4.89 and 0.70 – 0.71, respectively. F_{ST} for among region differentiation was extremely low but significant, 0.2% while F_{ST} among plots was 2.6% and strongly significant. Thus, most of the variation was within plots. There was support for three groups based on the structure analysis. The fixation index was estimated at 0.03 (west) and 0.064 (south).

The ABC analysis suggested that one asynchronous divergence scenario was most likely for the development unto the present situation with three populations. According to this scenario the three populations expanded from a smaller ancestral population. Further, the south population diverged from the other populations two generations (t2) ago while the east and west populations diverged one generation (t1) ago. Even if artificial regeneration had taken place in the south population it was stated that it was likely that local material was used for this regeneration. Finally, in this scenario changes in population size was allowed to occur both at one and two generations ago.

As regards the effective population size the analysis revealed that this was *never very small*. The estimated global increase of the population size from t2 to t1 was 43% but with a large imprecision of this estimate. The hypothesized bottlenecks in the history of this 4,000 hectares beech forest were not detected.

This investigation is unique since it combines historic demographic data with genetic diversity estimates in existing populations for understanding recent history of a large beech population.
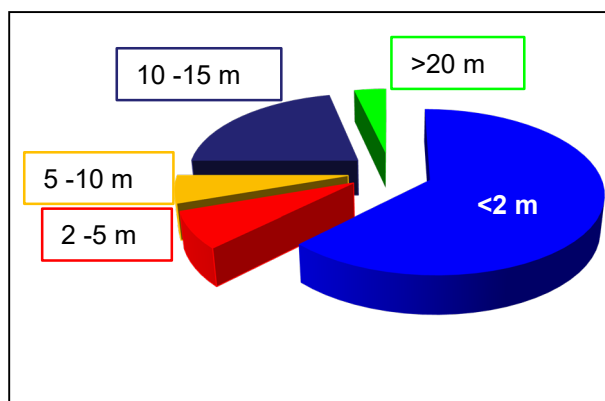

Figure 4-10. The partitioning of median estimate of beechnut dispersal in meters based on young trees approximately 30 years after agroforestry was abandoned and regeneration started. Six microsatellites were used for genotyping. Hassenkamp et al. 2011.

A pilot study of two German populations with the objective of development methods for seed source identification was carried out by Hassenkamp et al (2011). In the first population (P1) 5 – 6 beechnuts were collected within a radius of two meters beneath 19 trees. In addition leaf samples were taken from 334 trees in the 8.7 hectares stand. In the other population (P2) 100 beechnuts were randomly sampled in the whole stand comprising 56 hectares. Leaf samples were taken from 100 trees. Six microsatellites were used to genotype leaves and exocarps in order to enable tracing of the origin of the beech nuts. Seed dispersal was determined by comparison of the multilocus genotypes of exocarps and leaves using the Multilocus option Matches of the software GenAlEx version 6. The allelic richness varied in the ranges 10 – 30 and 8 – 23 in the two stands. The fixation indices of individual loci were in nine out of 12 cases positive with a wide range of estimates 0.002 – 0.351.

In P1 56 (according to Fig. 1 in the paper, 58 according to the text) of the 99 beechnuts could be assigned to a particular mother tree, which means that a little less than two thirds of the beechnuts were found beneath its mother tree (Fig. 4-10). Thus, another example of limited beechnut dispersal. In population P2 no beechnut could be assigned to any adult tree, which is not surprising considering the random sampling of the beechnuts.

4.2 *In situ* + analytical methods

Three hypotheses concerning previous and current mating pattern were tested in three French populations by Oddou-Muratorio et al. (2010):

1. Differences exist between historical and current gene dispersal
2. Difference in dispersal abilities in three localities with different demography
3. The effect of dispersal on present demography

Two populations from Mount Ventoux in southeastern France and one population from northeastern France were
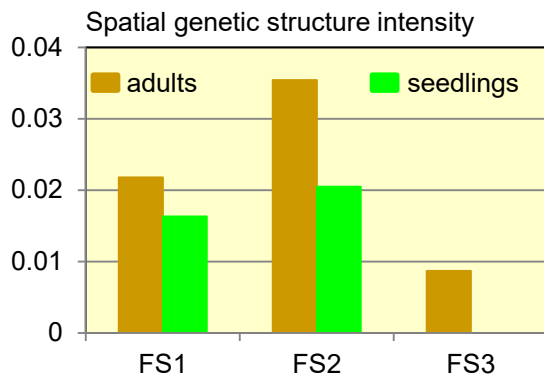
Figure 4-11. The intensity of the spatial genetic structure intensity (SGS) in two southeastern (FS1 and FS2) and one northeastern (FS3) French beech population. Five microsatellite markers were used in FS3 while eleven markers were used in FS1 and FS2. SGS could not be estimated in the FS3 seedling population . Oddou-Muratorio et al. 2010.

Figure 4-13. The gene dispersal cosidering tree densities in two southeastern (FS1 and FS2) and one northeastern (FS3) French beech population. Five microsatellite markers were used in FS3 while eleven markers were used in FS1 and FS2. Gene dispersal could not be estimated in the FS3 seedling population . Oddou-Muratorio et al. 2010.

included in this investigation. The first two populations (FS1 and FS2) are recolonizing pine forest areas while the third population (FS3) is located within an ancient beech forest area. Adult trees with a diameter >10 cm and seedlings <1 meter were mapped. Five (FS3) and eleven (FS1 and FS2) microsatellites were used for the genetic mapping. Kinship coefficients according to Loiselle et al. (1995) were calculated for estimation of the spatial genetic structure (SGS). A slightly modified neighborhood mating model according to Burczyk et al. (2006) was used to estimate neighborhood. Pollen and beechnut dispersal were estimated according to Oddou-Muratorio et al. (2005).

It was observed that both adults and seedlings were more related within distances up to 30 – 40 meters. The spatial genetic structure (SGS) was significant in all three populations. There was a significant difference in SGS among the populations. The SGS in the ancient FS3 population was smaller than SGS in the two colonizing populations (Fig. 4-11). This difference was attributed to *restricted*

overlap of seed shadows in FS1. The lower population density in FS2 is probably responsible for its higher SGS than SGS in the FS1 population. In FS1 a stronger spatial genetic structure among adults than among seedlings was proven while there was no significant difference in FS2. Similarly, the kinship coefficient for adults was lowest in the FS3 population (Fig. 4-12).

in Fig. 4-13 the estimated gene dispersal (d_e) is illustrated for the case that $d_e = d_{obs}/4$ in this long-living species. There was significantly lower gene dispersal in FS1 adults than in FS2 and FS3 adults. It was noted that gene dispersal in beech seems to be lower than in other low-density temperate tree species.

Fig. 4-14 illustrates that there is limited beechnut dispersal in FS1 and FS3, approximately 10.5 meters. The standard error of the estimate for beechnut dispersal in FS2 was larger than the estimated dispersal, which means that this estimate is very imprecise. This figure also shows that the pollen dispersal distances are considerably larger than beechnut dispersal in FS1 and FS3. It was pointed
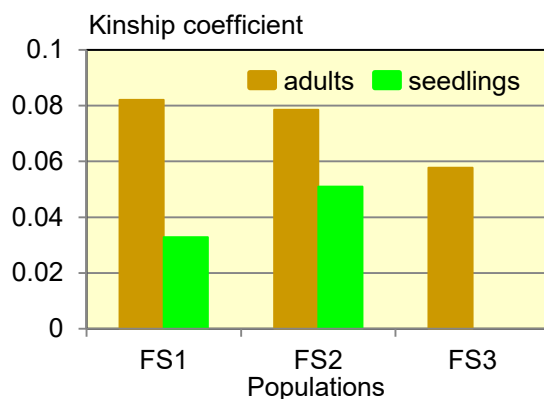

