Larix

Recent Genetic Research

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Preface

After the publications of a series of books entitled *Species - Recent Genetic Research* dr Seppo Ruotsalainen, Finland suggested that I should take on larch genetic research. I thought this was an inspiring challenge. Moreover, my forestry career started with larch species. My thesis dealt with the unique meiotic division in the three larch species *Larix decidua*, *L. kaempferi*, and *L. sibirica*. A few years ago Pâques et al. (2013) published the comprehensive article *Larches (Larix sp.) in Forest Tree Breeding in Europe 25, Current State-of-the-Art and Perspectives*. There is certainly some overlap between my summary and that article, which has a broader approach than I have in my summary of larch genetics research. Another difference is that I have included genetic studies of Asian and American larch species. As usual graphic illustrations are in focus in my summary. It should be noted that none of the illustrations were taken from the original papers. With a few exceptions, only literature from 1980 and onwards are included. As in the previous books, papers written in languishes that are not understood by the scientific society are not treated. For Japanese larch I have used *L. kaempferi* throughout the book even if some authors have used *L. leptolepis* for this species.

A generous grant from *Föreningen Skogsträdsförädling*, The Tree Breeding Association in Sweden, made this printing possible, which is much appreciated. My sincere thanks to Dr Björn Nicander for his willingness to swiftly solve any computer problems. A web version of this book is available at http://plantbio.vbsg.slu.se/webb/larix.pdf.

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Gösta Eriksson
Content

Preface

1. Population
   1.1 Metric traits
   1.1.1 Europe
   1.1.2 Asia
   1.1.3 America
   1.2 Disease tolerance
   1.3 Markers
   1.3.1 Isozymes
   1.3.2 Microsatellites, chloroplast DNA, mitochondrial DNA
   1.4 Species relationships
   1.5 Miscellaneous
   1.6 Summary
   1.6.1 Quantitative variation
   1.6.2 Markers
   1.6.3 Species relationships

2. Progeny testing
   2.1 Marker inheritance
   2.2 Quantitative traits
   2.2.1 Europe
   2.2.2 America
   2.3 Inbreeding
   2.4 Species hybrids
   2.5 Clone tests
   2.6 Summary

3. Breeding
   3.1 Breeding programs
   3.2 Reproduction
   3.3 Flowering stimulation
   3.4 Seed production in species hybrids
   3.5 Vegetative propagation
   3.6 Somatic embryogenesis
   3.7 Transformation
   3.8 Summary

4. References
Populations

1.1 Metric traits

1.1.1 Europe

Giertych (1979) summarized the results from 15 field trials belonging to an IUFRO provenance series of 43 populations of *L. decidua*. In addition, five other seed sources were included in the test plantations; populations outside the natural range of *L. decidua* or *L. kaempferi* and *L. sibirica* populations. The northernmost trial was located at latitude 62.00°N in Finland and the southernmost in USA, latitude 43.17°N. All populations were not represented in all trials. The ages of assessment varied between six years and 31 years. The graphic illustrations I have prepared are based on trials with a large number of populations and assessments at age ten years and older. Populations originated from collection of seeds from a few trees are not included in my scrutiny since their offspring may not be representative of their populations. Giertych preferred to illustrate the results separately for populations from different regions at 3-8 trials. He used standardized values of the populations in maps in order to enable a comparison of the results from different ages and growth conditions of the trial sites. This is a meritorious way of illustrating the performance of the populations but with 35 maps it is not easy to get a general overview of the results.

Fig. 1-1 reveals that there are identical and strong relationships between population latitudinal origin and tree height in two Central European trials. The second-degree polynomial relationships are surprisingly strong considering the broad elevational span of the populations, 30-1,900 masl. The similarity in performance suggests that there is no or limited population x trial locality interaction for these two trials. This is somewhat surprising since the difference in elevation between the two trials is > 600 meters. However, the mean tree heights of the two trials did not differ substantially, 10.5 m for the trial at 950 masl and 11.5 m for the trial at 330 masl. However, a few populations were unstable when the performance at all trials was considered, which is expected with such a broad range of trials. There were only weak relationships between population elevation origin and tree height in these trials (Fig. 1-2). This means that the latitudinal impact on growth is much stronger than the impact of population elevational origin in these two Central European trials.

![Figure 1-1](image1.png)

**Figure 1-1.** The relationship between *L. decidua* population latitude and tree height in meters at age 20 in two of the provenance trials belonging to IUFRO 1944 provenance trials series. 18Cz is a Czech trial at 950 masl; 19Pl is a Polish trial at 330 meters above sea level. The latitudes of the trials are indicated. Giertych 1979.

![Figure 1-2](image2.png)

**Figure 1-2.** The relationship between *L. decidua* population elevation and tree height in meters at age 20 in two of the provenance trials belonging to IUFRO 1944 series of provenance trials. 18Cz is a Czech trial at 950 masl in Czechia; 19Pl is Polish trial at 330 meters above sea level. Giertych 1979.
The oldest assessments emanate from an Italian trial, age 31. There was no relationship between tree height and latitudinal origin while a weak relationship was obtained after exclusion of one Italian high-elevation population (shown as red square in Fig. 1-3). The opposite situation was noted for the southern Swedish trial with a fairly strong relationship between population latitudinal origin and tree height (Fig. 1-4) while there was no relationship between elevational origin and tree height. It is evident that both the southernmost and northernmost populations were much inferior to the best performing populations at this northern trial. As seen from the latter two figures the number of populations is less than in the Czech and Polish trials. Giertych concluded that latitudinal origin played a greater role than elevational origin for height growth, which is confirmed in the above graphic illustrations.

The geographically furthest transfer of _L. decidua_ populations in this series took place to the USA trial in New Hampshire. For this reason I have illustrated the geographic relationships between tree height and latitude or elevation for this trial (Fig. 1-5 and 1-6). The relationship with latitude was fairly strong, $R^2 = 0.67$, while the fit to the third degree polynomial was somewhat weaker. Giertych stated that the Sudetan populations from the Czech Republic performed well in most trials. I have illustrated this for the six populations from this country in Fig. 1-7. As seen from this figure only twice Sudetan populations did not pass the mean performance. At the Italian test locality all three populations were close to the mean performance.

**Figure 1-3.** The relationship between _L. decidua_ population elevation and tree height in meters at age 31 in an Italian provenance trial belonging to the IUFRO 1944 series of provenance trials. The trial is located at latitude 44.02°N and 330 meters above sea level. A strongly deviating population, shown in red, from latitude 46.34°N is not included in the relationship. Giertych 1979.

**Figure 1-4.** The relationship between _L. decidua_ population latitude and tree height in meters at age 12 in a provenance trial at latitude 56.97°N and 175 masl in Sweden belonging to IUFRO 1944 series of provenance trials. Giertych 1979.

**Figure 1-5.** The relationship between _L. decidua_ population latitude and tree height in meters at age 12 in a provenance trial at latitude 43.17°N and 260 masl in USA belonging to IUFRO 1944 series of provenance trials. Giertych 1979.
The Swiss and Italian populations from the western part of the *L. decidua* distribution range performed poorly in several of the trials. As illustrated in Fig. 1-8 only in one case these populations were above the trial mean value; one high-elevation Italian population from 1,400 masl. Giertych made a strong statement *Neither elevational nor latitudinal transfer affect choice of best provenances. The same Sudetan provenances are the best producers wherever tried.* He further claimed that it would be desirable to have information on volume per hectare and that height is sufficiently correlated with most other important growth characteristics. Thus, it justifies the analysis of height only. Finally, the two Japanese larch populations were inferior to the Sudetan larch populations and introduction of this species was advised against.

In conclusion, this publication is one important contribution to the understanding of the genecology of *L. decidua* as regards growth.

In 1992 Weisgerber and Šindelár summarized the existing knowledge on genetic differentiation for growth, stem quality, and tolerance against larch cancer (*Trichoscyphella willkommii*). One focal point in this summary was the international provenance series with trials established in several Central European countries as well as in the USA. The information was presented in maps with different shading of circles or squares. This means that, observed figures of individual populations are not available, but trends can be seen in the published figures.

**Figure 1-7.** The relative tree height of six Czech populations (shown with different screens) in relation to the trial means in five trials belonging to the IUFRO series of *L. decidua* provenance trials. Red columns refer to populations originating from localities below 600 masl and blue to localities above 600 masl in the Czech Republic. Giertych 1979.

**Figure 1-8.** The relative tree height of two Italian and two Swiss populations in relation to the trial means in five trials belonging to the IUFRO series of *L. decidua* provenance trials. Pl = Poland, Cz = Czechia, It = Italia, Se = Sweden. Giertych 1979.
I have tried to summarize the results in the youngest series of trials at age 20 in Table 1-1. This table reveals that the best growth was noted for Sudetan and Polish populations followed by The Tatra region and eastern Alpine populations. It is evident that the further to south-west the poorer the growth. There might be a confounding of geography and elevation. Thus there are mainly high-elevation sources in the region with poor growing populations. An opposite trend is revealed for stem form, the slowly growing populations have the best stem form while the Polish populations showed the poorest stem form. With respect to tolerance against larch cancer the north-eastern populations and the low-elevation eastern Alpine populations had a high tolerance against this pathogen. Some seed sources obtained from planted stands have shown good characteristics in this provenance series.

Table 1-1. A summary of existing knowledge on growth, stem quality and disease tolerance of L. decidua populations. Weissgerber and Šindelár 1992.

<table>
<thead>
<tr>
<th>Region</th>
<th>Growth</th>
<th>Stem quality</th>
<th>Disease tolerance, 1 trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poland</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Sudeten</td>
<td>Good</td>
<td>Intermediate</td>
<td>Good</td>
</tr>
<tr>
<td>Tatra</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>Good</td>
</tr>
<tr>
<td>The Alps:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudes 6-7 °E</td>
<td>Poor</td>
<td>Intermediate</td>
<td>Poor</td>
</tr>
<tr>
<td>Longitudes 10-13 °E</td>
<td>Poor-intermediate</td>
<td>Intermediate</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Longitudes 13-17 °E</td>
<td>Intermediate</td>
<td>Good</td>
<td>High elevation poor</td>
</tr>
<tr>
<td>Longitudes 17-19 °E</td>
<td>Intermediate</td>
<td>Good</td>
<td>Low elevation good</td>
</tr>
</tbody>
</table>

Growth and quality of autochtonous as well as exotic German populations of L. decidua were studied at four Eastern German trials by Weiser (1992). Data on the performance at age 15 in the four trials (elevation range 85 – 410 masl) were pooled and no statistical evaluation of the results was presented. There was a large variation in growth and stem form with a good growth of exotic Eastern German populations while western German exotic populations showed poor growth. It was speculated that an adaptation to the existing low-elevation conditions in eastern Germany had taken place in the populations growing in this part of Germany. The most probable origin of these populations was Sudetan Mountains in the Czech Republic while the western German populations probably originated from The Alps. Finally there was a significant and negative relationship between stem form and tree height but the relationship did not explain much of the variation, r = -0.42.

Twelve larch populations from L. sukaczewii (5), L. sibirica (4), L. decidua (2), and L. gmelini (1) were studied with respect to spring frost tolerance under controlled conditions (Eysteinsson and Skulason 1995). After heat sum accumulation for 6.5 or 11.5 days at temperatures 9, 12, and 15°C the twigs were exposed to four freezing temperatures, -5, -10, -15, and -20°C. After freezing the twigs were kept at +20°C and frost damage was recorded in a scale 0 – 10 based on the degree of cambium damage. Only mean values of frost damage for all treatments were reported.

Figure 1-9. Mean frost damage score in 12 populations of four larch species after freeze testing at -5, -10, -15, and -20°C during bud flushing. Populations connected with bars are not significantly different. Eysteinsson and Skulason 1995.
As seen from Fig. 1-9 the *L. sukaczewi* and *L. sibirica* populations were most affected. These results might be attributed to lower heat demand for flushing in these two species than in *L. decidua* and *L. gmelini*. Threshold temperatures for flushing that was calculated in a complex way supported this interpretation. *L. decidua* had the highest threshold temperature, +5°C, while the corresponding temperature for most of the *L. sukaczewi* and *L. sibirica* populations was +1°C. It is regrettable that frost damage of individual treatments was not presented.

Pâques (1996a) reported on growth and quality traits in an old provenance trial with 17 *L. decidua* populations in Bretagne, France (Lat. 48.52°N, Long. 3.42°W, and 200 masl). The spacing was 2 x 2 meters and five replications. The following traits were assessed:

- Survival
- Height, circumference
- Height/DBH, straightness
- Pilodyn penetration, density, heartwood %

All traits assessed showed strongly significant population differences. At age 11 the populations from The French Alps had a poor survival, 20-25%, while the survival in populations from South-Eastern and Northern Alps had survival in the range 50-70%. The other populations had a satisfactory survival, i.e. >70%. Except for two populations from the South-Eastern Alps the variation in circumference was moderate, ±12% (Fig 1-10). The variation was somewhat smaller for density ±7%. The largest variability was noted for straightness with the best population being 17% above the average and the worst straightness was noted for a Sudetan population with a mean value 24.4% lower than the average. Seven of the nine Alpine populations deviated positively from the mean percentage while the four Sudetan populations had poor straightness, 75-83%.

The Pearson correlation between pilodyn penetration and density was significant, r = -0.68, which facilitates breeding. Except for density the percentage of heartwood was significantly correlated with all other traits. However, only in one case, tree height, the correlation coefficient exceeded 0.70.
Since the populations cover a large geographic range I found it of interest to test the relationship between certain traits and such variables as latitude and elevation at the origin of the populations. The relationships with circumference and these two geographic variables are presented in Figs. 1-11 and 1-12. It should be noted that the high-elevation French population from 1,730 masl showed an exceptional good growth. Such a growth performance deviates from results from other conifer species. Therefore, I found it justified to exclude this population from the relationships. It should be remarked that with respect to tree height this population did not deviate from the general pattern. I suspect that there is some error with the estimate of 11% above the mean for circumference while the estimate for height was 5% below the mean. It should also be noted that there was no latitude given for one of the Sudeten populations and no elevation was given for another Sudeten population. There were moderately strong relationships between population elevation or population latitude with circumference after exclusion of the deviating population. The relationships agree with results from *Picea abies* and *Pinus sylvestris* which show similar maxima for transfer of populations in latitude and elevation. Some age-age correlations for tree height, circumference, and straightness were reported (Fig 1-13). The correlation coefficient for height between ages 2 and 34 is surprisingly high suggesting that selection for height in nursery will substantially increase the genetic gain per time. As expected the correlation for circumference between ages 26 and 34 was almost perfect, $r = 0.94$.

A corresponding trial with twelve Japanese larch populations was reported by Pâques (1996b). The spacing was 4 x 4 meters and assessments were carried out at age 36. The following traits were included and the significant differences among the populations are indicated.

- Height*, circumference
- Height/DBH, straightness, branch angle, branch angle***
- Pilodyn penetration*, density*, heartwood%

Since there was no significant difference for circumference I have illustrated population relative tree heights in Fig. 1-14, which shows that the range is moderately large, 94.5-103.6. The narrow range of latitudinal origins of the populations made it less meaningful to test any relationships between traits and latitudinal origin. It was stated that there were no significant correlations between geo-
graphic variables and traits. However, a detailed analysis of these relationships reveals that some outlier data are the reason for the observed non-significances. Thus, the relationship between tree height and elevation origin shows the same pattern as for L. decidua but with some lower degree of explanation, $r = 0.64$ (Fig. 1-15). It is obvious that the high-elevation population had a great impact on this relationship. Branch angle showed the largest variation among populations. The relationship between population elevation and branch angle was strong after exclusion of one outlier population (Fig. 1-16). It would have been useful to have a discussion of the outlier population values in this and the previous paper.

In contrast to the situation in L. decidua, the age-age correlation coefficients for height increased considerably with age and reached 0.88 at age 20 (Fig. 1-17). The corresponding correlations for circumference and straightness for the age-20 – age-36 correlation were moderate. They are of the same strength as the corresponding for L. decidua 13-34 correlations.

These two reports are very important thanks to the age of assessments. Especially for the L. decidua report the large variation of most of the traits studied means that selection of reforestation material is important.
Phenological observations at five localities in the Italian Alps were carried out during two seasons by Magliavacca et al. (2008). At each locality three L. decidua stands in lower, middle, and upper part were included in this study. The elevational range of the localities was 1,350-2,160 masl. Since no classification system of larch phenology existed it was necessary to develop systems both for flushing and growth cessation, each with five stages. During two years a large number of observations were carried out during flushing and growth cessation, 15-21 and 12-15, respectively. Based on these observations the length of the growing season was calculated and the driving environmental conditions for flushing and growth cessation were calculated with advanced modelling.

In Fig. 1-18 the relationships between population elevation origin on one hand and bud flushing or growth cessation on the other hand are shown for the observation dates with the largest resolution among the five populations. Both relationships are extremely strong but it should be remembered that the data from the lowest elevation population weighs heavily in these relationships. In the paper instructive relationships are given for the relationships between population elevation origin and three phenological traits; onset of flushing ($R^2=0.93$), yellow spot decolouration ($R^2=0.85$), and growth period duration ($R^2=0.94$). The data were pooled from the two years of observation. The strong relationship involving bud flushing was attributed to the variation in temperature at different elevations. The somewhat lower strength of the relationship as regards growth cessation was attributed to the interaction between temperature and photoperiod. The results observed suggested that the higher autumn temperatures during the second year of observation caused a delay of growth cessation that year. With respect to duration of the growth period the difference between the low elevation population and the high elevation population was approximately 50 days.

One of the prediction models, coined spring warming, resulted in a difference of 0-7 days between predicted and observed flushing date, which must be regarded as remarkably good. Another model, coined growing season index, predicted the growing season with five days accuracy. The models suggested that daily mean temperatures above +2°C do not limit flushing. Night lengths longer than 15.9 hours trigger the growth cessation. It was also observed that the photoperiod effect on growth cessation decreases with elevation (cf. Fig. 1-19). There might be a confounding of temperature and night length at high elevations. It was stated that the precision of the models was improved if the population at 1,330 masl is excluded, which grows outside the natural range of L. decidua in northern Italy, 1,500-2,200 masl.

In conclusion, a most important investigation on the relationship between environmental conditions, and foremost temperature, on phenology in L. decidua alpine populations.

Detailed anatomical examination of the course of wood formation in seven Swiss populations of L. decidua from one valley was carried out by Moser et al. (2009). Three of the populations were growing on a south-east facing slope and the four others were north-west facing. The populations grow on steep slopes in the elevational range of 1,350-2,150 masl. Microcores from four trees in each population were taken every week between April and October. The data from the populations at the same elevation at the two slopes were pooled since the aspects of the slopes did not influence the development. Flushing was also recorded.

In Fig. 1-20 I have illustrated the relationship between population elevation and three of the traits studied. As expected, flushing starts at valley bottom and proceeds in a linear way upwards. The opposite trend is noted for the end of the growth season estimated as thickening of the cell wall. The duration of the growth period estimated as the number of days between onset of cell enlargement.

**Figure 1-18.** Bud flushing and growth cessation in L. decidua at five localities in northern Italy. Migliavacca et al. 2008.

**Figure 1-19.** The relationship between L. decidua population elevation and photoperiodic limiting effect. The latter is derived from the model of growth cessation. Migliavacca et al. 2008.
and cell wall thickening of the ring showed a different relationship with population elevation. It is striking that all three relationships are exceptionally strong in spite of the low number of trees from each elevation; eight for 1,600, 1,900, and 2,150 masl, while only four for 1,350 masl. It was concluded that the similar responses of the south-west and north-east facing populations suggest that site specific conditions had not influenced the results.

Based on the observed temperatures at the population localities and the assessment of the flushing and wood formation, the impact of a temperature change of 1°C was calculated for four traits (Fig. 1-21). Flushing takes place 7.2 days later with a decrease of the temperature of one degree Celsius. The corresponding figures for the anatomical traits are 5.8-7.4 days. These data are most useful to predict changes following global temperature change.

Twigs from 28 populations, 2 seed orchards, and 2 seed stands growing in a field trial in northern Sweden at latitude 65.18°N and 410 masl were tested for spring and autumn frost tolerance by Eysteinsson et al. (2009). Four larch species were represented in this field trial L. sukaczewii (15), L. sibirica (6), L. cajander (4), and L. gmelini (3). Twigs were collected on May 7-8 and September 26-27. Freeze testing was carried out after fast decrease of temperature to +4°C and then a slow decrease by two degrees per hour to the freezing temperatures -8, -12, -16, and -20°C. The freezing temperatures were kept for two hours. The thawing took place at a rate of two degrees per hour. The proportional damage to cambium was classified into 12 classes; 1 being no damage and 12 being no undamaged tissue.

In spite of the significant differences between treatments the authors did not present results from the four freeze tests separately but mean values for all four freezing treatments. The focus of the presentation was the relationships of autumn and spring frost damage with geographic variables of the populations from all four species. Besides, results from progenies of two seed orchards and two seed tree stands were presented.

Both for spring frost and autumn frost damage the populations from the eastern species, L. cajanderi and L. gmelini, had higher damage rates than the two other species. This influences the relationships with geographic variables strongly. Therefore, I have preferred to study the geographic relationships excluding the two eastern species, L. cajanderi and L. gmelini. I have also excluded data from the L. sibirica Altai population since its elevation is 1,000 meters higher than all other populations. It was noted that even the lowest temperatures tested were not severe enough to induce frost damage in some populations. However, significant differences among populations were obtained.
For all species included in the analysis Eysteinsson et al. (2009) reported a strongly significant relationship between spring frost damage and longitude, $R^2 = 0.76$. As seen from Fig. 1-22 this relationship is weak in the joint analysis of *L. sukaczewii* (blue) and *L. sibirica* (green) populations, $R^2 = 0.27$. This difference in strength of the relationship between my calculation of and the estimate for all species cannot be explained by the exclusion of the two eastern species. The authors did not find any significant relationship with longitude when data from *L. sukaczewii* were analyzed separately. Latitude origin had only a weak impact on spring frost damage in the joint analysis of all four species. Nor did I find any strong relationship with latitude and spring frost damage for *L. sukaczewii* and *L. sibirica* (Fig. 1-23). The authors reported a significant relationship between longitude and spring frost damage in the separate analysis of *L. sukaczewii*; no $R^2$ estimate was given. It was noted that populations from localities with maritime influence were less damaged by spring frosts than populations from localities with continental climate. This was seen as an adaptation to local climatic conditions, which had a maritime character.

In the multiple regression analysis of the relationship between autumn frost damage and latitude, longitude, and elevation origin Eysteinsson et al. (2009) reported significant relationships with latitude and longitude. The strongest was with latitude, $R^2 = 0.53$ while the $R^2$ for longitude was 0.36. In agreement with this I found a fairly strong relationship between longitudinal origin and mean spring frost damage to cambium of *L. sukaczewii* and *L. sibirica* (green) populations after freeze testing in the range -8°C - -20°C. A 12-degree scale was used with 1 = no damaged tissue and 12 = no undamaged tissue. Eysteinsson et al. 2009
The strong relationship with latitude and autumn frost damage is easy to understand. The more northern the origin the earlier the frost tolerance must be attained. The significant effect of longitude on autumn frost damage must be attributed to inclusion of the eastern *Larix* species in the analysis by Eysteinsson et al. (2009).

In Fig. 1-26 the relationship between spring and autumn frost damage is illustrated. Less than 30% of the variation in autumn frost damage is explained by the variation in spring frost.

The authors pointed out that the studied populations were not ideal for studies of relationships of damage with geographic variables. Thus, the relationship with longitude suffers from the lack of a large number of populations from the same latitude over a large longitudinal range. However, this investigation gives important information on the potential of populations for cultivation in northern Sweden in order to avoid risks for spring and autumn frost damage.

Growth, survival, and quality traits at age 25 in two Romanian provenance trials were presented by Mihai and Teodosiu (2009). There were offspring from six natural stands, four artificial stands, and five seed orchards in the provenance trials. In addition, data from a seed orchard with 50 clones from four different Romanian populations were presented. However, in Figs. 2-3 of the paper data from the same populations as in trials 1 and 2 are presented, which does not agree with the statement of four populations in the seed orchard. The design was three replications with 5 x 5 trees per plot. In one table it was stated that the design was 4 x 4 trees per plot. The main focus of the paper was identification of material with good performance as regards growth, survival and quality traits. One trial was located at lat. 45.48°N, long. 22.67°E, and 650 masl while the other was located at lat. 46.17°N, long. 21.84°E, and 300 masl.

The results from the ANOVAs that were carried out separately for each trial reveal that the population differences were strongly significant for all traits in trial 2 (Table 1-2). There was only one trait that showed significant population differences in the seed orchard. With only four populations in the seed orchard, significant difference among the populations might be hard to prove. No joint ANOVA comprising data from the two provenance trials was presented, which is regretted. As seen from Fig. 1-27 it is highly likely that there is a significant population x trial interaction for stem volume. Noteworthy is the good growth of three seed orchard progenies in the southern trial; one of them being German.

**Figure 1-26.** The relationship between spring frost damage score and autumn damage score to cambium of 15 *L. sukaczewii* populations (blue) and five *L. sibirica* populations (green) after freeze testing in the range -8 to -20°C. A 12-degree scale was used with 1 = no damaged tissue and 12 = no undamaged tissue. Eysteinsson et al. 2009

**Figure 1-27.** The stem volume at age 25 in two Romanian provenance trials outside the natural range of *L. decidua*. SO = seed orchard progeny; AS = artificial stand; the six other columns refer to natural stands. Mihai and Teodosiu 2009

<table>
<thead>
<tr>
<th>Stem volume</th>
<th>Branch diameter</th>
<th>Branch number</th>
<th>Stem straightness</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1 Bâutar</td>
<td>***</td>
<td>ns</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>Trial 2 Radna</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Seed orchard</td>
<td>ns</td>
<td>-</td>
<td>ns</td>
<td>***</td>
</tr>
</tbody>
</table>

**Table 1-2.** The differences observed in two Romanian field trials at lat, 45.48°N and at 46.17°N as well as a Romanian seed orchard. Data from natural populations only. Mihai and Teodosiu 2009.
The relationships between tree height and DBH with geographic variables were significant in one case only; tree height and altitude in trial 2. As pointed out in the paper one reason for the absence of significant relationships might be that populations from planted stands and seed orchards were included in the material. Therefore, I tested the relationships between stem volume and latitude for the natural stands only and found a significant relationship in trial 2 (Fig. 1-28). The low number of populations makes it hard to prove any geographic relationships. There were no strong relationships with the other two geographic variables, longitude and altitude.

In spite of the better growth in trial 1, the survival was higher in this trial than in trial 2, 53.5 versus 34.6%. The better growth might have led to stronger competition and death of trees for this reason. The highest survival was noted for the seed orchard offspring, 60.9%. No discussion of the reason for the poor survival was presented. Also for survival a strong population x trial interaction probably exists (Fig. 1-29). Only in one case there was a strong and negative relationship between survival and a geographic variable; latitude in the seed orchard.

Stem straightness was generally better in trial 2 in spite of the lower competition between trees in this trial; mean value 1.2 versus 1.60. Value 1 means a straight stem and value 3 is sinuous stem.

As expected, there were strong relationships between the three growth traits, height, DBH, and stem volume. The Pearson correlation coefficients varied between 0.78 and 0.97. These three traits also showed strong relationships with branch diameter, the range of correlation coefficients being 0.64-0.82. Stem straightness and survival did not show any relationship with other traits.

Four papers in the thesis of Antti Lukkarinen (2013) treat various aspects of growth and phenology of *L. sibirica* and *L. gmelinii* in Finnish field trials and in greenhouse. Two field trials were established at latitudes 61.82°N and 66.32°N in 2005. Assessments of growth and phenology were carried out up to an age of five years from seed (Lukkarinen et al. 2010, 2013, and 2014). Data from the experiment in the greenhouse were presented in Lukkarinen et al. (2009). Both field trials had five *L. gmelinii* populations and five check lots; one being *L. decidua*. The northern field trial had 12 and the southern trial 15 *L. sibirica* populations. With such a mix of material it might be questioned whether or not the evaluation should be based on all populations. Therefore, my scrutiny was focused on offspring from native *L. sibirica* populations.

Lukkarinen et al. 2009. An experiment with five replications was carried out in greenhouse with 20 populations, 11 *L. sibirica*, 5 *L. gmelinii*, and 4 check lots. Seedling heights were measured on July 5-6 and on October 5. The difference in height between these two occasions was coined as late summer growth. Terminal bud formation in three classes and coloration in four classes was assessed during September and October.

There were significant differences for all traits assessed. However, as judged for the data for phenology traits presented in Table 3 of the paper it was likely that the significances obtained could be attributed to a difference between four of the *L. gmelinii* populations and the other populations. The 100% homogeneity in autumn coloring both in *L. sibirica* and *L. gmelinii* populations was one
strange observation. Only in the check lots there were variation among the individuals in each population. It was speculated that the small difference in plant height between the populations might be attributed to the good growth conditions in the greenhouse and larger differences were projected for growth under field conditions. Late summer growth comprised around 75% of the total growth in the _L. sibirica_ populations with limited variation among the populations. I tested a few relationships between phenology traits and latitudinal or longitudinal origin of the _L. sibirica_ populations. Most of them did not turn out to be strong. Bud stage at week 36 (Fig. 1-30) became strong after exclusion of the high-altitude population from Altai Mountains, 1,630 masl. 

Ideally, when testing relationships with one geographic variable any other variable should be constant. This requirement is hardly ever possible to achieve in forest genetics studies. Data for all populations showed significant relationships with latitude for the following traits: Length of the growth period -0.86 Autumn coloring weeks 40 and 41 +0.91 and +0.93 Late summer growth -0.63 

The strength of the relationships between traits was estimated. I have illustrated some of them in Fig 1-31. Relationships between the same type of trait, such as autumn coloring at week 40 and week 41, are not included in this figure. As seen from this figure three of the six relationships were significant. The relationship between late growth in centimeters and final height was strongly significant but the same relationship including late growth in percent was non-significant (0.18 not shown in the figure). I tested the relationship between bud stage at week 36 with final height after exclusion of the high-altitude Altai Mountain population for the _L. sibirica_ populations only. Fig. 1-32 reveals that there is a relationship but not particularly strong.

For the _L. sibirica_ populations there was no relationship between 1000-grain weight and seedling final height, even with exclusion of the 66.00°N population that stopped growth early at the southern photoperiodic conditions in the greenhouse.

_Lukkarinen et al. (2010)_ presented data from two Finnish field trials. As far as I can see in this paper, the statistical evaluations were based on all populations including the check lots. The mean survival based on all populations was estimated at 59% in both trials. It was noted that the large pine weevil caused serious damage already the first year in field in the southern trial. There was a significant
A strong relationship between survival and population origin was noted for *L. sibirica* in the northern field trial when data from the deviating population from longitude 97.50°E was excluded (Fig. 1-33). A strong relationship between survival and population origin was noted for *L. sibirica* in the northern field trial (Fig. 1-33) while there was no such relationship in the southern field trial. This relationship for all larch populations explained less than 70% of the variation in survival. There was also a strong relationship between population longitudinal origin and survival percentage in the northern trial when data from the easternmost population were excluded (Fig. 1-34). The population from longitude 97.50°E deviated strongly from the general pattern with extremely high survival, 75%. This population had also the highest survival in the southern trial, 87%. Its locality had the most continental type of climate of all populations included in this study. The northern *L. gmelini* population from latitude 59.84°N had an exceptionally high survival at the northern field trial, 90%. In contrast to this, the *L. decidua* check lot had only 25% surviving plants in the northern trial. The final height at age five of the twelve *L. sibirica* populations common to the two field trials shows the typical relationship with latitudinal origin as in many other cases (Fig. 1-35). In both trials the curve has its maximum south of the trial latitude. This maximum was most pronounced in the southern field trial. The populations transferred to a southern latitude suffer from an early growth cessation leading to reduced growth. It should be noted that there was no southwards transfers at the northern trial. The poor growth of the three high-altitude *L. sibirica* populations from the Altai Mountains, 1,580-1,630 masl, was attributed to harsh climatic conditions in combination with their southern latitudes. Also for tree height there was a strong relationship with population longitudinal origin when data from the deviating population from longitude 97.50°E was excluded (Fig. 1-36). The four *L. gmelinii* populations from latitudes 49-52°N showed the best growth in the southern trial, all mean values exceeding 2 meters, while the check lots were close to the trial mean of 147 cm. The northernmost *L. gmelinii* population from lat. 59.84°N showed the best height growth in the northern trial, ≈75 cm.

The percentages of frost damage at age 5 were 16 (southern trial) and 32 (northern trial). No significant differences among the populations were noted. Forking occurred to a high extent, especially in the northern trial amounting to 52%. It was speculated that weather conditions, insects, fungi, and browsing mammals were responsible for this high percentage. There was a strongly significant difference among populations for forking in the northern trial. Mammal and saw fly damage was noted in the southern trial amounting to 16 and 18%, respectively. At the southern trial there were significant relationships between these two types of damage and latitude, $r = 0.70$ and 0.47 and a strong negative relationship between...
mammal damage and longitude, -0.85. The relationship between saw fly damage and altitude was negative, -0.70. The *L. gmelini* populations had significantly lower percentage of mammal damage than *L. sibirica*.

Pearson correlations were calculated for the relationships between the same trait in the two trials. With one exception, leader length at age 5 (r = 0.61), all other relationships between the same trait in the two trials were weak. Except for relationships between the same type of trait such as height at age 2 and height at age 5 there were few strong correlations. The significant correlations are shown in Fig. 1-37. The relatively high number of significant correlations between forking and other traits can partly be attributed to the high percentage of forking that was noted for the northern trial. Fig. 1-38 reveals that there was a strong relationship between survival percentage and tree height in the northern field trial.

As far as I understand only linear relationships between observed data and geographic or climatic variables were calculated. It is regrettable since the *a priori* expectation of linear relationships might be invalid. As seen from Fig. 1-35 one strong polynomial relationship between latitude and tree height at age five was noted for the southern trial. It is surprising that strong relationships between latitudinal origin and survival or height were obtained in spite of the large difference in longitudinal origin of the populations. There should be large climatic variation from west to east that might disturb latitudinal relationships.

Onset of growth and shoot elongation were assessed in the field trial at latitude 61.82°N (Lukkarinen et al. 2013). Five classes of bud flushing were used to determine to onset of growth. Three and two assessments were used at age four and five, respectively. At both ages shoot elongation was measured ten times during the growth periods. Temperature sums for the different occasions of assessment were calculated.

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**Figure 1-36.** The relationship between longitudinal origin and height at age 5 of 12 *L. sibirica* populations in two field trials at latitudes 61.82°N and 66.32°N in Finland. Lukkarinen et al. 2010.

**Figure 1-37.** Relationships with significant between trait correlations within each of two Finnish field trials with Larix populations. Blue columns refer to a trial at 66.32°N and the red column refers to a trial at 61.82°N. Lukkarinen et al. 2010.

**Figure 1-38.** The relationship between survival percentage and plant height in a *L. sibirica* trial at latitude 66.32°N in Finland. Lukkarinen et al. 2010.
Population mean values for bud flushing of the individual populations were given for the two recordings during 2009. Since only a low percentage of plants was noted at the first date of recording any trends of relationship with geographic variables would be impossible to trace. At the second occasion the opposite was the case with high percentages of all populations. Significant differences among all 25 populations studied were noted for three of the occasions of assessment, one in 2008 and both in 2009. At the observation on May 5 in 2008 the populations had the highest percentage of trees in bud flushing stages 3-5. A separate analysis of the L. sibirica populations showed an erratic pattern. In Fig. 1-39 I have illustrated some of the correlation coefficients between

**Figure 1-39.** Correlation coefficients for the relationship between bud flushing and longitudinal origin of the L. sibirica populations or two temperature variables. 08 stands for age 4 and 09 stands for age 5 from seed. Significant differences are indicated. Lukkarinen et al. 2013.

budding and latitude after exclusion of the three high-altitude populations. As seen from Fig. 1-40 there were strong curvilinear relationships at both ages with longitude after exclusion of the three high-altitude populations from Altai Mountains. More or less identical relationships were noted between population origin and temperature sums; +5°C as limit value. Also the R²-estimates were the same. The strong relationship between reaching of 90% of growth at ages 4 and 5 (Fig. 1-41) indicates a strong genetic regulation of this trait. The growth patterns of populations were illustrated in detail in the paper, which gives good insights in the individual performances of the populations.

Generally, the possibility to detect any relationship with physical variables is dependent on the proper time for such a study. The highest probability for this occurs when the resolution of the data is at their maxima. The problem is that this is not known beforehand. To find the maxima, observations must be carried out at frequent occasions. The absence of significant relationships might solely be due to too long intervals between observations.

Lukkarinen et al. (2014) studied growth cessation during two years (ages 4 and 5 from seed) in the larch field trial at altitude 61.82ºN in Finland. Three estimates of growth cessation were used:

- absence or presence of an apical bud
- coloring of needles in five classes
- needle shedding in five classes

Budset was recorded four times each year while color-
ing was recorded twice each year. Leaf shedding was assessed three times each year.

There were strongly significant differences among the populations for all three traits at all occasions. As stated above I focused on the performance of the *L. sibirica* populations. Strong relationships between population latitudinal origin and budset were obtained after exclusion of the high-altitude-populations from Altai Mountains (Fig. 1-42).

Similarly, for both ages strong relationships were noted for autumn coloring and latitudinal origin (Fig. 1-43).

For both traits there was a strong relationship between the two years of observation, $R^2$ 0.75 and 0.89. There was a difference in point of time for onset of growth cessation the two years. This was attributed to the different weather conditions during the summers of 2008 and 2009. A difference caused by age can probably be ruled out since the onset was earlier at age 4 than at age 5, which is opposite to what has been observed in other species (Ununger et al. 1988). Neither for budset nor for autumn coloring there were any relationships with longitude. The *L. decidua* population with its origin in the Czech Republic had the latest onset of growth cessation.

The results on the relationship between growth cessation traits and geographic or climatic variables for all populations (including *L. gmelinii* and check lots) are summarized in Fig. 1-44. Budset at age 4 had significant relationships at all three observations with latitude in agreement with the result for *L. sibirica* (Fig. 1-42). However, at age 5 only one of the three relationships was significant with latitude. Contrary to the results for the 15 *L. sibirica* populations, the entire material showed significant and negative relationships with longitude. This means that the eastern *L. gmelinii* populations with their late onset of growth cessation had a strong impact on this relationship. As regards autumn coloring, both observations at age 4 were significant and negatively correlated with population altitudes.
Growth rhythm of seven larch populations (4 *L. sukaczewii*, 2 *L. sibirica*, 1 *L. cajanderi*) from Russia was studied in a Russian field trial at latitude 61.65°N and 50.68°E by Fedorkov (2012). Growth was followed at eight occasions during the fourth growth period.

With the wide geographic origin of the populations – 13 degrees of latitude and 83 degrees of longitude - it was not surprising that strongly significant population differences were noted for annual elongation, growth rate, onset of growth, and cessation of growth. Onset of growth defined as the attainment of ten percent of elongation correlated significantly with climatic variables and latitude and longitude. The relationship with annual mean temperature was strongest, $R^2 = -0.96$. To trace the relationship with geographic variables Figs. 1-45 and 1-46 are presented. I used one date related to onset of growth, June 8, and one date with maximum resolution among the populations, June 28. As seen from both figures the relationships with longitudinal origin were stronger than for latitudinal origin. It is also clear that the Far East and high latitude *L. cajanderi* population had a great impact on the relationships. Exclusion of this population causes dramatic drops of the $R^2$ estimates; only the relationship between onset of elongation and longitude was moderately strong, 0.72. The absence of a series of latitudinal populations along one longitude and the vice versa absence of longitudinal populations along one latitude are great constraints for estimations of geographic relationships with growth rhythm traits.

Vitality in four classes and tree height at age five in the same field trial in Russia were presented by Fedorkov (2014). The population effect for tree height was strongly significant while vitality was non-significant. There was a good fit of tree vitality to a second degree polynomial while continentality did not explain much of the variation in tree height (Fig. 1-47). As anticipated from Fig. 1-47 there was a fairly strong relationship between tree height and vitality, $R^2 = 0.78$. Noteworthy is the observation that the two extreme populations with respect to continentality had the best vitality. As far as I can understand, only linear relationships were tested in the paper while I tested polynomial relationships. Linear relationships do not always explain biological variation. Besides, no data from *L. cajanderi* were included in my relationships.

### 1.1.2 Asia

Zhang et al. (2013) studied the variation in growth and markers in populations from seven regions in the Heilongjiang province in China. The trees were assessed after 23 years in field. Twenty-one ISSRs (inter-simple sequence repeats) primers and 23 RAPDs (random amplified polymorphic DNA) were used to estimate variation among and within populations. The gene diversity index according to Nei and the Shannon information index were calculated.
There was a considerable difference in stem volume (Fig. 1-48). However, there were no replications in the field, which means that site conditions might be responsible for part of the differences observed. There was only a week relationship between stem volume and the two diversity indices (Fig. 1-49). The two markers showed that around ten percent of the variation was attributed to among-population differences. For ISSR markers the mean genetic distances between the seven populations did not differ much, 0.058-0.071. The corresponding figures for the RAPDs were almost identical, 0.058-0.072. Several correlations between growth traits and geographic or climatic data were presented but there were disagreements between text and figures in the tables. Since no geographic or climate data for the populations were presented it was impossible to calculate any correlations between these data and growth traits.

Yang et al. (2002) presented a summary in English of growth, which is dependent on environmental conditions. (Table 1-3). It was stated that most of the growth is free growth, which is dependent on environmental conditions. This explains the large difference in performance at the three test localities. The locality at 700 masl is a frost pocket. In spite of these differences among the test localities the population x test locality was only significant in one case; plant height (Table 1-3). Nor was the interaction effect significant in the freeze testing (Table 1-4). It was stated that most of the growth is free growth, which is dependent on environmental conditions.

Table 1-3. The significances of different variables for bud flushing, budset, and seedling height at age 2 based on three provenance trials with 82 L. occidentalis populations from north-western USA. Rehfeldt 1982.

<table>
<thead>
<tr>
<th></th>
<th>Flushing</th>
<th>Budset</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Population</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>L x Pop.</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 1-4. The significances of different variables for frost damage after freeze testing at four temperatures; -35°C - -50°C. the freeze testing comprised 82 L. occidentalis populations from north-western USA. Rehfeldt 1982.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>**</td>
</tr>
<tr>
<td>Population</td>
<td>*</td>
</tr>
<tr>
<td>Pop x Temp</td>
<td>ns</td>
</tr>
</tbody>
</table>
The mean predetermined needle complement (PNC) was obtained by examining the buds. The number of needle scars on the 1982 terminal shoots was also determined to obtain the annual shoot needle complement (ASNC). The mean PNC amounted to 167 needles or 85% of the total needle complement. The free growth needle complement (FGNC) was obtained by subtraction of PNC from ASNC. There was a large variation in FGNC among trees, 0 to 94 needles but a limited variation in FGNC needles among stands, 30-34.

Both PNC and ASNC increased with elevation (Fig. 1-51). The ANOVAs showed that there were significant stand and tree within stand effects for the 1981 shoot length and ASNC in 1982. Although there was limited variation in FGNC this trait was negatively correlated with PNC (no correlation coefficient was given). It was also stated that FGNC was a better predictor of annual shoot growth within stands than PNC, r = 0.48 and 0.39, respectively. However, both traits explained a limited part of the variation in annual shoot growth.

Four wood properties - growth rate, latewood ratio, density, and tracheid length, were studied in 12 L. laricina in situ populations in northern Ontario by Yang and Hazenberg (1987). Two stands per locality, each with ten trees were selected for this study. Two increment cores from each tree were extracted at breast height. Growth rate was defined as the width of a growth ring. The ratio between latewood and the total ring width was defined as latewood ratio. Density is the mean of density of individual rings of the juvenile wood. The mean tracheid length of early-wood was based on 25 tracheids.

Table 1-5. The significances of different variables for four wood traits of wood samples from ten trees per stand in each of two stands at 12 localities of L. laricina in northern Ontario. Yang and Hazenberg 1987.

<table>
<thead>
<tr>
<th></th>
<th>Growth rate</th>
<th>Latewood ratio</th>
<th>Density</th>
<th>Tracheid length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality 12</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Stand/locality 2</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
</tr>
<tr>
<td>Trees/stand</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Figure 1-50. The variance in percent for bud flushing, budset, seedling height, and frost damage explained by elevation, longitude and latitude based on 82 L. occidentalis populations studied at three test localities and in freeze testing of these populations. Rehfeldt 1982.

Figure 1-51. Mean number of predetermined needle complement (PNC) and annual shoot needle complement (ASNC) in five populations of L. occidentalis from different elevations in northern Idaho, USA. Joyce 1987.
Growth rate \times 10^{-1}

These differ

from northern Ontario. For definition of the traits see text. Yang and Hazenberg 1987.

The results from the ANOVAs for each of the four traits are shown in Table 1-5. This shows that locality was significant for three of the four traits while the variation of trees within stands was significant for all traits. Growth rate declined with increasing latitude while latewood ratio showed an opposite trend (Fig. 1-53). These differences among localities were attributed to duration of the growth period and climatic differences among the localities.

As seen from Fig. 1-52 the relationship between latwood ratio and growth rate was negative and I estimated the \( R^2 \) to 0.63 (Fig. 1-53). In the paper the correlation coefficient \( r \) between these two traits was reported as -0.39, which must be a misprint. Except for the relationship latewood ratio – tracheid length, all the pairwise correlations between the four traits were significant. However, the coefficients were all in the range -0.40 - +0.40, which means that the degree of explanation of the relationships was small, <16%.

Survival, growth, and wood characteristics up to an age of 25 in 20 \( L. kaempferi \) populations in a provenance trial were reported by Park and Fowler (1983) and Fowler et al. (1988). Two \( L. laricina \) and three \( L. decidua \) populations were also included in this trial, which was located close to Fredericton, New Brunswick, Canada. There were three replications of 7 x 7 tree plots with a spacing of 3 x 3 meters. Thinning was carried out at age 25 leaving 25 trees per plot. Their DBH was determined. Volume per hectare was obtained from the following multiplication: tree volume x number of trees planted x percentage survival x 7.56 (to consider the size and spacing of each plot). In the first paper information on flowering up to age eight was included and recorded in classes. No genetic analysis of the wood properties was presented.

The mean survival of the \( L. kaempferi \) populations dropped from approximately 100% at age four to 78.6% at age 25. At age 25 the variation in survival of individual populations was significant and varied in the range 59.2 – 95.2% (Fig 1-54). Up to age 19 the two \( L. laricina \) populations had higher survival than \( L. kaempferi \), but at age 25 it was in the same range as \( L. kaempferi \), 75.2%, while the \( L. decidua \) had a mean value of 63.5% at this age. The lower survival of the \( L. decidua \) was attributed to preferential feeding of porcupines on this species. I could not verify the statement that survival at ages 8, 12 and 19 was highly correlated. Rather, the relationships were poor. In ten of the 20 populations the survival at age 19 was higher than at age 12; in some cases much higher. Different number of trees per plot was used for estimation of survival at different ages; probably owing to thinning between ages 12 and 19. This condition has certainly affected the strength of the correlations. If data from \( L. decidua \) are included in the estimates of the correlations, they strongly influence the strength in a positive way since the \( L. decidua \) data were outside the range of \( L. kaempferi \) data.
The relationship between stem volume at ages 19 and 25 populations but no significant correlations as regards species performance since L. decidua and L. laricina were represented by only two and three populations varied between 2.7 and 3.4 while the worst quality was noted for one European larch population with an estimate of 2.2. There was a strong correlation between stem straightness and tree heights at ages 8, 12, and 19. The variation in density among the L. kaempferi populations was limited. Stem straightness was significantly correlated with tree growth and diameter according to Park and Fowler (1983).

There was a significant difference in male flowering among the L. Kaempferi populations but no significant differences as regards female flowering were observed. The broad span in each class might lead to underestimation of population differences. Fowler et al. (1988) warned for drawing far-reaching conclusions as regards species performance since L. decidua and L. laricina were represented by only two and three populations. The large variation found among the L. kaempferi populations supports this statement. The yields of the three populations from Mount Asama were 159, 233, and 339 m³/ha. It was stated that elevation alone cannot explain the large difference between the two extreme populations from this mountain since the difference in elevation was just 200 meters. This might be one example of random genetic drift as suggested above. Owing to the large variation in growth it was stated that any recommendations of populations for commercial forestry is risky to come up with. However, use of tested L. kaempferi populations might be useful for short rotation fiber production in New Brunswick.

Zhang and Marshall (1994) carried out a study of water-use efficiency in 14 L. occidentalis populations from the entire range of distribution of the species. Two watering regimes were used. Well-watering treatment; the one-year old seedlings were watered every other day. Water-stressed treatment; watering two days after signs of wilting were visually observed.
The experiment was carried out in an open-wall glasshouse. Four times during the growing season assessments of photosynthesis, stomatal conductance to water vapor, transpiration, and the ratio of intercellular CO₂ to ambient CO₂ were carried out. Instantaneous water-use efficiency, WUEI, was obtained by dividing photosynthetic rate by transpiration. Transpiration efficiency, WUET, was determined by dividing total dry matter by the cumulative amount of water used during the growing season. Ground needles were used for carbon isotope discrimination, 13°C and 12°C. The ratio δ13 - δ12/1 +δ12, which is coined delta Δ, is one means to estimate the water-use efficiency. Three seedlings from each population were exposed to the two watering regimes. The pots were rotated every other day, which means that no block effects could be included in the ANOVAs.

All traits measured or derived, except for Δ, showed non-significant population effects. Three reasons for the absence of population differences were presented:

Only three seedlings per population and treatment
Gas exchange is strongly influenced by short-term environmental fluctuations
Low level of variation among populations for other traits in L. occidentalis

It is highly likely that point 1 is a large contributor to the absence of population differences. It might also be anticipated that such basic physiological processes as studied in this investigation are stable across populations and species.

Significant populations x treatment effects were noted for maximum photosynthesis and Instantaneous water-use efficiency. The authors reported that 47% of the variation among the populations as regards WUET in the water-stressed treatment was attributed to population elevation. However, according to Fig. 2 in the paper there are seemingly two separate relationships in this treatment. Since the geographic data of the populations were not given it is impossible to find out if the populations in one of the groups originated from one side of a mountain chain and the other group from the other side of the mountain chain.

The information from provenance trial data might be combined with data from climatic models predicting future climatic conditions. Genetic data from two trials with L. occidentalis populations containing 143 populations covering the area of distribution were used for that purpose by Rehfeldt and Jaquish (2010). Elaborate methods for prediction of future distribution area of L. occidentalis were used. The results were presented in maps. The methods used in this report can serve as a useful template for predictions of future distribution of tree species under global change conditions.

Worrall (1993) reported on variation in phenology over four decades in L. lyalli stands. In bud flushing and leaf fall the range was six and four weeks, respectively. Most of the variation in flushing was attributed to spring temperature. Experiments under controlled temperature conditions showed that the threshold temperature for bud flushing was lower than +1.5°C and that such a low temperature sum as 75 degree days caused flushing. There was a relationship between early flushing and early leaf fall. Leaf fall was triggered both by high summer temperatures and photoperiodic conditions. High summer temperatures caused early leaf fall. It might be speculated that the high summer temperatures cause drought, which in turn causes leaf fall.

1.2 Disease tolerance

The susceptibility to Lachnellula willkommii of 20 Polish L. decidua populations at ages 20-35 years in a trial located at 785 masl, latitude 49.35°N, and 20.35°E was reported by Kulej (2006). This trial is located at a higher elevation than the elevational origin of any of the 20 populations. The percentage of trees with larch cancer symptoms on the tree trunks was determined.

The differences among the populations were strongly significant at all ages. At age 35 only three populations had less than 50% damaged trees. At this age the mean for this trial was 65%. In Fig. 1-57 I have plotted the percentage of affected trees against the latitudinal origin of the populations for ages 20, 25, 30, and 35 years. The R² estimates for the curves varied in the range 0.20-0.30, which means that the latitudinal origin of the populations explained a limited part of the variation in susceptibility to Lachnellula willkommii. The curves suggest that there was some stability in performance over years. Still weaker correlations were noted for the relationships between percentage of affected trees and elevational origin of the populations. It is evident that the geographic origin of the populations does not well predict the susceptibility to larch cancer. In a previous paper in Polish, the author

![Figure 1-57. The relationship between population latitudinal origin and percentage of trees affected by larch cancer. Lachnellula willkommii in a Polish provenance trial at latitude 49.35°N, 20.35°E, and 785 masl. Kulej 2006.](image-url)
had reported a negative but non-significant relationship between tree height and susceptibility to larch cancer. It would have been useful to have growth data published in English as well. Weight loss following exposure to Coniophora puteana of outer heartwood logs of L. decidua, L. kaempferi, and their hybrid was studied by Curnel et al. (2008). An overall view of the sampling might be obtained in Fig. 1-58. Samples were taken from one natural stand in Austria and one natural stand in France. Samples were also taken from provenance trials in Belgium, France, and Germany as well as from a progeny trial with L. decidua x L. kaempferi hybrids in Great Britain. One Sudetan population was sampled in three trials and another Sudetan population was sampled in two trials. From each log 12 samples from each of the inner and the outer heartwood of each log were prepared for the analysis of decay. Of the 24 samples eight were used as reference samples. Half of the remaining samples were incubated with Coniophora puteana and half of them with Poria placenta. The former fungus caused the largest decays and the further analysis refers to Coniophora infection. The wood sample were incubated in Kolle flasks and remained in a conditioning room for 16 weeks. At the end of the treatment all mycelium was removed from the wood samples, which then were oven dried until constant temperature was reached. The site conditions might influence the results (Fig. 1-58). This is particularly evident for the Czech Ruda population (red filled columns), which weight loss varied in the range 15-25%. However, according to the ANOVA run for the two Czech Sudetan populations (red columns) the site effect was non-significant. The decay resistance was much larger in the two natural populations from Austria and France than in their progenies in field trials. The resistance was stronger in the old trees in the natural stands. The Japanese larch population was significantly more resistant than the European and the hybrid larches. The ANOVA run to test the effect of origin (Alpine versus Sudetan) showed significance for populations within origins. The effect of origin was not significant. This analysis revealed a strongly significant effect for trees within populations. The large variation in weight loss within individual trees is a disturbing factor in this investigation. It was stated that local growth conditions, age and maturity of wood may be more relevant than genetic effects. It was also stressed that development of efficient methods for estimation of wood decay resistance is highly desired if breeding for this type of resistance should be included in breeding programs.

1.3. Markers

1.3.1 Isozymes

Eighteen isozyme loci were analyzed to study the variation among four Italian, four Czech, and three Polish populations of L. decidua (Lewandowski and Mejnartowicz 1991a). Fourteen of the loci were polymorphic in at least two populations. Gs estimates for individual loci varied in the range 0.01 – 0.12 with the highest estimate for Mdh1 locus. Owing to the discontinuous distribution of L. decidua it was expected that the differentiation might be larger in this species than in P. abies and P. sylvestris. However, in agreement with differentiation in other conifers the mean value for all 14 polymorphic loci was low and estimated at 0.04. It was suggested that L. decidua during the glaciation occurred in a vast refugia with a common gene pool. The differentiation within the Czech region was much larger than within the other two regions (Fig. 1-59). Anthropogenic effects of the Czech larch populations might be one explanation for the difference among the three regions. However, it was stated that a larger number of populations must be analyzed to confirm the present results. There was no relationship between geographic and genetic distances among populations.
The same eleven populations as studied by Lewandowski and Mejnartowicz (1991a) were included in a study of the relationship between L. decidua and L. sibirica by Lewandowski (1997). Two additional Polish populations were included and three L. sibirica populations growing much further east; (longitudes 94 – 107°E) than the L. decidua populations, which grew in the range of 10 – 21°E. Thirteen polymorphic loci were analyzed.

Eight alleles were specific for L. sibirica while 17 were specific for L. decidua. The observed difference was attributed to the low number of L. sibirica populations. It was noted that the most common Shdh allele had a mean frequency of 0.51 in the L. decidua populations while it was absent in the three L. sibirica populations.

The pairwise relationships between regions and species reveal that the differentiation is largest between the L. sibirica populations and the three regions with L. decidua populations (Fig. 1-60). Even if these $G_{ST}$ estimates are several times higher than the other estimates they never exceeded 7%. It is obvious that the differentiation estimated by isozyme loci is limited even over such a huge span of longitudes as in this material. A short duration of the isolation between the species was suggested as one explanation for the limited differentiation between the two species.

The observed data did not support the hypothesis that Polish L. decidua arose following hybridization between European and Siberian larches, which might have taken place during migration after the latest glaciation. Thus, there were no unique L. sibirica alleles found in the Polish populations, which would be required according to the hybridization hypothesis.

The mating pattern of 21 randomly chosen trees in an old L. decidua stand in Poland was investigated by use of six isozyme loci (Lewandowski et al. 1990). Some of the single-locus estimates of outcrossing exceeded 1.0. This was attributed to negative assortative mating, which in turn might be caused by phenological flowering differences among the trees. The multilocus estimate of outcrossing was estimated at 0.943, which means that inbreeding had occurred to 5.7%. The fairly good agreement between the fixation indices calculated for the progeny population and expected under random mating suggested that most of the observed inbreeding must be attributed to selfing.

An idealized variance effective population size amounted to nine females and two males in the present study. It was suggested that the low estimates might be explained by asynchronous flowering phenology and/or to asymmetric fertility. The absence of air sacs in larch pollen might reduce the pollen flow distances compared to spruces and pines.

**Figure 1-59.** The differentiation among L. decidua populations from three regions in Europe. Fourteen polymorphic isozyme loci were analyzed. The number of populations from each region is given. Lewandowski and Mejnartowicz 1991a.

**Figure 1-60.** The genetic distances between populations within regions, between regions, and between L. sibirica and three regions of L. decidua. Thirteen polymorphic isozyme loci were included in this study. Lewandowski 1997.
Maier (1992) used seven isozyme loci for a study of genetic variation among seven European populations of *L. decidua*. Two of the loci did not show any polymorphism. Except for some rare alleles, all alleles occurred in all seven populations. The mean pairwise genetic distances between these seven populations are illustrated in Fig. 1-61, which reveals that the western Alpine population differed most from the other six populations. It was speculated that the low sample size from the westernmost population might have resulted in its large distances from the other populations. However, it was stated that other data support the finding of its different genetic structure. In spite of the large geographic distances among the three eastern populations their mean $F_{ST}$ was extremely low, 0.007. Large gene flow among population is one possible explanation.

Seventeen polymorphic isozyme loci were analyzed in twelve approved seed tree stand populations of *L. decidua* in North Western Italy by Belletti et al. (1997). The elevation of the populations varied between 900 and 1,950 masl and all main directions (south, west, north, and east) of exposure were represented. The longitudinal and latitudinal variation of the populations was 1.5 and 2.1 degrees, respectively. Around six megagametophytes per tree from at least 30 trees were analyzed to obtain the population structure.

The mean $F_{ST}$ for the pairwise comparison of populations was estimated at 0.033. Such a low estimate suggests that there is considerable gene flow among the populations in spite of the origin of the populations on different aspects. The southernmost population which was growing isolated from the other populations had the highest mean $F_{ST}$ estimate, 0.054. There was a significant relationship between $F_{IS}$ and geographic distance but with a low degree of explanation of the variation, $r = 0.47$. The total number of pairs of comparison (59) explains the significance.

The $F_{IS}$ estimates for the twelve populations varied between -0.068 and 0.121. The highest estimate was noted for the population from the lowest elevation, 900 – 1,100 masl. I tested the relationship between population $F_{IS}$ and latitude, longitude or elevation without finding any strong relationship. Thus, there was a surplus of homozygotes in most populations, which was attributed to selfing or matings between related individuals.

Twenty isozyme loci were used in a study of five Romanian *L. decidua* populations by Mihai and Teodosiu (2009). They reported a high $F_{IS}$ estimate, 0.079, indicating a large deficit of heterozygotes. It should be noted that three loci deviated strongly from zero, two with a large deficit of heterozygotes and one with a large excess of heterozygotes (Fig 1-62). No discussion of the large variation in $F_{IS}$ estimates was carried out. The $F_{ST}$ was estimated at 0.098, which is a fairly high estimate for a wind pollinated conifer species. This estimate suggests a larger isolation among the five Romanian *L. decidua* populations than for other widely distributed conifers.

The isozyme profiles of 36 *L. laricina* populations from the range of distribution of this species were presented by Cheliak et al. (1988).

Three of the 19 examined loci did not show any variation in any of the populations. Another three loci were found to be polymorphic in all populations. The number of alleles per polymorphic locus varied between 1.5 and 2.3 with a mean value of 1.8. Observed and expected heterozygosities were both 0.22 with minimum and maximum estimates 0.16 and 0.29, respectively. It was stated that *There were no obvious geographic trends associated with population variation in heterozygosity*. Similarly, there was no geographic trends in allele frequency variation with one exception, the G6P (glucose-6-phosphate dehydrogenase) locus which showed negative relationships with longitude and latitude. The $G_{ST}$ of individual loci varied generally between 0.03 and 0.07 with an exceptional estimate of 0.17 for locus Mdh-4 (malate dehydrogenase). It would have been of interest to know if this high estimate could be attributed to monomorphism of different alleles in different populations and thus just owing to the way of calculation of $G_{ST}$. The deviating $G_{ST}$ for the mdh-4 the mean value was 0.055. Without the $G_{ST}$ for this locus the estimate was 0.041. The somewhat higher estimate for *L. laricina* than for other wide-spread conifers was thoroughly discussed. It was suggested that small populations and lack of continuity among populations might explain the higher $G_{ST}$ in this species. In spite of this, it was ruled out that genetic drift had played a major role during the evolution of this species. There were no significant relationships between the pairwise genetic distances and longitude, or latitude.

Pairwise collection of *L. laricina* material from five regions in Ontario was carried out to study the variation within and between regions (Dickinson et al. 1988).
Fourteen polymorphic isozyme loci were analyzed to determine the genetic setup of the 5 x 2 populations. Needle anatomy, needle shape, and cone morphology were also assessed.

The difference among the ten populations with isozymes was estimated as 4.6%. A slightly higher estimate of the difference between the two populations within a region was noted, 6.5%. Thus, there was a tendency that populations from the same region showed less resemblance than the resemblance with other regions. The differentiation of the populations did not follow a geographic pattern. The same pattern of variation was noted for the cone morphology data while the two data sets for needle traits showed a geographic differentiation. It was suggested that the isozyme loci were not affected by selection. It was further suggested that the regeneration of the populations had taken place after forest fire and with a limited number of parents for each population. This suggestion was supported by the relatively low outbreeding rate for a wind-pollinated species, 0.73. Three of the populations originated from random distribution of seeds from neighboring stands.

Spatial autocorrelation was studied in two L. laricina populations with different origin by Knowles et al. (1992). The population designated as cutover was clear-cut approximately 20 years prior to collection of samples from 465 trees while in the other called field, the 573 trees had occupied an abandoned hay field. Three polymorphic isozyme loci were used in this study. In general, the distance classes for the autocorrelation study spanned five meters. The main focus of this investigation was to analyze whether the regeneration history of the stands influenced the distribution of genotypes in them.

It was stated that the allele frequencies in the two populations were remarkably similar. The F\textsubscript{is} estimate was somewhat higher in the cutover population than in the field population, 0.021 versus 0.003. This investigation showed that the mode of regeneration might have an impact on the pattern of genotypes in a stand.

Twenty-three isozyme loci were studied to estimate the genetic variation among 19 populations of L. occidentalis in NW USA (Fins and Seeb 1986). Each population was represented by nine or ten trees. Only seven of the 23 loci were found to be polymorphic. The authors grouped the populations into four sets according to geographic origin. Both the mean number of alleles per locus and expected heterozygosity was low. The differentiation among the four geographic groups amounted to 9% and thus 91% of the variation within groups. The southermost population deviated most from the three other groups with a mean G\textsubscript{st} of 0.012 while the corresponding estimate for the three other groups was much smaller, 0.005. Two ex-
Planning were given for the low variability in *L. occidentalis*:

Reforestation frequently takes place after forest fire that has left a few trees which are responsible for the coming generation.

The refugia during the latest glaciation contained a low number of trees.

Both these conditions were thought to have caused the limited variability in this species.

The hypothesis that tree density plays a role for the mating pattern in a population was tested at two localities in British Columbia, Canada by El-Kassaby and Jaquish (1996). It is expected that inbreeding occurs to a larger extent in a seed tree stand than in natural populations with a high density of trees per hectare (Rudin et al. 1974, El-Kassaby and Namkoong 1994). However, low density may result in less sibling relationships in low-density tree stands, which might lead to the opposite expectation. Thus, there would be limited inbreeding in a seed tree stand.

One natural stand and one seed tree stand at each of two localities were included in this study. The *L. occidentalis* natural stands studied were even-aged and more than 100 years old; probably developed after forest fires. Both localities are at high elevations, 1,200 and 1,380 masl, respectively. In the mixed stand at 1,200 masl there were 240 trees/ha (21% of the trees in this stand) and 60 trees/ha in the seed tree population. At the other locality the natural population consisted of 940 trees/ha (75% of the trees in this stand) while the seed tree stand had approximately 30 trees/ha. Seven isozyme loci were analyzed in multilocus outcrossing rate in four *L. occidentalis* populations at two localities in British Columbia, Canada. At each locality there was one natural stand and one seed tree stand. Seven isozyme loci were used to estimate *F* <sub>IS</sub> El-Kassaby and Jaquish 1996.

megagametophytes and embryos to assess the population structure in the growing stand and the pollen that had participated in mating of the trees.

Figure 1-63 reveals that there was one positive estimate of *F* <sub>IS</sub> in one of the stands, the natural stand at Flathead (blue column), suggesting some inbreeding in this stand. The negative estimates of *F* <sub>IS</sub> in the three parental populations are best explained by selection against homozygotes during the course of development of the stands. This selection is probably both natural and artificial in the case of seed tree stands. All four progeny populations showed positive estimates of *F* <sub>IS</sub> suggesting that there was some inbreeding in the progeny. The outcrossing in the two localities showed different patterns (Fig. 1-64). In the Flathead stand, the hypothesis by El-Kassaby and Namkoong (1994) is supported while this was not the case for the Becker lake locality. The low percentage of larch trees in the Becker lake population (21%) with a large percentage of other conifers might according to the authors have prevented a free movement of larch pollen in this stand leading to a low outcrossing percentage. Another explanation is high occurrence of matings among siblings in the natural stands. It should be noted that the 25% of trees which were not larch trees at Flathead were deciduous trees, which had no leaves at the time of larch pollination. Thus, they would not constitute a physical barrier and thus prevent outcrossing to the extent as occurred in the Becker lake stand. The skew distribution of outcrossing rates among individual trees in the Becker Lake natural stand supports the interpretation of a physical barrier in this stand.

Since the results were conflicting, a final answer whether the hypothesis put forward for this investigation is correct or not remains open. Such a study as this one is of great interest from an ecological genetic point of view and ought to be carried out for many forest tree species.
With the use of 20 isozyme loci Jaquish and El-Kassaby (1998) estimated the differentiation ($G_{ST}$) among nine Canadian L. occidentalis populations at 0.07. Two of the loci were monomorphic in all populations. The percentage of polymorphic loci within populations varied between 40 and 70%. Based on the 36 possible combinations a strongly significant relationship between genetic and geographic distances was reported. However, the correlation coefficient was only 0.42, which means that the relationship between geographic and genetic distance did not explain much of the variation. To identify any unique population, i.e. a population that deviates much from the rest of the populations, nine $G_{ST}$s were estimated after elimination of one population at a time. It turned out that population No 9, which grows isolated from the main area of distribution of L. occidentalis, deviated most from the other populations (Fig. 1-65).

1.3.2 Microsatellites, chloroplast DNA, mitochondrial DNA

A successful attempt to amplify several microsatellites simultaneously in so called multiplex reactions was reported by Wagner et al. (2012). Their analysis comprised 13 loci with 9-36 alleles per locus. Material from 18 populations covering the distribution area of L. decidua was analyzed. No information on population differentiation was presented.

In a series of papers nucleotide diversity in four alpine tree species (Abies alba, Larix decidua, Pinus cembra, Pinus mugo) was studied (Mosca et al. 2012a and b). One focus was to detect outliers that might be related to adaptation of its carrier. Twenty-two natural populations of L. decidua from the natural range of distribution in the Italian Alps were included in this study. The elevation range of the populations was 1,123-2,218 masl. The analysis comprised 233 high-quality polymorphic SNPs in 151 genes. Geographic and climatic data for the populations were gathered for studies of relationships with SNP data. Almost 50% of the candidate genes were polymorphic. The total numbers of SNPs for candidate and control genes were 219 and 88, respectively. There were four and three SNPs per gene in candidate and control genes. The diversity was approximately 70% higher for candidate genes than for control genes. The total number of segregating sites was 80, of which 26 was non-synonymous.

In another report by the same senior author single nucleotide polymorphisms, SNPs, were used for a comparison of four alpine tree species from their natural range of distribution in the Italian Alps and Apennines (Mosca et al. 2012b). L. decidua was one of these four species, in which 24 populations were sampled. The genetic data were related to 35 geographic and climatic variables. Thus, the number of variables was larger than the number of populations, which is somewhat problematic. (Principal component analysis was used to evaluate the observed data.) The data set for L. decidua consisted of 267 SNPs. The mean $F_{ST}$ estimate was negative, -0.042. Thus, none of the populations suffered from inbreeding depression. Three different $F_{IS}$s were estimated, which all three were low: Among localities 0.011 Among geographic areas 0.009 Among localities within geographic areas 0.007 The low $F_{IS}$s suggest that the gene flow among the 24 populations is substantial. A separation of western and eastern Alps was noted and separated from the Apennine populations. Winter precipitation, seasonal temperature maximum, seasonal temperature minimum, and longitude showed strong correlations with the genetic data. It was concluded that several SNPs originated from genes encoding proteins, which are related to plant responses to abiotic stresses.

In the paper from 2014 (Mosca et al. 2014) one focus was on isolation by distance (IBD) and isolation by adaptation (IBA) in Larix decidua and Abies alba. Another focus was to detect outliers that might be related to adaptation of the species. Twenty-two natural populations of L. decidua from the natural range of distribution in the Alpine Alps were included in this study. The elevation range of the populations was 1,123-2,218 masl. The analysis comprised 233 high-quality polymorphic SNPs in 151 genes. Geographic and climatic data for the populations were gathered for studies of relationships with SNP data. Three geographic groups within The Alps were distinguished, western, central, and eastern Alps. Hierarchical AMOVAs were used to identify genetic differentiation among geographic groups as well as differentiation among populations within the three geographic groups. Loci that had extremely low or high $F_{ST}$ estimates were classified as outliers. Outliers according to the finite island or the hierarchical methods were separated. A third method was also used, in which specific population and locus effects were considered.
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It was stated that

further investigations are necessary to confirm the involvement in adaptation. This is certainly

true. Since most of the SNPs must be regarded as neutral markers and their role in adaptation is limited or absent and significances observed on population differentiation with minute parts of the variation attributed to population differentiation should not be over-interpreted. A joint analysis of common garden experiments and markers would give a better understanding of the adaptive variation and its molecular genetic causes. The use of SNP data are most important for estimates of gene flow and

IBD was investigated using a Mantel test to correlate pairwise physical and spatial distances and pairwise genetic differentiation ($F_{ST}$). The association between genetic distances and environmental variables (elevation, temperature and precipitation variables, and soil type) were calculated without using spatial distance as a covariate. Fig. 1-66 reveals that most of the variation occurred within populations even if the variation among populations as well as the variation among populations within groups were significant.

The positive and significant outcome of the pairwise genetic and geographical distance matrices suggests that isolation by distance is more important than the isolation by adaptation. In support of previous reports a significant relationship between genetic distance and precipitation was found.

Based on the finite island method six outliers were detected while the hierarchical method identified two outliers when classification was based on geographic grouping. (It should be noted that a much larger number of outliers were detected but the numbers presented are a secure number of real outliers.) For the environmental groups only the hierarchical method could be used to identify outliers, in which seven outliers were noted. Most outliers were located in genes encoding unknown proteins or with unspecific roles. Conclusions as regards their involvement in adaptation could not be drawn.

It was stated that further investigations are necessary to confirm the involvement in adaptation. This is certainly true. Since most of the SNPs must be regarded as neutral markers and their role in adaptation is limited or absent and significances observed on population differentiation with minute parts of the variation attributed to population differentiation should not be over-interpreted. A joint analysis of common garden experiments and markers would give a better understanding of the adaptive variation and its molecular genetic causes. The use of SNP data are most important for estimates of gene flow and

In their study focusing on dendrochronology King et al. (2013) reported on genetic variation in three L. decidua populations in Switzerland. Five nuclear microsatellites were included in their study, which revealed that 99.4% of the variation occurred within populations. It was concluded that gene flow among the populations is substantial. It was stated that the high within-population variability will be useful under changing environmental conditions. This was solely based on the neutral microsatellites. The situation might be totally different if traits of adaptive value are studied.