Figure 4-12. Kinship coefficients at individual distance of <10 meters between two individuals in two southeastern (FS1 and FS2) and one northeastern (FS3) French beech population. Five microsatellite markers were used in FS3 while eleven markers were used in FS1 and FS2. Oddou-Muratorio et al. 2010.
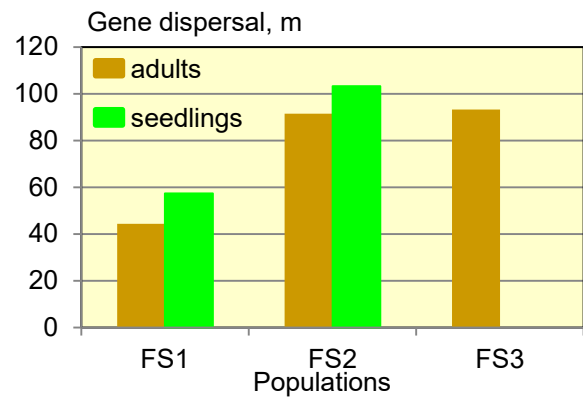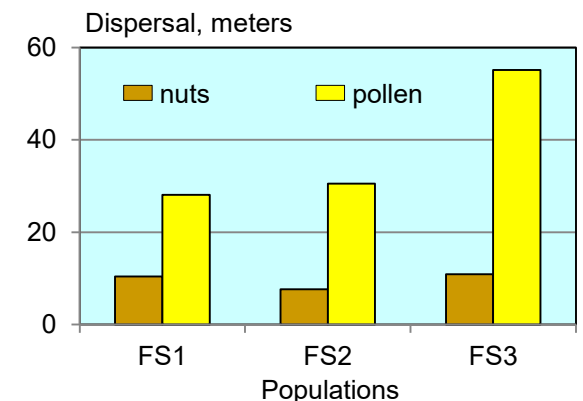

Figure 4-14. Beechnut and pollen dispersal in two southeastern (FS1 and FS2) and one northeastern (FS3) French beech population. Five microsatellite markers were used in FS3 while eleven markers were used in FS1 and FS2. Oddou-Muratorio et al. 2010.

out that old seedlings, >4 years, were over-represented in FS2, which probably attributed to the high estimate. The beechnut dispersal of a sub-sample of young FS2 seedlings had a lower beechnut dispersal estimate ($\approx$ 8 m) than in FS1 and FS3. Generally, the beechnut dispersal in beech is very restricted even if some long-distance dispersal may occur. The restricted distribution was attributed to heavy beechnuts and short-distance distribution by small rodents. However, when looking at beechnut migration from outside sources fairly high beechnut dispersal was observed; 27%, Disturbances in the populations with recent expansions in FS1 and FS2 contribute probably to this result. A storm in 1999 caused a large canopy opening, which allowed establishment of seedlings from outside sources.

The mean pollen dispersal for all three populations was estimated at 38 meters. Since only compatible parent pairs within each locality were included in the estimations of pollen distribution these estimates might underrepresent the pollen migration distances. Based on the beechnut and pollen dispersal, stronger spatial genetic structures in the three populations were expected. One reason for weaker SGS than expected was attributed to the high estimates of gene flow via beechnuts and pollen; 27 and 68%. Such a gene flow masks the SGS if gene flow from outside populations occurs.

The low selfing rates in all three populations, 0.01 – 0.04, are noteworthy.

The impact of the obtained results on mitigating global climate change by management was discussed and three suggestions for increase of genetic diversity at local scale were presented:

1. Regular spatial spacing of seed trees
2. Favor regeneration over several years
3. Favor regeneration plots evenly distributed within the regeneration area

In conclusion, detailed information on the existing genetic structures based on pollen and beechnut dispersal in populations with different demography was obtained. Credits should be given to the authors for the cautions for various types of weaknesses of the data presented.

Four questions related to pollen and beechnut dispersal and migration were addressed by Oddou-Muratorio et al. 2011:

1. Correlation in space and time between seedling density and survival
2. Spatial restriction of contemporary pollen and beechnut dispersal
3. Which are the major factors shaping seedling genetic structure?
4. Impact of mortality on the genetic structure of seed lings

A north-eastern French beech stand with a size of 7.8 hectares (lat. 48.54N and 6.10 E) was selected for this investigation. Sampling of seedlings took place in three plots with reduced light availabilities 45, 69, and 94% for plots A, B, and C, respectively. In all, 462 seedlings were
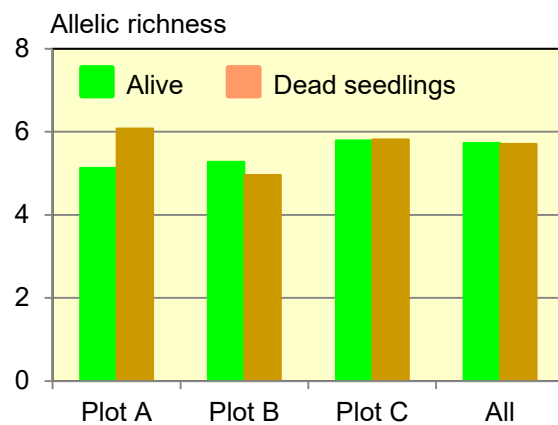

Figure 4-15. Allelic richness in alive and dead seedlings in three plots in a 7.4 ha beech stand in north-eastern France. The light reduction compared to an open field was 45, 69, and 94% in plots A, B, and C, respectively.. Oddou-Muartorio et al 2011...

mapped, of which 254 were genotyped with five microsatellites. The recruiting year for the period 1993 to 2005 was determined by examination of the seedlings. Besides, 372 beechnuts from 29 trees (5 – 16 nuts/tree) were collected and genotyped.

Hierarchical AMOVAs were used for identifying the spatial and temporal components of the seedling genetic structure. The fine-scale spatial genetic structure within plots was focused on the between-generation component of the spatial genetic structure *by computing coefficients of genetic relatedness (F_{ij}) among all pairs of individuals that involved one seedling*. Such a computation reflects the parent-offspring component of genetic structure. The kinship coefficient F_{ij} according to Loiselle et al (1995) was computed to estimate the relatedness. The impact of mortality was estimated by aid of hierarchical AMOVA as above but replacing the average effect of year with dead/alive.

The spatially explicit mating model (SEMM) according to Burzyk et al. (2006) and Oddou-Muratorio and Klein (2008) was used to estimate pollen and beechnut dispersal shape and range *from genotypes and positions of established seedlings and their potential parents*. In addition, SEMM was also used for estimation of pollen dispersal kernel based on *genotypes and position of maternal trees, progeny arrays, and potential fathers*.

Year 2002 was found to be a masting year while earlier years 1993 – 2001 showed little recruitments. The seedling densities varied between 12.7 (plot A) and 31 (plot B) per m². It was stated that germination of beechnuts was almost independent of light conditions. The mortality rate 2004 – 2005 was 22.7% and dropped to 8.2% for years 2005 – 2006. The mortality rate for years 2004 and 2005 varied in the range 15 – 27% with the lowest estimate in plot A and the highest estimate in Plot C with its strongly reduced light conditions. Thus, mortality was dependent on the light availability in the plot. As regards allelic richness (Fig. 4-15), number of alleles, and expected heterozygosity there were no significant differences

among plots. Inbreeding was ruled out as explanation for the mortality of seedlings since the difference in fixation indices between alive and dead seedlings was limited; 0.073 for alive versus 0.058 for dead seedlings.

The fixation index differed with higher estimate in adults than in seedlings, 0.131 versus 0.067. However, when the three loci containing null alleles were excluded, this difference disappeared.

According to the hierarchical AMOVAs the effect of plots was significant while the effect of years was non-significant. Plot C with its strongly reduced (94%) light availability was significantly different from the plots A and B. Most of the genetic variation occurred within plots. This is reflected in a low F_{ST}, 2.6%, for plot differentiation. However, 2.6% must be regarded as large considering the limited geographic area investigated with an average distance among plots of approximately 100 m only.

A fine-scale analysis of the genetic structure within plots was carried out by plotting seedling-adult coefficients of genetic relatedness, F_{ij}, against distance. The spatial genetic structure was in all cases (the entire material or individual plots) strong within distances up to 30 meters and after 40 meters no significance was noted. The genetic variation among different age groups within plots was non-significant in spite of the large variation in recruitment different years. It might be questioned whether this lack of differentiation might be attributed to low number of seedlings in several of the age groups leading to imprecise estimates. It was evident that genetic drift had limited impact on the genetic structure across years. Even if there was a marked difference in the spatial genetic structure among plots no significant difference among plots was noted.

The SEMM method was used for estimates of dispersal and migration. Pollen dispersal was much longer than nut dispersal 57 versus 11 meters, and so was migration percentage, 72 versus 20% (Fig. 4-16). As seen from this figure the observed results for pollen dispersal and migration did not differ in a pronounced way whether estimated via seedling or beechnut genetic structure. This consistency between the two ways of estimating pollen dispersal indicates that pollen origin (outbreeding or inbreeding) does not lead to any selection between beechnut release and seedling establishment. This is also reflected in the relationships between distance and the adult-seedling differentiation, which was almost identical.

According to the method developed by Oddou-Muratorio and Klein (2008) a real-time gene flow was estimated at 51 meters.
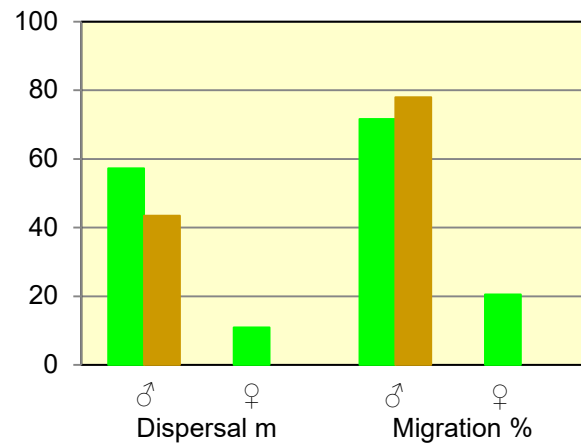

Figure 4-16. *Pollen and nut dispersal in meters & pollen and nut migration in % in a beech stand in north-eastern France. Green columns refer to estimates based on seedlings and brown columns refer to estimates in beechnuts.. Oddou-Muartorio et al 2011...*

In conclusion, no difference in genetic diversity was noted for the below comparisons:

   Adults – seedlings
   Dead – alive seedlings

A significant difference among plots was noted. The pollen dispersal distances were much larger than beechnut dispersal. Similarly, the pollen migration was much larger than beechnut migration.

A simulation study with the objective to compare Bayesian approaches with other estimations of male fecundity and to *understand how variance in fertility differs from variance in fecundity* was carried out by Klein et al. (2011). Two scenarios, low density (LD) and high density (HD) of the stands were studied.

For the LD scenario, the center part in 50 populations with a Poison distribution and a tree density of 0.35/hectare constituted the starting material. For the HD scenario, the center part in 50 populations with a Poison distribution and a tree density of 3.5/hectare was the starting material. In both scenarios, 60 mother trees were selected. The 60 trees were randomly assigned a genotype at six microsatellite loci with 6 – 24 alleles per locus. No selfing and linkage equilibrium were assumed.

Log-normality distribution of the fecundity and gamma distribution of fecundity were tested in Bayesian simulations. Besides the Bayesian methods, the classical maximum likelihood method and two indirect methods, KinDist and Two-Gener (Robledo-Amuncio et al. 2006) were
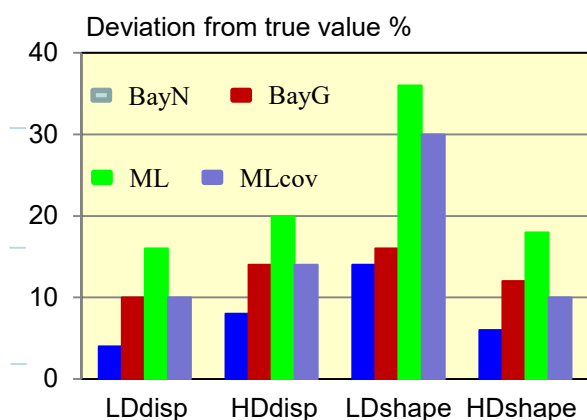
Figure 4-17. Deviations in % from true values in simulation with four methods with respect to pollen dispersal and shape of the pollen dipersal curve. Two types of populations were used LD = low density 0.35/hectare and 3.5/hectare = high density (HD). The simulation methods used were:

BayN = Bayesian with Poison distribution
BayG = Bayesian with gamma distribution
ML = maximum likelihood
ML_{cov} = maximum likelyhood with covariate to study how much variance in fecundity influence the pollen dispersal parameters.-

Klein et al. 2011.

used in the simulations. The variance in fecundity might influence the pollen dispersal parameters. Therefore, covariates were also considered in the multiple likelihood simulations (ML_{cov}).

The results as regards deviations from the "true" values are summarized in Fig 4-17, which shows that the BayN simulation resulted in the lowest deviations in all four cases and that the ML method had the largest deviations. Of the four cases presented in Fig 4-17 the deviations were largest for the shape of the pollen dispersal curve in the low-density population. Contrary to this, the lowest deviations were found for pollen dispersal distance in the low-density population. It should be noted that the KinDist method showed large deviations from the true values.

The correlation coefficients for the relationships between the individual fecundities and the observed individual fertilities were rather strong for the BayN method; r = 0.92 (LD) and 0.90 (HD), and almost identical for the BayG method, 0.92 and 0.89, respectively. In spite of these strong relationships the authors stressed the distinction between individual fecundity and individual fertility. The former is the amount of pollen produced while the latter is the realized fertilizations with pollen from a donor tree. Especially, if one tree is out of phase with the other

trees in a population the difference between fertility and fecundity might be large. Differences in phenology were not considered in the scenarios tested. The importance of phenology was stressed since differences in phenology might be more important than distance as regards realized fertilizations. The methods tested might be extended to consider phenology but it would require much information on variation in phenology.

In conclusion the Bayesian methods were superior to the other methods in estimating pollen dispersal and pollen dispersal curve.

4.3 Summary

Owing to the weights of beechnuts their dispersal is limited. Estimates of dispersal by embryos and mesocarp resulted in almost identical dispersal distances. In one study more than 50% of the beechnuts were found in a radius of two meters around the target tree. In other studies dispersal distances up to 50 meters were found. Estimated kinship in trees within a radius of ten meters was high with higher estimates in adult trees than in young seedlings. Different methods for estimations of beechnut dispersal were presented. Mostly, the methods agreed fairly well. Curiously, in one case an up-slope dispersal of beechnuts was revealed. This might be attributed to animal transport of beechnuts. Finally, to a limited extent rodents and jays extend the dispersal of beechnuts.

Widely varying estimates of pollen dispersal was noted; from 25 meters to over 300 meters. In one colonizing population estimates of pollen dispersal based on beechnuts, seedlings, and young trees was carried out. The pollen dispersal based on young trees was ten times higher than based on beechnuts, 337 versus 32 meters. The young seedling population had an estimate of 100 meters. At the start of the colonization most pollination took place with immigrant pollen. Over time more trees started to produce pollen leading to an increased pollination within the stand.

Selfing was studied in beechnuts, young seedlings, and somewhat older young trees in one population. Selfing was approximately three times higher in beechnuts than in the two types of seedlings. It is evident that culling of selfed individuals takes place during the course of development up to young trees. Another investigation reported no or minor genetic difference between alive and dead seedlings suggesting that purging of inbred seedlings had not taken place to any great extent.

No impact of light availability for nut germination was noted in one investigation while mortality was highest in the plot with lowest light availability.

5. Management impact on genetic diversity

The impact of management regimes on mating success in four populations, two in Austria and two in France, was studied by aid of four highly polymorphic microsatellite loci (Piotti et al 2012). (Data on these populations and three other pairs of populations from Germany, Italy, and The Netherlands were published in 2007 by Buiteveld et al. 2007). The distance between the two Austrian populations was 500 m while the distance between the two French populations was 100 km. Three microsatellites were common in the studies in Austria and France while the fourth was different. All microsatellites were unlinked and showed Mendelian inheritance. The number of trees per hectare in the four populations was:

Austrian unmanaged	57
France unmanaged; an isolated population	150
Austria shelterwood	163
France recently colonized	68

Two methods were used for paternity analysis: a maximum likelihood (Gerber et al., 2000) and a mating model approach (Burczyk et al. 2002).

Pollination distances were calculated as the Euclidean distance between pollen donors and maternal trees. When two (or more) pollen donors with the same LOD scores were the most likely ones, the mean of their distances from the maternal tree was considered as the pollination distance.