From three slopes of Mount Fuji in Japan Nishimura and Setoguchi (2011) collected material for studies of nuclear microsatellites. The trees along the three slopes were classified phenotypically according to the schematic illustration in Fig. 1-67. Eleven microsatellite markers were used, of which seven showed deviations from Hardy-Weinberg equilibrium.

At elevations below 2,000 masl only phenotype 1, erect trees, were present. With increasing elevation the number of distorted trees increased (Fig. 1-68 and 1-69). Whether or not this is due to the site conditions or to genetic differentiation cannot be determined based on this study. With such a broad span of elevation, 1,286-2,715 masl, it is likely that the climatic conditions have influenced the genetic set-up of the different populations. As seen from Fig. 1-70 the observed heterozygosity was rather similar across trials and without differences between low and high elevation populations. Only the YO2 population had a non-significant $F_{IS}$ estimate, all others were significantly different from 0. At high elevation the number of flowering trees becomes fewer, which in turn
IS populations in three elevational trails (Pluess 2011).

Figure 1-68 and 1-69. L. kaempferi tree phenotypes along Subachiri (SU above) and Yoshida (YO below) slopes of Mount Fuji in Japan. Phenotypes: 1 = regular, erect tree, 2 = semi-flag tree with most branches on the down-wind side, 3 = flag-tree with all branches on the wind-down side, 4 = semi-krumholtz mat tree with some horizontally growing branches, 5 = krumholtz mat tree with all branches growing horizontally. Nishimura and Setoguchi 2011.

might cause some selfing. At low elevation L. kaempferi grows in mixed stands with other conifers. This may constitute physical obstacles for pollen flow. However, the analysis of molecular variance among the six populations was extremely low, 0.58%. Similarly, the variance among trails, elevational groups, and phenotypic classes were all low; 0.18, 0.23, and 0.23 respectively. All molecular data suggest that the gene flow among the six populations was high. Recent colonization from one common founder population could also cause a limited molecular differentiation among the six populations.

Alleles in nine microsatellite loci with 115 alleles were used to test whether or not there was a difference in variability between early successional subpopulations (ESSP) and late successional subpopulations (LSSP) of L. decidua in a Swiss alpine forest (Plüss 2011). The glacier close to the sampling site had retreated approximately 2.75 km since 1850. The expansion into new ground of European larch could be estimated by aid of maps for

est cover over time. This allowed an estimate of the maximum age of the larch forests. Trees were sampled across its entire range at the mountain slope selected for investigation. This area was approximately 350 hectares. A dense sampling of trees was carried out in one ESSP and one LSSP, each subplot around 0.8 hectares having 140 and 190 trees, respectively. In all 730 trees were sampled.

To estimate spatial genetic structure (SGS) sampling of trees at distances of 20 meters took place to maximize the numbers of tree pairs at short distances. A separation of the trees into nine subpopulations with different ages was carried out. Mean pairwise kinship coefficients were estimated to estimate the spatial genetic structure (SGS). Four distance classes up to 120 meters distance, each 30 meters, were analyzed with respect to kinship.

The observed heterozygosity of the old subpopulations (> 160 years old) varied in the range 0.57-0.66 while the corresponding range for the young populations (80-100 years old) was 0.62-0.69. There was no difference between the age classes with respect to genetic diversity or F_{is}. Eleven and twelve rare alleles were noted for the ESSP and LSSP populations. As in many other conifers from the temperate and boreal areas there was a high variability within populations. Contrary to the expectation, the genetic diversity was not smaller at the population expanding frontier. The results suggest that long-distance dispersal to the expanding frontier had occurred. Strong winds during winter could promote seed distribution on the snow covered surface in the forests. The large genetic diversity of the ESSP population was attributed to seed supply from a large number of trees for establishment of the expanding population.

Forty-eight of the pairwise 66 F_{is}’s were below 0.02; with the highest estimate being 0.066. One population deviated with eight of its eleven pairwise estimates exceeding 0.030. It was speculated that seeds from other forests might have contributed to its establishment after the
withdrawal of the ice after 1850. Three distinct clusters were detected containing 11, 29, and 690 individuals. Ten saplings from the ESSP population belonged to the smallest group while 25 saplings from the LSSP population belonged to the 29-individual group.

The analysis of kinship in the entire material showed that the kinship coefficient lost significance at distances above 80-160 meters. Both for young individuals and adults in the LSSP population the kinship correlation decreased with distance. The estimated dispersal distance was low in the LSSP population, 17.8 meters, while it in some cases was more than two kilometers in the ESSP population. The LSSP population consists of grown-up trees in a dense forest, which constitute physical obstacles to pollen and seed dispersal.

It was concluded that a species expanding rapidly as a consequence of climate warming can maintain levels of genetic diversity with no indication of founder events. It should be remembered that this statement is built on neutral markers while the important matter for predictions of the ability of populations to cope with climate change is the diversity of genes contributing to fitness.

The differentiation of four *L. decidua* populations along an elevational gradient (1,350 – 2,300 masl, latitude 54.87°N, longitude 6.68°E) in the French Alps was studied by aid of the variability in eleven microsatellite loci (Nardin et al. 2015).

In the 788 trees analyzed eleven microsatellite loci with 125 alleles were analyzed. The number of alleles per locus varied considerably: 4 - 22. There were between two and four private alleles in the four populations. There was a significant number of linkage disequilibrium (LD) between pairs of loci in the 2,300 masl population. In the other populations no significance for LD was noted. The observed heterozygosity did not vary much among the populations, 0.739 – 0.761. The *F*<sub>IS</sub> estimates were positive and significant in the two intermediate populations, 0.02 and 0.0286, respectively. The pairwise *G*<sub>ST</sub> estimates are shown in Fig. 1-71, which shows that the 2,300 masl population is most differentiated from the other populations. It was stated that there was no geographic trend related to the differentiation between populations. The low differentiation among the four studied populations was attributed to large gene flow between the populations. Recent colonization might be another contributing factor to the limited differentiation.

For the population at 2,300 masl a significant spatial genetic structure (SGS) was noted. A difference in stand density between the populations could be excluded as the cause of the SGS since the density was almost the same in all four populations. Differences in *F*<sub>IS</sub> were also excluded as reason for the difference in SGS. As mentioned above the *F*<sub>IS</sub> estimates were either negative or had low positive values. Human intervention might have had an impact on SGS in the two lowest elevation populations. In conclusion, it is remarkable that the differentiation among the populations is so limited in spite of an elevation difference of almost 1,000 meters. The climatic conditions must be quite different over such an elevational gradient. It suggests that genes in the loci analyzed do not contribute to fitness.

### 1.4. Species relationships

The genetic variation in 16 isozyme loci in nine *Larix* species from the northern hemisphere was studied by Semerikov and Lascoux (1999) and had a focus on phylogeny. Three species were represented by one population, *L. kamtschatica*, *L. kaempferi*, and *L. lyalli*. *F*<sub>ST</sub> and *F*<sub>IS</sub> estimates were presented for four of the species, *L. gmelinii*, *L. laricina*, *L. occidentalis*, and *L. sibirica*.

The lowest estimates of number of alleles, the percentage of polymorphic loci, expected and observed heterozygosities were noted for the species with limited distribution ranges, *L. lyalli* and *L. olgensis*. It was suggested that the narrow range of distribution of small populations and specific niches occupied by these two species were responsible for the limited genetic variation. In three loci there was a significant heterozygote deficit in *L. occidentalis* and one locus in each of *L. laricina* and *L. lyalli* also showed a heterozygote deficit.

The lowest *F*<sub>ST</sub> estimates were noted for *L. gmelinii* and *L. laricina* (Fig. 1-72). The relatively narrow range of the *L. laricina* populations was suggested as an explanation for the low *F*<sub>ST</sub> estimate. The Siberian larch populations covered a latitudinal range of 16 degrees and a longitudinal range of 45 degrees. In spite of this large geographic variation the *F*<sub>ST</sub> was only 0.079 while the highest estimate, 0.100, was noted for *L. occidentalis* with a much smaller geographic range. The *F*<sub>IS</sub> estimate for *L. occidentalis* was high, 0.178, but not discussed. There was an extreme variation in the *F*<sub>IS</sub> estimates for individual loci, -0.137 – 0.784, and a much larger range than in the three other species. This calls for some caution of the interpretation of this result.

Table 1-6 was compiled to get an overview of the results as regards differentiation among species from several re-
ports treating this topic. The minor differences that exist within certain groups are indicated by different colors in the box. L. amurensis, L. cajanderi, L. gmelinii, and L. olgensis are closely related and they differ from L. ochotensis (orange in Table 1-6) mainly by the frequency of the Pgi-B-79 allele. The frequent hybridization among the larch species complicates unambiguous classification. As regards the phylogeny of Larix as revealed by isozyme pattern it was stated:

A clear difference between the American and Eurasian species
A limited differentiation among the Eurasian species
A close relationship between L. iyalli and L. occidentalis

The results in this paper, which indicate a relationship between the two western North American species and L. kaempferi, are in conflict with some morphological data. Contrary to this, L. laricina shows relationships with Eurasian species. This discrepancy was attributed to recent demographic events being responsible for variation in isozyme pattern while morphological variation was attributed to long-term evolution in fitness-contribution traits.

Semirikov et al. (1999) reported on the relationship among the Eurasian species and populations in the above paper. Two additional species were included in this report, L. amurensis and L. czekanovskii. The latter species was not included in any dendrogram. In all 15 isozyme loci were analyzed. The population differentiation was estimated by $F_{ST}$. To test the relationship between genetic and geographic distance a multi-scaling analysis was carried out.

The expected heterozygosities for some of the populations were presented in the previous paper. The figures in this paper deviate marginally from the previous figures, which might be attributed to analysis of 15 instead of 16 isozyme loci. Two marginal populations had the lowest heterozygosities, which was attributed to their low number of trees. The mean $F_{ST}$s for differentiation within L. sibirica and L. gmelinii were 0.079 and 0.021, respectively. It should be noted that the L. sibirica populations covered latitudinal and longitudinal ranges much larger than the L. gmelinii populations; 16 degrees of latitude and 49 degrees of longitude for L. sibirica while the corresponding for L. gmelinii were 6 and 23 degrees. Two clear groups of L. sibirica populations were observed. One eastern-northwestern group and one southwestern group were distinguished. Five populations took an intermediate position as regards their isozyme genotypes. There was a significant relationship between isozyme profile and longitude but no significant relationship with latitude.

In Fig. 1-73 I have illustrated the $F_{ST}$s between western and eastern L. sibirica with the other species as well as the $F_{ST}$ for the difference between these two groups of L. sibirica populations. Species represented by one population only are not included in the comparisons. It is evident that the differentiation between L. decidua and the two groups of L. sibirica populations is most pronounced. The western L. sibirica group is more differentiated from the three other species than the eastern group. It is evident that geographic distance plays a role for the differentiation. The differentiation between L. olgensis and the eastern L. sibirica populations was lower than its differentiation from L. gmelinii, $F_{ST}$s being 2.0 and 2.8% respectively. This result, which does not follow the expectation according to geographic distances between the three taxa, was thoroughly discussed. L. czekanovskii had its lowest $F_{ST}$ estimate with L. amurensis and should probably be included in box 2 in Table Larix but it is not included since it was not included in any dendrogram in the paper.
and each of the four other species. Among populations
L. sibirica extended their previous ST. In this report nuclear DNA
ST Among populations is an extension
and a second the other four
and its hybrids
Still expected, this figure illustrates that species differentia-
population within species differentiation in
I have summarized the results as regards species and
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Also the joint analysis of the three types of DNA markers
The AFLP analysis resulted in six groups; the eastern and
two Siberian larch groups with respect to cpDNA.
Siberian larch there was no big difference between the
mitochondrial haplotypes between eastern and western
species. In contrast to the large difference in number of
The cpDNA analysis revealed two groups of haplotypes.
the first being L. sibirica and a second the other four
species. In contrast to the large difference in number of
mitochondrial haplotypes between eastern and western
Siberian larch there was no big difference between the
two Siberian larch groups with respect to cpDNA.
The AFLP analysis resulted in six groups; the eastern and
western L. sibirica and each of the four other species.
Also the joint analysis of the three types of DNA markers
resulted in division of the studied species into six groups.
I have summarized the results as regards species and
population within species differentiation in Fig. 1-74. As
expected, this figure illustrates that species differentia-
tion is larger than population differentiation and that the
largest differentiation was noted for mtDNA. The cpDNA
estimate of among-population differentiation it is exception-
tionally low compared to many other species (cf Eriksson
et al. 2014). With the low number of cpDNA haplotypes
and with each of them occurring in different taxa it is
expected to result in high FST estimates. Noteworthy is
the remarkably high differentiation between western and
eastern L. sibirica for AFLP markers, FST among groups
= 0.14. It was discussed that the differentiation noted for
AFLP markers might be overestimated.
It was suggested that there were two L. sibirica refugia
during the latest glaciation, one in southern Ural and
northern Kazakhstan and the other in southern Siberian
Mountains. The results indicated that hybridization
among the eastern Larix species occurred to a large ex-
tent.
The report by Semerikov et al. (2003) is an extension of
the previous paper to some more Larix species with
limited or no focus on within-species variation. The con-
flicting phylogenies based on nuclear and organelle DNA
remained. It was suggested that the split between the
American species and the Eurasian species occurred first
and probably during the late part of the Tertiary. During
this cold period larches were able to reach some southern
and western mountains. This led to the South Asian clade
and somewhat later to the ancestors of present day L. de-
cidua. Later migrations led to the differentiation into L.
sibirica, L. kaempferi and L. principes-rupprechtii. Still
later on there was a split of L. sibirica into one western
and one eastern branch as a consequence of the existence
of two refugia, one in Southern Siberia and the other in
The Ural Mountains. Finally, it was stated that separation
within the L. olgensis–L. gmelinii is still an ongoing
process.
Five mitochondrial marker loci were used by Semerikov
et al. (2007) to study the phylogeny of L. sibirica. Sam-
ple from 99 populations of L. sibirica and its hybrids
with L. gmelinii were analyzed. As a reference two popu-
lations of L. decidua were sampled. Each population was
represented by 3–30 individuals. In one population fifty-
seven trees along a transect was sampled to study the spatial
structure of genetic variation. The distance between
the trees in this transect was approximately 50 meters.
Four groups of populations were distinguished:
1. Populations of Eastern European Russia, The Urals,
the western part of western Siberia, in which haplotype
S1 is dominating.
2. Altai Mountains and Tien Shan populations, in which
haplotype S0 occurs in high frequency while haplo-
types LN, L0, and L1 are absent,
3. The region adjacent to southern Lake Baikal, in
which haplotype L0 is dominating.
4. The rest of Siberia, in which all haplotypes specific
for L. sibirica occur, except for haplotype L20.
Based on the haplotype diversity it was suggested that
one refugium of Siberian larch existed in The Southern Ural Mountains. It was stated that the Tien Shan populations most likely originated from the Altai Mountains. The dominance of haplotype L0 south of Lake Baikal suggested a glaciation refugium of larch in this region. The origin of populations under point 4 was discussed in detail without coming to any definite conclusion as regards probable refugium/refugia.

In the maps presented in Fig. 2 of the paper hypothetical routes of the spreading of the populations after the latest glaciation were presented. The \( F_{ST} \) for population differentiation according to mitochondrial haplotypes was estimated at 45.7%. Of the five populations along the northern timberline, two were monomorphic. The total number of monomorphic populations was 12. Monomorphism for different haplotypes contributes strongly to high \( F_{ST} \) estimates. \( R_{ST} \) could only be calculated for one locus, \( R11 \), and was estimated at 38.6%, which was close to the \( G_{ST} \) estimate, 35.5%, for this locus. The analysis of the transect reveal mosaicism for the mitochondrial haplotypes. It was concluded that the size of groups of trees related to each other was 50-100 meters. As a consequence of this, sampling of populations for mitochondrial diversity should include trees at a wider distance than 100 meters.

Kisanuki et al. (1995) found four cpDNA haplotypes in their study of eight Larix species; \( L. gmelinii \) was represented by six varieties. As far as I can understand only one tree per species or variety was studied, which does not permit general conclusions about the phylogeny of these larch species.

Seventeen polymorphic isozyme loci were analyzed in twelve approved seed tree stand populations of \( L. decidua \) in North Western Italy by Belletti et al. (1997). The elevation of the populations varied between 900 and 1,950 masl and all main directions (south, west, north, and east) of exposure were represented. The longitudinal and latitudinal variation of the populations was 1.5 and 2.1 degrees, respectively. Around six megagametophytes per tree from at least 30 trees were analyzed to obtain the population structure.

The mean \( F_{ST} \) for the pairwise comparison of populations was estimated at 0.033. Such a low estimate suggests that there is considerable gene flow among the populations in spite of the origin of the populations on different aspects. The southernmost population which was growing isolated from the other populations had the highest mean \( F_{ST} \) estimate, 0.054. There was a significant relationship between \( F_{IS} \) and geographic distance but with a low degree of explanation of the variation, \( r = 0.47 \). The total number of pairs of comparison (59) explains the significance. The \( F_{IS} \) estimates for the twelve populations varied between -0.068 and 0.121. The highest estimate was noted for the population from the lowest elevation, 900 – 1,100 masl. I tested the relationship between population \( F_{IS} \) and latitude, longitude or elevation without finding any strong relationship. Thus, there was a surplus of homozygotes in most populations, which was attributed to selfing or matings between related individuals.

Chloroplast DNA differences among eight Larix species were studied by restriction fragment length polymorphism by Qian et al. (1995). Generally there were small differences among the eight species. Three groups were distinguished:

1. \( L. griffithiana \)
2. \( L. laricina, L. occidentalis, and L. sibirica \)
3. \( L. decidua, L. gmelinii, L. kaempferi, and L. potaninii \)

\( L. griffithiana \) differed most from the other species. The above grouping differs from other groupings of the \( L. \) species, mainly based on cone characteristics. It was pointed out that the uniparental mode of chloroplast DNA inheritance might be the cause of the deviating classification of the species based on cpDNA.

Eight isozyme loci were used to study the separation of three Chinese larch species, \( L. gmelini, L. olgensis, \) and \( L. principes-rupprechti \) by Hu and Ennos (2001).

The genetic distance among the taxa was very limited, 0.01 and the \( F_{ST} \) for among-population variation within species was estimated at 0.002. The two \( L. principes-rupprechti \) populations differed most from the 14 other populations. This was interpreted as a result of earlier separation of these populations from the other populations following cooling of the climate. There was a relationship between \( F_{ST} \) and geographic distance among the \( L. olgensis \) populations. It was suggested that the occurrence of the populations on high elevation has resulted in isolation and restricted gene flow among the populations. However, the limited \( F_{ST} \)s do not support this explanation. It was concluded that these three species should be regarded as subspecies rather than separate species.

Wei and Wang (2003a) used the trnT and trnF regions of the chloroplasts to disclose the relationship between all recognized Larix species except \( L. lyallii \). In all 46 trees were included in the analysis. Forty-four informative nucleotide sites were identified.

Three distinct groups were distinguished (Table 1-6):

1. \( L. decidua, L. gmelini, L. kaempferi, L. olgensis \)
2. \( L. griffithii, L. himalaica, L. mastersiana, L. potaninii, L. sibirica, L. speciosa \)
3. \( L. laricina, L. occidentalis \)

These results support the view that the separation of the long-bracted (section \( Multiserialis \)) and short-bracted (section \( Larix \)) took place as early as the separation between North American and Eurasian species. The disagreement in the grouping of \( L. griffithii \) species based on bract traits and markers was discussed in detail. It might be concluded that a grouping solely on the morphological traits might be misleading as regards the evolution of genus \( Larix \).
Wei and Wang (2003b) used internal transcribed spacer (ITS) to study the relationship between Larix species. They sampled material from all Larix species except for L. lyallii. In three cases there were more than one tree examined per taxon.

At least four distinct ITS clones were detected from each sample except Larix griffithii, L. olgensis and one individual of Larix gmelinii. The total number of distinct clones amounted to 101. Below the distinguished three types of clades (ITS clones) are presented and their belonging to the two sections Larix and Multiserialis are shown with different colors (Table 1-6):

1. L. decidua, L. gmelinii var. gmelinii, L. gmelinii var. principis-rupprechtii, L. kaempferi, L. olgensis, L. sibirica (3). All belonging to section Larix.
3. L. lariicina, L. occidentalis

It is noteworthy that the two American species are molecularly closely related while they taxonomically belong to separate sections of Larix. The intragenomic sequence divergence was much lower in L. lariicina than in L. occidentalis, 0.005 versus 0.016. It was suggested that L. lariicina had experienced several bottlenecks during the Pleistocene glaciations.

The intragenomic sequence divergence was small, varying between 0.003 and 0.025 for the Multiserialis species.

Wei and Wang (2004) studied the evolution of the 4-coumarate:coenzyme A ligase (4CL) gene and the phylogeny of larch species. L. gmelinii var. principis-rupprechtii had seven distinct 4CL gene members and all other Larix species had two to four 4CL gene members. The parsimony analysis revealed two clades designated 4clA1 and 4clA2. The former was further divided into two subclades 4clA1 and 4clA2. The subclade 4clA1 consisted of all Eurasian species of Larix, L. speciosa, and the North American species L. lariicina and L. occidentalis. An especially strong relationship between L. gmelinii and the two North American larch species was found. The subclade 4clA2 consisted of all Eurasian species belonging to section Multiserialis. It should be noted that L. speciosa, which is endemic to South-Western China, appeared in both subclades. The authors stated that the phylogeny based on the 4CL gene differ from the phylogeny revealed by all other molecular analyses as well as fossil events. This caused the authors to stress the difference between a gene tree, such as the one based on the 4CL gene, and a species tree. As a consequence of this, they stated that the present 4CL phylogeny does not mirror the species phylogeny of Larix. The authors deserve respect for tuning down the own results for identification of the phylogeny of larch species.

To obtain species diagnostic markers for the four larch species L. decidua, L. laricina, L. kaempferi, and L. sibirica RAPDs, SNPs, mtDNA, and cpDNA analysis of needles were carried out by Gros-Louis et al. (2005). The needles were collected in a provenance trial in Québec, Canada. Besides, samples from the natural habits of L. decidua and L. sibirica were included in the study. The L. sibirica populations originated from localities far away from the zone of contact with L. gmelinii. For a study of the phylogeny of the genus Larix analysis of L. gmelinii, L. griffithiana, L. lyallii, L. mastersiana, L. occidentalis, and L. potaninii were also included in this investigation. In a first step five trees from each provenance of the four main species were screened for several genomic regions. Then lack of polymorphism at the intraspecific level for each marker was ascertained in a larger number of trees per species, 12-33. The number of primers for the rapid analysis was 130. Twelve EST primer pairs were used for detection of expressed sequence tag (EST) polymorphism. As regards mtDNA five introns were amplified and sequenced. One intron and two intergenic regions of the chloroplast genome were amplified and sequenced. Cleaved amplified polymorphic sequence markers (CAPs) were developed in order to facilitate analyses of relationships between taxa.

Most of the paper dealt with development of DNA fragments that could be used in discrimination between the four main species of this investigation.

Khatab et al. (2008) studied nucleotide changes in two nuclear gene regions in 19 populations of seven Larix species. Each species was represented by 1-6 populations. The nuclear gene regions analyzed were: 4-coumarate ligase (4CL) Coumarate-3-hydroxylase (C3H)

The L. gmelinii varieties and L. kaempferi were represented by one population only. The western Siberian larch (L. sibirica) was represented by six populations while all other species were represented by three populations. In the 4CL region 26 haplotypes were found while 17 haplotypes were found in the C3H region. Neighbor joining trees were presented for the nucleotide patterns for each of the two nuclear regions. It was stated that the haplotypes differed only by small steps and the differences did not correspond to the taxonomic classification. L. kaempferi was the only exception to this. Of more importance for the present publication is the result from the pairwise estimates of FST. In Figs. 1-75 and 1-76 I have illustrated the mean FST for comparisons, in which at least one species in the comparison is represented by more than one population. Negative FST estimates were regarded as zero in the calculations. The within and between FST’s for the two groups of Siberian larch were low for the 4CL and C3H regions studied. Fig. 1-75 clearly shows the large difference between L. kaempferi and the other species and populations. The
within-species $F_{ST}$s of *L. cajanderi* and *L. gmelinii* var. *olgensis* were low while they were moderately different from the Siberian larch populations. However, two of the *L. cajanderi* populations that grew only two kilometers apart differed significantly from each other for both markers, $F_{ST}$s = 0.16 and 0.22, respectively.

Fig. 1-76 shows that the *L. gmelinii* population, which originated from northern Siberia, differed strongly from its comparison partners. This population had a unique 4CL haplotype, which was not found in any other population. Thus, this haplotype contributed strongly to a high $F_{ST}$ estimates. The differentiation between *L. gmelinii* var. *olgensis* and the two other varieties of *L. gmelinii* was limited. Almost the same pattern was noted for the differentiation between the two latter populations and *L. cajanderi* while they are more differentiated from the two Siberian larch groups.

In conclusion the $F_{ST}$ estimates reflect to a large extent the geographic distances among the populations. In some cases the location of the populations close to the margin of distribution has probably caused a larger differ-

**Figure 1-75.** Mean $F_{ST}$ estimates for within species and among species differentiation based on nucleotide variation in two nuclear genes, 4-coumarate ligase (4CL, blue columns) and coumarate 3-hydroxylase (C3H green columns), in larch species. Within species $F_{ST}$s have a yellow background. *LGo* = *L. gmelinii* var. *Olgensis*, *LC* = *L. cajanderi*, *LS-E* = *L. sibirica* east of Ural mountains, *LS-W* = *L. sibirica* west of Ural mountains, *LK* = *L. kaempferi*. The latter species was represented by one population only. Khatab et al. 2008.

**Figure 1-76.** Mean $F_{ST}$ estimates for among species differentiation based on nucleotide variation in two nuclear genes, 4-coumarate ligase (4CL, blue columns) and coumarate 3-hydroxylase (C3H green columns), in larch species. *LGo* = *L. gmelinii* var. *Olgensis*, *LC* = *L. cajanderi*, *LS-E* = *L. sibirica* east of Ural mountains, *LS-W* = *L. sibirica* west of Ural mountains, *LG* = *L. gmelinii* var Kamtchatica, *LGj* = *L. gmelinii* var. *Japanica*. The three latter species were represented by one population only. Khatab et al. 2008.

entiation from more centrally located populations; the *L. gmelinii* and the southernmost *L. sibirica* populations are examples of this. The authors stated that more populations, especially of *L. gmelinii* ought to be studied to enable more far-reaching conclusions as regards the species relationships.

Araki et al (2008) studied six populations of *L. sukaczewii* and three populations of *L. sibirica* with respect to the variation in 5.8 S rDNA including two internal transcribed spacers (ITS) as well as sequences of the 4-coumarate:coenzyme A ligase (4CL). The main focus was on the status of *L. sukaczewii* and *L. sibirica* as a single species with two subspecies or as two separate species. In the ITS1 and ITS2 regions 29 and two segregating sites were found, respectively. Eleven segregating sites were found in the 4CL region. In the ITS region 25 haplotypes were found, of which only one was common to the two species. Some of the haplotypes showed small differences; one mutational step. In the 4CL region 13 haplotypes were observed; five of them occurred only in *L. sukaczewii* while one was unique to *L. sibirica*. Seven haplotypes occurred in both species.
The mean pairwise $F_{ST}$ estimates shown in Fig. 1-77 for population differences within *L. sukaczewii* and *L. sibirica* reveal that five of the *L. sukaczewii* populations are not much differentiated for the two regions tested. The southernmost population No. 6 differed much from the other *L. sukaczewii* populations. It was suggested that this population had been isolated from other regions. Support for this assumption was found in other investigations of adjacent populations. The high estimates for the Siberian larch populations might be explained by the wide origin of them in latitude (50-58°N), longitude (87-97°E), and elevation (158-1,630 masl) and that they have evolved in different directions.

With one exception for population 7 (ITS), all other between-species $F_{ST}$ estimates were higher than the within-species estimates (Fig. 1-78; note the different scales in the two figures!). The large difference between the IST and 4CL estimates for population 7 in its comparison with the six *L. sukaczewii* populations is disturbing. Based on these results it might be justified to regard *L. sukaczewii* and *L. sibirica* as two separate species. Further support of this was the finding that some 4CL haplotypes that were frequent in *L. sibirica* occurred rarely in *L. sukaczewii* and vice versa as regards other haplotypes. It was pointed out that more *L. sibirica* populations must be studied to get a full understanding of the phylogeny of these two taxa.

Chloroplast haplotypes and mitochondrial DNA were used by Zhang et al. (2014) to determine species relationships among *Larix* species in Eastern Asia, mainly China. Fifty trees from 15 populations (1–5 trees per population) of *L. gmelinii*, *L. mastersiana*, *L. olgensis*, *L. potaninii*, and *L. sibirica* were analyzed. They originated from three different regions in China; north east, north west, and south west. In addition, *L. decidua*, *L. kaempferi*, and *L. laricina* were included for comparison. Two estimates of diversity were calculated; nucleotide diversity (ND) according to Nei (1987) and haplotype diversity (HD) according to Nei and Tajima (1983). Six haplotypes were identified. Both the HD and ND in the north eastern region did not show any differentiation while both HD and ND were highest in the south western region. In the north eastern region only eleven trees in the four populations from this region were analyzed. Except for one of the eight populations from the north eastern region they were monomorphic for one haplotype, which was not found in any other populations. This monomorphism is reflected in the high estimate of variance among regions as illustrated in Fig. 1-79. Based on the observed data, present and potential distribution of the species following climate change was estimated. Mean temperature of the coldest months of the year and precipitation seasonality were found to be the most important factors both for present and future distribution of the species.

The chloroplast trnK intron sequences of *L. czekanovskii*, *L. decidua*, *L. gmelinii*, *L. sibirica*, and *L. sukaczewii* were used by Bashalkanov et al. (2003) to study the phylogeny of the latter species. Whether or not more than one tree per species was analyzed was not reported. The authors reported a clear divergence between *L. sibirica* and *L. sukaczewii* for the trnK intron.

### 1.5 Miscellaneous

Karyotypes of tissue cultures and of root tips of *L. decidua*, *L. kaempferi*, and their hybrid were assessed by Nkongolo and Klimaszewska (1995) to disclose any relationships between these three taxa. The heterobrachial chromosome No 7 in *L. decidua* with a secondary constriction can be used to identify the *L. decidua* genome. Chromosome No 9 of *L. kaempferi* can be used to separate this species from *L. decidua*. In the hybrid embryonic lines as many as 70% of the cells had one extra chromosome. Based on the own study and earlier presented karyotypes from *L. sibirica* made it possible to state that *L. decidua* is closer related to *L. kaempferi* than to *L. sibirica*.
1.6 Summary

1.6.1 Quantitative variation

Strong latitudinal differentiation in growth was noted in several studies. Typically, a maximum in transfer was observed with the best performance of populations transferred northwards. In some cases a similar dose-response curve was observed for the relationship between elevation and growth while there was no relationship of this kind in other studies. One Pearson age 2 - age 34 correlation for tree height was unexpectedly strong, $R^2 = 0.80$.

Generally, Sudeten larch populations grew well and had a satisfactory disease tolerance. But their stem quality was in some cases unsatisfactory. Survival at a northern trial increased with latitude, $R^2 = 0.83$. Plant height at this trial dropped with longitude, $R^2 = 0.77$. There was a strong consistency between growth cessation over years, $R^2 = 0.82$. Artificial freeze testing resulted in weak relationships between spring frost damage and latitude in Siberian larch. A stronger relationship between latitude and autumn frost damage was reported, $r = 0.78$.

Spring frost scores after freeze testing were higher in L. sibirica and L. sukaczewii than in L. decidua and L. gmelinii. Low-elevation populations had earlier bud flushing dates than high-elevation populations in phenology studies in situ. The opposite was true for growth cessation. In one investigation it was estimated that one degree temperature difference resulted in 6-7 days change of phenological traits. Large population variation in wood density and tolerance against Coniophora puteana between populations was reported.

1.6.2 Markers

In the early investigations including isozyme markers, focus was on population or species differentiation. Later, ecology-oriented studies and phylogenetic studies were carried out. Generally, the isozyme studies revealed limited population differentiation independent of species and number of populations involved in the individual investigations. The lowest estimate in Fig. 1-80 was noted for a study of L. gmelinii while the highest was reported for scattered Romanian L. decidua populations. In some cases geographic differentiation was noted but equally frequent no such differentiation was found. $F_{is}$ estimates varied between 0.003 (L. laricina) and 0.178 (L. occidentalis).

Substantial gene flow among L. kaempferi populations growing on a slope with a difference in elevation of more than 1,400 meters was observed. Similarly, variation in SNP and microsatellites among alpine L. decidua populations from an elevation range of 1,100 meters was limited. In one case the high-elevation population was more differentiated than populations from low elevations. In studies including populations from huge geographic areas relationships between geographic distance and isozyme profile were observed; especially longitudinal relationships for Siberian larch. Geographic distance also played a role in species comparisons.

Substantial population differentiation were noted in studies including mtDNA and cpDNA. A major reason for these results is monomorphism of different markers in different populations. Nine microsatellite loci were used in a comparison of the genetic variation between an early successional subpopulation and late successional subpopulations. Only low $F_{st}$ were noted indicating that there was no differentiation related to successional stage.

![Figure 1-79. The partitioning of the variance based on cpDNA in a study of 15 Larix populations from three regions in Eastern Asia; north western (including one adjacent Russian population), north eastern, and south western China. The following species were represented in this investigation: L. gmelinii, L. mastersiana, L. olegensis, L. potaninii, and L. sibirica. Zhang et al. (2014).](image)

![Figure 1-80. A summary of the $F_{st}$s or $G_{st}$s reported for among-population differentiation within species by use of isozymes.](image)
Table 1-6. A synthesis of the results as regards species relationships studied with different markers. The grouping is based on the individual reports. It should not be read such that all species in a specific column belong to the same group of species. Minor differences that exist within certain groups are indicated by different colors in the box.

<table>
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<th>Reference</th>
<th>Markers used</th>
<th>Number of groups distinguished</th>
<th>Branch diameter</th>
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<td>Semerikov et al. 1999</td>
<td>isozymes L. sibirica western populations</td>
<td>L. sibirica eastern populations L. cajanderi L. olgensis L. amurensis L. gmelinii L. ochotensis</td>
<td>L. Kamtchatica L. decidua L. kaempferi</td>
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<td>Qian et al. 1995</td>
<td>cpDNA L. decidua L. gmelinii L. kaempferi L. potaninii</td>
<td>L. griffithiana</td>
<td>L. laricina L. occidentalis L. sibirica</td>
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<td></td>
<td>ITS L. decidua L. sibirica</td>
<td>L. kaempferi L. olgensis L. principes-Ruprechtii</td>
<td>L. griffithiana L. potaninii L. laricina L. lyalli L. occidentalis</td>
</tr>
<tr>
<td>Semerikov and Lascoux 2003</td>
<td>mtDNA L. decidua and most eastern L. sibirica haplotypes</td>
<td>western L. sibirica and a few eastern L. sibirica haplotypes</td>
<td>L. gmelinii and a few haplotypes in L. olgensis</td>
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<td></td>
<td>cpDNA L. deecidua L. gmelinii L. kaempferi L. olgensis</td>
<td>L. sibirica</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AFLP L. decidua L. sibirica west L. sibirica east</td>
<td>L. gmelinii L. olgensis L. kaempferi</td>
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Table 1-6 continued. A synthesis of results as regards species relationships studied with different markers.

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<th>Branch diameter</th>
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<td>2004</td>
<td></td>
<td>L. gmelinii var.</td>
<td>L. occidentalis</td>
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<tr>
<td></td>
<td></td>
<td>gmelinii</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>L. olgensis</td>
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<tr>
<td></td>
<td></td>
<td>L. kaempferi</td>
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<td></td>
<td></td>
<td>L. sibirica</td>
<td></td>
</tr>
<tr>
<td>Khatab et al.</td>
<td>4CL and C3H</td>
<td>L. sibirica</td>
<td>L. cajanderi</td>
</tr>
<tr>
<td>2008</td>
<td>genes</td>
<td></td>
<td>L. gmelinii</td>
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<td></td>
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<td>L. gmelinii var. olgensis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>L. gmelinii var. Japonica var. Kamtchatica</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>cpDNA</td>
<td>L. sibirica</td>
<td>L. potaninii var. potaninii</td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td>L. gmelinii var.</td>
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<td>gmelinii</td>
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<td>gmelinii</td>
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<tr>
<td></td>
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<td></td>
<td>princes-ruprechtii</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L. olgensis</td>
</tr>
</tbody>
</table>
The impact on mating pattern on stand density was studied at two localities, each with one natural population and one seed tree stand. In one of the natural stands a positive $F_{ST}$ was noted while all four progeny populations had positive $F_{IS}$ estimates.