The impact of degree of phenological overlap between female and male development, distance, and aspect (north, east, south, and west) between trees was evaluated for the two Austrian populations according to Burczyk et al. (1996). In addition, the relationships between mating success and breast height diameter, tree height, crown area, and nearby maternal trees were estimated.

There was a high genetic variability in all four populations with observed heterozygosities in the range 0.568-0.649. This results in high probabilities, ≈ 98%, for exclusion of males in individual nuts. The selfing rates were extremely low in the two French populations, 0.01 while the highest selfing rate was noted for the Austrian shelterwood population, 0.09. The unmanaged Austrian population took an intermediate position and had an estimated selfing rate of 0.04.

Fig. 5-1 reveals that external pollen accounted for around 80% of the matings in three of the four populations. Only the unmanaged French population had a lower contribution from outside, 53%. This was attributed to long distance to closest beech stand, around 60 km. The high influx of matings with pollen from other populations means that the variability in the recipient populations increase and offers good opportunities for change of populations genetic constitution by natural selection.

The expected and reduced pollen dispersal distance in the Austrian shelterwood population compared to the unmanaged Austrian population (Fig. 5-2) was attributed to higher tree density in this population compared to the unmanaged population, 163 versus 57 trees per hectare. The data from the two French populations show the same trend with highest pollen dispersal distance in the unmanaged populations.
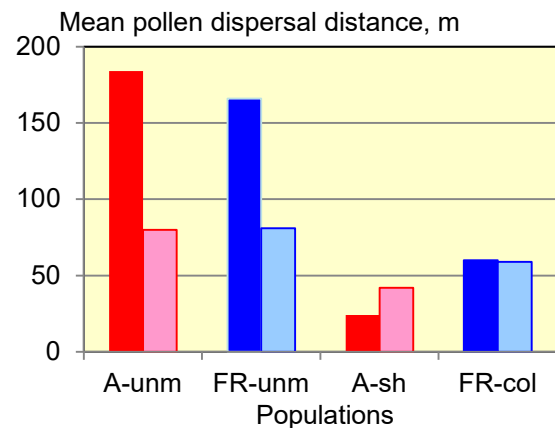
Figure 5-2. Figure Piotti12-2. Two estimates of mean pollen distance for successfull matings within four populations (A = Austria, FR = France) with different management regimes:
unm = unmanaged
sh = shelterwood
col = recently colonized.
Filled columns refer to estimates by mating paternity model analysis and light colors refer to the maximum likelyhood paternity analysis. Piotti et al. (2012)
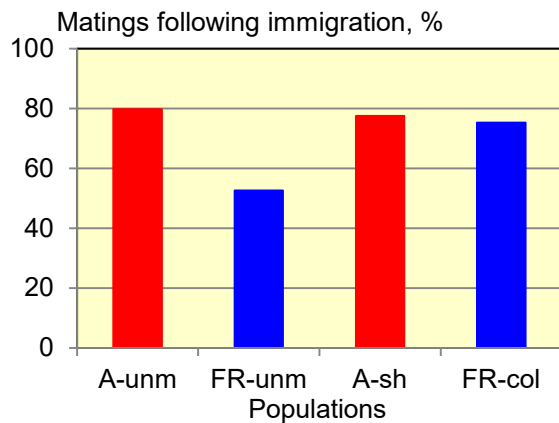

Figure 5-1. The percentage of beechnuts not assigned to local males in four populations (A = Austria, FR = France) with different management regimes:
unm = unmanaged
sh = shelterwood
col = recently colonized.
Piotti et al. (2012).

Figure 5-3. The fraction of matings in female trees with pollen from different distances. Two unmanaged populations; one in Austria (red) and one in France (blue).. Piotti et al. 2012.

The relationships between mating success and pollen donor distance are shown in Figs. 5-3 and 5-4., which reveals that there is a marked difference between the unmanaged populations and the two other populations. The unmanaged populations show a flat maximum while there is a s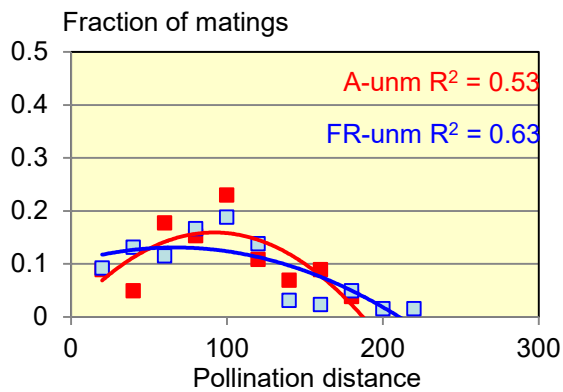ubstantial decrease of successful matings with distance from the female tree in the two other populations. Differences in tree densities cannot explain the different patterns since it was higner in the unmanaged French stand than in the colonizing population while the opposite was the case for the Austrian populations.

The reproductive success of individual males, measured as number of gametes assigned to each pollen donor was more evenly distributed in the two unmanaged populations, (0.30±0.54 and 0 32±0.6) than in the other two populations (0.26±0.76 and 0.7±1.25).

One striking observation was the high gene flow into the four studied populations. Another observation from this investigation was the difference in the relationship between pollination distance and fraction of matings in the unmanaged populations and the other two populations (Fig.5-3 and 5-4).

In conclusion, the management did not influence the diversity to any large extent, a result which was shared with the preceding study by Buitenveld et al. (2007) while the
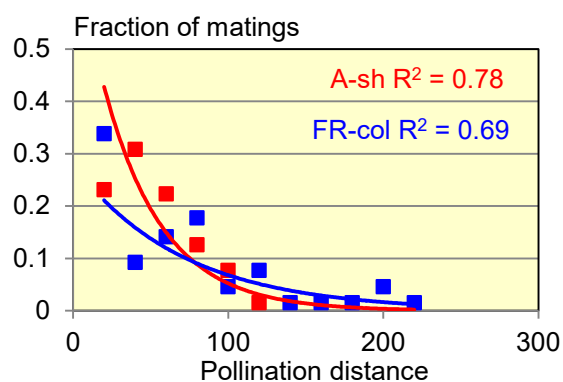

Figure 5-4. The fraction of matings in female trees with pollen from different distances. Two unmanaged populations; one in Austria red and one in France blue.. Piotti et al. 2012.
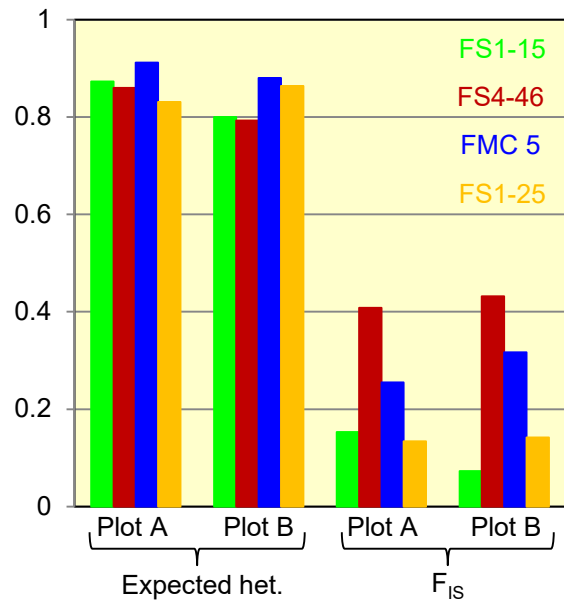

Figure 5-5. Expected heterozygosity estimated by four polymorphic nuclear microsatellites (FS1-15, FS4-46, FMC 5, FS1-25) in one unmanged old-growth forest, plot A, and in one shelterwood forest system, plot B, and fixation index, F_{IS}, in these plots. Pafetti et al. 2012.

pattern of pollination success differed between the two types of management.

Another study treating the genetic impact of different stand treatments were carried out in two central Italian populations by Pafetti et al. (2012). (Data on these populations and three other pairs of populations from Austria, Germany, Italy, and The Netherlands were published in 2007 by Buiteveld et al. 2007). Four polymorphic nuclear microsatellites and RAPD markers were used for genotyping of 100 trees in the unmanaged old-growth forest (plotA) and 93 trees in the managed shelterwood plot (B). The distance between the plots is 2 km and they are located at an elevation range of 1,100- 1,400 masl. Breast height diameter and tree height were measured. Number of stems per hectare, basal area per hectare, and volume per hectare were computed. Estimates of clusters within each plot were analyzed by STRUCTURE and *geneland* software. Samples from the regeneration were analyzed by RAPDs to investigate whether or not there is a difference between the parental and filial generation.

Fig. 5-5 reveals that there are marginal differences between expected heterozygosities and fixation indices between the two plots. No significant differences were noted. The total number of alleles and rare alleles was somewhat higher in the unmanaged forest. Rare alleles in plot A were not found in plot B. The regenerated population in plot A had a somewhat higher diversity estimate than in the parental generation, 0.297 versus 0.257. The increase of diversity was less pronounced in plot B, 0.257 versus 0.246.

F_{ST}s for the individual microsatellites were all four low, 0.011-0.014. The diversity was also studied by 87 RAPDs

The diversity was also studied by 87 markers, which did not indicate any diversity difference between the two plots, 0.257 in plot A versus 0.246 in plot B.

In the spatial separation of plot A seven clusters were identified in the CLUSTER and Geneland analyses while only two clusters were identified in the managed stand, plot B. Fairly high positive F_{IS} estimates, > 0.20, were noted in four clusters, which might be attributed to inbreeding. In spite of these high estimates, the pairwise F_{ST}s among the clusters varied in the range 0.007 and 0.093.

The structural diversity in the unmanaged A-plot was reflected in:

Tree size differentiation
Vertical crown distribution
Horizontal pattern distribution
The managed B-plot had more even-size trees

The difference between the two plots was attributed to history of the stand and the stand age. In the A-plot a wide variation in DBH was noted with a maximum of 70 cm while 75% of the trees in the B-plot had a DBH between 15 and 30 cm. Besides, the trees were mostly of the same age.

In conclusion, the management did not influence the diversity to any large extent. The most pronounced difference was the loss of rare alleles in the managed plot. Since rare alleles only marginally contribute to additive variance, this difference does not seem to be serious. (Additive variance in fitness traits is decisive for the future adaptation of the populations). Contrary to the genetic diversity, the management has influenced the structural character of the populations.

In Germany the impact on beech forests has been considerable for centuries. The consequences of such an impact were studied in three regions, southern, central, and northern Germany. In each region ten populations were selected, six or seven of them were managed and the other three to four stands were classified as unmanaged (Rajendra et al. 2014). Three of the populations were mixed beech and Scots pine stands, the other 27 were pure beech populations. The maximum distance between populations within regions was approximately 20 km. At least 100 samples were taken from each stand. Six genomic microsatellites and three EST-microsatellites were analyzed. The following parameters were computed:

total number of alleles,
number of rare alleles,
effective number of alleles,
allelic richness,
observed heterozygosity,
mean genetic diversity within populations,
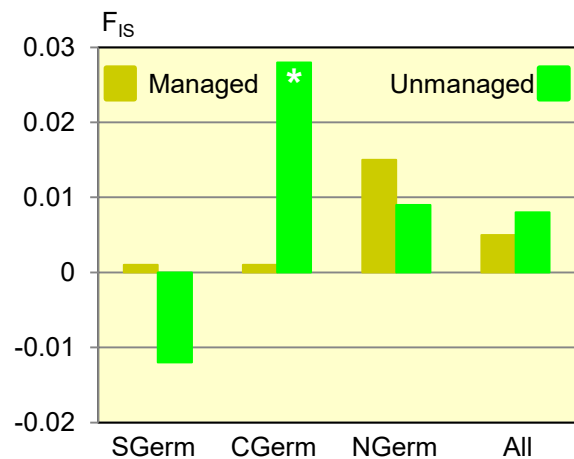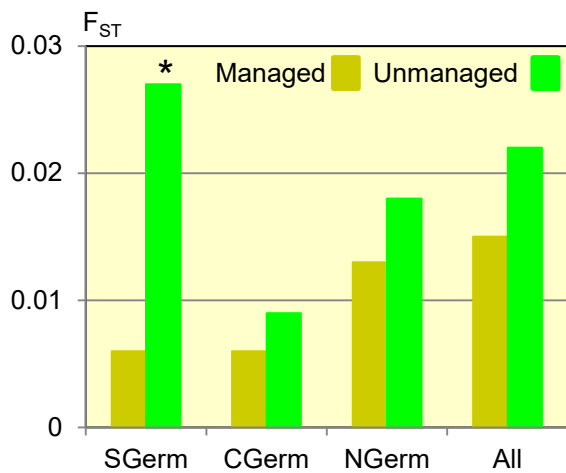fixation index, F_{IS}.

Figure 5-6. Fixation index, F_{IS}, for unmanaged and managed stands in three regions of Germany with ten stands per region. Six genomic microsatellites and three EST-microsatellites were analyzed. * = significant. S = southern, C = central, N = northern. All = pooled data from the three regions. Rajendra et al. 2014.

Diversity among populations, F_{ST}, was estimated as well as multilocus kinship coefficients to estimate the spatial genetic structure within each of the 30 stands.

Generally the genetic diversity was high. Thus, the mean value for all nine markers was 0.623. The genetic diversity was largest in the northern region in spite of the fragmented character of beech forest in this part of Germany. The EST-SSRs contributed most to this result. The site conditions in the northern region are characterized by sand or sandy loam and drier conditions than in the central and southern regions. This might contribute to the observed results. For the parameters listed above there was no significant impact of management. Only once there was significance for the fixation index estimate, F_{IS} (Fig. 5-6) and in this case there was an excess of heterozygotes in the unmanaged population.

The AMOVA revealed that almost all genetic variation occurred within populations and only approximately 1% each was attributed to variation among regions and populations within regions.

Figure 5-7. Population differentiation estimated as F_{ST} in three regions of Germany with ten stands per region for unmanaged and managed stands. Six genomic microsatellites and three EST-microsatellites were analyzed. * = significant. S = southern, C = central, N = northern. All = pooled data from the three regions. Rajendra et al. 2014.
Rajendra et al. 2014.

Approximately 50% of the pairwise F_{ST}s were significant and there were higher F_{ST}s in unmanaged populations (Fig. 5-7). The difference between the two management regimes was pronounced in the southern region. The age of the stands varied and it is impossible to relate the observed difference to any age differences between the two types of populations.

The spatial structure of the investigated populations varied considerably and was most pronounced in the southern region. Management reduced the spatial structure but significance for this trait was only noted for the southern region. Non-random mating in the unmanaged populations leading to complex family structures in unmanaged populations was suggested as explanation for difference in structuring between the two types of management. It was noted that one of the unmanaged populations in the southern region was strongly separated from the other po-

pulations in this region. Its elevation did not differ from the elevations of the other populations within this region and its DBH was 40.4 cm almost identical with the DBH of another unmanaged population in this region. These conditions cannot explain its outlier characteristic. The effect of age of this population cannot be evaluated since its age was unknown.

It was concluded that there was a limited impact of management as regards genetic diversity. Another conclusion was the high neutral genetic diversity in all populations. However, it varied with markers used. Therefore, it was recommended that a careful selection of markers should be done for this type of study.

5.1 Summary

Generally, the impact of management on within-population diversity or fixation index was limited. However, a larger loss of rare alleles was observed in the managed population.

The pollen dispersal distances for successful matings were larger in unmanaged stands than in managed stands, which was attributed to higher tree density in the latter. The relationship between pollination distance and fraction of matings in the unmanaged stands showed a curve with a flat maximum while there was a substantial decrease of successful matings with distance from the female tree in the two other populations.

Rather high estimates of fixation indices, >0.2, were noted for molecular markers in one investigation. The high estimates were attributed to occurrence of clusters with related individuals with preferential matings among the related individuals in such clusters. In another study of ten populations from each of three regions in Germany, a significant excess of homozygotes was noted for the central German region, but the F_{IS} estimate was much lower in this case, 0.028. The genetic distances among the unmanaged populations within the three regions were up to 4.5 times larger than in the managed populations, but still very low estimates, <0.03.

6. References

Aranda1,I., Cano, F.J., Gascó, A, Cochard, H., Nardini, A., Mancha1, J.A. López, R., and Sánchez-Gómez, D. 2014. Variation in photosynthetic performance and hydraulic architecture across European beech (*Fagus sylvatica* L.) populations supports the case for local adaptation to water stress. Tree Phys. 00-1-13. doi:10.1093/treephys/tpu101.