1.6.3 Species relationship

Table 1-6 was constructed to facilitate a comparison of the results from different investigations. It should not be read such that all species in a specific column belong to the same group of species. It is merely grouping of species in individual investigations. Another problem in the comparisons of different studies is the inconsistency in identification of species. Thus, Khatab et al. (2008) distinguished four different varieties of $L. gmelinii$ while others regarded the varieties as different species. One strange observation was noted in the study of mitochondrial DNA variation. Thus, $L. decidua$ was closer related to most eastern Siberian larch populations than to western Siberian larch.

One AFLP study reported a high $F_{ST}$ estimate, 0.14, for the difference between eastern and western Siberian larch. Analyses of isozymes revealed a clear differentiation between American and Euroasian species with a limited differentiation in the latter group. A close relationship between $L. occidentalis$ and $L. lyallii$ was noted. The low number of populations or even trees included in some of the studies is likely a source for conflicting results.

In spite of all shortcomings and several conflicting classifications there are some general patterns: The three North American species are usually different from the rest of the species. $L. kaempferi$ from Japan seems to deviate much from all other species. Exceptions occur; one study reported that $L. decidua$ and $L. kaempferi$ belonged to the same group of species.


1.1 Marker inheritance

For a study of inheritance and linkage of isozymes, seeds were collected from individual trees in a Canadian *L. laricina* provenance trial with populations covering the range of distribution of this species (Cheliak and Pitel 1985).

In all 21 loci with 49 alleles were detected with two loci without any variation. Inheritance and linkage at 13 loci with 29 alleles could be determined. There was generally an agreement with expectation for a single-gene inheritance according to the Mendelian law. Two loci were monomorphic and two of the loci seemed to be modified by the ambient condition and was discarded from the linkage study. Linkage was disclosed in the following four cases:

1. aspartate aminotransferase – superoxide dismutase
2. superoxide dismutase – aconitase
3. phosphoglucose isomerase 1 - phosphoglucose isomerase 2
4. aspartate aminotransferase - phosphoglucose

The latter two were found to be closely linked. The results obtained were compared with observations in other conifer genera and the evolution of the enzyme system was discussed.

Lewandowski and Mejnartowicz (1990) used megagametophytes and embryos to study the inheritance of isozymes in *L. decidua*. In the 70 trees from four populations and 25 seed orchard clones they found eleven polymorphic loci and four monomorphic loci. In addition, they observed rare alleles in four loci in a collection of seeds from eleven populations covering the distribution area of *L. decidua* in Poland. No deviation from the 1:1 segregation in the first mentioned eleven loci occurred.

Lewandowski and Mejnartowicz (1991b) reported linkage between two isozyme loci out of eleven studied in *L. decidua*.

Ying and Morgenstern (1990) sampled eight *L. laricina* populations from New Brunswick, Canada for an analysis of inheritance and linkage of isozyme loci. They found 15 loci with regular Mendelian inheritance. Two groups, one with two loci and another group with three loci, showed linkage within the groups.

Offspring from six crosses between *L. decidua* and *L. kaempferi*, three times with each species as female partner in the cross, were studied by Szmidt et al (1988) to disclose the pattern of inheritance of chloroplast DNA (cpDNA).

Of the five restriction enzymes used, two Bam-HI and Bci, disclosed differences in cpDNA between the two species. Five of the six families showed that there was a clear paternal inheritance of cpDNA. For one of the six families a maternal inheritance could not be excluded. Different explanations for this deviating pattern were discussed. The most likely might be the contamination of the pollen batch used for the crosses. The risk of pollen contamination is a constant risk in crosses involving wind pollinated species.

DeVerno et al. (1993) used several wheat mitochondrial probes for analysis of the transmission of mitochondria in eight *L. decidua* x *L. kaempferi* hybrid trees and eight reciprocal hybrids. With one exception the transmission was maternal.

Khasa et al. (2000) described 14 microsatellite loci in *L. lyalli* and *L. occidentalis*, which showed Mendelian inheritance according to analysis of megagametophytes and embryos. Two Markers were species-specific, which allows a rapid identification of these two species in hybrids.

Isoda et al. (2006) isolated 19 polymorphic microsatellite loci from 15 *L. kaempferi* trees. Another 45 trees were genotyped and the expected heterozygosity in these microsatellite loci varied in the range 0.57 – 0.95.

Semerikov et al. (2006) developed PCR-based mitochondrial DNA markers based on twelve individuals of each of the species *L. gmelinii* and *L. sibirica*. The relationship between the various mitochondrial haplotypes was presented.

Chen et al. (2009) isolated nine microsatellite loci with 5-19 alleles per locus in *L. occidentalis*. Forty-one clones in a seed orchard were sampled to test the polymorphism of these markers. For six of the loci significant deviations from the Hardy-Weinberg equilibrium were noted. This was attributed to presence of null alleles or limited sample size. Analyses of 15 open-pollinated families disclosed presence of null alleles in three loci.

A study of identification of hybrids between *L. decidua* and *L. kaempferi* by RAPDs was carried out by Scheepers et al. (2000). In a first step 180 primers were used in assay of 20 European and 20 Japanese larch trees. Fifteen fragments differentiated the two species; of them two were mitochondrial and can thus not be used for the identification of hybrids owing to their maternal inheritance. Later
Table 2.1. The number of linkage groups, numbers of markers assigned to a linkage group, unlinked markers based on AFLP, RAPD, and ISSR markers in a hybrid full-sib L. decidua x L. kaempferi family with 112 individuals. Arcade et al. 2000.

<table>
<thead>
<tr>
<th>Linkage groups</th>
<th>Marker No</th>
<th>Unlinked markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. decidua</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>L. kaempferi</td>
<td>21</td>
<td>107</td>
</tr>
</tbody>
</table>

it was stated that eleven species-specific bands were tested in eight trees in each of 17 L. decidua populations as well as eight trees in one Japanese larch population. This screening confirmed that the RAPDs identified were species-specific and thus suitable for identification of hybrids between the two species under study. Two RAPD primers specific to each of the two species were used to identify hybrids in four different seed lots of varying origin. The percentage of hybrids varied from 4% to 84% in these lots. The seed lot with the highest percentage of hybrids originated from a French lot which was produced from a special type of fertilization with pulverized Japanese larch pollen on European larch trees. Evidently, it was a fairly successful technique. In passing it might be noted that the percentages of hybrids in Table 3 of the paper suggested high precision of the estimated percentages of hybrids. Thus the percentage of hybrids in a Danish seed lot was given as 9.315. Since it was stated that around 96 seeds were analyzed such a precision is unjustified.

Another study to develop methods for secure identification of L. decidua x L. kaempferi hybrids by markers was reported by Acheré et al. (2003). Twenty-six native populations of European larch and 12 Japanese populations of larch were used to find diagnostic markers of these two species. Seed lots were obtained from a Belgian seed orchard with 19 L. decidua clones and 16 L. kaempferi clones. The seed lots analyzed originated from 12 L. decidua clones and three L. kaempferi clones. In all 97 individuals from the L. decidua clones were analyzed while the corresponding number for L. kaempferi clones was 108.

One mitochondrial and one chloroplast marker were found to be suitable markers for detection of larch hybrids. The percentages of hybrids in the two seed lots (L. decidua and L. kaempferi females) were 52.6 and 42.6. With total random mating in the seed orchard the percentage of hybrids would be close to 50 %. Considering that this is an interspecific seed orchard with two species with differences in flowering phenology and crossing barriers the hybrid percentages must be regarded as surprisingly high.

Genetic linkage maps for L. decidua and L. kaempferi were developed by Arcade et al. (2000). A full-sib family with 112 hybrids between these two species was screened with respect to 266 markers; 114 AFLPs, 149 RAPDs, and three ISSR markers. One parent was heterozygous and the other parent had a null allele leading to a testcross configuration. A high LOD score was used in the linkage determination. AFLP. Five out of 64 primer combinations, which generated numerous polymorphic bands, were selected for the mapping. An 1:1 segregation was noted for 111 markers and a 3:1 segregation was observed for four markers. There was almost equal numbers of markers in the two species, 58 and 56.

RAPD. Six hundred primers were tested; of them 95 revealed 155 polymorphic fragments, of which 147 showed 1:1 segregation while six others showed 3:1 segregation. European larch contributed with 64 markers and the Japanese larch with 83 markers.

ISSR. Out of the eleven primers tested, four produced at least one polymorphic marker. One primer amplified five polymorphic markers, of which three were clearly scorable. Only these three were used in genotyping of the 112 individuals.

The results as regards number of markers and linkage groups are summarized in Table 2.1. Although there are only 12 pairs of chromosomes in Larix, the number of linkage groups was much higher than 12. Since there was a large number of unlinked markers, the higher number of linkage groups must be attributed to too low number of markers or strongly varying number of markers on each of the 12 pairs of chromosomes. However, it was stated that there was a good distribution of markers to the linkage groups, which would rule out the second explanation. Several linkage groups had low numbers of markers. Thus, linkage groups 11-17 in L. decidua had 2-4 markers each. In L. kaempferi linkage groups 11-21 had 2-4 markers each. It was estimated that the maps covered 80% of the genome size.

There was a direct homology between linkage groups decidua 1(D1) and kaempferi 1(K1). Similarly, linkage groups D2 and K2 also showed strong homology. Generally, there was a good homology between the two linkage maps. The genome sizes in the two species were estimated at 2,537 cM in L. decidua and 2,997 cM in L. kaempferi.

In conclusion, an important first step to get a genetic linkage map in Larix.

2.2 Quantitative traits

2.2.1 Europe

Data from 14 German trials with offspring from 77 clones of L. decidua and L. kaempferi were presented by Hering (1994). Eight trials had progenies from partial factorial matchings with four females and four males. (The author writes dialleles crosses but my interpretation of the illustration of the mating design is that it is a factorial matching
design.) Other trials had polycross progenies with a pollen mix of four males. Tree height, breast height diameter, and stem form were assessed at ages 7-27. Narrow sense and broad sense heritabilities were estimated separately for each trial and in some cases for two ages of assessment in the same trial. A few illustrations on general combining ability of clones with progenies at several trials were presented but no joint ANOVA seems to have been calculated, which is to regret.

The narrow sense heritabilities in the progeny trials with full-sibs are illustrated in Fig. 2-1. As regards tree height all estimates, except for trial 29 at age 13, are below 0.25. It was mentioned that a selective thinning for tree height took place between ages 13 and 17, which explains the levelling of the genetic differences. The extremely high heritability of 0.50 for tree height in a conifer must be attributed to strong competition. Such a competition means that differences are broadened. The increase of the heritability for stem form may also be explained by the selective thinning. In the polycross trials the heritabilities for tree height increased between years seven and ten, which must be attributed to increased competition in the trials. The illustrations presented in the paper suggest that there was a negative relationship between the growth traits and stem form. As stated above no joint analysis of the trials took place and no estimation of the correlation between the two growth traits and stem form was presented, which should have been most valuable for breeding.

Growth and survival of open pollinated progenies from 25 seed orchard clones of *L. sibirica* were studied at age ten in ten progeny trials in Finland (Mikola and Vakkari 1995). Six of the test localities were at forest sites and coined as field tests while the rest of the trials were coined as test gardens. The test gardens were characterized by weed control, protection, and fertilization much more intensive than in normal silviculture. The clones were selected in three stands with Raivola larch in Finland.

The variation in tree height was less than 5% among the families. However, it was significant according to the joint ANOVAs. It was concluded that there was limited variation among the families as regards all traits studied. As seen from Fig. 2-2 there was a large difference in tree height between latitudinal origins of the trials. One trial deviated with exceptionally good growth. When data from this trial was excluded the relationship between trial latitude and tree height became strong, $R^2 = 0.90$. ANOVAs were carried out for height, survival and height sum (= survival % + mean tree height). Significances were noted for family and family x test locality for all traits whether the deviating test site was included or not in the analysis.

![Figure 2-1](image1.png) **Figure 2-1.** Narrow sense heritability for tree height, breast height diameter, and stem form in eight German trials with partial factorial crosses including *L. decidua* and *L. kaempferi* plus tree clones. For two of the trials, 29 and 30, assessments from two years are available. Estimates are missing for some traits in some trials. Herking 1994.

![Figure 2-2](image2.png) **Figure 2-2.** The relationship between trial latitude and tree height or per cent survival at age 10 in ten field trials with *L. sibirica* progenies. Brown frame of the squares indicate that the trial is a so called test orchard with intensive care of the trial. Filled brown square = test orchard on old farm land. Blue line is the relationship between tree height and field locality origin for the eight trials on forest sites. Mikola and Vakkari 1995.
Since site effects were tested as random effects, the variance components for family and family x site interaction were very small. In all cases the interaction component was larger than the family component (Fig. 2-3). Thus was expected since the trials covered a latitudinal span of almost six degrees of latitude in Finland. A large family x site interaction is expected. As a rule of thumb this ratio should not be larger than one to avoid separate breeding zones. Even if it was stated that there was good agreement in family performance over the ten tests the ratio suggests that separate breeding zones ought to be distinguished.

The impact of the genetic difference between European and Japanese larch parents and quantitative traits was studied by Arcade et al. (1996). Following RAPD genotyping the distance between the 12 parents of each species was estimated by three different techniques; Jaccard, Sokal and Michener, and Nei and Li. The different techniques yielded similar results. The correlations with quantitative traits were limited to the distances obtained from the Jaccard technique. Fifty-six families from a partial factorial 12 L. decidua x 12 L. kaempferi mating design were planted in a field trial at an elevation of 540 masl in France (no latitudes or longitudes were given). Some of the 12 Japanese larch males were full-sibs. Tree height was measured almost annually up to age eleven. Several wood quality traits were recorded at ages 6-11. The genetic distances according to the Jaccard technique were for L. kaempferi (unrelated parents) and L. decidua 0.39 and 0.45, respectively. The distance between the two species was estimated at 0.72.

Some of the correlation coefficients between genetic distance and growth or quality traits are shown in Fig. 2-4. Of the 24 relationships calculated nine were significant. The relationship between parental genetic distance and quantitative traits performances was thoroughly discussed. It was stated that the genetic distances between the parents have to be large to obtain significant relationships since no significant relationship was observed for individual families. Even if there were several significant relationships none of them explained more than 40% of the variation in the growth or quality traits. The large number of families tested also contributed to the significances observed.

As regards tree height the largest regression coefficient was noted at age six, 0.55, and the highest regression coefficients of all was noted for increment at age six, 0.62 (Fig. 2-4). The peak in the relationship was much discussed. However, I doubt that there is a significant difference between the two relationships at ages 6 and 11 (r = 0.45 versus r = 0.55).

The authors concluded This result represents a potential selection criterion in a breeding program if growth is the requested character. This statement is based on strongly significant relationships and not on the more important; the degree of explanation for the relationship.

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**Figure 2-3.** The ratio of the family x site interaction variance component over family variance component based on:
- all 10 sites included in the study
- after exclusion of an outlier site
- the six forest sites.

Mikola and Vakkari 1995.

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**Figure 2-4.** The correlation coefficient between growth or quality traits on one hand and the genetic distances of the parents in an interspecific partial factorial mating of L. decidua females and L. kaempferi males at ages 2-11 in a field trial in France at 540 masl. Red column indicates that the relationship is negative. NBr = number of branches, Str = straightness. Arcade et al. 1996
2.2.2 America

Rehfeldt (1992) studied ten open-pollinated families from each of five *L. occidentalis* populations from northern Idaho and Montana in one field trial at 1,150 masl (Test I). There was a span in elevation of 700 meters of the ten populations. Height at age 5, the annual growth during the eighth season that was completed at May 28, June 16, July 20, and final growth were recorded.

The elongation before May 28 was coined early growth. Elongation up to June 16 was designated spring growth. Elongation after July 20 was coined as late growth.

Rate of elongation during the period June 16 – July 20 was calculated.

Another field trial (Test II), which was planted at an elevation of 670 masl, contained 58 open-pollinated families from three populations of wide latitudinal origin. The elevational span of these three populations was 790 meters. This trial was intensively managed including fungicide treatment to avoid needle cast infection. Height assessments were carried out at 17 occasions during the fourth growing season. Tree heights at ages 2 and 3 as well as needle cast infection at age 4 were also recorded.

The following growth rhythm traits were determined:

- Initiation of growth was the day by which 2 cm of growth had occurred.
- Start of elongation was defined as the day by which 8 cm of growth had occurred.
- Cessation of elongation was defined as the day when all but 2 cm of growth had occurred.
- Duration of growth = number of days between initiation and cessation
- Growth rate = the growth per day during the period of 20 to 80% of the growth

One focal point of this investigation was to estimate genetic parameters that would guide breeding of *L. occidentalis*.

Test I. Except for rate of shoot elongation and amount of spring growth there were significant differences among the populations in Test I. Fig. 2-5 reveals that there was a large difference in late growth at age 8 among the populations and somewhat smaller difference in relative tree height at that age. The tallest population from 945 masl was 3.70 meters while the lowest population from 1,100 masl was 3.16 meters. For tree height there was a moderately strong relationship with population elevation. The ANOVAs showed significant differences among families for all traits. The family variance components for growth rhythm traits were larger than the population variance components for these traits (Fig. 2-6). The opposite was noted for tree height and growth rate.
reveals that height showed moderately strong relationships with the growth rhythm traits, the range of genetic correlation coefficients being 0.64-0.72. As expected the strongest relationship was noted for early growth and spring growth. Noteworthy is the absence of any relationship between the early growth traits and late growth as well as the weak relationship between late growth and growth rate.

Test II. Fig. 2-8 shows that the differences in height among the populations increase from age 2 to age 4. There were significant population differences for all variables. In agreement with Test I all variables in Test II showed significant family differences. Except for start of growth the family variance components were much smaller than the population components (Fig. 2-9). This shows that the latitudinal difference among the population played a great role in the past evolution of the populations. Needle cast was weakly or negatively correlated with the other traits (Fig. 2-10). Height at age 4 was negatively correlated with initiation of growth while there were moderately strong correlations with growth cessation, duration, and growth rate.

Genetic changes following selection for growth or growth rate were estimated for a selection intensity of 1.5 for selection of families and a selection intensity of 2.0 for individuals within families (Figs. 2-11; note that there are different scales for the traits). In Test I there was a positive change in the other traits when selection for tree height was carried out. When the selection concerned growth rate the change in the other traits is smaller than for selection for height. The changes are dependent on the additive variance of the traits and their genetic correlations. Since the latter were positive there were positive changes in all cases. Since there was a negative genetic correlation between tree height and start of growth in test II there was a negative change (=earlier start) for the latter trait when selection for height was carried out. Contrary to this, the duration of the growth period was prolonged with 6 days after selection for tree height. The impact on other traits in selection for height was greater than selection for growth rate. It was noted that the consequences of univariate selection within populations is similar to population transfer. Therefore, Rehfeldt stated that It is ironic, that in the mountains of the west, provenance transfer is considered to be dysgenic in general and abhorred in practice, while selective breeding within populations is considered eugenic.

In conclusion this study was carefully carried out and presented in a good way resulting in useful information for breeding L. occidentalis.

**Figure 2-7.** Genetic correlation coefficients between traits in a field trial with five L. occidentalis populations. Each population was represented with 9-10 open-pollinated families. H8 = height at age 8, EG = early growth, SpG = spring growth, LG = late growth, GR = growth rate. Rehfeldt 1992.

**Figure 2-8.** The tree heights at ages 2-4 of the three L. occidentalis populations included in Test II. The origin elevation is indicated 1992.

**Figure 2-9.** The population and family variance components in percent for various traits studied in a field trial in Idaho, USA with three L. occidentalis populations. Each population was represented by 14-20 open-pollinated families. Rehfeldt 1992.
Zhang et al. (1994) carried out a genetics-physiology study at age 12 comprising five L. occidentalis families from three North-Western USA states. Photosynthetic rate, stomatal conductance to water vapor, water-use efficiency, and carbon isotope discrimination were assessed. Tree height, breast height diameter, stomatal conductance, and carbon isotope discrimination showed significant family differences while photosynthetic rate deviated little among families. Family 1 deviates much from the other four families in stomatal conductance and growth (Fig. 2-12). As pointed out by the authors the number of families is too low to allowing any far-reaching genetic conclusions. However, the limited variation in photosynthetic rate is in agreement with other studies in other species.

![Genetic correlation coefficient](image)

**Figure 2-10.** Genetic correlation coefficients between traits in a field trial with three L. occidentalis populations. Each population was represented by 14-20 open-pollinated families. H4 = height at age 4, Ini = growth initiation, Cess = growth cessation, Dur = duration of growth GR = growth rate, NC = needle cast. Red columns = negative coefficients. Rehfeldt 1992.

![Genetic change](image)

**Figure 2-11.** Trial I (above). The genetic change in growth and growth rhythm traits following selection for height at age 8 and growth rate with selection intensities of 1.5 for families and 2.0 for trees within families. Trial II (below). The same selection intensities as for trial I but the selection was based on data from age 4. Rehfeldt 1992.

Zhang et al. (1994) carried out a genetics-physiology study at age 12 comprising five L. occidentalis families from three North-Western USA states. Photosynthetic rate, stomatal conductance to water vapor, water-use efficiency, and carbon isotope discrimination were assessed. Tree height, breast height diameter, stomatal conductance, and carbon isotope discrimination showed significant family differences while photosynthetic rate deviated little among families. Family 1 deviates much from the other four families in stomatal conductance and growth (Fig. 2-12). As pointed out by the authors the number of families is too low to allowing any far-reaching genetic conclusions. However, the limited variation in photosynthetic rate is in agreement with other studies in other species.

![Genetic change](image)

**Figure 2-12.** Breast height diameter, tree height, and stomatal conductance of water vapor in mol$^2$ x s$^{-1}$ x 10$^2$ of five L. occidentalis families from North-Western USA studied at age 12 in a field trial at Moscow, Idaho, USA. Zhang et al. 1994.
Fourteen *L. occidentalis* families from five populations in North-Western USA were included in a detailed study of lateral shoot growth components by Zhang and Fins (1995). There were large differences in latitude and longitude of the populations as well as large elevation differences. Data from ages, two, eight and nine in a trial located at Moscow, Idaho were presented. The traits analyzed are listed in Table 2-2. The contribution of component traits to the target traits (lateral shoot length and number of stem units) was estimated by path coefficients according to Wright (1968). The number of free growth stem units was close to 20% in both years. The joint ANOVAs for estimation of population and family effects revealed no significant population effect for any of the traits while more than half of the traits showed significant family effects (Table 2-2). The path analysis revealed a strong contribution of mean number of stem units to the target trait, lateral shoot length at age 8 while its contribution at age 9 was less dominating (Fig. 2-13). The large contribution of free growth at age 2 to number of stem units was expected since free growth constitutes a large part of the juvenile growth. Fig. 2-14 reveals that predetermined growth stem units and number of stem units are strongly correlated between years while mean stem unit length is strongly influenced by the environment. The latter observation caused a weak relationship between years for total and lateral shoot length. It was speculated that the low precipitation during the growth period at age 9 influenced the elongation of the stem units negatively at this age. Free growth stem units took an intermediate position to the above two categories of traits. A few of the traits were assessed at age 2 and generally there were no strong relationships with them and their performance at ages 8 or 9 even if some were significant (Fig. 2-15). One major objective of dissecting growth into its components is to identify components that predict final growth. The major findings of Pearson correlations of family means between growth components and tree height at age 9 (target trait) can be summarized in the following way:

### Table 2-2. The significances of the family effect of the lateral shoot growth traits in a study of 14 families from five *L. occidentalis* populations at ages 8 and 9. Zhang and Fins 1992.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Definition</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 8</td>
<td>Age 9</td>
</tr>
<tr>
<td>FGSU</td>
<td>Free growth stem units on LSL, ages 8 and 9</td>
<td>ns</td>
</tr>
<tr>
<td>LSL</td>
<td>Lateral shoot length, ages 8 and 9</td>
<td>ns</td>
</tr>
<tr>
<td>MSUL</td>
<td>Mean stem unit length on LSL, ages 8 and 9</td>
<td>*</td>
</tr>
<tr>
<td>NSU</td>
<td>Total number of stem units on LSL, 8 and 9</td>
<td>**</td>
</tr>
<tr>
<td>PGSU</td>
<td>Number of primordia in terminal bud of LS</td>
<td>**</td>
</tr>
<tr>
<td>TSL</td>
<td>Total shoot length</td>
<td>*</td>
</tr>
<tr>
<td>H9</td>
<td>Tree height at age 9</td>
<td>ns</td>
</tr>
</tbody>
</table>
Family x site
Family
Age 9
Age 8
Age 7

Correlation coefficient trait – height 9

![Figure 2-15. Pearson correlation coefficients between traits at ages 2, 8, and 9 and tree height at age 9. TSL = total shoot length, LSL = lateral shoot length, PGSU = predetermined growth stem units, FGSU = free growth stem units, NSU = number of stem units, MSUL = mean stem unit length. Zhang and Fins 1995.](image)

1. Number of stem units showed moderately strong relationships with the target trait at all young ages; range of r, 0.69-0.81
2. Predetermined growth stem units had fairly strong correlation coefficients >0.60
3. Mean stem unit length cannot be used to predict later tree height; this trait is evidently too much influenced by the current environmental conditions.
4. Lateral shoot length is less reliable for prediction of the target trait. The impact of the environmental condition is too strong.
5. As expected, total shoot length is a good predictor of the target trait.
6. Free growth stem units are not good predictors since they are dependent on the environmental conditions.

In conclusion a laborious study, which has delivered much basic information of great value for breeding of *L. occidentalis*.

Ratcliff et al. (2014) focused on wood quality traits at age 20 in a series with three progeny trials including 25 open-pollinated families of *L. occidentalis* in British Columbia, Canada. The latitudinal range of the trials was 49.35-49.57°N, the longitudinal range 115.35-116.04°W, and elevational range 1,000-1,700 masl. In each family two or three trees were selected for assessment.

The following wood quality traits were assessed:

1. VDir = acoustic velocity in meters per second; *in situ*
2. WDRes = drilling resistance amplitude with Director ST300 and resistograph F300; *in situ*
3. WXray = X-ray densitometry by QTRS-01X tree ring analyzer; kilograms per cubic meter
4. MOE_D = Dynamic modulus of elasticity *VDir x WXray*

Besides the quality traits, tree height and breast height diameter were measured and stem volume was calculated. Usually it is assumed that open-pollinated families estimate one quarter of the additive variance. In the calculations of heritability the authors assumed that this variance estimates a third of the additive variance owing to some inbreeding. Thus, overestimations of the heritabilities were avoided.

I have illustrated the family variance and family x site interaction variance components for the four quality traits and one growth trait, stem volume, in Fig. 2-16. For all traits the family variance components were much larger than the interaction component. Whether the lack of significance of the G x E interaction can be attributed to the absence of differences in mean values among the trials cannot be disclosed since data from the individual trials were not presented.

It was concluded that the WDRes method was the best surrogate estimate of western larch wood density. Moreover, it permits a rapid screening of wood density in field trials.
The genetic correlations between the traits are of great importance for breeding. Fig. 2-17 reveals that the correlation coefficients between growth traits and the four quality traits were approximately 0.40 or lower but they were all positive. Striking are the perfect relationships between WD<sub>res</sub> and WD<sub>D</sub> as well as between MOE<sub>D</sub> and V<sub>Dr</sub>. It should be noted that V<sub>Dr</sub> is a component of MOE<sub>D</sub> and for this reason are expected to show a strong relationship. According to the authors, the perfect correlation (r<sub>g</sub> = 1.0) between WD<sub>res</sub> and WD<sub>D</sub> agrees with data from several other conifer species. As expected the correlations between the three growth traits were strong, r<sub>g</sub> varying between 0.95 and 0.99.

It might be concluded that there are good possibilities for improvement of all traits thanks to the relatively high family variance components. Since there were no negative genetic correlations between growth traits and the wood quality traits, selection for growth traits will not lead to substantial decrease in wood quality.

### 2.3 Inbreeding

In four subpopulations of *L. laricina* in New Brunswick, Canada, Park and Fowler (1982) carried out selfings and crosses with neighboring trees growing at a mean distance of 22 meters from four other trees in the own subpopulation. The crosses were carried out such that four disconnected groups of 5 x 5 dialleles were created. In addition, crosses were carried out with males from two distance groups at 59 and 135 meters from the female group of trees leading to two sets of progeny each with four groups of disconnected factorial crosses. Crosses with pollen mixes of each female were carried out. Open-pollinated materials from all females were also studied. Seed set, germination and plant height at age 2 in nursery were recorded. The self-fertility was estimated by dividing the percentage of full seeds after selfing with the percentage of full seeds after pollination with a pollen mix.

As seen from Fig. 2-18 the seed set and mortality of the selfed material deviates strongly from the other types of mating. The seed set is extremely low, 1.6%, and the mortality of the remaining seeds is close to 40%.

Generally, the seed set following artificial crosses is lower than in the open-pollinated material while the mortality is almost the same. Fig. 2-19 reveals that there is slightly poorer growth of the selfed material during the first year in greenhouse. This difference was strengthened during the second year with approximately 17% smaller plants than the mean of the crosses. These observations follow the general trend that inbreeding depression is least pronounced under favorable growth conditions. Based on the observations of empty seeds the number of embryonic le-
thal equivalents for individual trees was estimated to vary between 3.0 and 19.3. The coefficients of relationship for the trees in the 22m-group was estimated at 0.167 and it dropped to 0.115 for the 59m-group and to zero for the 135-group.

The estimates of variance components showed that the non-additive components were much larger than the additive variance components (Fig. 2-20). Noteworthy is the limited differentiation of the subpopulations. It was pointed out that the estimates of the non-additive effects might be exaggerated owing to the observed relationship among the trees in each of the subpopulations. This investigation and the presentation of the data are done in a way worthy of imitation.

### 2.4. Species hybrids

Reck (1977) reported on growth, survival, and trunk quality in a German field trial with four full-sib families of each of *L. decidua* and *L. decidua x L. kaempferi*, and four families of *L. decidua* after pollination with a pollen mix of Sudeten larches. Two males were used for generation of the full-sib families, one for the hybrid families and the other for the intra-specific crosses. The major findings are illustrated in Fig. 2-21. This figure reveals that the hybrids showed a better growth than the other two categories of progeny. The superiority in DBH of the hybrid was more than 20%. Similarly, the hybrids were superior with respect to the percentage of good quality trees but inferior with respect to survival.

It is regrettable that different males were used for the two types of full-sib progeny, which means that the comparison is not straightforward. This study calls for a better designed mating scheme to verify the superiority of the hybrids over *L. decidua*.

![Figure 2-19.](image1.png)
**Figure 2-19.** The mean plant height at age 2 in selfing, open-pollination, pollination with a pollen mix and pollination with males growing at various distances from the female tree. Park and Fowler 1982.

![Figure 2-20.](image2.png)
**Figure 2-20.** Variance components for plant height at age 2 in a disconnected diallele and a disconnected factorial mating in four subpopulations of *L.laricina* in New Brunswick, Canada. Park and Fowler 1982.

![Figure 2-21.](image3.png)
**Figure 2-21.** The mortality in %, breast height diameter (DBH) in cm, and the percentage of good quality trunk form in a German progeny trial with three categories of progeny. Full-sibs LD = mean value of four *L. decidua* families, Mix = mean of a pollen mix of Sudeten larch males used for crosses with four *L.decidua* females, Hybrid = mean value of four *L. decidua x L. kaempferi* families. Reck 1977.
Bastien and Keller (1980) presented mean values for growth and quality traits at age 19 for provenances of *L. decidua* and *L. kaempferi* as well as for their hybrid growing in a field trial at latitude 48.52°N, 3.42°W, and 200 masl. European larch was represented by a Czech population and Japanese larch was represented by a high-elevation population from Ina, Japan. The hybrid was obtained from Denmark.

As seen from Fig. 2-22 the hybrid showed superior growth compared to the two pure species. As regards quality traits the hybrid was either superior to the pure species or intermediate to them. A disadvantage with the hybrid in this study was its larger heterogeneity than the pure species. No general conclusions can be drawn on the data presented since only one population of each species was represented. Furthermore, the hybrid were obtained from crosses between parents from other populations than those included in the trial. This means that no general conclusions can be drawn on the data presented. However, the results suggest that further studies of the hybrid and the pure species would be worthwhile to carry out.

In a later paper by Ferrand and Bastien (1985) the focus was on volume production of three entries from the same trial as treated in the above paper. The three were one Sudetan population of *L. decidua*, one *L. kaempferi* from Ina in Japan, and one Danish hybrid population. The superiority at ages 21 and 26 of the *L. decidua x L. kaempferi* hybrid with respect to circumference, tree volume and volume per hectare is evident from Fig. 2-23. There is no sign of drop of the superiority as regards annual increment with the current growth rate at age 26 being 28.6 m³/year (Fig. 2-24). Such a sustained superiority of the hybrid differs from many other investigations. It was noted that initially there was loss of plant material in this trial. The extrapolations to volume per hectare based on circumference under such conditions might have influenced the results. However, the authors regarded the results as promising for hybrid larch breeding in France.

In a brief report by Einspahr et al. (1984) data from a trial in Wisconsin, USA, with *L. decidua* and *L. kaempferi* and their hybrids were presented (Fig. 2-25). Both parental species served as females and males. As seen from the height at age 4 the hybrids are taller than both of the parental species and the domestic species, *L. laricina*. Details about the number of parents included in the crosses were not given.

Matyssek and Schulze (1987b) reported on superior growth of two *L. decidua x L. kaempferi* hybrids compared to the parental species in a commercial forest in Germany. The DBH superiority of the hybrids against *L. decidua* was 65% and against *L. kaempferi* 50%. There was no information on the parents to the two hybrids. Therefore, a true hybrid vigor cannot be proven.

Several physiological characteristics were studied in the

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**Figure 2-22.** Tree height and stem volume of the pure species *L. decidua* and *L. leptolepis* as well as their hybrid in a French trial at latitude 48.52°N, 3.42°W, and 200 masl. Bastien and Keller 1980.

**Figure 2-23.** Tree circumference in cm, stem volume in m³, and volume per hectare in m³ at ages 21 and 26 of the best growing entries in each of the pure species *L. decidua* and *L. kaempferi* as well as their hybrid in a French trial at latitude 48.52°N, 3.42°W, and 200 masl. Ferrand and Bastien. 1985.

**Figure 2-24.** The mean annual increment/hectare in m³ and current increment at age 26 of the three best growing entries in each of the pure species *L. decidua* and *L. kaempferi* as well as their hybrid in a French trial at latitude 48.52°N, 3.42°W, and 200 masl. Ferrand and Bastien. 1985.
There was no difference in CO$_2$ assimilation per leaf area, nitrogen use, and stomatal conductance. Nitrogen content of the needles and maximum assimilation rate was higher in the hybrid than in the parental species. More populations must be studied to verify any genetic differences in these physiological traits.

Pâques (1989) reviewed the state of the art of the European x Japanese larch hybridization. Fig. 2-26 is a summary of the height superiority of the hybrids over the parental species. It should be noted that the results originate from widely varying ages and varying number of comparison material; in some cases there was just one parental entry. In all studies there was an increase of tree height of the hybrid over at least one of the parental species and the cases when the hybrids were compared with both parental species, the hybrid showed superiority against both of them. It was noted that mostly the hybrids were compared with the best material available, which means that the superiority cannot be interpreted as due to heterosis. The growth superiority of the hybrids was in some studies reduced over time. However, the rapid growth during the phase of establishment might be an advantage in competition with weeds and may also lead to an earlier harvest of hybrid larch stands. Comparisons were in most cases with data from one trial only which does not allow any estimate of genotype x environment interaction. It was noted in one study that the F$_2$ progenies still were superior to their parents but to a lesser extent than the F$_1$ progenies. In some cases the hybrids were characterized by high uniformity, which in one case was reduced over time. It was noted that the hybrids were superior for stem form and wood mechanical properties. As regards wood physical properties the hybrids took an intermediate position between the two parental species. It was stated that most data emanate from young material and the cases of superiority might be relevant for young ages but might disappear with age. Pâques concluded that for satisfactory estimation of heterosis there is a need for long-term, multisite, well-designed experiments with appropriate parental references.
Families from two incomplete factorial matings between European larch females and Japanese larch males were assessed up to ages 15-16 years to study the inheritance of various growth and quality traits (Pâques 2004). All female clones included in the matings were unrelated. The Japanese larch clones were selected in a provenance trial while eleven of the European larch clones were selected in Sudetan Mountains and one in The Alps. Owing to problems in completing the mating schemes, 30 families from factorial mating No1, 12 x 6, and 29 families from another factorial mating No 2, 9 x 7, could be studied in the field trial located at 45.75°N, 1.84°E, and 540 masl. In Factorial 1 the male clones were unrelated while the clones for the second factorial were related. The mating design was not illustrated but I assume that the number of females and males are the given figures. Single-tree plots were used in the field trials and the spacing was 3 x 3 meters.

Based on the ANOVAs the author grouped the traits into the following categories.

1. Female and male variance components of similar size; total height
2. Traits under strong male control; breast height girth, stem straightness, branching, stem volume (Fig. 2-27)
3. Traits under strong female control; wood density, heartwood proportion (Fig. 2-28)

The development of the variance components over time could be followed for tree height, which was more than nine meters at age eleven in both factorials. As seen from Fig. 2-29 there was a steady increase of the female and male variance components for height over time in Factorial 1. In contrast to this, the opposite was found for the female x male interaction component up to age eleven. It is hard to know whether the increase of the interaction component at age 16 is due to random events or to a lasting increase of this component. The latest assessment in factorial 2 was carried out at age 11, which means that comparison with data from this factorial at age 16 cannot be done (Fig. 2-30). In factorial 2 the interaction component was non-significant from age 6 and estimated at 0.0% for ages 8-11. The reason for the change of the components over time was not discussed. With the tall trees, more than nine meters, and a spacing of 3 x 3 meters suggest that there was an increasing competition with time. This might explain the increase of the genetic effects over time.

The heritabilities based on half-sibs were generally high and in many cases consistent between the two factorials. Male heritabilities for heartwood proportion varied most between the two factorials with an extremely low estimate in factorial 1.

In conclusion, this report brought important information for future breeding of hybrid larch.

Figure 2-27. Partitioning of variance components for stem volume at age 15 for female, male, and female x male interaction in two French factorial matings progeny trial with L. decidua x L. kaempferi. The progeny trial is located at latitude 45.75°N, longitude 1.84°E, and 540 masl. Pâques 2004.

Figure 2-28. Partitioning of variance components for for wood density at age 13 for female, male, and female x male interaction in two French factorial matings progeny trial with L. decidua x L. kaempferi. The progeny trial is located at latitude 45.75°N, longitude 1.84°E, and 540 masl. Pâques 2004.
In an investigation mainly focusing on the feasibility of using a rigidimeter for estimates of modulus of elasticity (MOE) of standing trees, Pâques and Rozenberg (2004) studied ten trees from each of 11 families of *L. decidua* × *L. kaempferi*. These estimates of MOE were compared with MOE obtained from sawn boards and clear-wood specimens.

The breast height diameter of the eleven families varied between 15.7 and 19.8 cm. As seen from Fig. 2-31 there was fairly strong relationship between the estimates of MOE by rigidimeter and the corresponding for standard specimens ($R^2 = 0.73$, Fig. 2-31). This investigation showed that the less laborious estimates of MOE by rigidimeter on standing trees may be carried out to identify trees with high MOE.

In 1914 a trial with *L. decidua* and the hybrid *L. decidua* × *L. kaempferi* was planted in Poland, which was analyzed with respect to ring width up to an age of 51 years (Oleksyn and Fritts 1991). There were certainly no replications in this trial and the number of trees assessed was only 25. The age of the assessment justifies a presentation of the observations. Owing to larch cancer attacks the original number of trees was considerably reduced during the existence of this trial.

In Fig. 2-32 the mean ring with for four 10-year periods - ages 10-19, 20-29, 30-39, and 40-49 – are shown. The development is similar in the two genetic entries with a slightly better growth of the hybrid that was non-significant. These data suggest that the superiority of the hybrid is not only expressed at the juvenile age.
The survival and growth at age five in a progeny trial in west-central Maine at approximately 600 masl including intra-specific and interspecific families of *L. decidua, L. laricina*, and *L. kaempferi* were reported by Baltunis et al. (1998). In all 52 full-sib families and three check lots were included in the trial.

I have illustrated height increments for the different taxa in Fig. 2-33, which shows the superiority in growth of the hybrids. Height increment is probably a better estimate of the growth potential since height at such a young age harbors planting shocks. The highest survival was noted for the *L. laricina x L. laricina* families, 91% while the mean survival of the interspecific hybrids was 81%. The ANOVAs for height and DBH showed strong significant effects for taxa. No ANOVA was carried out for height increment.

The ANOVA that was run for estimation of family effects (ignoring taxa) showed strong family effects both for tree height and DBH. There was a reasonable stability in height growth over the years. However, the pure *L. laricina* families lost in rank while the opposite was the case for the *L. kaempferi x L. decidua* families.

A selection of the five tallest families would lead to substantial gains (Fig. 2-34) presumed that the growth differences are sustainable. Since there was a large variation within taxa it was stressed that progeny testing is important for identification of the best families.

As far as I can understand the paper by Baltunis and Greenwood (1999) concerns phenology recordings from the same trial as in the Baltunis et al. (1998) paper. The exact location of the trial was not given in the 1999 paper and observations from *L. kaempferi x L. kaempferi* crosses were not presented owing to severe spring frost damage in this material. This is unfortunate since this prevents a comparison of the *L. kaempferi* interspecific hybrids with both parental species. For each type of cross the two bottom and two top-ranking families with respect to age 5 tree height were selected for the phenological observations. These comprised ten observation dates of three lateral shoots at breast height on 5-7 trees per family. The shoots were growing at approximately 120° difference between each other. The following traits were measured or determined:

<table>
<thead>
<tr>
<th>Trait Description</th>
<th>Symbol(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth initiation</td>
<td>20 mm of growth</td>
</tr>
<tr>
<td>Start of growth</td>
<td>start of linear growth</td>
</tr>
<tr>
<td>Growth cessation</td>
<td>the day when all but 20 mm of the elongation was completed</td>
</tr>
<tr>
<td>Growth duration</td>
<td>number of days between initiation and cessation</td>
</tr>
<tr>
<td>Rate of elongation</td>
<td>elongation per day during the period of most rapid elongation</td>
</tr>
</tbody>
</table>

Growth increments for each tree were fitted to a sigmoidal growth curve and the traits could be read from these curves. Besides the phenological traits, data on 5-year heights were presented.

The ANOVAs revealed significant differences among the entries for all traits as well as among families within entries for all traits but growth cessation and growth duration. The absence of significant family differences is somewhat surprising as judged from Figs. 2 and 3 in the paper. With only four families it might be hard to find significant differences. The start of growth did not differ much among the entries (Fig. 2-35). The check lot had the highest estimate for this trait (38 days) and might have contributed much to the strongly significant differences among the entries. The interspecific hybrids had longer
duration of the growth period than the parental species, which they were compared with (Fig. 2-36). The same pattern was revealed for growth cessation but somewhat less pronounced.

Three strong correlations between traits were noted:
- Growth initiation – growth start $R^2 = 0.99$ positive,
- Growth initiation – rate of elongation $R^2 = 0.89$ negative,
- Growth start – rate of elongation $R^2 = 0.91$ negative (Fig. 2-37).

The relationships between tree height and the two onset of growth traits were weak and even negative. A fairly strong relationship between tree height and growth cessation or duration of growth is expected but these two relationships were only moderately strong (Fig. 2-38). It should be noted that the check lot had a relatively strong impact on the relationship with start of growth. The authors reported a significant relationship between total elongation and height increment of the trunk during the year of observation. However, this relationship did not explain more than 27% of the variation. It can be speculated that differences in phenology of lateral shoots have limited impact on terminal shoot growth in these entries. However, the authors recommended that lateral shoot growth studies could be used instead of terminal shoot examination. As in many other cases significances do not tell the degree of explanation for different relationships.

**Figure 2-35.** The start of growth of intra-specific and inter-specific (H) crosses of L. decidua (D), L. kaempferi (K), and L. laricina (L) at age 5 in a test locality in Maine, USA at Latitude 45.50°N and 70.08°W. Baltunis and Greenwood 1999.

**Figure 2-36.** The growth duration in days of intra-specific and inter-specific crosses of L. decidua (D), L. kaempferi (K), and L. laricina (L) at age 5 in a test locality in Maine, USA at Latitude 45.50°N and 70.08°W. Baltunis and Greenwood 1999.

**Figure 2-37.** The relationship between start of growth and rate of elongation of intra-specific and inter-specific crosses of L. decidua, L. kaempferi, and L. laricina at age 5 in a test locality in Maine, USA at Latitude 45.50°N and 70.08°W. Baltunis and Greenwood 1999.

**Figure 2-38.** The relationships between tree height and duration of the growth period in days or growth cessation in number of days from May 25 of intra-specific and inter-specific crosses of L. decidua, L. kaempferi, and L. laricina at age 5 in a test locality in Maine, USA at Latitude 45.50°N and 70.08°W. Baltunis and Greenwood 1999.
The report by Greenwood et al. (2015) is a follow-up of the previous papers. It deals with survival and growth at age 22. It should be remarked that the seed set of the two interspecific crosses with L. laricina as female was very low. The field trial had ten blocks and each family was represented by randomized 3-tree row plots at a spacing of 2.3 meters.

Figs. 2-39 and 2-40 were drawn such that it should be easy to see the performance of the species hybrids. This means that the intra-specific crosses are shown twice. Fig. 2-39 reveals a drop in survival between five and 22 years, which is particularly dramatic for the L. laricina x L. laricina families. The survival at age 22 of the three interspecific hybrids was intermediate to the survival of the corresponding intra-specific crosses. The superiority of the hybrids in stem volume at 22 years is impressive (Fig. 2-40). This is a good example of hybrid vigor. The poor growth of the intraspecific families of L. kaempferi and L. laricina is noteworthy. It is likely that design of the trial exaggerates the differences over time owing to strong competition among the randomized 3-tree row plots. This can be seen from Fig. 2-41, in which the development of growth from year five to 22 is illustrated. The good growth of the interspecific crosses, L. decidua x L. kaempferi and reciprocal, was attributed to selection of superior parents for the crosses. However, if this was the case the intraspecific crosses should benefit from this as well. Sometimes it was speculated that interspecific crosses might outperform the parental species under non-native conditions. Since neither L. decidua nor L. kaempferi are native to USA this explanation might be an alternative explanation for the hybrid vigor. Around 5% of the interspecific L. decidua x L. kaempferi had defects in terms of broken or forked tops while 54% of the intraspecific L. laricina crosses were affected, which certainly contributed to the poor growth of these families.

A study of identification of hybrids between L. decidua and L. kaempferi by RAPDs was carried out by Scheepers et al. (2000). In a first step 180 primers were used in an assay of 20 European and 20 Japanese larch trees. Fifteen fragments differentiated the two species; of them two were mitochondrial and can thus not be used for the identification of hybrids owing to their maternal inheritance. Later it was stated that eleven species-specific bands were tested in eight trees in each of 17 L. decidua populations as well as eight trees in one Japanese population. This screening confirmed that the RAPDs identified were species-specific and thus suitable for identification of hybrids between the two species under study. Two RAPD primers specific to each of the two species were used to identify hybrids in four different seed lots of varying origin. The percentage of hybrids varied from 4% to 84% in these lots. The seed lot with the highest percentage of hybrids originated from a French lot which was produced from a special type of fertilization with pulverized Japanese

**Figure 2-39.** Percentage survival at ages 5 and 22 of inter- and intraspecific crosses between the three larch species L. decidua, L. kaempferi, and L. laricina in a trial in west-central Maine, USA at 600 masl. The species hybrids have intermediate colors to the pure species crosses. Baltunis et al. 1998 and Greenwood et al. 2015.

**Figure 2-40.** Stem volume at age 22 of inter- and intraspecific crosses between the three larch species L. decidua, L. kaempferi, and L. laricina in a trial in west-central Maine, USA at 600 masl. The species hybrids have intermediate colors to the pure species crosses. Greenwood et al. 2015.

**Figure 2-41.** The relative height increment at age five and stem volume at age 22 for different intra- and interspecific crosses between the three larch species L. decidua, L. kaempferi, and L. laricina in a trial in west central Maine, USA, at 600 masl. Baltunis et al. 1998 and Greenwood et al. 2015.
Development of late-wood density heritability at latitude 43.28°N, longitude 141.88°E, and 250 masl at age seven was 0.42 g/cm³ at age 12. After that there was no further increase of the density. Overall density at age seven was 0.42 g/cm³ and it increased gradually to 0.50 g/cm³ at age 18. After this age there was no further increase of the overall density. The proportion of late-wood during the whole period increased from approximately 15 to 30%.

The development of the heritability for late-wood density over time is illustrated in Fig. 2-42. (The curve is a third degree polynomial.) There was a rapid increase of this heritability between years 10 and 14 and then it levelled off. A similar trend was noted for the heritability of late-wood proportion but at a higher level. The heritability for overall density increased almost linearly from eight years until age 28 (approximately 0.40 to 0.55).

The early-wood heritability fluctuated around 0.40 during the years of observation. From year nine and onwards the dominance variance components were zero for all four traits. It was pointed out that the partial factorial mating design makes it hard to prove dominance effects.

At the young age there was a strong negative genetic correlation between wood density and radial diameter. Over the time this relationship became weaker approaching $r = -0.50$.

The peak of the efficiency for genetic gain per year was noted at age 8 for overall wood density and at ages 8-10 for early-wood density and late-wood proportion (Fig. 2-43). The peak efficiency of the late-wood density appeared somewhat later; at age 14. Since no data before eight years were available it was speculated that the peak in efficiency might occur still earlier.

In conclusion, this investigation gave promising results as regards the time of selection for wood density. However, the negative relationship between wood density and growth is a constraint to breeding.

Another study to develop methods for secure identification of *L. decidua* x *L. kaempferi* hybrids by markers was reported by Acheré et al. (2003). Twenty-six native populations of European larch and 12 Japanese populations of larch were used to find diagnostic markers of these two species. Seed lots were obtained from a Belgian seed orchard with 19 *L. decidua* clones and 16 *L. kaempferi* clones. The seed lots analyzed originated from 12 *L. decidua* clones and three *L. kaempferi* clones. In all 97 individuals from the *L. decidua* clones were analyzed while the corresponding number for *L. kaempferi* clones was 108.

One mitochondrial and one chloroplast marker were found to be suitable markers for detection of larch hybrids. The percentages of hybrids in the two seed lots (*L. decidua* and *L. kaempferi* females) were 52.6 and 42.6. These percentages are much higher than what is expected from complete random mating in such a seed orchard. Considering that this is an interspecific seed orchard with two species with differences in flowering phenology and crossing barriers the hybrid percentages must be regarded as surprisingly high.

Three reports on genetic variation in wood quality traits were presented by Fujimoto et al. (2006a, b, and c). Nineteen families from a partial factorial mating between four *L. Gmelinii* females and six *L. kaempferi* males in a trial at 43.28°N, 141.88°E and 250 masl were studied. Fujimoto et al. (2006b) used X-ray densitometry to determine wood density in early-wood and late-wood of all annual rings except for the first and last ring. Average density was calculated and proportion of late-wood of each annual ring was assessed. Optimum age of selection, i.e. to obtain the highest gain per year was calculated.

The early-wood density at age seven was 0.35 g/cm³ and decreased more or less linearly to 0.32 g/cm³ at age 28. Contrary to this, late-wood density increased from 0.75 g/cm³ at age seven to 0.85 g/cm³ at age 12. After that there was no further increase of the density. Overall density at age seven was 0.42 g/cm³ and it increased gradually to 0.50 g/cm³ at age 18. After this age there was no further increase of the overall density. The proportion of late-wood during the whole period increased from approximately 15 to 30%.

**Figure 2-42.** Development of late-wood density heritability over the years 10-28 in a trial with *L. gmelinii* x *L. kaempferi* hybrids at latitude 43.28°N, longitude 141.88°E, and 250 masl in Japan. Fujimoto et al. 2006b. larch pollen on European larch trees. Evidently, it was a fairly successful technique.

In early selection of wood traits in a Japanese trial with *L. Gmelinii* x *L. kaempferi* hybrids by markers was reported by Acheré et al. (2003). Twenty-six native populations of European larch and 12 Japanese populations of larch were used to find diagnostic markers of these two species. Seed lots were obtained from a Belgian seed orchard with 19 *L. decidua* clones and 16 *L. kaempferi* clones. The seed lots analyzed originated from 12 *L. decidua* clones and three *L. kaempferi* clones. In all 97 individuals from the *L. decidua* clones were analyzed while the corresponding number for *L. kaempferi* clones was 108.

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Three reports on genetic variation in wood quality traits were presented by Fujimoto et al. (2006a, b, and c). Nineteen families from a partial factorial mating between four *L. Gmelinii* females and six *L. kaempferi* males in a trial at 43.28°N, 141.88°E and 250 masl were studied. Fujimoto et al. (2006b) used X-ray densitometry to determine wood density in early-wood and late-wood of all annual rings except for the first and last ring. Average density was calculated and proportion of late-wood of each annual ring was assessed. Optimum age of selection, i.e. to obtain the highest gain per year was calculated.

The early-wood density at age seven was 0.35 g/cm³ and decreased more or less linearly to 0.32 g/cm³ at age 28. Contrary to this, late-wood density increased from 0.75 g/cm³ at age seven to 0.85 g/cm³ at age 12. After that there was no further increase of the density. Overall density at age seven was 0.42 g/cm³ and it increased gradually to 0.50 g/cm³ at age 18. After this age there was no further increase of the overall density. The proportion of late-wood during the whole period increased from approximately 15 to 30%.

The development of the heritability for late-wood density over time is illustrated in Fig. 2-42. (The curve is a third degree polynomial.) There was a rapid increase of this heritability between years 10 and 14 and then it levelled off. A similar trend was noted for the heritability of late-wood proportion but at a higher level. The heritability for overall density increased almost linearly from eight years until age 28 (approximately 0.40 to 0.55).

The early-wood heritability fluctuated around 0.40 during the years of observation. From year nine and onwards the dominance variance components were zero for all four traits. It was pointed out that the partial factorial mating design makes it hard to prove dominance effects.

At the young age there was a strong negative genetic correlation between wood density and radial diameter. Over the time this relationship became weaker approaching $r = -0.50$.

The peak of the efficiency for genetic gain per year was noted at age 8 for overall wood density and at ages 8-10 for early-wood density and late-wood proportion (Fig. 2-43). The peak efficiency of the late-wood density appeared somewhat later; at age 14. Since no data before eight years were available it was speculated that the peak in efficiency might occur still earlier.

In conclusion, this investigation gave promising results as regards the time of selection for wood density. However, the negative relationship between wood density and growth is a constraint to breeding.

**Figure 2-43.** The efficiency as regards gain per year for early selection of wood traits in a Japanese trial with *L. Gmelinii* x *L. kaempferi* at latitude 43.28°N, 141.88°E, and elevation 250 m. The year(s) of peak efficiency is(are) indicated. It should be remarked that no estimate of efficiency was carried out before eight years. Fujimoto et al. 2006b.
The grain angle decreased more or less gradually from the pith to ring No. 25. In contrast to the other wood quality traits, the estimates for inner, outer, and mean specimen were noted in rings 2-3 and 7-10 from the pith. There was a peak in the heritability at rings 3 and 4 from the pith, 0.42 and 0.45. In the following rings the heritability dropped to approximately 0.20 at ring No. 8. From ring No 10 and onwards there was a trend of increase of the heritability that reached 0.33 at ring No 25. The genetic correlation coefficients between the innermost ring and the inner specimen had the lowest estimate. It was suggested that the increase of heritability from inner to outer specimens might be attributed to change of control of these quality traits over time. The genetic correlation coefficients illustrated in Fig. 2-45 reveal that all correlations of the quality traits with breast height diameter are high, which implies that there is some concern as regards very early selection.

Butt logs from each of 96 L. gmelinii x L. kaempferi trees were analyzed at age 29 by Fujimoto et al. (2006c). The following traits were assessed:

1. Dynamic modulus of elasticity of the green logs = $E_{log}$
2. Clearwood modulus of elasticity, separately for innermost, outermost, and average specimen; MOE
3. Clearwood modulus of rupture, separately for innermost, outermost, and average specimen; MOR
4. Clearwood compression strength, separately for innermost, outermost, and average specimen; CS
5. Wood density, separately for innermost, outermost, and average specimen; DEN
6. Breast height diameter. = DBH

Dominance effects were absent in all ANOVAs for all traits. It was stated that the mating design with few full-sib families per half-sib family was not the best for estimation of dominance variance. Heritabilities for all traits are presented in Fig. 2-44. Heritabilities are shown separately for inner and outer specimens together with their mean values for the four quality traits. Density showed the highest heritability while the MOE heritability for the inner specimen had the lowest estimate. It was suggested that the increase of heritability from inner to outer specimens might be attributed to change of control of these quality traits over time. The genetic correlation coefficients illustrated in Fig. 2-45 reveal that all correlations of the quality traits with breast height diameter were very high, approximately 0.90. Then it dropped to a minimum of less than 0.50 at ring No. 5. At ring 11 and onwards the genetic correlation coefficients were all > 0.90. The selection efficiency based on the genetic correlations peaked at ring No. 3 from the pith with a relative value of approximately 3.0. After this age it dropped to a value slightly above one at ring No. 7. The results suggest that a selection for spiral grain angle could be done very early. However, the precision of the estimates was not high, which implies that there is some concern as regards very early selection.

Figure 2-44. The mean estimates of heritability for wood quality traits and breast height diameter based on 19 full-sib families of L. gmelinii x L. kaempferi at age 29 in a Japanese field trial in Hokkaido. For four of the quality traits the estimates for inner, outer, and mean specimen are given. $MOE_A$ = clearwood modulus of elasticity, $MOR_A$ = clearwood modulus of rupture, $CS_A$ = clearwood compression strength, $DEN_A$ = wood density, $DBH_A$ = breast height diameter, $E_{log}$ = dynamic modulus of elasticity of green logs. Fujimoto et al. 2006c.

Figure 2-45. L. Gmelinii x L. kaempferi. The genetic correlation coefficients between four quality traits and breast height diameter. Red bars refer to negative correlation coefficients. MOE_A = average clearwood modulus of elasticity, MOR_A = average clearwood modulus of rupture, CS_A = average clearwood compression strength, DEN_A = average wood density; DBH = breast height diameter, Elog = Dynamic modulus of elasticity of the green logs. Fujimoto et al. 2006c.
were negative but weak and non-significant. Contrary to this, perfect correlations were noted for the MOE, MOR, and CS. $E_{log} = \text{dynamic modulus of elasticity of green logs}$, MOE = average clearwood modulus of elasticity, MOR = average clearwood modulus of rupture, $CS = \text{average clearwood compression strength}$, DEN = average wood density, DBH = breast height diameter. Fujimoto et al. 2006c.

![Figure 2-46](image)

**Figure 2-46.** The percentage gain in five quality traits after selection of the five best performing families of this particular trait is shown in green. The effect of selection of the five best families for each of these five traits on MOE, MOR, and CS. $E_{log} = \text{dynamic modulus of elasticity of green logs}$, MOE = average clearwood modulus of elasticity, MOR = average clearwood modulus of rupture, $CS = \text{average clearwood compression strength}$, DEN = average wood density, DBH = breast height diameter. Gauchat and Paques 2011.

It was concluded that non-destructive methods or surrogate traits like wood density could be used for indirect assessment of stiffness and strength in hybrid larch progeny trials. The obtained results mean that the costs for breeding can be reduced by using assessments on the growing trees rather than on sawn specimens. Finally, it was concluded that most tree breeding programs in Japan were focused on improvement of growth. This will result in a regression in the mechanical wood traits.

Data on flushing in three French progeny trials was compared with flushing of the same families under controlled conditions in growth chamber (Gauchat and Paques 2011). One objective of this investigation was to determine how well flushing under controlled conditions agree with *in situ* observations of flushing. The three trials contained different families of European larch, Japanese larch, and their hybrid. Twigs were collected from the trees in early January and stored for 25-30 days at 2°C in darkness. After that the twigs were put in jars with water and placed in growth chamber with a temperature of 11°C or 16°C and with an 8h photoperiod. The criterion for flushing was the Julian day when 50% of the buds had flushed. Temperature sums for flushing was calculated according to the Thermal Time model (Cannell and Smith 1983) assuming a linear relationship between rate of development and temperature. In the field trials terminal bud flushing and proportion of green crown were assessed. When 50% of the crown of a tree had flushed it was defined as having a green crown. The authors stated that the limited number of families per taxon is a constraint for generality of the results obtained.

I have summarized the results from the ANOVAs as regards family and taxon differences for flushing, temperature threshold, and temperature sums for bud flushing in the two growth chamber treatments in Table 2-3. Too far reaching conclusions cannot be drawn owing to the limited number of families of some taxa. Trial 3 with similar representation of taxon showed strongly significant differences for both family and taxa for all traits but threshold temperature. The single Japanese larch family had a significantly lower threshold temperature in trial 1 than the nine hybrid larch families, 1.8°C versus 3.2°C.

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**Table 2-3.** The significant differences among families or taxa for traits related to bud flushing under two temperature conditions in growth chamber; 16°C and 11°C. EL = Larix decidua, JL = L. kaempferi, HL = hybrids between these two species. The numbers of the different taxa are indicated. Gauchat and Paques 2011.
The Spearman correlations between the family flushing dates in the two treatments was moderately strong for families in trial 1 (0.60) and strong in trials 2 and 3 (0.94 and 0.95).

According to Fig 2 in the paper, the threshold temperatures for flushing varied in the range 1.8 – 3.2°C with lowest value for the Japanese larch family growing in trial 1 and the highest value for the hybrid larch families from trial 3. The accumulated heat sums to flushing varied between 250 and 330 degree days. Only in two cases there was a significant difference between taxa. It was noteworthy that the Japanese larch family from trial 1 had one of the highest heat sums for flushing while the mean of the five Japanese families from trial 3 had the lowest heat sum requirement for flushing. The latter families deviated significantly from the two other taxa from this trial. The low number of families studied might contribute to this difference.

Heat sums for flushing and greening showed higher family variance components than for the dates of flushing or greening both in growth chamber and field observations (Figs. 2-47 and 2-48). These estimates were based on hybrid families only, which mean that inclusion of different taxa could not explain the high estimates for heat sum related traits.

In all 24 correlations between growth chamber traits and field traits in two of the trials were presented. The correlation coefficients for the seven significant relationships are illustrated in Fig. 2-49. There is some consistency in the correlations in trial 2. Thus, flushing in the two growth chamber treatments showed almost the same strength in their relationships with threshold temperature and green crown in this field trial. Contrary to this, flushing in the 11°C growth chamber was negatively correlated with heat sum for flushing and green crown in field trial 1. The assumption of linear relationship between temperature and phenological stages might not be valid, and thus contributing to the two negative relationships.

In conclusion this investigation gave some support to the hope that growth chamber observations could be used to predict field performance. The genetic information from this investigation suffers from the low number of families of the three taxa studied.

Pâques et al. (2010) reported on genetic variation in modulus of elasticity, MOE, from two progeny trials and one clone trial with 100 clones selected for plant height at age two in a nursery. Thirty-two of the clones were included in this study. The numbers of full-sib families in the two

![Figure 2-47. The family variance components for bud flushing in 16°C and 11°C treatments, heat sum to flushing in growth chambers (green bars) and for in situ observations in trial 1 of green trees (G50), terminal bud flushing (TB2) as well as heat sum (HS) for these two traits. Gauchat and Pâques 2011.](image)

![Figure 2-48. The family variance components for flushing (FL) and greening in two French field trials and for the heat sums (HS) for these traits. Trial 1 contains one Japanese larch family and nine European × Japanese hybrid families. Trial 2 contains five European larch families and one hybrid family. Gauchat and Pâques 2011.](image)

![Figure 2-49. The significant correlation coefficients between traits assessed in field in green and traits assessed in growth chambers at 11°C or 16°C in black. TF1 = Threshold temperature for flushing, HSFl = Heat sum for flushing, HΣGr = Heat sum for green crown, Fl11 = Days to flushing in the growth chamber at 11°C, Fl16 = Days to flushing in the growth chamber with 16°C, Gr = Days to green crown. Gauchat and Pâques 2011.](image)
Larch heartwood has a higher durability and decay resistance than sapwood. This has been attributed to different types of compounds in the wood such as phenols. Also the color of wood has been linked to its durability. Several larch genetic studies have focused on extractives and/or wood color.

Gierlinger et al. (2004A) studied the color of larch heartwood and its relationship with extractives in two L. decidua x L. kaempferi families, nine L. decidua populations, and one L. kaempferi population. One old population (160 years) was included while all others were 38 years old. The wood samples were collected in different trials and countries, but no differences were noted for the same population grown in different countries. A wood decay test was carried out after inoculation with Poria placenta or Coniophora puteana.

Figure 2-50. Family and clone variance components for MOE, stem volume, and wood density in two progeny trials (filled bars) and one clone trial in France. All material has hybrid origin, L. decidua x L. kaempferi. Filled columns = progeny trials, striped columns = clone trial. The ages of assessment are indicated. Pâques et al. 2010.

The variability among families and clones was noted for MOE in two of the trials (cf Fig. 2-50). The best family had a MOE of 8.81 and the poorest had 5.18 MPa. The family effect of all traits was significant in the two progeny trials. Clonal MOE was almost significant in the clone trial, p = 0.067. The occurrence of G x E interaction was studied by calculation of so called B-type genetic correlations and of rank correlations of family means. Seventeen of the full-sib families were common to both trials. From Fig. 2-51 it is evident that G x E interaction is strong for DBH and stem volume. It was suggested that the larger mortality in one of the trials had affected the diameter growth much more than in the other trial.

The genetic relationship between MOE and wood density was strong in all three trials (Fig. 2-52) but it was weak or even strongly negative with stem volume. The strong G x E interaction is a constraint to breeding. Similarly, the weak correlations between growth traits and MOE are other constraints to joint breeding for these traits.

Figure 2-51. Two types of correlation coefficients between five traits in two progeny trials (latitude 45.75°N, 1.84°E, and elevation 550 masl and 47.38°N, 6.04°E and 470 masl) with L. decidua x L. kaempferi full-sib families. Pâques et al. 2010.

Figure 2-52. The genetic correlation coefficients between MOE and stem volume or wood density in two progeny trials (filled bars) and one clone trial in France. All material has hybrid origin, L. decidua x L. kaempferi. Filled columns = progeny trials, striped columns = clone trial. Pâques et al. 2010.
In the young material (age 38) the Japanese and the hybrids were significantly redder than the young European larches while the old European larch and the hybrid had similar color (Fig. 2-53). The two hybrids had the highest values in the blue to yellow axis while one Austrian and one French population had lightest heartwood.

There was a strong relationship between reddishness and amount of phenols (r = 0.93) while the relationship between reddishness and brown rot decay was strongly negative (r = -0.82, Fig. 2-54).

In conclusion, it is not possible to verify whether the observed differences in color parameters among the different taxa are general since there were too few hybrids and only one Japanese population included in this investigation. There is a call for extension of this study.

Gierlinger et al. (2004b) studied extractives from hot-water or acetone extraction and lignin content to identify differences between three taxa, European larch, Japanese larch, and their hybrid. The lignin content in extract-free heartwood was also determined. In all 106 trees at age 39 from a French trial were analyzed. The European larch was represented by three origins while Japanese larch was represented by one origin. The hybrid trees belonged to a full-sib family. In addition, a wood decay test was carried out by inoculation of eight samples of heartwood of each of the two fungi, *Porzia placenta* and *Coniophora puteana*. The mass loss of weight was determined after 16 weeks of exposure to the mycelium.

In spite of the large tree to tree variation there were significant differences among the taxa for phenols, lignin, and acetone extractives according to the ANOVAs run. For phenols the hybrid took an intermediate position to the pure species (Fig. 2-55). It was stated that *The influence of the Japanese parent on the amount of phenols in the hybrid larch (L. decidua x L. kaempferi) seems to be dominant.* However, as seen from Fig. 2-55 the dominance is far from complete.

Several relationships between the traits included in this investigation were presented. Relationships including one population as well as pooled data from all populations except for the hybrid family were calculated. The overall correlation coefficients are shown in Fig. 2-56. All correlations were significant but half of them had coefficients less than 0.50. Both high lignin and high phenol contents reduced the loss of weight after inoculation with the two fungi, *Porzia placenta* and *Coniophora puteana*. The resistance against the two fungi by phenol compounds was almost identical and strong, r varying between 0.81 and 0.83. The strong and negative relationships between phenol content and tolerance against the two rot fungi are promising for breeding for disease tolerance.
So called Fourier-transform near infrared spectra of the heartwood of the three taxa, European, Japanese, and their hybrid was determined by Gierlinger et al. (2004c). Samples were taken from 15 trees of each taxa and were analyzed in the region 6,700 cm\(^{-1}\) to 6,300 cm\(^{-1}\). Principal component analysis was carried out to identify differences between taxa. If particular wavelengths were used for the classification up to 100% successful classifications were obtained.

The same trials were used by Pâques et al. (2013) for a study of heartwood characteristics. In this investigation there was a focus on taxifolin (tax) and dihydrokaempferol (DHK). Color parameters were also determined on dry powder of heartwood by spectrocolorimeter in the 400-700 nm range. If there is a strong relationship between color parameters and phenol compounds assessment of color parameters could be used for prediction of phenols. Fig. 2-57 reveals that the hybrids took an intermediate position to the pure species for total phenols and taxifolin content in the pooled sample from inner and outer heartwood. There were significant differences among all taxa for all parameters. The data for these three parameters showed the same pattern in the separate analyses of inner and outer heartwood and the significances for differences among taxa remained. There were also strongly significant differences between outer and inner heartwood for total phenols and for taxifolin content while it was just significant for DHK. There were strong correlations between total phenol content and taxifolin content for inner, outer, and pooled values. The hybrid full-sib families varied considerably for the three extractives; with the largest range for DHK.

In spite of the limited differences for two of the three color parameters significant differences among taxa were observed for all three color parameters (Fig. 2-58). The Japanese larch had the highest red and blue hues.
All correlations between the three extractives and the light parameters were weak or moderately strong; all < 0.60 (Fig. 2-59). It was concluded that color parameters are weak predictors of phenolic content.

A Chinese paper dealing with species and species hybrid performances as regards phenology and growth with Chinese text except for abstract, tables and figures was published by Sun et al. (2008). The trial was located in North-Eastern China.

As seen from Fig. 2-60 there was large variation in all three traits illustrated in this figure. *L. kaempferi* families as well as the *L. kaempferi* species hybrids were characterized by late flushing, late budset and the best growth. These entries had the longest growth periods of all entries. Moreover, they had among the highest percentages of survival in the trial. Remarkably poor growth was noted for *L. sibirica* probably owing to its early growth cessation. Bud flushing showed moderately strong correlates with budset (r=0.78), height increment (r=0.72), and tree height (r=0.64).

**Figure 2-59.** The correlation coefficients between three extractive compounds and heartwood color traits of *L. decidua*, *L. leptolepis* and their hybrid. Ramets of the pure species were growing in two French clone archives while the hybrids were growing in one French progeny trial. Empty columns = non-significant relationships; red columns = negative relationships. Tax = taxifolin, DHK = dihydrokaempferol, Lumin. = luminosity. Pâques et al. (2013).

**Figure 2-60.** Bud flushing, bud-set, and tree height in a trial in North-Eastern China. G = gmelinii, O = olgensis, P-R = principes-rupprechtii, S = Sibirica, D = decidua, K, K84, and K81 are kaempferi parents; KxG, KxP-R, and KxO are species hybrids with K81 as female parent. Bud flushing = days after April 1, Terminal budset is days after July 1, tree height = dm at age 5. Sun et al. 2008.

**Figure 2-61.** Tree height percentage variance components for family, clone, and clone x test location interaction based on seven clones from each of ten families originating from three populations in Canada and planted at two test localities. Mixed = mixed populations with other conifers. Park and Fowler 1987.

relations with budset (r=0.78), height increment (r=0.72), and tree height (r=0.64).

**2.5 Clone tests**

Vegetative propagation of seven seedlings from each of 30 families of *L. laricina* was carried out by Park and Fowler (1987). The 30 families originated from three populations growing 80 km apart. One population was a pure stand of *L. laricina* while the others were mixed stands with other conifers. Seven clones from each family were planted in two field trials. Assessments were carried out at age 5. ANOVAs were run for each of the populations as well as a joint analyses including populations. To compensate for any relatedness of the trees in the populations it was assumed that the family variance components should be interpreted as estimating one third of the additive variance instead of the usual $\frac{1}{4}$.