Aranda, I., Sánchez-Gómez,D., de Miguel, M., Mancha, J.A.,, Guevara, M.A., Cadahía, E., Fernández de Simón, M.B. 2017. *Fagus sylvatica* L. provenances maintain different leaf metabolic profiles and functional response. Acta Oecol. 82:1-9.

Basler, D. and Körner, C. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agr. For. Meteor. 165:73-81.

Baudis, M., Ellerbrock, R.H., Felsmann, K., Gessler, A., Gimbel, K., Kayler, Z., Puhlmann, H., Ulrich, A., Weiler, M., Welk, E., and Bruelheide, H. 2014. Intraspecific responses to rainshelter-induced drought and competition of *Fagus sylvatica* L. across Germany. For. Ecol. Manag. 330:283-293.

Bilela, S., Dounavi, A., Fussi, B., Konnert, M., Holts, J., Mayer, H., Rennenberg, H., and Simon J. 2012. Natural regeneration of *Fagus sylvatica* L. adapts with maturation to warmer and drier microclimatic conditions. For. Ecol. Manag. 275:60-67.

Bontemps, A., Klein, E.K., and Oddou-Muratorio, S. 2013. Shift of spatial patterns during recruitment in *Fagus sylvatica*: Evidence from seed dispersal estimates based on genotypic data. For. Ecol. Manag. 305:67-76. Doi.org/10.1016/j.foreco.2013.05.033.

Bontemps, A., Lefevre, F., Davi, H., and Oddou-Muratorio, S. 2015. *In situ* marker-based assessment of leaf trait evolutionary potential in a marginal European beech population. J. Evol. Biol. 29. Doi.org/10.1111/jeb.12801

Božič, G. Ivanković, M., and Kutnar, L. 2013. Genetic structure of european beech (*Fagus sylvatica* L.) seed stands from different forest sites of Gorjanci Mountains as revealed by isoenzymes. Šumarski List, 1–2: 25–32

Bresson, C.C., Vitasse, Y., Kremer, A., and Delzon, S. 2011. To what extent is altitudinal variation driven by genetic adaptation in European oak and beech? Tree Phys. 31:1164-1174.

Buiteveld, J., Vendramin, G.G., Leonardi, S., Kamer, K., and Geburek, T. 2007. Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. For. Ecol. Manag. 247:98–106.

Burczyk, J., Adams, W.T., Birkes, D.S., and Chybicki, I.J. 2006. Using genetic markers to directly estimate gene flow and reproductive success parameters in plants on the basis of naturally regenerated seedlings. Genetics 173:363-372.

Burczyk, J., Adams W.T., Moran G.F., Griffin A.R. 2002. Complex patterns of mating revealed in a *Eucalyptus regnans* seed orchard using allozyme markers and the neighbourhood model. Mol Ecol 11: 2379–2391.

Burczyk J., Adams W.T., and Shimizu, J.Y. 1996. Mating patterns and pollen dispersal in a natural knobcone pine (*Pinus attenuata* Lemmon.) stand. Heredity 77: 251–260.

Caffarra, A. and Donelly, A. 2011. The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. Int. J. Biometeorol. 55;711-721.

Capblancq, T., Morin, X., Gueguen, M., Renaud, J., Lobreaux, S., and Bazin, E. 2019. Climate associated genetic variation in *Fagus sylvatica* and partial responses to climate change in the French Alps. Preprint BioRxiv. Doi.org/10.1101/849406

Carsjens, C., Ngoc, Q.N., Guzy, J., Knutzen, F., Meier, I.C., Müller, M., Finkeldey, R., Leuschner, C. and Polle, A. 2014. Intra-specific variations in expression of stress-related genes in beech progenies are stronger than drought-induced responses. Tree Phys. 34:1348-1361.

Chubicki, I.J., Trojankiewicz, M., Oleksa, A., Dzialuk, A., and Burczyk, J. 2009. Isolation-by-distance within naturally established populations of European beech (*Fagus sylvatica*). Botany 87:791-798.

Ciocîrlan, E., Sofletea, E., Ducci, F., and Curtu, A.L. 2017. Patterns of genetic diversity in European beech (*Fagus sylvatica* L.) at the eastern margins of its distribution length. I. Forest 10:916-922. Doi: 10.3832/ifor2446-010.

Comps, B., Gömöry, D., Letouzey, J., Thiébaut, B., and Petit, R.J. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. Genetics157:389-397.

Corander J., Siren, J., and Arjas, E. 2008. Bayesian spatial modeling of genetic population structure. Comput. Stat. 23:111-129.

Cornuet, J.M., Santos, F., Beaumont, M.A., Robert, C.P., Marin, J.M,, Balding, D.J., Guillemaud, T., and Estoup, A. 2008. Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. Bioinformatics, 24:2713–2719.

Csilléry, K., Lalagüe, H., Vendramin, G.G., Gonzalez-Martinez, S.C., Fady, B., and Oddou-Muratorio, S. 2014. Detecting short spatial scale local adaptation and epistatic selection in climate-related candidate genes in European beech (*Fagus sylvatica*) populations. Mol. Ecol. 23:4496-4708.

Čufar, K., De Luis, M., Saz, M.A., Črepinšek, Z., and Kajfež-Bogataj, L. 2012. Temporal shifts in leaf phenology of beech (*Fagus sylvatica*) depend on elevation. Trees 26:1,091-1,100. DOI 10.1007/s00468-012-0686-7.

Cvrčková, H., Máchová, P., Poláková, L., and Trčková, O. 2017. Evaluation of the genetic diversity of selected *Fagus sylvatica* L. populations in the Czech Republic using nuclear microsatellites. J. For. Sci. 63:53-61.

Davi, H., Gillmann, M., Ibanez, T., Cailleret, M., Bontemps, A., Fady, B., and Lefèvre, F. 2011. Diversity of leaf unfolding dynamics among tree species: New insights from a study along an altitudinal gradient. Agr. For. Met. 151:1504-1513.

Dounavi, A., Koutsias, N., Ziehe, M., Hattemer, H.H. 2010. Spatial patterns and genetic structures within beech populations (*Fagus sylvatica* L.) of forked and non-forked individuals. Eur. J. For. Res. 129:1191-1202. DOI 10.1007/s10342-010-0409-9.

Eilmann, B., Sterck, F., Wagner, L., de Vries, S.M.G., von Arx, G., Mohren, G.M.J., den Ouden, J., and Sass-Klaassen, U. 2014. Wood structural differences between northern and southern beech provenances growing at a moderate site. Tree Phys. 34:882-893.

Eriksson, G., Clapham, D., and Ekberg, I. 2013. Genetics Applied to Forestry. An introduction. 206pp.

Falusi, M. and Calamassi, R. 2003. Bud dormancy in beech (*Fagus sylvatica* L.). Effect of chilling and photoperiod on dormancy release of beech seedlings. Tree Physiol. 6:429-438

Foll, M. and Gaggiotti, O. (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics 180: 977–993.

Fu, Y.H., Campioli, M., Deckmyn, G., and Janssens, I.A. 2013. Sensity of leaf unfolding to experimental warming in three temperate species. Agr. For. Meteor. 181:125-132.

Garza, J.C. and Williamson, E.G. 2001. Detection of reduction in population size using data from microsatellite loci. Mol. Ecol. 10:305-318.

Gauzere, J., Klein, E.K., and Oddou –Muratorio, S. 2013. Ecological determinants of mating system within and between three *Fagus sylvatica* populations along an elevational gradient. Mol. Ecol.22:5001-5015. Doi 10.1111/mec.12435.

Gerber S., Mariette S., Streiff R., Bodénès, C., and Kremer, A (2000). Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. Mol Ecol 9: 1037–1048.

Gömöry, D and Paule, L. 2011. Trade-off between height growth and spring flushing in common beech (*Fagus sylvatica* L.). Ann. For Sci. 68:975–984.