The mean tree heights of the populations did not vary much among the populations, 138 -155 cm, while the survival did not vary at all, 82%. Except for the pure population, the variance components family x locality was smaller than the clone x locality component. Figure 2-61 reveals that the clone component for tree height was the largest in all three populations. The family component...
was larger than the clone x locality population in only one of the populations. The clone component for survival in one of the mixed populations was estimated at 11.4% while no other variance component reached 5%. The joint ANOVA also showed that the clone component was the dominating variance component for height and also for survival (Fig. 2-62). Most of the interaction components including height were smaller than the non-interaction effects.

From Fig. 2-63 it is evident that the pure stand population deviates from the other two populations with a low proportion of additive variance for tree height. Contrary to this, no non-additive variance was detected for survival in the pure stand population. It was speculated that inbreeding in the pure stand population might have contributed to the obtained results. The gain in tree height following selection of the best 20% of clones based on clonal means was estimated at 11 and 18% in the two mixed populations while the pure stand population would result in a gain of six percent.

This paper presents a well-prepared investigation and similarly a good description of the obtained results.

Two experiments involving cuttings from open-pollinated families of *L. laricina* populations from northwestern Ontario, Canada, were reported by Farmer et al. (1993).
The differences in tree heights among the provenances were significantly different all years and in both experiments. Fig. 2-64 illustrates that the southern populations had taller trees than northern populations. Pearson correlations between final height and latitude were strong and negative (-0.83 in experiment 1, and -0.86 in experiment 2). There were strong positive relationships between tree height and mean daily temperature and latitude (0.83 in experiment 1, and 0.92 in experiment 2). The differences among provenances in growth were attributed to differences in late-season elongation while the large variation within population in tree height was attributed to both rate and duration of elongation. The partitioning of the variance for tree height into different effects (Fig. 2-65) reveals that the provenance effect was strongest of the genetic effects in experiment 1 while clone x replication was strongest in experiment 2. Effects with small percentages such as stand within provenance are included in “rest” together with the error term. The narrow-sense heritability in experiment 2 declined from 0.21 at age 2 to 0.13 at age 7.

At age five and six, 39 and 27% of the trees had sylleptic shoots. The clonal variance component for number of sylleptic shoots at age 6 was more than four times larger than the provenance component (Fig. 2-66). The other variance components were all less than 5%. At age 5 the clonal component was a little less than three times as large as the provenance component. As a corollary of this the broad-sense heritability was larger at age 6 than at age 5, 0.47 versus 0.37. Except for the error term the other variance components were 4% or smaller in both experiments. There was a large variation in the length of sylleptic shoots among populations, 2.6 – 89.4 cm. Although geographic coordinates were not given it seems as there was a relationship between this trait and latitude. It was stated that syllepsis is moderately correlated with tree vigor. In conclusion this investigation of detailed genetic analyses of survival and growth constitutes a good basis for breeding L. laricina.

Sylvestre-Guinot et al. (1999) studied Lachnellula willkommii susceptibility after inoculation of 275 clones of L. decidua x L. kaempferi and eight clones of L. kaempferi x L. decidua. All the clones originated from the families described by Paques (2004). The isolate of the inoculum was obtained from a natural cancer on a L. decidua tree. The inoculation took place during mid-April on grafts at ages 3-4. The evaluation of the inoculations took place 15 and 18 months after the inoculation. The percentage of successful inoculations was calculated. The success rates after 15 and 18 months were 24.9% and 27.1%, respectively, with a clonal variation from 0 to 100% symptoms at both assessments. A healing of the symptoms occurred such that the remaining infections 39 months after the inoculation had dropped to 2.2%. There was no proven difference between the two types of hybrid, nor was there any difference between the two origins of the L. decidua females.

Wood stiffness in 16 hybrid larch (Larix x eurolepis) clones in a Southern Belgium clone trial was investigated.
by different methods by Jacques et al. (2004). The modulus of elasticity in static bending according to French standards was used as a control method, MOE_{4PT}. Other estimates shown below were compared to this method. It is beyond the scope of this publication to present the complexity of these methods.

\[ V_{US} = \text{the ultrasound propagation speed in the longitudinal direction expressed in meters per second} \]

\[ \text{MOE}_{US} = V_{US}^2 \rho 10^{-6} \]

\[ \text{MOE}_{GR} \text{ and } F_r \text{ are obtained from a Grindo-Sonic device measuring the transient vibration caused by a mechanical disturbance of the tested object} \]

Pilodyn = the penetration depth into wood by a low-dimension striker pin propelled by a fixed energy.

Some of the so called clonal heritabilities are all high except for one of the Grindo-Sonic methods, F_r (Fig. 2-67). The gains in wood stiffness after selection with a selection intensity of 1.0 are illustrated in Fig 2-68. A comparison with Fig. 2-67 shows that there is not full correspondence between genetic gain and heritability. This is attributed to the difference in variability among the traits. The clonal heritabilities for pilodyn penetration obtained from assessment at ages 11 and 14 were high, 0.62 and 0.77, respectively. With a selection intensity of one, the genetic gains from the two pilodyn assessment were estimated at 8.9 and 8.5%, respectively. The three genetic correlation coefficients between the modulus of elasticity traits, MOE_{4PT}, MOE_{US}, and MOE_{GR} were all three almost perfect, > 0.98.

Thanks to the high heritabilities of the ultrasound method applied at different stages of sawing of the material, MOE_{aprt} is the standard French method for assessment of modulus of elasticity. Jacques et al. 2004.

\[ V_{US} \text{ and pilodyn assessments took place in field. Other assessments were taken during the sawing process of the wood. The following categories were examined: Normalized and long specimen, heartwood and sapwood logs, and boards.} \]

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Thanks to the high heritabilities of the ultrasound method applied at different stages of sawing of the material, MOE_{aprt} is the standard French method for assessment of modulus of elasticity. Jacques et al. 2004.
Variation in resistance against *Coniophora puteana* was studied in 53 *L. sibirica* clones in a Finnish seed orchard located close to latitude 62°N (Venäläinen et al. 2001). The clones were selected in stands in northern Finland north of latitude 65°N. These stands originated from seed collected in Raivola in southeastern Finland. Increment cores were taken in four directions on the growing seed orchard grafts and from eleven plus trees in the stands in northernmost Finland. The brown rot fungus *Coniophora puteana* was grown on agar in Petri dishes. The bore cores were placed on the surface of agar medium. After six weeks of incubation weight loss was determined as percentage loss and as mg/cm³. Each clone was represented by five grafts. Besides disease resistance, growth and wood quality traits were assessed.

The two estimates for clonal repeatability of weight loss were almost identical and were intermediate to wood density and the two growth traits (Fig. 2-70). It was pointed out that clones in the seed orchard can with high probability not be regarded as a randomly selected population since they were selected as plus trees. The generality of the estimates was therefore warned for. As in many other studies of confiers the repeatability for wood density was high, 0.56. The percentage of latewood showed a high repeatability too, while the lowest repeatabilities were noted for the two growth traits.

In Fig. 2-71 I have illustrated most of clonal mean correlations that were presented. As seen from this figure many of the correlations were significant. However, only two of them had estimates higher than 0.60. Even if the relationships were significant it means that only a limited part of the variation can be attributed to the relationship. The high number of clones tested contributes to significant relationships even if the coefficient is as low as 0.20 (DBH – WD). It is evident that the two ways of estimation of the weight loss owing to fungal attacks give almost similar results, *r* = 0.79. The lack of strong relationships between the two resistance estimates and other wood quality traits mean that indirect selection for wood decay cannot be carried out. Noteworthy are the low correlation coefficients between breast height diameter and the two estimates of brown rot decay.

Only for wood density the relationship between the grafts and the plus trees was significant. As stated above significances are dependent on the number of pairs studied. Since only 11 pairs were included in this part of the study, even a correlation coefficient of 0.52 (WL%) was non-significant.
2.6 Summary

With the objective of finding species diagnostic markers, studies on inheritance of markers were carried out. Isozyme markers were in focus during the last decades of the previous century. Lateron inheritance of nuclear markers as well as chloroplast and mitochondrial markers were studied. Diagnostic markers were identified. Paternal inheritance of cpDNA was disclosed. Significant between family differences were noted for growth, phenology, and quality traits as well as some physiological traits. The population variance component was larger than the family variance component in trials with populations from a broad span of localities. Large female x male interaction variance components were noted in a progeny trial with *L. laricina*. Substantial inbreeding depression was observed in *L. laricina*.

Several studies on species hybrids were carried out, mainly with *L. decidua*, *L. kaempferi*, and *L. laricina* included in the interspecific crosses. Hybrid superiority in growth over the parental species means are shown in Fig. 2-72. In several cases the hybrids showed substantial superior growth, not only in the juvenile phase. It should be noted that the comparisons are not always straightforward since the intraspecific crosses do not always have the same parents as the hybrid families.

Family and clonal differences in wood quality traits and in extractive substances from wood were observed. Frequently the relationships between growth traits and wood quality traits were weak or even strongly negative. This is a great constraint for joint improvement of growth and quality traits.

![Figure 2-72. Percentage gain in growth traits of larch hybrids over parental species’ mean values. DK = decidua x kaempferi, DL = decidua x laricina, KL = kaempferi x laricina, H4 = tree height at age 4, V22 = stem volume at age 22.](image)

Substantial gains following direct selection of growth and quality traits may be obtained. High estimates of clonal repeatability for resistance against fungal disease were observed.

In passing it might be noted that estimates with many digits are shown even if the number of entries is rather limited. This suggests higher precision of the estimates than the observed data permit. As one example the percentage was once reported as 9.315. This figure was based on 96 individuals, which does not justify such a precision.
3. Breeding

3.1 Breeding programs

In their summary of the state of the art of inter-population and intra-population variation in *L. decidua* Weisgerber and Šindelár (1992) reported that several seed orchards were established during the 50-ties – 80-ties. The hope was that individual selection should lead to improvement of growth, stem quality, and disease tolerance. Outstanding growth of many progenies from The Sudetan region was noted in some progeny trials. The potential gain in height growth was estimated at 15-20%. Moreover, superiority of some families over commercial seed sources had given incentives to establishment of advanced generation seed orchards. The risk for self-fertilization was discussed and it was reported that outcrossed progenies had 56% lower mortality than selfed progenies. A similar difference between the two categories was noted for trees with straight stems. Crosses should be carried out to test if good combinations of the three types of trait can be achieved by crosses between trees from different populations.

The famous Dunkeld larches in Scotland, which are hybrids between *L. decidua* and *L. kaempferi*, are superior to both parental species and have stimulated to carrying out species hybridization. The first successful hybridization should have taken place in 1914. However, it was noted in long-time progeny trials that the initial superiority of the hybrids over the pure species diminishes over time. The timber quality of the hybrids might be inferior to the pure species. It was stated that *F*$_2$ and *F*$_3$ hybrids showed vigorous growth during the juvenile phase and thus superiority over the pure species. More empirical data are required to verify these results.

Seed orchards were established with the incentive to obtain species hybrids with one clone of one species for seed production and several clones from the other species for pollen production. In such orchards it is important that the pollen dispersal is matched with the receptivity of the female clone. The authors warned for a strong narrowing of the genetic setup in the seeds from such female clones. Vegetative propagation via cuttings or via somatic embryogenesis was also discussed in this report. The authors stressed the need for international cooperation to solve problems related to vegetative propagation in order to enable a successful larch tree breeding.

Temperature recordings in larch strobili buds in a polythene house. Photograph Kjell Lännerholm.
Li and Wyckoff (1992) presented a breeding strategy for *L. decidua*, *L. kaempferi* and their hybrid for a Breeding Cooperative in The Lake States and North-Eastern USA. The strategy was based on empirical data for these taxa as well as on tree breeding strategies in general. Plus tree selection was carried out in field trials and plantations prior to the establishment of the Cooperative.

Factors critical for successful plantations with *L. decidua* are:
- Depth of free rooting
- Available soil moisture
- Bud flushing date to avoid exposure to spring frosts
- Resistance to *Mycosphaerella laricina*
- Control of competing vegetation

**Figure 3-1. Schematic illustration of the breeding strategies for *L. decidua* and *L. decidua x L. kaempferi* hybrids. Li and Wyckoff 1992.**
The strategy is schematically outlined in Fig. 3-1. A subdivision of the breeding population into 20 groups each with ten trees was carried out. This grouping was done according to the four major sources of European larch; Sudeten Mountains in Czechia, Poland, Tatra Mountains in Slovakia, and The Alps. The former two have shown good performance in North-America as well as in Europe. The Tatra larch might confer larch cancer tolerance while the good stem form of the Alpine larch is valuable to incorporate into the base breeding population. For this reason the number of groups was highest for these two origins. In each group two 5 x 5 trees disconnected dialleles should be created. The progeny trials should cover the main planting site conditions and evaluation should be done at age five.

From each group of 10 families the two best performing trees in each of the three best performing families will be selected for the long-term breeding. New diallele matings will be based on five of the six selected trees from each group. Seed orchards will also be established to produce seeds for future plantations.

Generally, reciprocal recurrent selection is the best method in breeding for hybrid vigor. However, one major constraint with this method is that it takes two generations until commercial seed can be produced. It was stated that the few existing investigations on L. decidua x L. kaempferi hybrids showed that additive effects were important, which means that after the first creation of a hybrid breeding population future breeding might as well exploit the additive effects neglecting any possible non-additive effects. This was the reasoning behind the strategy suggested for hybrid breeding. As seen in Fig 3-1 it was suggested that 150 L. decidua and 150 L. kaempferi trees are crossed in single-pair matings. This will result in no relationship among the 150 families. As for the European larch breeding program evaluation in field trials shall take place at age five. The three best performing trees in the 50 best performing families are selected for the future breeding. These 150 trees will be mated in 75 single-pair matings. In the field trials the selection process is repeated, i.e. the three best performing trees in the 50 best performing families are selected for creation of the coming breeding population generation. Assortative mating is foreseen. However, this was not elaborated but one could imagine that high-ranked trees are crossed with other high-rank trees. Superior trees are also planted in seed orchards for commercial seed production. Vegetative propagation may also be used to produce cuttings for commercial plantations. After the first step in the hybrid breeding strategy, the strategy is simply a recurrent selection for general combining ability.

The limited differences from provenance trials with L. kaempferi suggested that just one breeding population with 150 trees of this species will be used. A pollen mix of ten male trees should be used for progeny testing of the selected trees. Seed orchards with the trees with highest breeding values will be established. Also for this species artificial assortative mass pollination might be used to produce the commercial seed. This strategy might be coined simple recurrent selection. The minor economic importance of this species is reflected in the suggested breeding strategy.

Pâques (1989) in his review of performance of hybrid larch discussed breeding programs for larch. Since micropropagation might be applied for production of commercial hybrid larch plants it is of importance that the technique does not cause genetic changes. Richard et al. (1995) used five isozyme loci to study the genetic stability of micropropagated material from Larix x eurolepis following different cultivation regimes. Treatments included type of growth regulators in culture media, periods in culture, and intervals between subcultures. Three isozyme loci showed the same pattern in in vitro shoots independent of composition of growth regulators while others caused variation. Similarly, subculture interval caused differences in isozyme pattern compared to the control. Even if there were differences during the course to acclimatized in vitro propagated plants, the end result did not suggest that the micropropagation technique had caused genetic changes of in vitro plants. Both for growth regulators and subculture periods the causes for differences from control plants were thoroughly discussed and it was suggested that the content in the media was responsible for the varying isozyme patterns observed.
To fulfill the projected need of hybrid larch in the Québec forest industry a reciprocal recurrent selection breeding program for production of *L. decidua* x *L. kaempferi* hybrid was presented by Perron (2008). The program includes activities related to the second and third generation of breeding hybrid larch. According to the information available at the time for the start of the program, only one breeding zone for the latitudinal range 45.62°N to 48.35°N in Québec in Canada would be required. To the benefit of tree breeders the program is very detailed with many numbers of trees to be selected at various occasions. I have tried to outline the essential parts of the program without being too detailed.

An initial selection of good growing trees at ages 9-15 years had taken place. A further scrutiny was carried out based on stem and crown quality as well as absence of pathogen infections. Pollen mixes of each species with a broad genetic origin will be used to generate the 80 half-sib families of each entry, *L. d* x *L. d, L. k. x L. k, L. d. x L. k, and L. k. x L. d* (Fig. 3-2). The progeny trials will be distributed to three ecogeographic regions. Part of the material will be planted in randomized blocks independent of genetic entry and another part will be planted with the four different entries kept apart but with randomized single-tree plots within each entry. In all 16 seedlings per half-sib family will be grafted; Based on 10-year data two trees from each of the 8 best performing families in each group will be used for crosses. For the two types of hybrid, *Ld x Lk* and *Lk x Ld* twelve trees will be selected based on GCA GHA and a combination of GCA and GHA. 2 interspecific crosses per selection strategy will be performed.

**Figure 3-2.** A reciprocal recurrent selection breeding program for production of *L. decidua* x *L. kaempferi* material for Québec province in Canada. Perron et al. 2008:
for the third generation based on general combining ability (GCA), general hybridizing ability (GHA) as well as joint evaluation of GCA and GHA. For each of the selection criteria 4 x 40 trees will be selected. This means that there will be a maximum of 480 trees. This number will be reduced if the same family also is selected for one or two other selection criteria. As regards *L. kaempferi* and the two hybrid larches only the GHA will be used in the selection for the next generation. This means that 160 trees will be selected for these three breeding populations. The results from the second generation progeny trials will also be used for delineation of breeding zones if the genotype x test locality effect is substantial. Nuclear microsatellites will be used for paternity analysis.

Eight elite trees per species will be selected based on the performance of the second generation breeding population and planted in an indoor seed orchard for commercial seed production. Open-pollination cannot be applied since this will result in many within-species matings. Mass pollination with pollen mixes using the electrostatic gun described below (Colas et al. 2008) was hinted at.

Supportive research for the breeding program was presented in the form of four experiments. As far as I can understand these experiments are parts of the breeding population.

The first experiment consists of a factorial mating between the ten phenotypically best trees of each species. This mating will result in 25 *L. d.* x *L. d.* and 25 *L. k.* x *L. k.* full-sib families, as well as 25 *L. d.* x *L. k.* and 25 *L. k.* x *L. d.* families. This makes it possible to estimate non-additive genetic effects.

A second experiment focused on the comparison between the two types of hybrid, *L. d.* x *L. k.*, and *L. k.* x *L. d.*. It was presented in the following way *We will compare only E x J and J x E HSFs that have both mother and father used in the crosses* (HSF = half-sib families). To my understanding this would require a diallelic mating design with full-sibs and not half-sib families as stated in the paper.

The third experiment aims at a comparison of the performance of the first generation hybrids (H1) with the progenies from H1 x H1 crosses, coined as H2. After selection at age 10, pollen mixes will be used to create 16 H1 hybrid progenies of each type and H1 pollen mixes will be used to create 32 H2 progenies.

Experiment 4 aims at a comparison of the selection methods, GCA, GHA, and a combination of GCA and GHA. Based on 5-year results a backward selection of 12 trees per species from experiment 1 will be carried out with respect to GCA and another 12 trees per species with respect to GHA. For each of the three types of selection polycross matings will be carried out to generate 12 of each type of cross, *L. d.* x *L. k.*, and *L. k.* x *L. d.*, resulting in 72 half-sib progenies. Two progeny trials in different ecogeographic regions are planned with measurements at ages 5 and 7.

**Figure 3-3.** A schematic illustration of the occurrence of meiosis in pollen mother cells of *L. decidua*. Eriksson 1968.

No studies of J-M correlations are mentioned in the program, which is surprising since selections are planned to take place at rather early ages without much knowledge of juvenile-mature correlations.

### 3.2 Reproduction

The peculiar meiotic division in larch pollen mother cells (PMC), which starts during the autumn, followed by a resting diplontene stage, and completion of the meiotic division during late winter or spring, may expose the sensitive PMCs to adverse environmental conditions (Eriksson 1968). Poor seed set was frequently reported in studies of seed crops from larch seed orchards without any reference to disturbances occurring during the meiosis in pollen mother cells (see below). Many larch seed orchards are located outside the natural distribution area of the species, which under certain weather conditions caused complete male sterility. The typical pattern of meiosis in PMC of *L. decidua* is shown in Fig. 3-3. The resting stage must be broken by low temperatures. Once the rest stage is broken the continued development diakinesis – telophase II will take place when mild temperatures occur. The Siberian larch requires just a few days with low temperature to break the dormancy while European larch requires a considerable cold period to break the dormancy. Japanese larch takes an intermediate position. Certain years the rest in Siberian larch PMCs is broken already in November resulting in a further development of the meiotic division if a few days of mild weather occur. If the mild weather is followed by another frost spell the sensitive meiotic stages, diakinesis – telophase II, will be damaged. Certain years complete pollen sterility in Siberian larch was observed. It is likely that there is no advantage of a long resting period in Siberian larch, which grows under continental climatic conditions without frequent fluctuations between mild and cold weather. Therefore, no selection for a large demand of cold weather for breaking the rest has taken place. For European larch, which grows under less continental climatic conditions with fluctuations between cold and mild periods, it is advantageous with a large demand for cold weather to avoid an early break of rest. The location of larch seed orchards must be done with care to avoid mismatching of PMC meiosis and weather conditions.
Different grafts were used in a Polish seed orchard. Hall 1985.

Grafts of two *L. decidua* clones were crossed with own species pollen or with *L. kaempferi* pollen for an analysis of viable seeds (Hall 1985). Seeds after open-pollination were also examined. This study was carried out during two years, 1974 and 1975. Several relationships between cone characteristics and seed viability were estimated. The percentages of viable seeds for the two clones are presented in Figs. 3.4 - 3.5. Different grafts were used for intra- and interspecific matings which means that the comparison between these two types of mating are not straightforward. Besides, for the clone in Fig. 3-5 data from all grafts were not available for both years and types of mating. The pattern is erratic; the open-pollination resulted in some cases in higher percentage of viable seeds while in others the opposite was found. The interspecific hybrid yielded in most cases the lowest percentage of viable seeds.

The relationships between cone weight and percentages of viable seeds and full seeds were weak. It was stated that cone size was useful as an index of seed quantity but of little value in prediction of seed quality. To obtain general information on seed viability and type of mating more clones need to be investigated and the experiments should be designed such that unambiguous comparisons can be done.

During two growth seasons Kosiński (1986a and b) studied the megagametogenesis and embryo development anatomically in two and four *L. decidua* clones growing in a Polish seed orchard.

In Kosiński (1987a) the focus was on four factors:
1. Lack of pollination
2. Female gametophyte degeneration
3. Lack of fertilization
4. Embryo degeneration
Based on the percentages of these stages the percent-

**Figure 3-4.** Percentage of viable seeds in two sets of grafts (Ld1 and Ld2) of one *L. decidua* clone after mating with *L. kaempferi* or another *L. decidua* clone. Open-pollination in the two sets of grafts are also shown. Data emanate from two years 1974 and 1975. Hall 1985.

**Figure 3-5.** Percentage of viable seeds in two sets of grafts (Ld3 and Ld4) of one *L. decidua* clone after mating with *L. kaempferi* or another *L. decidua* clone. Open-pollination in the two sets of grafts are also shown. Data emanate from two years 1974 and 1975. Hall 1985.

**Figure 3-6.** Percentage separation of factors influencing seed production in two clones of *L. decidua* in a Polish seed orchard. Kosiński 1987.
were also mentioned with one of the clones with abundant female strobili production and the other with abundant male strobili production.

The phenology of different steps of the megagametogenesis was focused on in a report by Kosiński (1987). Different stages during female meiosis and embryo development occurred at different dates the two years. Evidently, the weather conditions have an impact on the phenology. The differences among the clones were marginal but pollen germination of the four clones examined varied somewhat. It was stated that Lack of pollination, disturbances during megasporogenesis and female gametophyte development, failure of fertilization and embryo degeneration are the major factors resulting in empty seed.

The causes for poor seed set in the hybridization of *L. decidua* and *L. kaempferi* were addressed by Said et al. (1991). Pollen water content, pollen viability with different methods, and pollen germination in vitro were studied. Fresh pollen grains were collected at the time of pollen shedding and stored at 20°C in open air or in sealed vessels at 4°C. The water content of pollen was determined after drying for five minutes at 85°C of the freshly collected pollen and after one year of the pollen stored at 4°C. The so called 1H-NMR method was used for assessment of pollen water content. Pollen viability was tested by the fluorochromatic reaction test (FCRT). Pollen germination was tested on media with ovule substrate collected at pollination or at fertilization, i.e. six weeks later. Two staining methods were used to detect germination. The female strobili development was photographically monitored weekly during January and February and then daily to mid-March. From that date weekly monitoring was carried out. Scanning electron microscopy was carried out parallel to the photographic monitoring. The water content of the pollen at the time for pollination was estimated at 16% and at 8% after storage for one year in sealed vessels. It was found that approximately 25% of the water content was lost during the first day then it remained constant for 14 days. It was stated that the two germination tests on the two substrates from the ovules was difficult in larch and could not be used as a pollen viability test. The FCRT method revealed a pollen viability of 62% at pollen shedding and the viability dropped rapidly to 50% and kept this value for three months. The pollen that was stored for one year at 4°C for had a viability of 45%.

A few days after the strobili had got an upright position the bracts opened fully enabling pollen to enter the ovuliferous scales. The bracts remained open for 1-2 days. It was stressed that this time of strobili receptivity differed among the strobili on a tree. The most important result from this investigation was the short time of receptivity of the female strobili and the variation in receptivity among strobili on a single tree. This variation is a great constraint to successful artificial pollination in *L. kaempferi* and probably other larch species as well.

The percentage of full seeds in different parts of the tree crowns of 22 *L. decidua* grafts was studied by Burczyk et al. (1991). They classified the grafts in two groups, those with higher filled seed percentage in the upper part of the crown, type A > 2 m height (A) or below 2 m height (B). Upper = > 2m height, lower < 2m height. Burczyk et al. 1991.

The main results are summarized in Fig. 3-7, which shows that selfing was higher in the lowest part of the tree crowns, and particularly in the type B grafts. This calls for collection of cones from the upper parts of the tree crowns to avoid selfed seeds for commercial plantations. There was a negative F<sub>is</sub> in the parental population while it was positive in the offspring population. Finally, there was no relationship between outcrossing percentage and percentage of full seeds.

![Figure 3-7][1]

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[1]: Figure 3-7. Percentage of full seeds in *L. decidua* grafts in a Polish clone archive. Grafts were referred to two types, grafts with highest percentage of full seeds above 2m height (A) or below 2 m height (B). Upper = > 2m height, lower < 2m height. Burczyk et al. 1991.
Nine isozyme loci were used for a study of outcrossing rate in 27 L. decidua clones in a Slovak seed orchard at 12-14 years after establishment of the orchard (Gömöry and Paule 1992). In seven of the nine loci there was a significant difference between the pollen pool of the orchard and the progeny. The observed heterozygosity was higher in the seed orchard than in the offspring population; 0.283 versus 0.206. The fixation index was positive in the offspring population, 0.176, while it was negative in the clone population, -0.052. This means that there was a larger homozygosity in the offspring population than in clone population. The reason for this might be attributed to a higher degree of heterozygosity among plus trees selected for seed orchards than in the offspring to this orchard.

Differences in fecundity and flowering phenology may also contribute to the variation in outcrossing rates. In a series of papers Slobodnik presented data on flowering biology of four L. decidua clones in a Slovak seed orchard (Slobodnik 2000, Slobodnik 2002a and b, Slobodnik and Guttenberger 2003 and 2005, Krížo and Slobodnik 1996). The last paper treats pollen dispersal in the seed orchard but no study of genetic differences.

The numbers of pollen grains attached to the stigmatic apparatus and percentage of full seeds in four L. decidua clones were studied by Slobodnik (2002a). He sampled three open-pollinated strobili and three strobili following selfing from four grafts of each clone and the strobili from base, middle, and top parts of the tree crowns were kept apart. The collection took place immediately after the pollination period. Similarly, mature cones were collected for analysis of full seed percentage in the different parts of the crown. With a scalpel longitudinal sections of normal-locking seeds were halved and examined.

The ANOVA revealed that there were strongly significant differences as regards pollen attached to the stigmatic apparatus for:

<table>
<thead>
<tr>
<th>Clone</th>
<th>Selfing or open-pollination</th>
<th>Part of the crown</th>
</tr>
</thead>
</table>

The interactions clone x type of pollination and type of pollination x part of tree crown were also strongly significant. A scrutiny of Fig. 3-9 suggests that the interactions are significant but the strongly significant effect of clones was less expected.

The percentages of full seeds in the open-pollinated material varied strongly, 2.25 – 42.24%, with a mean value of around 30%. To reveal differences among clones with respect to full seeds in different parts of the crown I calculated the standard deviation for percentage of full seeds for each clone (Fig. 3-10). This figure reveals that the percentage of full seeds is lowest in the top parts of the crowns in all clones and is highest in the middle part with one exception, clone Š14. According to the ANOVA clonal differences were non-significant while the type of pollination and part of the tree crown were significant as well as all interactions. It was pointed out that lack of pollination was not the only contributing factor to the low percentage (around 30%) of full seeds since the proportion of ovules without any pollen grains varied in the

![Figure 3-8](image)

**Figure 3-8.** The number of clones in different classes of outcrossing among 27 L. decidua clones in a L. decidua seed orchard in Slovakia based on analysis of nine isozyme loci. Gömöry and Paule 1992.

![Figure 3-9](image)

**Figure 3-9.** Percentage of pollinated ovules following selfing (striped columns) and open-pollination (filled columns) in four L. decidua clones growing in a Slovak seed orchard. Separate data from the base (brown), middle (blue) and top parts (green) are shown. Slobodnik 2002a.
range 3-30%. Rather genetic load leading to degeneration of embryos was suggested as a major contributing factor to low percentages of full seeds. The low percentage of full seeds in the selfed material was partly attributed to asynchrony between pollen dispersal and ovule receptivity. Also genetic load can contribute much to a low full seed percentage after selfing. It should be remembered that selfing was accomplished by isolation of female and male strobili in the same isolation bag.

The correlations between the percentages of ovules pollinated with 3, 5, 7, or 9 pollen grains and full seed percentage were calculated. These relationships were all weak $r < 0.5$.

Slobodnik (2002b) reported on low percentages, <20%, of irregularities during microsporogenesis in *L. decidua* grafts from a Slovak seed orchard. There was a variation between years as regards the date for the further development from the late diplotene stage in the pollen mother cells.

Slobodnik and Guttenberger (2003) followed the sequence of events taking place from pollination until fertilization in the four Slovak *L. decidua* clones in a Slovak seed orchard. The process was documented by microphotographs. For the open-pollinated material it was noted that the percentages of unfertilized ovules were larger than the percentage of unpolminated ovules, 16.2% versus 34.1%. The corresponding figures for selfed material were 35.2% versus 54.9%.

The embryogenesis following open-pollination and selfing was followed in the four clones (Slobodnik and Guttenberger 2005). For selfing female and male strobili were enclosed in the same isolation bag. The occurrence of simple polyembryony and degenerating embryos was recorded during the first three weeks of embryo development.

The frequency of polyembryony was somewhat higher after open-pollination than selfing, 12.1 versus 5.4%, but non-significant. This frequency increased to a threshold value of four pollen grains per pollinated ovule. The percentage of fertilized ovules was more than 50%.

Fig. 3-11 shows that there is a large difference in percentage of degenerated embryos among the clones and between open-pollination and selfing. The latter difference was significant at the 1.0 percentage level. Clonal differences were not tested.
The seed formation after different types of matings of *L. gmelinii* var. *japonica* clones in a clone archive in Hokkaido was studied by Sato (1997). Three types of mating were applied:

- Pollination with one unrelated male
- Pollination with a mix of own pollen and pollen from one unrelated male
- Selfing

The numbers of seeds formed as well as their germinability were recorded. To illustrate the effects of selfing and the impact of own pollen in a pollen mix of own pollen and pollen from an unrelated clone the following calculations were carried out:

1. The ratio of full seed number over the number of full-size seeds for the offspring from the pollination with one unrelated clone was determined
2. The same ratios for the two other types of mating were calculated
3. The ratios from point 2 of the individual observations were divided by the ratios according to point 1

The ratios for pollen mix and selfing are illustrated in Fig. 3-12. Before a scrutiny of this figure, it is important to mention that the remarkable results found for clone *Sapp* must be attributed to the low number of full seeds, four, in the offspring from the cross with an unrelated pollen donor. It was suggested that the pollination had taken place when the receptivity of the ovules was low. The open-pollinated material had much higher proportion of full seeds. At least at four occasions the proportion of full seeds after selfing was much lower than after unrelated matings. Except for clone 5 these differences were significant. In three cases the proportions for the mixed matings varied in the range 60-80%. This must be attributed to some success of the own pollen in the pollen mixes, which according to the author means non-random mating. There was a strong linear relationship between full seed proportion and germinated seeds, $R^2 = 0.98$.

The difference in performance between years 1993 and 1995 in clone *Sapp* was attributed to the use of different males during the two years.

In conclusion there was no uniform pattern among the clones as regards selfing and pollen competition in pollen mixes. Direct estimates with markers would give more direct information on pollination success than what is possible with indirect estimates via full seed or germinability percentages. However, the results call for further analyses of the mating pattern in vivo and under artificial conditions.

A detailed study of the pollination mechanism and early seed development in *L. occidentalis* was presented by Owens et al. (1994). This paper included instructive light and transmission electron micrographs. Five female clones were included in the study but only general statements about clonal differences were given.

Twelve microsatellite markers were used to identify the seed crop of 41 clones in a *L. occidentalis* seed orchard in Canada (Funda et al. 2008). The male contribution to the progeny of 14 clones was estimated. Offspring with segregation pattern similar to the maternal genotype were excluded to avoid bias of the results. The *multilocus mixed-mating model* by Ritland (2002) was used to estimate mating system parameters.

The following estimates of outcrossing rates based on three different methods were obtained:

1. Single locus  85%
2. Multi-locus  97%
3. Selfing occurrence  91%

These high outcrossing rates are in the same range as found in natural populations of *L. occidentalis*. The participation of many males in the fertilization was inferred from the estimate of multi-locus correlated paternity, amounting to 0.07.

The paternity could be identified in 430 of the 551 analyzed embryos. Part of the non-identified males was attributed to gene flow. Non-informative genotypes of the embryos for identification might also contribute to non-assignment of the males. In all 221 full-sib families were noted in the 14 female clones. The numbers of full-sib families varied between 12 and 22. This result was regarded as an indication that the pollination within the seed orchard was efficient.

The variation in male contribution was regarded as slight, which does not agree with the data presented in Fig. 3-13. Actually the contribution of the clone with the highest fecundity was 10.7% of the offspring while four clones had no progeny at all. The number of grafts per clone var-

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**Figure 3-12.** The percentage of full seeds after selfing and after pollination with a pollen mix of own pollen and non-related pollen in equal amounts as related to pollination with non-related pollen. *L. gmelinii* var. *japonica*. Sato 1997.
ied and the phenology differences contributed to the variation observed. The male effective population size was estimated at 21, which is approximately half of the census number of clones. It was stated that the genetic gain was not much affected by the skewness in contribution to the progeny. Further it was stated that the paternity identification by microsatellites is a more accurate method to study the pollination pattern in seed orchards than just counting strobili numbers. A statement that is easy to endorse.

3.3 Flowering stimulation

Bonnet-Masimbert (1982) studied flowering in 5–7-year old trees of *L. decidua* and *L. kaempferi* following gibberellin, naphthalene acetic acid (NAA), and girdling. In the first series of experiments two different concentrations and different points of time of application of gibberellin $GA_{4/7}$ were used, 50 and 100mg/liter; the latter together with 10mg NAA. In one treatment $GA_{4/7}$ was combined with $GA_{3}$. The application of gibberellin was done by injections. In half of the material girdling was carried out. Strobili production two years after application of gibberellins was assessed. Plastic mulching was carried out and its effect on strobili production was assessed the year after application.

In the second series of experiments spraying with 400mg $GA_{4/7}$ was included and separate treatments on upper and lower branches were carried out.

**Figure 3-13.** The percentage of fertilizations of groups of five males in a Canadian *L. occidentalis* seed orchard with 41 clones. The groups are displayed according to descending order of pollination success. The paternity was assessed by nine microsatellites. Funda et al. 2008.

**Figure 3-14.** Female and male strobili number in *L. decidua* (*Ld*) and *L. kaempferi* (*Lk*) clones in 1979 following gibberellin $GA_{4/7}$, $GA_{4/7} + girdling$ treatment in 1977. The mulching treatment was carried out in 1978. Note the logarithmic scale. Bonnet-Masimbert 1982.

Experiment 1. The treatments in this experiment did not cause any flowering during the following year but had an effect two years after the treatment. This was surprising since the gibberellins are known to have short biological life time. It was speculated that the gibberellins might have had an indirect effect on the vigor of the shoots and in this way have influenced the flowering two years after treatment.