Gregorius, H.R., 1974: Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. Silv. Gen, 23: 22–27

Harter, D., Nagy, L., Backhaus, S., Beierkuhnlein, C., Fussi, B., Huber, G., Jentsch, A., Konnert, M., Thiel, D., and Kreyling, J. 2015. A comparison of genetic diversity and phenotypic plasticity among European beech (*Fagus sylvatica* L.) populations from Bulgaria and Germany under drought and temperature manipulation. I. J. Plant Sci. 176:232-244. DOI:101086/679349.

Hassenkamp, N., Ziegenhagen, B., Mengel, C., Schulze, L., Schmitt, H-P., and Liepelt, S. 2011. Towards a DNA marker assisted seed source identification: a pilot study in European beech (*Fagus sylvatica* L.). Eur. J. For. Res. 130:513-519. DOI 10.1017/s10342-010-0493-3

Herbette, S., Wortemann, R., Awad, H., Huc, R., Cochard, H., and Barigah, T.S. 2010. Insights into xylem vulnerability to cavitation in *Fagus sylvatica* L.: phenotypic and environmental sources of variability. Tree Phys. 30:1448-1455.

Hill, W.G., and Robertson, A. (1968). Linkage disequilibrium in finite populations. Theor. Appl. Genet. 38:226–231.

Jochner, S.C., Sparks, T.H., Estrella, N., and Menzel, A. 2012. The influence of altitude and urbanization on trends and mean dates in phenology (1980 – 2009). J. Biomet 56:387-394. DOI 10.1007/s00484-011-0444-3

Jones, F.A., Chen, J., Weng, G.J., and Hubbel, S.P. 2005. A genetic evaluation of seed dispersal in the neotropical tree, *Jacaranda copaia* (*Bignoniaceae*) Amer. Nat. 166:543-555.

Jones, F.A, and Muller-Landau, H.C. (2008) Measuring long-distance seed dispersal in complex natural environments: an evaluation and integration of classical and genetic methods. J. Ecol. 96:642–652.

Kempf, M. and Konnert, M. 2016. Distribution of genetic diversity in *Fagus sylvatica* at the north-eastern edge of the natural range. Silva Fenn. 50. 17 pp. http://dx.doi.org/10.14214/sf.1663.

Klein, E.K., Carpentier, F.H., and Oddou-Muratorio, S. 2011. Estimating the variance of male fecundity from genotypes of progeny arrays: evaluation of the Bayesian forward approach. Meth. Ecol. Evol. 2:349-361. Doi:10.1111/j.2041-210X.2010.00085.x

Knutzen, F., Meier, I.C., and Leuschner, C. 2015. Does reduced precipitation trigger physiological and morphological drought adaptations in European beech (*Fagus sylvatica* L.)? Comparing provenances across a precipitation gradient. Tree Phys. 25:949-963.

Kraj, W. and Sztorc, A. 2009. Genetic structure and variability of phenological forms in the European beech (*Fagus sylvatica* L.). Ann. For. Sci. 66:203. 7pp. DOI:/forest/2008085.

Kramer, K., Degen, B., Buschbom, J., Hickler, T., Thuiller, W., Sykes, M.T., and de Winter, W. 2010. Modelling exploration of the future of European beech (*Fagus sylvatica* L.) under climate change – range, abundance, genetic diversity and adaptive response. For. Ecol. Manag. 259:2213-2222.

Kramer, K., Ducousso, A., Gömöry, D., Kehlet Hansen, J., Ionita, L., Liesebach, M., Lorent ̧ A. Schüler , S., Sulkowska, M., Vries, S., and von Wühlisch, G. 2017. Chilling and forcing requirements for foliage bud burst of European beech (*Fagus sylvatica* L.) differ between provenances and are phenotypically plastic. Agr. For Meteorology 234:172-181.

Kreyling, J., Thiel, D., Nagy, L., Jentsch, A., Huber, G., Konnert, M., and Beierkuhnlein, C. 2012. Late frost sensitivity of juvenile *Fagus sylvatica* L. differs between southern Germany and Bulgaria and depends on preceding air temperature. Eur. J. For. Res. 131:717–725

Kreyling, J., Buhk, C., Backhaus, S., Hallinger, M., Huber, G., Huber, L., Jentsch, A. Konnert, M., Thiel, D., Wilmking, M., and Beierkuhnlein, C. 2014. Local adaptations to frost in marginal and central populations of the dominant forest tree *Fagus sylvatica* L. as affected by temperature and extreme drought in common garden experiments. Ecol. Evol. 4(5):594-605.

LaFontaine, G., Ducousso, A., Lefèvre, S., Magnanou, E., and Petit, R.J. 2013. Stronger spatial genetic structure in recolonized areas than in refugia in the European beech. Mol. Ecol. 22:4397-4412. Doi: 10. 1111/mec.12403

Lalagüe, H., K. Csilléry, K., Oddou-Muratorio, S,. Safrana, J., de Quattro, C., Fady, B., González-Martínez, S.C., and Vendramin, G.G. 2014. Nucleotide diversity and linkage disequilibrium at 58 stress response and phenology candidate genes in a European beech (*Fagus sylvatica* L.) population from southeastern France. Tree Gen. Genom. 10:15-26.

Lander, T.A., Oddou-Muratorio, S., Proullet-Leplat, H., and Klein, E. 2011. Reconstruction of beech population bottleneck using archival demographic information and Bayesian analysis of genetic data. Mol. Ecol. 20:5182-5196.

Lefèvre, S., Wagner, S., Petit, R.J., and de LaFontaine, G. 2012. Multiplexed microsatellite markers for genetic studies of beech. Mol. Ecol. 12:484-491. Doi: 10.1111/j.1755-0998.2011.03094.x

Leonardi, S., Piovani, P., Scalfi, M., Piotti, A., Giannini, R., and Menozzi, P. 2012. Effect of habitat fragmentation on the genetic diversity and structure of peripheral populations of beech in Central Italy. J. Her. 103:408-417.

Liesebach, M. 2012. Wachstum und phänotypische Variation von sechs Herkünften der Rot-Buche (*Fagus sylvatica* L.) an einem Standort in Schleswig-Holstein. Appl. Agric. For. Res. 4:179-192.

Loiselle, B.A., Sork, V.L., Nason, J., and Graham, C. 1995. Spatial genetic structure of a tropical understory shrub, *Psycotria officinalis* (Rubiaceae). Am. J. Bot. 82:1420—1425. Doi:10.2307/2445869.

Lynch, M. and Ritland, K. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152: 1753–1766.

Manzanedo, R.D., Schanz, F.R., Fischer, M., and Allan, E. 2018. *Fagus sylvatica* seedlings show provenance differentiation rather than adaptation to soil in transplant experiments. BMC Ecology 18:42. https://doi.org/10.1186/s12898-018-0197-5

Matyás C., Bozic, G., Gömöry, D., Ivankovic, M., and Rasztovits, E. 2009. Juvenile growth response of European beech (*Fagus sylvatica* L.) to sudden change of climatic environment in SE European trials. Forest – Biogeosciences and Forestry 2:213-220. Doi: 10.3832/ifor0519-002.

Maurer, W., Cremer, E., Degen, B., Konnert, M., Tröber, U., Jolivet, C., Liesebach, H., Fladung, M., Schneck, V., Scholz, F., Köhl, M., Brandt, R., Jenner, R., Naujoks, G., Stauber, T., Weckfort, G., and Münch, E. 2008. Erfassung der genetischen Struktur der Rotbuche (*Fagus sylvatica*) als Grundlage für ein genetisches Monitoring wichtiger Waldbaumarten in Deutchland. Final report BLE 82pp.

Millerón, M., López de Hereida, U., Lorenzo, Z., Alonso, J., Dounavi, A., Gil, L., and Nanos, N. 2013. Assessment of spatial discordance of primary and effective seed dispersal of European beech (*Fagus sylvatica*) by ecological and genetic methods. Mol. Ecol. 22:1531-1545.

Millerón, M., López de Hereida, U., Lorenzo, Z., Perea, R., Dounavi, A., Alonso, J., Gil, L., and Nanos, N. 2012. Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus sylvatica* L.). Plant Biol. 219:1715-1728. Doi 10.1007/s.11258-012-0125-2

Moran, E.V. and Clark, J.S. 2011. Estimating seed and pollen movement in a monoecious plant: a hierarchical Bayesian approach integrating genetic and ecological data. Mol. Ecol. 20:1248-1262.