In spite of the large increase of the mean values after gibberellin treatment in experiment 1, only the increase of male flowering in *L. kaempferi* in the gibberellin + girdling treatment was significant (Fig. 3-14). The lack of significance was attributed to the large individual variation. The plastic mulching resulted in significant increases compared to the control material and in some instances also significantly more abundant flowering than following gibberellin treatment. Mulching might change the growth conditions in two ways; firstly a reduction in water availability to the trees, secondly by increasing the soil temperature.

In *L. kaempferi* the flowering response was highest after treatment in May while it was strongest after the July treatment in *L. decidua*. 
Experiment 2. Figs. 3-15 and 3-16 reveal that gibberellin + girdling had a strong impact on the number of strobili in both species. Even spraying with gibberellin resulted in increases of strobili production in upper and lower branches. The treatment effect and the effect of timing were in most cases significant. The interaction effects among treatments were non-significant.

The partitioning of the variation in female strobili production based on the ANOVAs run for each species is illustrated in Figs. 3-17 and 3-18. These figures reveal that the impact of genotype was larger in L. decidua than in L. kaempferi. The partitioning as regards male strobili production but slightly more effect of type of branch. In contrast to experiment 1, there was no effect of gibberellin treatment on strobili production the second year after application.

Studies on flowering stimulation, pollen collection, and pollination success were reported by Bonnet-Massimbert et al. (1998). Flowering stimulation with girdling or girdling + gibberellin A, (GA) was carried out during four years and the number of strobili was counted the following year. GA, 60 mg, was applied once in 1989, four times with 20 mg GA in 1991, twice in 1993 and 1995 with 50 mg GA.

The viability of pollen lots was tested by studying its conductivity, respiration, germination, and the 2,3,5-triphenyl tetrazolium chloride test (TTC). The correlations of pollen viability of these testing methods with the number of full seeds per cone were estimated. The results from three of the treatments are summarized in Fig. 3-19. The treatments resulted in smaller strobili with a lower number of seeds per cone. I have tried to compensate for this reduction of seeds per cone by dividing the observed numbers with the ratio of cones per liter in treatment/cones per liter in control. I am aware that such a correction might be far from true but it is likely a
better estimate of an improved seed production after the two flowering stimulation treatments. Such a correction could not be done for the observations during 1994 since the number of female strobili was too low for an estimation of strobili per liter. A strong stimulation of flowering was noted for both treatments and the seed production is expected to be much higher after the treatments than in the control. It was noted that the treatments had negative impact on the growth of the grafts, which means that flowering stimulation should be carried out at intervals rather than every year. The study on the effect of time on treatment application showed that the strongest stimulating effect was achieved with two combined treatments, girdling + GA, at an interval of one month during the shoot elongation period.

**Figure 3-19.** The number of female strobili in one clone of L. decidua following girdling or girdling + injection of gibberellin GA$_4$. C stands for a correction based on the reduced size of the strobili following treatment. Such a correction could not be done in 1994 owing to poor flowering in the control this year. Bonnet-Masimbert et al. 1998.

A study of the hand picking of male strobili resulted in 441 grams of pollen, with a rate 3.4 grams pollen per hour. The pollen harvester used increased the efficiency of pollen collection to 30 grams per hour. Thus collected pollen does not need any extraction or cleaning. A pollen drier was developed in France to obtain pollen of the desired moisture content of 7-7.5%. Crosses within species and reciprocal crosses between L. decidua and L. kaempferi were carried out with low seed sets; 4.0-13.8 filled seeds per cone. The filled seeds per cone were correlated with pollen germination and pollen conductivity. Only the intraspecific type of cross with L. decidua resulted in strong relationships (Fig. 3-20) with pollen germination ($r = 0.87$) and pollen conductivity ($r = -0.91$). All other relationships explained less than 30% of the observed variation. It was suggested that the low correlations with L. kaempferi as female parent was attributed to other reasons than poor pollen viability. A short period of drying and storing of the pollen before pollination was applied to test pollen viability in three lots of L. kaempferi. The prehydration caused a drop of conductivity and an increase of pollen germination; in one lot a considerable increase in germination (Fig. 3-21).

**Pollination techniques.** The access of pollen from larch is frequently limited. Therefore, dilution of larch pollen by mixing with talc was tested. Based on the results from this study it was recommended that dilution with 50% talc could be used in artificial pollinations. It was hypothesized that cold night temperatures in the isolation bags would influence the success of pollinations. During the pollination season low night temperatures are frequent. Pollinations at 10.00 am resulted in higher numbers of full seeds per cone (28.3%) than pollinations at 4 pm (19.4%) in one year. The corresponding percentages another year were 10.7 and 4.3, respectively. Pollination with paint brush was compared with a cyclone pollinator (according to Matthews and Bramlett 1981) with or without misting of water into the isolation bag. The misting was done to mimic adverse pollination conditions. The number of visible pollen grains per cone was higher after
artificial treatment than in the control while the opposite was the case for the percentage of ovules with pollen. The number of pollen grains per ovule was highest in the control material, 4.4 and lowest in the misting treatment (Fig. 3-21). The percentage of full seeds was significantly lower in the misting treatment than in the other two treatments. Unfortunately, too few cones were available in the control to permit an estimate of percentage of full seeds. The success of supplemental mass pollination in different parts of the tree crown was studied during three seasons. Pneumatic hand gun, electrostatic machine (see Philippe and Baldet 1997 below), these two techniques combined was used one year (Y2). During another year these three were complemented with electrostatic hand gun (Y3). In the year with three treatments the percentage of full seeds in the bottom of the crown was significantly lower than in the other two treatments PHG and EM+EHG. It was stated that the pollinations took place during the end of the receptivity period, which might have had an impact on the percentage of full seeds. In spite of this, these percentages were higher than in open-pollinated material. One advantage with the electrostatic machine pollination is that it required less pollen than the other treatments. As was concluded in the report important progress was made to improve the low percentage of filled seeds following artificial pollinations in larch but much remains to be done.

Gibberellin \(A_{4/7}\) treatments of \(L. laricina\) grafts to promote flowering were carried out by Eysteinsson and Greenwood (1990). Three-year old grafts from four families in an indoor seed orchard were treated. Half of the grafts were obtained from 16-24 years old trees and the other half from one-year open-pollinated seedlings from the old trees. Starting on May 11 first-order lateral branches were given five, seven or nine biweekly gibberellin \(A_{4/7}\) applications. In a second group of grafts three branches received five biweekly applications of gibberellin \(A_{4/7}\), starting on May 11 or June 6. A third group was used as control. Root pruning was also carried out as one treatment. Parallel to the indoor treatments, outdoor applications were carried out: five times biweekly foliar spray applications on first-order lateral branches starting on June 6 or June 20. The control group received ethanol-Aromox spraying.

Significant family differences in female and male flowering were noted without showing any figures in the paper. Evidently there was an interaction family x location (indoor-outdoor) since the least flowering family inside the greenhouse was the most abundant flowering outdoors and vice versa.
Fig. 3-24. Number of female and male strobili after treatment with gibberellin A$_{4/7}$, root pruning (Rp), or these two treatments in combination of L. laricina grafts grown in an indoor seed orchard. Eysteinsson and Greenwood 1990.

Fig. 3-23 shows that the May 11 gibberellin A$_{4/7}$ treatments had a strong effect on female strobili production. This caused a significant increase of the female/male strobili ratio. In spite of the large variation in male strobili number the treatment effect in the indoor material was non-significant. The high male strobili number following nine applications of GA$_{4/7}$ was attributed to few branches with high numbers of male strobili. For the outdoor material the female strobili number was significantly higher after the June 6 treatment than in the two other treatments. It is evident that starting time of application is more important than the number of times of application. The scion age influenced the flowering differently indoors and outdoors. The juvenile grafts had the highest strobili productions in the indoor material while the opposite was true for outdoor materials. Root pruning alone did not improve female and male strobili production while it in combination with gibberellin GA$_{4/7}$ treatment resulted in a significantly higher female strobili production (Fig. 3-24).

The most important information from this study was the increased female strobili production by application GA$_{4/7}$ to juvenile material.

Another study of flowering stimulation of 32 potted L. laricina grafts originating from scions collected from material of different age was reported by the same authors (Eysteinsson and Greenwood 1993). Twelve first-order branches from the upper parts of the crown were selected for spraying with GA$_{4/7}$. The treatments were five weekly, five biweekly, and nine biweekly sprays with 200 mg GA$_{4/7}$. Half of the grafts were root pruned after short-shoot bud flushing but before the long-shoot extension had started. The number of strobili of both sexes was counted on the treated as well as on non-treated branches. Since the branches selected for treatment were of different lengths the number of strobili per cm was also presented. The material was classified in four groups with mean ages at treatment, 4, 8, 20, and 50 years. The GA application caused a significant increase of female and male strobili. The five weekly sprayings improved the female strobili number from 0.9 per branch in the control to 19 strobili per branch. The effect of root pruning was non-significant. It was suggested that the point of time for root pruning was not optimal or that intensity of pruning was too low in combination with the watering regime applied. Since the trend as regards relationship between age of ortet at treatment and number of strobili was approximately the same in the three GA$_{4/7}$ treatments I calculated the mean values for the GA$_{4/7}$ treatments. Fig. 3-25 shows that the number of female strobili dropped with age and with a surprisingly good fit to the second degree polynomial curve. Similarly, the fit to the curve in the control material was extremely good. Several traits related to branch development were recorded to understand the background to the observed effects of GA$_{4/7}$ treatment. Most of the variation in branch characteristics was non-significant - terminal increment, laterals per branch, and terminal increment/cm of initial branch. Contrary to this, total lateral increment was significantly higher in the five weekly and five biweekly treatments. The increased number of laterals that elongated and the increase of reproductive bud initiation might be responsible for the effect on flowering of the gibberellin treatments. The increase was proportionately stronger as regards female strobili than on male strobili. It was stated that there seems to be a real decrease of flowering potential with age since the observed flowering could not be related to branch characteristics. A decreasing endogenous level of gibberellin by age might be responsible for the reduced flowering with increasing age.
The drop in response with age of female strobili in the untreated parts of the tree crowns was similar to the drop of flowering in treated branches (Fig. 3-26). It was also speculated that this age difference might be attributed to the reproductive strategy of L. laricina. At times when there is open space for germination of L. laricina seeds, abundant flowering should be advantageous for the species. Once the stand is closed the conditions for L. laricina regeneration are less advantageous.

In conclusion, this investigation resulted in important information for breeding of L. laricina.

Philipson (1995) used one-and-a-half-meter tall grafts of L. decidua and L. kaempferi cultivated in pots in four experiments aiming at flowering promotion. The essence of the experiments is summarized in Table 3-1. GA$_{4/7}$ was injected into the stems. Heat treatment was accomplished by having the grafts in a polythene house. Six clones from each species were included in this investigation.

Experiment 1. There was a significant difference between the species; no female strobili were produced on the L. kaempferi clones while there were few strobili on the L. decidua clones. The opposite was found for male strobili with higher numbers in L. kaempferi. Heat and GA$_{4/7}$ treatments caused an increase of male strobili in L. kaempferi. It was remarked that the heat treatment was very weak since the temperature difference was only 2°C between indoor and outdoor temperatures.

Experiment 2. Fig. 3-27 shows that all treatments caused a drop of female strobili in L. kaempferi, but only root pruning turned out as significant.

Experiment 3. A significant difference between the duration of heat treatments was noted for male strobili in L. kaempferi. All other effects were non-significant. However, for both species approximately twice as many female strobili were noted after four months of heat treatment as compared to two months of heat treatment. In spite of this large difference it was not significant.

Table 3-1. The design of four flowering promoting experiments with grafts of L. decidua and L. kaempferi. Philipson 1995.

<table>
<thead>
<tr>
<th>Experiment and number of clones</th>
<th>Gibberellin A$_{4/7}$</th>
<th>Temperature</th>
<th>Drought</th>
<th>Girdling root pruning</th>
<th>Significance female strobili</th>
<th>Significance male strobili</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 10 clones/species</td>
<td>0 and 2mg, applications at May 25 and June 19</td>
<td>Inside and outside polythene house</td>
<td>-</td>
<td>-</td>
<td>All NS</td>
<td>Species, temperature, GA$_{4/7}$</td>
</tr>
<tr>
<td>2. 10 clones/species</td>
<td>0 and 2mg, applications once per month during 4 months</td>
<td>-</td>
<td>-</td>
<td>With or without root pruning</td>
<td>Root pruning</td>
<td>All NS</td>
</tr>
<tr>
<td>3. 6 clones/species</td>
<td>2 and 10 mg; application once per month in polythene house</td>
<td>Duration of heat treatment, May - June May-August</td>
<td>With or without root pruning</td>
<td>All NS</td>
<td>Drought in L. kaempferi</td>
<td></td>
</tr>
<tr>
<td>4. 6 clones/species</td>
<td>0 and 2mg, applications once per month in polythene house</td>
<td>Inside and outside polythene house</td>
<td>With or without drought</td>
<td>Heat drought</td>
<td>Heat</td>
<td></td>
</tr>
</tbody>
</table>
The increase of female strobili number compared to the control after three treatments, 50mg gibberellin A4/7, girdling and a combination of these two treatments. The L. decidua control materials had 11 female strobili and L. kaempferi had one strobilus/graft. Philipson 1996.

Experiment 4. Heat and drought treatments individually or in combination caused an increase of both female and male strobili. The effect of the combined treatment on female strobili production was dramatic; from the mean value for the two species of less than 40 strobili per graft to more than 140 strobili per graft. In some of the treatments a large fraction of the male strobili did not “flush”. In experiments 2-4 the flowering was abundant without any species differences. The lack of promotion of flowering by the gibberellin treatment was discussed in detail and it was speculated that the injection method might be one reason for the lack of response. Particularly the Japanese larches were affected with yellowing of needles in the polythene house and it was most expressed after gibberellin treatment.

It was concluded that heat and drought treatment of potted trees in polythene house promoted flowering, which can be used in larch breeding.

Philipson (1996) carried out a similar study in an outdoor clone bank with grafts of L. decidua and L. kaempferi. Six clones of each species were included with grafts < three meters tall. Gibberellin GA4/7 was injected at June 22 and July 27, each time with 50 mg GA4/7. Two semicircular bands of bark at a difference of 6-7 cm and somewhat overlapping were used in the two treatments with girdling.

The strobili production after subtraction of the number in the control grafts is visualized in Fig. 3-28. In both species there was a strong effect of girdling alone or in combination with GA4/7 treatment but limited effect of GA4/7 treatment alone. The clonal difference was significant; three of the L. decidua clones did not have any female strobili neither in the control nor in any treatment. For L. kaempferi there was a significant drop in male strobili number after GA4/7 treatment.

As in the previous study there was a considerable number of male strobili that did not flush (Fig. 3-29). The treatments including GA4/7 had a significantly higher percentage of such strobili. This percentage was higher in L. decidua than in L. kaempferi.

It was noted that girdling might be a useful technique in breeding programs and that girdling did not cause any serious effects on graft vitality.

An equipment for harvesting of large quantities of larch pollen was developed by Philippe and Balde (1992). A big tank (approximately 4.7 m³) was built on a tractor. At pollen collection the tank encloses part of the tree to be harvested. By whipping of the tree, pollen is released into the tank and then sucked into a filtration unit and collected at the bottom of this unit in vessels. The pollen collection equipment was tested during three seasons. The pollen catch amounted to 1.3, 3.9, and 10.2 g per tree. It was remarked that lowest amount of pollen was obtained during a year with poor flowering. One advantage with this technique of pollen harvesting is that it is labor saving and that pruning of the trees is avoided. One disadvantage with this way of collecting pollen is that collection cannot take place before release of pollen. When pollen release there is always a risk that contamination may take place. However, if the collection takes place in a hybrid larch seed orchard with one female clone of L. decidua and many clones of L. kaempferi contamination is less of a problem. Besides, differences in phenology between the two species reduce the contamination with L. decidua pollen.
A technique for mass pollination via electrostatic dusting of pollen was reported by Philippe and Baldet (1997). They developed a tank that enclosed the trees during the pollen application. In a first experiment the effect of voltage on the success of pollination was studied. In a second experiment numerous grafts of a self-incompatible *L. decidua* clone was pollinated by a pollen mix from 150 grafts of *L. kaempferi* clones. Three treatments were used: 1. manual pollination of 13 grafts using compressed air 2. electrostatic pollination of 10 grafts; three times less pollen used than in treatment one. 3. control treatment = electrostatic treatment without pollen

First experiment. The electrostatic dusting of pollen had a significant effect on number of pollen grains per bract, especially in the upper part of tree crowns (Fig. 3-30). The number of pollen grains per bract decreased to low level at distances further than one meter away from the dusting unit. It was noted that the pollen spreading was more homogenous at 40 kV than at 15 kV.

Second experiment. Fig. 3-31 shows that the electrostatic dusting promoted an increase of full seeds in the upper part of the tree crowns and at lower number of pollen used. The seed germination did not differ among the three treatments and was estimated at approximately 80%. It was stated that huge amounts of pollen was seen on strobili from the uppermost part of the tree crowns. In spite of that, the percentage of full seeds per cone never exceeded 60%. Thus, lack of pollen cannot be the reason for not reaching 100% full seeds per cone.

Colas et al. (2008) described the development of tunnel seed orchards for production of *L. decidua* x *L. kaempferi* hybrids in Quebec province in Canada. The tunnel is 4.4 m wide and 4.6 m high at the highest point and was coined as cathedral tunnels (Fig. 3-32). The roof is rather steep to avoid snow compaction on the roof during winter. A tunnel of 30 m length can accommodate 47 pots and a tunnel of 60 m 77 pots. It was assumed that grafts could remain in these orchards up to an age of 15 years. A pot-in-pot cultivation technique is used with holes in the inner pot, which facilitates root pruning. The pots are buried in the soil in the orchard, which allows for more above-ground growth of the grafts in such a type of orchard. Before pollen dispersal, branches with male strobili are tied together and enclosed in large Kraft paper bags. To facilitate the collection of pollen the entire graft is put at an angle and vacuum is used to collect the pollen. The pollen is dried in a conventional oven at 40°C for four hours. The conductivity of the pollen is measured to estimate the pollen vitality. Pollen may be stored under vacuum in sealed bottles or polyethylene bags and kept at -25°C in freezer.

Pollination of non-isolated female strobili takes place by the aid of an electrostatic pistol. Before pollination the grafts are carefully emasculated. Pollination takes place twice at peak receptivity of the female strobili. The receptivity of the strobili occurred two to three weeks earlier inside the tunnels than under outdoor conditions, which considerably reduces the risk for contamination. The tunnel cover is removed when the risk for spring frost has passed and after the closure of the strobili. Cone harvests take place four to five months later.

The percentage of female flowering clones in 2005 was over 60% in both European and Japanese larch while male flowering was somewhat lower in Japanese larch (50%) and substantially lower in European larch (26%). It should be noted that the age of the grafts was not specified; grafting had taken place in 1984, 1986, and 1995. The yield of filled seeds varied in the range 3.4 – 19.5 seeds per cone. The germinability of the filled seeds was satisfactory good, 64 – 85% (Fig. 3-32). One commercial seed orchard utilizing this concept was established in 2006.

Owing to the difficulties in production of hybrid larch seeds the development of the cathedral tunnel orchards is a good means to commercially produce hybrid larch
seeds. Once hybrid larch seedlings are available vegetative propagation of them may take place. The generation time in one breeding cycle is approximately 15 years. This means that the current grafts can be substituted at an age, at which the grafts have reached the maximum height for keeping them in the tunnel.

3.4 Seed production in species hybrids

Occurrence of hybrid seeds in a German seed orchard with one clone of each species, *L. decidua* and *L. kaempferi*, was determined by Bergmann and Ruetz (1987). Seeds were collected at ages 12 and 15 years after establishment of the orchard. At age 12 only the seeds from the *L. decidua* clone were collected. Two species specific alleles in the isozyme SKDH-locus were used to detect species hybrids. Only full seeds were examined.

The percentages of species hybrids in the seed crops from *L. decidua* were estimated at 19 and 43% for years 12 and 15, respectively. The corresponding figure for *L. kaempferi* was 71%. If pollinations took place with pollen produced within this seed orchard it means that selfing varied in the range 29-81%. Such high percentages of selfing seem highly unlikely. There was no way to identify how much outside pollen sources had contributed to the within-species crosses. Even if larch pollen is not dispersed over such large areas as spruce and pine pollen it is likely that there was considerable pollen pollution from outside sources.

Production of hybrid seeds in seed orchards with one *L. decidua* clone and numerous (79-261) *L. kaempferi* clones was studied by Häcker and Bergmann (1991). Two isozyme loci with allele combinations unique for the two species enabled an estimation of hybrid seeds as well as selfed seeds in the offspring from three species hybrid seed orchards.

The results from this investigation are shown in Fig. 3-33, which shows that the hybrid seed formation was high in all cases, 69-85% and that the contamination was low, <8%. Selfing was high and varied in the range 10-31%. It was suggested that the limited pollen dispersal in larch explains the low percentage of contamination. The difference in flowering phenology of the two species leads to limited *L. kaempferi* pollen during female receptivity of the *L. decidua* female clone and in this way promote selfing.
The percentage of filled seeds varied in the range 22-60%. There was no trend to have less hybrid offspring at low percentages of filled seeds, rather the reverse. One year the percentage of filled seeds was 22% and this seed crop had the highest percentage of hybrids, 85%. It was noted that the use of two enzyme systems avoids the overestimation of hybrids compared to the use of one system only. Some offspring, which is the result of contamination, might be classified as hybrid offspring. However, even with two enzyme systems there may be some overestimation of the hybrid percentage but to a lesser extent than with the use of one system only. Even if there is some overestimation of the hybrid percentages in the three seed orchards, these percentages are remarkably large.

A similar type of study was carried out by Burczyk et al. (1997) in a Finnish species hybrid seed orchard with one L. sibirica female clone and six L. decidua clones. However, the flowering was so poor in two of the European larch clones that they could not be included in the study. Four isozyme systems were used for the paternity study. Three methods were used for estimation of the outcrossing rates:

- Mixed-mating model
- Mating model
- Fractional paternity model

The outcrossing rate of the Siberian larch clone was extremely low, <10% while the outcrossing rates for the other clones were close to 100%. Independent of the method for estimation of the mating pattern, it turned out as extremely skew in this seed orchard (Fig. 3-34). This figure gives the data from the only L. decidua clone that had a substantial pollination contribution to the offspring. It is somewhat alarming that the estimates differ so much between the two methods used. Another alarming figure is the extremely high selfing rate (90% and 68%) for the two methods of calculation) and thus low outcrossing rate (0.096) in the L. sibirica clone. The skewness of the mating pattern might be attributed to:

- Eight times higher pollen production of the L. sibirica clone
- The low percentage of full seeds in the L. sibirica clone, 6.34% as compared to 13-31% in the L. decidua clones
- The difference in flowering phenology between the European and the Siberian clones

It was concluded that the hybridization pattern was close to unidirectional in this seed orchard. The objective of using the Siberian larch clone as female was not fulfilled. If hybrid seeds should be collected in this orchard it has to be taken from the L. decidua clones.

In an earlier report based on data from the same seed orchard Lewandowski et al. (1994) reported that the proportion of hybrid seeds with L. sibirica as male parent amounted to 93% while the L. decidua clones produced 18% of hybrids. In this case only one isozyme locus was used, Mdh3. The large difference was attributed to differences in male fecundity between the grafts in the orchard with several times higher pollen production of the L. sibirica grafts than the L. decidua grafts.

In a German seed orchard consisting of one clone of L. kaempferi and four clones of L. decidua cones were collected 27 years after establishment of the seed orchard for estimation of hybrid seed formation (Tröber and Haasemann 2000). Separate collections on the southern and northern sides of the grafts were carried out. Each row of L. kaempferi grafts had two rows of L. decidua on each side to promote hybridization. The SKDH isozyme locus was used for identification of species hybrids.

With one exception, Fig. 3-35 shows that the percentages of hybrid seeds and full seeds were somewhat higher in the southern parts of the tree crowns. It was suggested that the temperature on the southern side of the grafts are...
higher, which contributes to better development of seeds and earlier flowering with more pollen available during the female strobili receptivity. The proportion of hybrids in the eastern part of the seed orchard was larger than in the middle and western parts of the orchard. This was attributed to the dominating wind direction from west to east. The results from age 27 were compared with the results from the same orchard at age 18 presented by Braun et al. (1990). The weather condition at age 18 caused an extended period of pollen dispersal and receptivity while this period was condensed at age 27. These conditions explained the larger variation in hybrid occurrence at age 18 than at age 27.

Two parts of a Japanese seed orchard (Latitude 43.75°N and longitude 143.75°E) aimed for production of *L. gmelinii* × *L. kaempferi* and their reciprocal hybrids were planted with different designs (Moriguchi et al. 2008). One has a traditional design with random planting of the two species; the other has replications of four rows of *L. kaempferi* and one row of *L. gmelinii*. This latter design was referred to as SMC = single maternal clone. The latter species is regarded as female species for hybrid production. Seed crops from the two parts of the orchard were harvested in 2004 and 2005 for analysis of the hybrids in the traditional type of seed orchard separately and one row of hybrids in the reciprocal type of seed orchard separately.

The latter species is regarded as female species for hybrid production. Seed crops from the two parts of the orchard were harvested in 2004 and 2005 for analysis of the hybrids in the traditional type of seed orchard separately and one row of hybrids in the reciprocal type of seed orchard separately. Only data from the females harvested both years are included in this Fig. 3-36. The frequency of hybrids was significantly higher in the SMC orchard both years. The opposite trends between years as regards hybrid production in the two species were attributed to varying ratios in the production of pollen. The two species had higher pollen production than *L. kaempferi*. Pollen abundance was suggested as the most limiting factor for production of the hybrids, while difference in phenology was regarded as a less limiting factor. With high self-incompatibility of the *L. gmelinii* female clones the hybrid production could feasibly be produced in SMC seed orchards.

![Figure 3-36](image)

**Figure 3-36.** The percentage species hybrids in two Japanese seed orchards with *L. gmelinii* (Lg) and *L. kaempferi* (Lk). Lg-T and Lk-T stand for traditional seed orchard design with random planting of the two species; Lg-R stands for a seed orchard with *L. gmelinii* trees planted in rows surrounded by four rows of *L. kaempferi*. Moriguchi et al. 2006.

![Figure 3-37](image)

**Figure 3-37.** The percentage of meiotic irregularities in *L. gmelinii* (Lg), *L. kaempferi* (Lk), and their two reciprocal hybrids. D-M = diakinesis metaphase I, A-T = anaphase I – telophase I. Zhang et al. 2010.

...
3.5 Vegetative propagation

Mid-crown branches were collected on July 20-21 from *L. laricina* trees 19-41 years old for cutting propagation (Carter 1984). The cuttings were dipped in commercial rooting powder (Rootone containing naphthaleneacetic amide, 2-methyl-1-naphthaleneacetic acid, 2-methyl-1-naphthaleneacetamide, and indole-3-butyric acid as active ingredients) before being inserted into the rooting medium. Three rooting substrates were used; peat, perlite, and 50% of each of them in the third treatment. The cuttings were kept in greenhouse until end of November. No significant difference in rooting percentage between the three media was detected. The rooting percentage varied in the range 62-73%. There was a significant difference among ortets with respect to rooting percentage. Similarly, the ortet x medium interaction was significant. The relationship between ortet age and rooting success was weak (Fig. 3-38) and even after exclusion of the rooting for the 41-year old ortet (red square) the relationship explained less than 60% of the variation.

The effect of age, clone, and point of time for success in rooting of *L. laricina* cuttings were studied by Morgenstern et al. (1984). Ten seedlings/tree characterized by superior growth and stem form were selected for cutting propagation. Four age classes (3-4, 5-6, 7-8, and 9-10 years) and four points of time (May 23, July 3, July 23, and August 14) during the growth period were included in this investigation. At each occasion and age class ten cuttings were collected from each ortet. Cuttings 8 cm long were dipped in water followed by a dip in rooting powder consisting of Seradix 3 and 0.08 % 3-indol butyric acid. Three months after treatment five characteristics were recorded:

- Rooting %
- % cuttings with major roots, >3cm
- Number of major roots per cutting
- Length of the longest roots
- Shoot phenology of the cuttings in four classes. Only correlations with the other traits were presented.

Separate broad-sense heritabilities were estimated as clonal variance components/clonal rooting percentage + within clone variance for each collection date. For all age classes the rooting percentage peaked for the 22 July collection (Fig. 3-39). This figure also illustrates that the oldest ortets had the lowest percentage of rooted cuttings.

![Figure 3-38](image1.png)  
*Figure 3-38. The relationship between ortet age and rooting success of *L. laricina* cuttings. The extremely high rooting percentage of the 41-year old ortet (red square) is excluded from the response curve. Carter 1984.*

![Figure 3-39](image2.png)  
*Figure 3-39. The rooting percentage of *L. laricina* cuttings in four different age classes 3-4, 5-6, 7-8, and 9-10 years taken at four different points of time May 23, July 3, July 23, and August 14. Morgenstern et al. 1984.*

![Figure 3-40](image3.png)  
*Figure 3-40. Percentage of *L. laricina* cuttings with major roots in four different age classes 3-4, 5-6, 7-8, and 9-10 years taken at four different points of time May 23, July 3, July 22, and August 14. Morgenstern et al. 1984.*
cuttings. Since presence of major roots is of great significance for the future success of the cuttings I have illustrated the same relationships for this trait in Fig. 3-40, which shows that the best results were obtained for cuttings collected on May 23 and July 22. Fairly similar relationships were noted for the number of major roots per cutting and the length of the roots. It would have been of interest to have a discussion of the dip in the relationships for the July 3 collection. The effects of ortet age, date of collection, and clone, were all strongly significant for the four root related traits. All clone x collection date interactions were also significant and in two cases strongly significant.

Rooting percentage and percentage of cuttings with major roots had the highest estimates of broad-sense heritabilities (Fig. 3-41). The low broad-sense heritabilities for the number of major roots and root length, 0.17 and 0.12 for the collection date with the highest rooting percentage are disappointing from an application perspective. The clonal mean correlation coefficients for all traits and for collection date July 22 are presented in (Fig. 3-42), which shows that root length was strongly related to all other four traits while rooting percentage had two strong and two moderate correlations. The relatively weak correlation between rooting percentage and percentage of cuttings with major roots is also disappointing. In conclusion a valuable investigation for elucidation of some critical issues related to clonal forestry of L. laricina.

The height growth of the cuttings two to five years after their collection was presented in a follow-up paper by Morgenstern (1987). Strangely, the ortet age was non-significant for tree height at age 5 while it was significant at age 3 (Fig. 3-43). This analysis was based on pooled data for the four collection dates. The large difference in rooting at the different collection dates is probably responsible for the absence of significance at age five.

The Morgenstern (1987) paper is a follow-up of the paper Morgenstern (1984) with a focus on rooting techniques and cultivation conditions for large-scale application of L. laricina cutting propagation. An experiment with cuttings from 25 clones 5-6 year old was carried out. The collection of cuttings took place on July 24. By October 11 the rooting was 86%. After that date the temperature in the greenhouse was lowered and the photoperiod was changed to the natural conditions. After one month the cuttings were split into three different conditions. The percentages of survival after overwintering in the individual treatments are presented:

- Unheated polythene house and kept under sealed conditions until end of February or early March, 98%
- Outside shade hall and under snow, 100%
- Lightly heated greenhouse at 5-10°C at natural photoperiod, 89%

In conclusion the methods applied may be used in commercial production of L. laricina cuttings.
Plants 3-12 years old from two stands at each of 12 localities in Ontario were transplanted and pruned to stimulate lateral shoot development for studying rooting formation in cuttings (Farmer et al. 1986). Cuttings were collected in early summer and midsummer and treated with 1% IBA and 1% PPZ (1-phenyl-3-methyl-5-pyrazolene) and 5% captan. Early summer rooted cuttings were moved to a lath house after a “hardening” of the cuttings. The midsummer cuttings were placed in a cooler at 3°C. The early summer and midsummer cuttings were put into a greenhouse on December 5 and January 4, respectively and bud flushing was recorded. As a comparison rooting of cuttings from young seedlings were also studied. All localities had high rooting percentages, 83-98%.

The effect of maturation on rooting success, growth and DNA methylation was studied in four families of L. laricina by Greenwood et al. (1988).

Experiment 1. Scions were collected from the upper one third of four sexually mature trees in a natural stand of L. laricina. Scions were also collected from one-year old seedlings in four open-pollinated families, one from each of the four mature trees. The terminal buds were removed from the scions. The stem segments used in this study had a length of 20 cm. Side grafting of twelve scions from each mature tree and OP-family was carried out. Five grafts from each tree and OP-family were used in long-term experiment. Female and male strobili were counted two and three years after the grafting. Height and diameter were recorded and DNA methylation as well as chlorophyll content was measured. Finally the plagiotropism was visually inspected.

Experiment 2. Scions were collected from nine trees in three age classes; 3-7, 16-19, and 33-74 years respectively. The trees were growing in a natural stand of L. laricina in Maine, USA. In addition, scions from one-year old seedlings in OP-families were included in the grafting experiment. From each ortet five scions were grafted. Height and diameter were recorded and DNA methylation as well as chlorophyll content was measured. Finally the plagiotropism was visually inspected.

In experiment 1 the mean heights of the scions and the Methylation of DNA did not differ between juvenile and mature trees. Scion diameter was somewhat larger in juvenile scions than in mature ones while the chlorophyll content was higher in mature than in juvenile scions. Methylation of DNA, which has been expected to be responsible for ageing, did not differ between juvenile and adult scions. For three of the traits that showed large differences between juvenile and mature scions, the family performances are shown in Fig. 3-44. This figure reveals that mature scions showed large family differences. The mean number of male and female strobili increased dramatically from year one to year 2 after grafting; both for juvenile and mature scions. Fig. 3-45 reveals that the sex ratio differed between juvenile and mature scions. Particularly for the mature scions the family variation in sex ratio was pronounced.

In experiment 2 with four age classes there was a drastic drop in orthotropic growth between ages one and five (Fig. 3-46). Decreases over age were noted for most traits except for chlorophyll content, which increased linearly with scion age.
This study showed that the mature nature of scions remains after grafting. The results are of significance for seed orchard establishment of *L. laricina*.

The above material was used in a physiology-molecular genetics study by Hutchison et al. (1990), in which needle morphology, net photosynthesis, cab and rbcS cDNA clones were studied. Differences in needle morphology, chlorophyll content, and net photosynthesis capacity were noted between age classes (1, 2, 3, and 4 years) but data from individual families were not presented. The level of cab RNA was higher in juvenile plants than in older plants while rbcS RNA did not differ with age. No family differences in expression were reported.

Pâques and Cornu (1991) reported on rooting success, root quality scored in four classes, and plantable cuttings of hybrid larch *L. decidua* × *L. kaempferi*, as well as the reciprocal cross. A comparison on these traits in trees originating from ortet ages of two and ten years was also presented. In a first experiment softwood cuttings from 101 two-year old ortets belonging to 14 full-sib families were followed. The cuttings were treated with 0.5% indol butyric acid to promote rooting. After one year in greenhouse the cuttings were transferred to nursery, in which they were kept for two years. Rooted cuttings were planted in a French trial at lat. 45.74°N, 2.69°E at 1,000 masl. In addition seedlings of two full-sib families were included in the trial for a comparison of seedling and cut-

Figure 3-46. The relationship between ortet age and fraction of orthotropic scions following grafting with scions of *L. laricina* originating from Maine, USA. Greenwood et al. 1989.

Figure 3-47. The percentage of rooting, good root quality, plantable cuttings, and remaining clones in the field trial. One hundred and one *L. decidua* × *L. kaempferi* clones and 25 *L. kaempferi* × *L. decidua* clones were selected at ages two and three, respectively. Data from a trial at Latitude 45.74°N, 2.69°E and 1,000 masl evaluated at age eight. Pâques and Cornu 1991.
Some of the results from the comparison between seedlings and cuttings from the same family are shown in Fig. 3-48. The seedlings were superior to the cuttings in all traits except for field survival for the *L. decidua* x *L. kaempferi* family. However, this difference was not significant. Besides the significant difference shown in Fig. 3-48 there was a significant difference in height increment between ages 4-8 in the same family. In the paper it was claimed that cutting performance was superior to seedlings, which does not agree with the data presented in Table V of the paper. It was stated that more comparisons are required to reach any far-reaching conclusions.