Oddou-Muratorio, S., Bontemps, A., Klein, E.K., Chybicki, I., Vendramin, G.G., and Suyama, Y. 2010. Comparison of direct and indirect genetic methods for estimation seed and pollen dispersal in *Fagus sylvatica* and *Fagus crenata*. For. Ecol. Manag. 259:2151-2159. Doi:10.1016/j.foreco.2010.03.001

Oddou-Muratorio, S. and Davi, H. 2014. Simulating local adaptation to climate of forest trees with a physio-demo-genetics model. Evolutionary Applications ISSN 1752-4571. 15pp.

Oddou-Muratorio, S. and Klein, E.K. 2008. Comparing direct vs. indirect estimates of gene flow within a population of a scattered tree species. Molecular Ecology, 17, 2743–2754.

Oddou-Muratorio, S., Klein, E.K., and Austerlitz, F. 2005. Pollen flow in the wild service tree; *Sorbus torminalis* (L.) Cranz. II. Pollen dispersal and heterogeneity in mating success inferred from parent-offspring analysis. Mol. Ecol. 14.4441-4452.

Oddou-Muratorio, S., Klein, E.K., Vendramin, G.G., and Modefluga, B. 2011. Spatial vs. temporal effects on demographic and genetic structures: the roles of dispersal, masting and differential mortality on patterns of recruitment in *Fagus sylvatica* Mol. Ecol. 20:1997-2010

Ohta, T. 1962. Linkage disequilibrium due to random genetic drift in finite subdivided populations. Proc. Nat. Acad. Sci. 79:1940-1944.

Pafetti, D., Travaglini, D., Buonamici, A., Nocentini, S., Vendramin, G.G., Giannini, R., and Vettori C. 2012. The influence of forest management on beech (*Fagus sylvatica* L.) stand structure and genetic diversity. For. Ecol. Manag. 284:34-44.

Peuke, and Rennenberg, H. 2011. Impacts of drought in mineral macro- and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings. Tree Phys. 31:196-207.

Piotti, A., Leonardi, S., Buiteveld, J., Geburek, T., Gerber, S., Kramer, K. Vettori, C., and Vendramin, G.G. 2012. Comparison of pollen gene flow among four European beech (*Fagus sylvatica* L.) populations characterized by different management regimes. Heredity 108:322-331.

Pluess, A.R. and Weber, P. 2012. Drought-adaptation potential in *Fagus sylvatica*: linking moisture availability with genetic diversity and dendrochronology. PLoS ONE 7(3): e33636. doi:10.1371/journal.pone.0033636

Pritchard J.K, Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–

Pšidová, E. Ditmarová, I., Jamnická, G., Kurjak, D., Majerová, J., Czajkowski, T., and Bolte, A. 2014. Photosynthetic response of beech seedlings of different origin to water deficit. Photosynthetica 53:187-194.

Rajendra, K.C., Seifert, S., Prinz, K., Gailing, O., and Finkeldey, R. 2014. Subtle human impacts on neutral diversity and spatial patterns of genetic variation in European bech (*Fagus sylvatica*). For. Ecol. Manag. 319:138-149.

Ribbens, E., Silander, J.A., and Pacala, S.W. 1994. Seedling recruitment in forests – Calibrating models to predict patterns of seedling dispersion. Ecology 75:1794-1806..

Ritland, K. 1996. Marker-based method for inference about quantitative inheritance in natural populations. Evolution 50:1062-1073.

Robledo-Amuncio, J.J., Austerlitz, F, and Smouse, P.E. 2006. A new method of estimating pollen dispersal curve independent of effective density. Genetics 173: 1033-1045.

Robledo-Arnuncio, J.J. and Garcia, C. 2007. Estimation of the seed dispersal kernal from exact identification of source plants. Mol. Ecol. 16:5998-5109

Robson, T.M., Rasztovits, E., Aphalo, P.J., Alia, R., and Aranda, I. 2013. Flushing phenology of European beech (*Fagus sylvatica* L.) provenances from a trial in La Rioja, Spain, segregate according to their climate of origin. Agr. For. Meteor. 180:76-85.

Robson, T.M., Sánchez-Gómez, D., Cano, F. J., and Aranda, I. 2012. Variation in functional leaf traits among beech provenances during a Spanish summer reflects the difference in their origin. Tree Gen. Genom. 8:1111-1121.

Rose, L., Leuschner, C., Kökkemann, B., and Buschman, H. 2009. Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? Eur. J. For. Res. 128:335-343.

Sánchez-Gómez, D., Robson, T.M., Gascó, A., and Gil-Pelegrin, E. 2013. Differences in the leaf functional traits of six beech (*Fagus sylvatica* L.) populations are reflected in their response to water limitation. Env. Exp. Bot. (7:110-119.

Schueler, S. and Liesebach, M. 2015. Latitudinal population transfer reduces temperature sum requirements for bud burst of European beech. Plant Biol. 216:111-122.

Seifert, S., Vornam, B., and Finkeldey, R. 2012. DNA sequence variation and development of SNP markers in beech (*Fagus sylvatica* L.). Eur. J. For. Res. 131:1761-1770. DOI 10.1007/s10342-012-0630-9.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

Stojnic, S., Sass-Klaassen, U., Orlovic, S., Matovic, B., and Eilmann, B. 2013. Plastic growth response of European beech provenances to dry site conditions. IAWA Journal 34:475-484.

Thiel, D., Kreyling, J., Backhaus, S., Beierkuhnlein, C., Buhk, X., Egen, K., Huber, G., Konnert, M., Nagy, L., and Jentsch, A. 2014. Different reactions of central and marginal provenances of *Fagus sylvatica* to experimental drought. Eur. J. Forest Res. 133:247-260.

Valladares, F., Sanches-Gomez, D., and Zavala, M.A. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J. Ecol. 94:1103-1116.

Vitasse, Y. and Basler, D. 2013. What role for photoperiod in the bud burst phenology of European beech. Eur. J. Forest Res. 132:1-8. DOI 10.1007/s10342-012-0661-2.

Vitasse, Y., Bresson, C.C., Kremer, A.R., and Delzon, S. 2010. Quantifying phenological plasticity to temperature in two temperate tree species. Funct. Ecol. 24:1211-1218. Doi:10.1111/j.1365-2435.2010.01748.x.

Vitasse, Y., Bresson, C.C., Michalet, R., and Kremer, A., Delzon, S. 2009a. Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. Can. J. For. Res. 39:1259-1269. Doi:10.1139/X09-054.

Vitasse, Y., Delzon, S., Dufrêne, E., Pontailler, J-Y., Louvet, J-M., Kremer, A., and Michalet, R. 2009b. Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? Agr. For. Meteor. 149:735-744.

Vitasse, Y., Hoch, G., Randin, C.F., Lenz, A., Kollas, C., Scheepens, J.F., and Körner, C. 2013. Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. Oecologia 171:663-678.

Vitasse, Y., Porté, A.J., Kremer, A., Michalet, R., Delzon, S. 2009c. Response of canopy duration to temperature changes in four temperate tree species: contribution of spring and autumn phenology. Oecologia 161:187-198.

Wang, J.L. 2002. An estimate for pairwise relatedness using molecular markers. Genetics 160:1203-1215.

Weber, P., Bugmann, H., Pluess, A.R., Walthert, L., and Rigling, A. 2013. Drought response and changing mean sensitivity of European beech close to the dry distribution limit. Trees 27:171-181.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistic for the analysis of population structure. Evolution 38:1358-1370.

Wortemann, R., Herbette, S., Barigah, T.S., Fumanal, B., Alia, R., Ducousso, A., Gömöry, D., Roekel-Devret, P. and Cochard, H. 2011. Genotypic variability and phenotypic plasticity of cavitation resistance in *Fagus sylvatica* L. across Europe. Tree Phys. 31:1175-1182.

Železnik, P., Westergren, M., Božič, G., Eler, K., Bajc, M., Helmisaari, H-S., Horvath, A., and Kraigher, H. 2018. Root growth dynamics of three beech (Fagus sylvatica L.) provenances. For. Ecol. Manag. Doi.org/10.1016/j.foreco.2018.o6.024.

BioCenter
Department of Plant Biology SLU
Box 7070, 750 07 Uppsala
Sweden