The cuttings from the 21 *L. decidua* x *L. kaempferi* clones selected at age two showed a significantly better growth than the cuttings from old ortets (Fig. 3-49). It should be noted that the comparison is not straightforward since the genetic constitution of the two materials is not the same.

Data from one of the field trials presented in the above paragraph (lat. 45.74°N, long. 2.69°E at 1,000 masl) were presented by Pâques (1992). Nine blocks with 95 plants each were established; each entry was represented by two or three non-contiguous plots within each block. Survival, growth traits, basal sweep in five classes, and frequency of straight stems were studied. Cuttings from the following materials were included:

- *L. decidua* x *L. kaempferi* 14 full-sib families
- *L. kaempferi* x *L. decidua* 1 seed orchard progeny
- *L. laricina* x *L. kaempferi* 1 full-sib family
- *L. laricina* x (*L. decidua* x *L. kaempferi*) 1 full-sib family
- (*L. dec. x *L. kaempf.*) x (*L. dec. x *L. kaempf.*) 1 full-sib family

Two populations of *L. laricina* 20 ortets

The crosses with *L. laricina* as one of the parents yielded few seeds and the number of living seedlings in nursery was low. It was suggested that the weather conditions as well as differences in flowering phenology contributed to this limited success with *L. laricina* as one parent. It was noted that the survival was satisfactory for most of the entries except for the *L. laricina* population. The test locality is close to the margin of distribution for larch. Fig. 3-50 reveals that the rooting percentage was moderately good to good in the different genetic entries. However, the percentage of plantable cuttings dropped considerably in some of the entries. The *L. decidua* x *L. kaempferi* cuttings had the tallest trees and the largest height increment of all entries with strongly significant differences among entries (Fig. 3-51). The parents of the double hybrid family (DK) x (DK) were full-sibs. Therefore, it was speculated that cuttings from this family suffered from some inbreeding depression. However, the ortets did not reveal any signs of inbreeding depression. The growth of almost one meter of the *L. decidua* x *L. kaempferi* cuttings during...
the last growth period was impressive. It seems as differences among the entries increase over time, which might be attributed to increasing competition in the trial. There were also significant differences among ortets within entries for all growth traits. The percentage of trees with straight stems did not exceed 50% in any of the entries. The low percentages of straight trees in the \textit{L. laricina} population and the cross \textit{L. laricina} \textit{x} \textit{L. kaempferi} are conspicuous. The large percentage of trees affected by basal sweeps in the best growing entry is disturbing from a breeding point of view.

As stated in the paper far-reaching conclusions as to the usefulness of the different hybrids included in this study cannot be drawn until more families of the different entries are studied.

Verger and Pâques (1993) studied forcing methods for obtaining large numbers of scions in four experiments with 1-4 open-pollinated progenies from hybrid larch trees (\textit{Larix} \textit{x} \textit{eurolepis}). The first experiment concerning promotion of branchiness had five treatments of the cuttings besides the control: 50 ppm or 100 ppm of BAP, those treatments combined with topping of the cuttings, and finally topping without any BAP treatments. Experiment 2 comprised a comparison of cuttings with different sizes, 3-4 cm or 6-8 cm. No BAP treatment or topping were carried out. Experiment No 3 included three treatments of non-lignified scions, control, 50 ppm BAP, and topping. Experiment 4 comprised four open-pollinated progenies with the control treatment. All mother plants were kept in greenhouse and they reached a mean height of one meter after one year of cultivation. The basal part of the scions was treated with 0.1% butyric acid. The number of scions per square meter was 710. The percentage of rooting and the multiplication factor were presented. The latter is the product of percentage rooting and number of rooted cuttings per mother plant.

The treatment with 50 ppm BAP in Experiment 1 had significantly higher percentage of rooting and largest multiplication factor (Fig. 3-52). The high rooting capacity in this experiment compared to literature data was attributed to the juvenile character of the mother plants. In experiment 2 the multiplication factor for the 3-4 cm cuttings was more than twice as large as for the 6-8 cm cuttings. The multiplication factor for the non-lignified cuttings studied in experiment 3 was low in all treatments, 30-36. These figures were smaller than for the corresponding treatments of lignified cuttings in experiment 1. Also in this experiment, the BAP 50 ppm treatment had a significantly higher rooting percentage than the other treatments. Within the treatments there was no correlation between rooting percentage of the two types of cuttings, lignified or non-lignified.

**Figure 3-51.** Tree height increment between ages 4 and 8, height at age 8, percentage of trees with basal sweep, and percentage of trees with straight stems in a French trial at latitude 45.74°N, longitude 2.68°E and 1,000 masl. Cuttings from interspecific hybrids and two populations are included. D = decidua, K = kaempferi, and L = laricina. Pâques 1992.

**Figure 3-52.** The percentage of rooting of scions and the multiplication factor in five treatments and control:
1. 50 ppm of BAP (benzyl-amino-purine)
2. 100 ppm of BAP
3. Topping
4. Topping + 50 ppm BAP;
5. Topping + 100 ppm BAP.

The multiplication factor is the number of rooted cuttings x percentage of rooting. One open-pollinated family of \textit{L. kaempferi} \textit{x} \textit{L. decidua} was used. Verger and Pâques 1993.
There was a significant difference in rooting percentage among the mother plants. In experiment 4 the height growth and rooting percentage among the four progenies varied in the range, 90-102 cm and 60-76%, respectively, both being significant (Fig. 3-53). Strangely enough, the difference in multiplication factor was non-significant. A comparison of the two sizes of the cuttings was carried out for OP-1 and again the multiplication factor for the small cuttings was more than twice that of the large cuttings, 166 versus 70. It was concluded that the forcing of mother plants to increase the number of cuttings per mother plant is promising for rapid bulk propagation of hybrid larch. However, the observed differences among OP-families and clones within families with respect to cutting numbers per tree, rooting percentage, and multiplication factors constitute problems in bulk propagation. For application, the multiplication factor must be large enough to compensate for the high cost of vegetative propagation.

Peer and Greenwood (2001) reported on effects of rooting from:

1. Watering regime; Four treatments were used: misting for 10 seconds every ten minute (M1), misting for 6 seconds every 16 minute (M2), M1 + hand watering, and M2 + hand watering
2. Crown position; top, middle, and bottom third of five-year old trees. Five full-sib hybrid larch families comprising *L. decidua*, *L. laricina*, and *L. kaempferi* were used.
3. Age of orte; 1 – 7 years old trees. One hybrid European x Japanese larch family growing in Maine but originating from Germany was included. Misting for 12 seconds every 10 minute
4. Date of cutting collection; July and early September. The same five families as used above were included.

In all experiments 5cm scions were collected for rooting. The percentage of rooted cuttings was determined and the quality of the root system was grouped into five classes; 0 = no roots to 4 = multiple roots radially arranged around the base of the cutting.

There were significant effects of the watering regime on the percentage of rooted cuttings as well as on percentage of cuttings with good root quality (classes 3 and 4; 3 = more than one root but on the opposite side of the cutting Figs. 3-54 - 3-55). The low percentage of cuttings with good root quality is particularly pronounced in the M2 + supplemental watering treatment (Fig. 3-55, red squares). A fairly strong linear relationship between rooting percentage and root quality was noted for the M1 treatments (Fig. 3-54). To figure out whether there was a

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**Figure 3-53.** The height of mother plants, percentage of rooting of scions, and the multiplication factor in four open-pollinated families. Mean values for these three characteristics are shown. No BAP treatment or topping were carried out. The multiplication factor is the number of rooted cuttings x percentage of rooting. Four open-pollinated (OP-1 – OP-4) families of *L. kaempferi* x *L. decidua* were included. Verger and Pâques 1993.

**Figure 3-54.** The relationship between percentage rooting and root quality in five hybrid larch families exposed to two rooting regimes. M1 = misting for 10 seconds every ten minute (blue squares), M1 + H.0 = misting for 10 seconds every ten minute + supplemental hand watering (red squares). Peer and Greenwood 2001.

**Figure 3-55.** The relationship between rooting percentage and root quality in five hybrid larch families exposed to two rooting regimes. M2 = misting for 6 seconds every 16 minute (blue squares), M2 + H.0 = misting for 6 seconds every 16 minute + supplemental hand watering (red squares). Peer and Greenwood 2001.
family x treatment effect I calculated the relative rooting percentage for each family in each treatment (Fig. 3-56). It should be noted that the low percentage of rooting in the M2 without supplemental watering treatment causes the percentage deviations from the mean to be substantial. As seen from Fig. 3-56 the ranking changes for the other treatments were not pronounced, which means that the family x treatment effect is of less importance than the family effect. This was also stated in the paper. The effect of crown position of scions on the rooting percentage varied among the families. The purely European family (DD, Fig. 3-57) showed an increase in percentage from bottom to top while the opposite was the case for the L. laricina x L. decidua family (LD). As expected there was a significant family x crown position interaction for percentage rooting. Such an interaction was also observed for the root quality of the cuttings. With the large variation in rooting percentages of the families presented under the columns in Fig. 3-57 it is not surprising that the family effect was strongly significant too. Power curves were fitted to the observed data from the study of the effect of ageing on cutting performance (Fig. 3-58). This figure illustrates the rapid decline of the cutting survival and percentage of root quality with age. Even if the percentage of survival was around 40% at age seven, the percentage of high quality cuttings was less than 5%. The authors reported a linear decrease in rooting percentage over time with an r-estimate of 0.72.
Fig. 3-59. The relative rooting percentage of five hybrid larch families after cutting collection at July 14 and at September 2. E = European larch, J = Japanese larch, and T = tamarack larch. Peer and Greenwood 2001.

Fig. 3-60. The rooting percentage and number of cuttings per ortet from L. olgensis ortets of different ages. The ortets were growing in hedges. Wang et al. 1997.

3.6 Somatic embryogenesis

Nagmani and Bonga (1985) collected immature female strobili from L. decidua trees at weekly intervals from May until mid-August. Gametophytes were cultivated on media containing different concentrations of casein hydrolysate, glutamine, N-6-benzyladenine, naphthaleneacetic acid as well as 2% sucrose. Only five out of 1,440 explants produced callus. Callus from these five explants produced a large number of immature embryos, 2,505 – 4,197. The percentage of plantlets obtained varied in the range 3.7 – 12.1%.

Megagametophytes from weekly collection of female strobili from L. decidua trees were used as starting material for induction of haploid and diploid somatic embryogenesis by von Aderkas and Bonga (1988). Callus clones multiplied in 30 subcultures for more than two years were used as starting material for induction of embryoids. The focus of the paper was on detailed description of the development via microscopic observations rather than quantification of success rates of induction of embryoids. The haploid plantlets obtained were several times smaller than the diploid plantlets at a comparable stage of development.

In a follow-up report by von Aderkas et al. (1990) material was collected at times expected to be optimal for induction of haploid and diploid somatic embryoids. 2,4-dichlorophenoxy-acetic acid was used for induction of both haploid and diploid embryoids. For the diploid induction benzyladenine was also used. Different solidifying agents of the growth media were tested on Murashige – Skoog medium. The diploid embryogenic response after use of agar or Gellan gum was tested starting with 60 explants per treatment (Fig. 3-61). The success rate was much lower on Litvay medium solidified with agar. Few of the haploid cultures had well-developed embryoids.

Wang Qiuyu et al. (1997) studied the effect of hedging and ortet age on cutting production, cutting survival, and rooting success in L. olgensis. They reported that cutting survival peaked at the collection on August 10; one earlier and two later collection dates resulted in lower survival. Hedges kept at 60 cm gave larger number of shoots per cutting than hedges 20 cm or 100 cm. As seen from Fig. 3-60 the rooting percentage drops with age and the number of shoots per plant increases with ortet age.
Three cell lines with different ability to produce somatic embryos were included in a study on embryogenesis from haploid protoplasts of _L. decidua_ (von Aderkas 1992). The embryogenic tissue underwent plasmolysis with enzyme mixtures and then protoplasts were isolated from undigested tissue by passing the mixture to a series of screens. The efficiency estimated as number of colonies per 100 cells varied between 17 and 26 in the three cell lines. The development was arrested at an early stage in the line that previously was shown to produce limited numbers of somatic embryos. The efficiency of different compounds involved in regulation of the osmotic pressure was tested to promote production of somatic embryos but no significant differences between the compounds were noted.

Female strobili from reciprocal hybrids between Japanese and European larch were collected weekly from June 1st to July 13th at Petawawa research station by Klimaszewska (1989a) for induction of somatic embryogenesis of megagametophytes. Three cultivation media were tested. All three media contained 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4D), 0.5 mg/l benzyladenine (BA), and 2% sucrose. The further development of the somatic embryos was done by culturing them on media containing 0.2 mg/l kinetin 0.1 mg/l abscisic acid (ABA) but without the growth regulators. The sampling time had a great impact on the induction success with the highest percentages for June 22nd. This corresponds to 2-3 weeks after fertilization when the embryos are at the precotyledonary stage. The highest initiation percentage, 25%, was noted for the modified Murashige and Skoog medium with 1.46 g/l glutamine. Subculturing took place on media with ABA or kinetin. After the third subculture embryoids were developed and they developed primary roots and secondary needles. Abnormal morphology was noted in some cases. At the time of publication 35 somatic embryo plants were in cultivation in soil. Chromosome counts confirmed that embryoids were diploid.

Different methods for production of protoplasts and somatic embryos were studied by Klimaszewska (1989b). She used two cell lines of _Larix x eurolepis_ with different abilities to produce somatic embryos according to the above report. It was noted that the yield of protoplasts was higher in cell suspensions than in callus cultures. The characteristics of the protoplasts differed between the two cell lines. The percentage of uninucleate protoplasts with large vacuoles differed; 5% (line 1) versus 35% (line 2). The opposite relationship was noted for uninucleate protoplasts with dense cytoplasm; 50% (line 1) versus 25% (line 2). Three fractions of protoplasts were obtained from the density gradient centrifugation that was carried out. Fraction 2 protoplasts, in which 80% of the protoplasts were uninucleate and had a dense cytoplasm, continued their development to some extent. Cell divisions in line 1 were higher than in line 2 but the latter line had higher number of somatic embryos. These embryos gave rise to plants that were cultivated in soil. Finally, low osmotic pressure was critical for obtaining somatic embryos.

Cornu and Geoffrion (1990) induced somatic embryogenesis in two cell lines of _L. decidua_ using 10mg/L of 2,4D, BA (concentration not given) and 5mg/L kinetin under darkness. Maturation took place at 16 h day length. Fifteen times higher number of somatic embryos was obtained in day length treatment for six weeks than under six weeks of darkness. The number of somatic embryos peaked at 300g PEG/L.

Pitel et al (1992) focused on changes in enzyme activity and protein patterns during the course of development of somatic embryos in the two cell lines studied by Klimaszewska (1989a and b). Ten enzyme systems were studied with a decrease of the enzyme activity over time. The protein pattern differed between callus tissue and somatic embryos but without any difference between the two cell lines.

The first successful production of somatic embryos in _Larix_ from isolated embryos was reported by Thompson and von Aderkas (1992). They used embryos from _L. occidentalis_ cones harvested at three different dates. The effect of ABA concentration (0.025-100 mM) and duration of the treatment (1-4 weeks) in the maturation media was studied in seven cell lines. Five different concentrations of ammonium nitrate, 5-25 μM were tested on material collected at July 13. Only the highest concentration gave a lower percentage of embryogenic cultures, 60% versus 72-93% for the other four concentrations. These rates of success were much higher than in the material collected on June 27 and July 5, 21-32%. The stage of embryo development was suggested as the cause of this difference. It was also noted that within cones there was a large variation in stage of embryo development. Only four of the seven cell lines in the ABA study produced any somatic embryos.

**Figure 3-61.** The impact of solidifying medium on the range of number of diploid embryoids out of 60 explants per each family. Three intraspecific ( _L. decidua_ and _L. kaempferi_) and three interspecific families ( _L. decidua x L. kaempferi_) were included. Von Aderkas _et al._ 1990.
The line that produced the largest number of somatic embryos had an optimum production at the two lowest concentrations of ABA, 0.025 and 0.1 μM, for one to two weeks of treatment. One line had a peak at 40 μM ABA and four weeks of treatment. Another produced somatic embryos only in absence of ABA. Finally, the fourth line produced few somatic embryos that any pattern could not be revealed. Plants were only obtained from the line with the highest production of somatic embryos.

This investigation illustrates well the difficulties in obtaining plants from somatic embryos. Standardized methods are hard to reach when there is variation in stage of embryo development at a certain collection date, even within a cone. Furthermore, there seems to be large variation in response of individual cell lines to the treatments they are exposed to.

Strobili from one cross between *L. kaempferi* and *L. decidua* were collected at three different dates, May 31st, June 12th and June 20th by Lelu et al. (1994b). The immature zygotic embryos were cultivated either on Murashige – Skoog medium (Becwar et al. 1990) or Gupta and Durzan (1986) medium. The media contained 9μM 2,4-D and 4.5 μM ABA and 90 mM sucrose. In one experiment seven lines were exposed to four levels of ABA, 0 - 60 μM, and three levels of sucrose, 0.1 – 0.4 M. Fig. 3-62 shows that the first collection date, May 31st, had the highest initiation of embryonal mass. The induction of embryonal mass in this investigation, 55%, was higher than in the earlier study by Klimaszewska (1989). This was attributed to the dissection of embryos; in this investigation no gametophytic tissue was present in the dissected material. The amount of sucrose in the medium influenced the number of somatic embryos per gram fresh weight strongly (Fig. 3-63). Thus, in treatments with 0.4 M sucrose no somatic embryos at all were found. Similarly, the control without ABA did not produce any somatic embryos. Both the effect of ABA and sucrose concentra-

- **Figure 3-62.** Initiation of embryonal mass %.

- **Figure 3-63.** The number of mature somatic embryos per gram fresh weight in different treatments after induction of somatic embryogenesis in a *L. kaempferi* x *L. decidua* hybrid. Lelu et al. 1994b.

- **Figure 3-64.** Number of mature somatic embryos in four treatments of embryos of seven lines (LB – 83) of *L. deci-

dua* x *L. kaempferi* hybrids:

  - 40μM ABA + 0.1 M sucrose
  - 40μM ABA + 0.2 M sucrose
  - 60μM ABA + 0.1 M sucrose
  - 60μM ABA + 0.2 M sucrose

*Note the logarithmic scale.* Lelu et al. 1994b.
embryos were removed from the embryonic mass after three or four weeks of cultivation on maturation medium. A part of these somatic embryos was then transferred to the surface of the germination medium. These treatments are referred to as 3W and 4W, respectively. Other somatic embryos were placed for one week on a nylon mesh on top of MSG (Becwar et al. 1990) medium containing 1 μM indol butyric acid, 40 μM ABA, and 0.1 M sucrose. These treatments are referred to as 3W + 1 and 4W + 1. Each treatment had 30 somatic embryos. Germination took place on growth regulator free MSG medium. Radi
cle emergence was used for classifying a somatic embryo as germinated. A germinated somatic embryo with an epicotyl was considered as a plantlet. Growth functions of germination and plantlet production were focused on in this investigation. The maturation process was studied after transfer of embryonic mass to MSG medium with six different treatments:

- 1 μM IBA + 20 μM ABA + 0.1 or 0.2 μM sucrose
- 1 μM IBA + 40 μM ABA + 0.1 or 0.2 μM sucrose
- 1 μM IBA + 60 μM ABA + 0.1 or 0.2 μM sucrose

To illustrate the dynamics of germination and plantlet production different growth functions were tested. The best fit of the functions for germination and plantlet frequency was obtained for a Weibull function with four parameters.

After a 5-week germination period the plantlets were transferred to glass jars with sterile substrate. Each glass jar contained ten plantlets and was closed. The plantlets were kept for one month in glass jars and then exposed to 16-h day length under controlled growth conditions and the jars were gradually opened. After another three to four weeks the acclimatized plantlets were potted into containers with ordinary substrate. Two to three weeks later the plantlets were placed in green house.

Fig. 3-65 reveals that the 3W treatment had the highest percentage of germination and plantlet production while the 4W+1 treatment had the lowest percentages. The effect of treatment was strongly significant.

To get an impression of the dynamics of germination and plantlet production the number of days for reaching 90% of the maximum frequency of germination and of plantlets were determined. The 90% germination was reached extremely fast in the 3W+1 treatment (Fig. 3-66). The 4W+1 with 60 μM of ABA treatment showed the slowest development (Fig. 3-66).

Reaching of 90% of plant production was slower in the 60 μM of ABA treatment than in the corresponding treatments with 20 μM ABA (Fig. 3-67). It should be noted that percentage of plantlets had not levelled at the end of the experiment in the two 3W+1 treatments.
The plantlets transferred to glass jars resumed growth within one week. Also with respect to the percentage of plantlets transferred for outdoor cultivation the 4w + 1 treatment resulted in the lowest percentage (Fig. 3-68). The three other treatments did not vary much, 75 – 84%. In all 893 plantlets of the 923 planted survived.

It is evident from this investigation that plantlet frequency and time required to get plantlets from somatic embryogenesis is strongly affected by the treatment and that a considerable number of somatic embryo plants were generated within a limited time; 18 – 20 weeks after onset of maturation.

Genetic differences in induction of somatic embryos of L. decidua, and its two hybrids with L. kaempferi were presented by Lelu et al. (1994c). The collection of material was carried out at two occasions; the first date included embryo stages between no primordia visible to presence of cotyledonary primordia (stage I). The second date collection comprised embryos with elongating to fully developed cotyledons (stage II). Induction of somatic embryo-genesis from mature zygotic embryos and from cotyledons and needles was also studied.

As seen from Fig. 3-69 induction of embryonal mass was larger in the material collected at stage I than at stage II. It is evident that there are family differences in the success rate of embryogenic mass induction. However, it cannot be excluded that the observed differences can be attributed to differences in developmental stage of the material collected. The difference in response between the reciprocal crosses involving clones 267d and 3026k is striking. In the three seed lots used for induction of embryogenic mass from mature zygotic embryos only one produced embryogenic mass in a low proportion, 5%. It was stated that there might be genetic differences in this case. The low number of seed lots does not permit too far-reaching conclusions.

Embryonal masses from cotyledons and needles were obtained after one week of treatment with 4.5 M BAP in low frequencies, 8 and 3%, respectively. Without pre-treatment the response was meagre, just in one case out of 193 (needles) embryonal mass was obtained.

Maturation capacity was estimated as embryos per gram fresh weight of embryonal mass. Once embryonal mass is initiated the maturation treatment led to a large number of somatic embryos (Fig. 3-70). This means that there are great possibilities to raise a huge number of uniform materials.

The role of ABA and its glucose ester for germination of somatic embryos and plantlet production was investigated by Lelu and Label (1994). They used one line of hybrid larch (L. kaempferi x L. decidua), which had responded positively to induction of embryonal masses. After initiation of embryogenic masses actively growing masses were transferred to growth-regulator free MSG media.

Embryos/g fresh weight embryonal mass

![Figure 3-70. Maturation capacity estimated as number of somatic embryos per gram fresh weight of embryonal mass. The embryonal mass was initiated in four types of material. Lelu et al. 94c.](image)
medium and kept for a week at 16h light conditions. Then the pieces were transferred to two maturation media, one containing no ABA or 60 μM ABA. Both MSG media contained 1μM IBA and 0.2 M sucrose. After four weeks the characteristics of the somatic embryos was recorded. Germination and plantlet development were studied after cultivation on growth regulator free MSG medium. After 2, 3, 4, and 5 weeks on maturation medium (MSG 60 μM ABA) the germination of somatic embryos and plantlet production were followed for six weeks. Somatic embryos from ABA-free MSG medium were removed from the embryonal masses after four weeks of culture. There was a clear morphological difference between the somatic embryos from the two different maturation media. After four weeks on the MSG 60 μM ABA almost all (97%) somatic embryos had reached the cotyledonary stage and showed resemblance with zygotic embryos. Contrary to this, the somatic embryos cultivated on the ABA-free medium did not show any homology with a zygotic embryo.

In Fig. 3-71 I have illustrated the percentage of somatic embryos leading to germination and plantlet production in one hybrid line of L.kaempferi x L. decidua following different durations of the treatment with 60μM ABA. The percentages emanate from recording after 6 weeks. The ABA and ABA glucose ester (ABAGE) content in pM is also shown. Lelu and Label 1994.

After one week on maturation medium MSG 60μM ABA, the level of ABA in the embryogenic mass dropped from 100 pM ABA at the shortest treatment (2 weeks) to 60 pM ABA at the longest maturation treatment (5 weeks). Contrary to this, the ABA level in the somatic embryos increased rapidly from approximately 20 pM ABA at week 3 to 150 pM ABA at week 5 (Fig. 3-71). The corresponding estimates of ABA in the material cultivated on ABA-free media were several times lower and close to zero in the somatic embryos. As regards the ABAGE, a similar pattern for the somatic embryos was noted. In this case the strong increase occurred between weeks 4 and 5 (Fig 3-71). The level at week 5 was approximately three times lower than for pure ABA, 50 pM.

The role of ABA for germination and plantlet production of somatic embryos of hybrid larch (L. Kaempferi x L. decidua) was further analyzed by Label and Lelu (1994). In this study the ABA content in the maturation medium varied; 0, 20, 40, and 60 μM ABA. The treatment lasted for four weeks. As seen from Fig. 3-72 ABA promoted germination and plantlet production but no increase was obtained beyond 20 μM ABA, rather there was a slight decrease in plantlet production. The ABAGE showed an almost identical response pattern as ABA.
A histochemical investigation on the same material was presented by Gutmann et al. (1996). In Table 3-2 I have compiled some details that differ between the 60 μM ABA treatment and the control material. Already two weeks after the end of ABA treatment there was a noticeable difference. The ABA treated somatic embryos passed through a fairly normal embryo development while the control embryos expanded without cell divisions. Storage proteins were only detected in the ABA treated embryos. The presentation was accompanied by excellent micro photographic illustrations.

Another publication treating desiccation tolerance in the same material was presented by Lelu et al. (1995). Somatic embryos were grown in darkness for two days on growth regulator free medium with 0.1 or 0.4 M sucrose (preculturing). After that somatic embryos were transferred to a special type of empty containers and other somatic embryos were transferred to containers with distilled and sterile water. This treatment lasted for one week at a temperature of 4°C. The treatment at 0.1 M sucrose was designated as D1 (D = desiccation) while the other on 0.4 M sucrose was designated as D2. In a third treatment (D3) the temperature was 25°C instead of 4°C and took place without any preculturing. To obtain the desiccated state of seed embryos, the somatic embryos were either kept in empty vessels or exposed to a supersaturated salt solution for one week in darkness at a temperature of 4°C. The maturation treatments were the same as in Lelu et al. (1994b).

In Figs. 3-73 and 3-74 I have summarized the final percentages of germination and plantlet production. It is obvious from these figures that the longer maturation treatments (4W and 4W +1) had a negative impact on the percentages in the non-dried material. Contrary to this, the 3W treatment had the lowest percentages in the D2 treatments with the highest content of sugar, 0.4 M sucrose. In the treatment with the highest temperature during dehydration, D3 25°C, there were small differences among the four maturation treatments. Most differences among treatments were significant. It was concluded that: Drying somatic embryos under high RH (= relative humidity) suppressed the unfavorable effects of prolonged maturation treatment and improved germination rates. This was attributed to a decrease of the inhibitory levels of ABA during drying. Except for plantlet percentages in the D2 and D3 treatments, the rate of reaching the final percentages of germination and plantlets was highest in the treatment.

Table 3-2. Features of the histological examination of somatic embryo development in control and 60 μM ABA during maturation of somatic embryos of hybrid larch (L. kaempferi x L. decidua). Gutmann et al. 1996.

<table>
<thead>
<tr>
<th>weeks</th>
<th>60μMol ABA</th>
<th>No ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>Size 0.2-0.4 mm; continuity between hypocotyl and root cap</td>
<td>Essentially similar to the ABA treated material</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Overall elongation of the embryo; Differentiation of cotyledons; Phenolic deposits in the basal region</td>
<td>Globular instead of cylindrical shape of the embryos; The innermost cells highly vacuolated and expanded; Starch mainly in subapical and lateral regions</td>
</tr>
<tr>
<td>3 weeks</td>
<td>Development of a shoot apex; Cotyledons ≈ 150 μm in lengths; Root meristem developed; Starch grains in the peripheral regions; Phenolic deposits in the basal region</td>
<td>Enlargement owing to cell expansion without cell division; Poorly developed root meristem; Basal region with a thick layer of polyphenol-accumulating cells of the root cap</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Well-developed root organization; Storage proteins in some embryos; Catechins in polyphenol-accumulating cells</td>
<td>Stubby embryos had germinated; Contrary to the ABA-treated embryos, polyphenols and starch grains accumulated in peripheral cells at base of the hypocotyl; Catechins and proanthocyanins were present; Storage proteins absent</td>
</tr>
</tbody>
</table>

Figure 3-73. The final level of germination of somatic embryos in a hybrid line of L. kaempferi x L. decidua exposed to different drying treatments (for explanation see text) after four maturation treatments with 60 μM ABA for three or four weeks and with one growth regulator free week before start of germination (3W + 1 and 4W + 1). Lelu et al. 1995.
ments with the highest percentages of germination and plantlets. The differences in rate were significant. The test of the tolerance of somatic embryos against drying under low relative humidity, somatic embryos from treatments 3W + 1, 4W, and 5W and 0.2 M sucrose, were exposed to four levels of relative humidity in the range 59-100%. There was a drastic reduction in germination at the lowest humidity level and this was especially pronounced for the 3W + 1 treatment. The reduction in plantlet production at the low humidity levels was still more pronounced for the two shorter maturation treatments (Fig. 3-75). A rapid loss of water might explain the reduction of germination and plantlet formation at the low humidity level.

Immature embryos were collected from four open-pollinated families of _L. laricina_ growing in France (Klimaszewska et al. 1997). One family was used in 1992 and three families in 1994. The excised embryos were cultivated on MSG medium (Becwar et al. 1990) containing 1.45 g L-glutamine×L.1, 0.1 g inositol×L.1, 0.03 or 0.09 M sucrose, 9 mM 2,4-dichlorophenoxyacetic acid (2,4-D), 4.5 mM BA, and 0.4% gellan gum. Three embryonic lines were obtained from the 1992 collection. The maturation took place on MS media containing various concentrations of ABA, sucrose, and PEG. The cultures were grown at a day length of 16 hours at 24°C. After four weeks on the maturation media, the cotyledonary somatic embryos were transferred to MSG containing 0.06 M sucrose. After development of roots and secondary needles the plantlets were transferred to soil and kept at high air humidity for the first three days.

The production of embryonic mass was low in both years and for the two embryo stages at collection of the material for somatic embryogenesis. Only in the material collected at precotyledonary or cotyledonary primordia stages seven out of 16 explants were obtained. In the media containing 0.06 M or 0.1 M sucrose no or just a few somatic embryos were obtained.

The relationship between somatic embryo relative humidity and plantlet percentage in three maturation regimes with 60 µM ABA:

- 3 weeks + 1
- 4 weeks
- 5 weeks

Lelu et al. 1995

Fig 3-76 reveals that the highest number of somatic embryos was obtained for the medium combination: 0.4 M sucrose + 5% PEG + 40µM ABA.

It is evident that PEG promotes production of somatic embryos when the media contain 0.2 M sucrose. At 0.4 M sucrose there is an optimum at 5% PEG. The osmolality of the media was significant for the success of somatic embryo production. Osmolality values in the range 276-543 mM/kg promoted production of somatic embryos.

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The germination percentage of the somatic embryos did not exceed 50% and was low in the 10μM ABA treatment (Fig. 3-77). Twenty-five seedlings were planted in field.

Kim et al. (1999) carried out weekly collections (late June – early August) of immature embryos from 12 L. kaempferi clones in a Korean seed orchard. The excised immature zygotic embryos were cultivated on three different media:

- LM (Litway et al. 1985)
- LP (Quoirin and Lepoivre 1977)
- MS (Murashige and Skoog 1962)

9.0 μM 2,4D (2,4-dichlorophenoxyacetic acid) and 4.4 mM BAP (N6-benzylaminopurine) were added to each growth medium. Two replications each with 50 embryos were cultivated on the three media at each collection date. Recording of embryonic tissue was carried out after cultivation for eight weeks at 25°C in darkness. Subculturing for proliferation of embryonic tissue from the three media took place on ½ LM with 58.4 mM sucrose. The embryonic tissue was subdivided into pieces and then cultured in darkness.

Maturation experiments were limited to one embryonic line. In all, eight concentrations of ABA in the medium were tested, 0 – 81.2 μM. All media contained 87.6 mM sucrose and 6.8 mM L-glutamine. The cultures were kept on these media for 1-5 weeks at darkness and then cultured on ½LM medium without ABA and 16h day length. After 12 weeks the numbers of cotyledonary stage somatic embryos were recorded.

The collection of material on July 30 (day 60) had the highest percentage embryonic tissue (Fig. 3-78). The relationship between collection date and percentage of embryogenic mass did not differ much among the three media. In most cases proliferation stopped in material collected before July 22. In contrast with other taxa it was noted that the embryogenic tissue from LP and MS media failed to proliferate. It was suggested that L. kaempferi requires different composition of the growth medium than other taxa. Of the 294 embryonic lines initiated only one could be proliferated in subsequent culture, which must be regarded as distressing.

The mean values for number of somatic embryos obtained after different duration of the maturation treatment showed an extremely strong fit to a third-degree polynomial curve (Fig. 3-79). It seems as ABA treatment for three and four weeks gives the largest yield of somatic embryos. There was no clear relationship between ABA concentration and mean number of somatic embryos over the duration of the ABA treatment. There was a tendency for higher numbers of somatic embryos (mean = 10.0) for the lowest ABA concentrations, 0 – 4.4 μM than for the higher ABA concentrations, 8.1 – 81.2 μM (mean = 5.7). The biological interpretation of the curves in Figs. 3-78 and 3-79 is problematic.
3.7 Transformation

A study focusing on techniques for microprojectile bombardment of naked plastids of hybrid larch (L. decidua x L. kaempferi) was reported by Charest et al. (1991). They used B-glucuronidase, neomycin phosphotransferase II, and chloramphenicol acetyltransferase (CAT) genes in their study. The CAT gene was found to be the most useful. The transient gene expression was studied following the use of plasmid pCaMVCN containing a 35S-CAT-35S gene construction. The optimum conditions were found to be:

- 300V, 150 μF, and 300 μg/ml pCaMVCN DNA.

Eight promoter systems were used in experiments studying the transient expression of the GUS gene following microprojectile bombardment of embryogenic cell lines of hybrid larch (L. decidua x L. kaempferi) by Duchesne and Charest (1992). The wheat abscisic acid inducible Em gene promoter gave the highest β-glucuronidase expression of the promoters tested. The expression was increased by addition of ABA to the culture medium.

Twenty-one cell lines (5 haploid and 16 diploid) of four different taxa of Larix were exposed to bombardment with tungsten microprojectiles of four different sizes (Duchesne et al. 1993). The plasmid pRT99GUS containing genes coding for GUS (uidA) and neomycin phosphotransferase II were transferred by the biolistic particle delivery system. Bombardment took place at different times (0–13 days) after transfer to fresh culture medium. The success of the transfer was based on the number of expression units (= number of islands of cells showing blue coloration) per 100 mg fresh weight.

The increase in weight of the embryogenic mass varied in the range 20–638%. The mean weight increase of the haploids was estimated at 32.6%. The number of somatic embryos per 100 mg fresh weight varied in the range 0–49.3; with absence of somatic embryos in five cell lines; two of them being haploid. Tungsten particles with a diameter size of 1.11 μm led to the highest gene expression. The treatments after 5–6 days of culture in fresh medium gave the best response to treatment. There were no clear trends as regards GUS expression units and taxon (Fig. 3-80). The highest value was obtained for one haploid L. decidua cell line. The only haploid cell line of L. kaempferi had a high estimate of expression units. There was no clear relationship between increase of growth and GUS expression units for the 16 diploid cell lines. No somatic embryo plants were reported.

Two treatments that had been successful for production of mature somatic embryos and their germination were chosen for transformation experiments by Klimaszewska et al. (1997):

- 0.4 M sucrose + 40 μM ABA
- 0.2 M sucrose + 10% PEG + 40 μM ABA.

Microprojectile bombardment was done by use of the Biolistic PDS-1000/HE device. Three vectors were used:
- pRT66gus carrying hygromycin phosphotransferase
- pRT55gus carrying dihydrofolate reductase
- pRt99gus carrying neomycin phosphotransferase II (NPTII).

Plasmid vectors containing the ubiquitine promoter from sunflower, pCGUA0 and pCGUAi, and the promoters from maize Bronze 2 gene and its transcriptional activators, pC1 and pR, as well as pGUSINT with a 35S promoter were used.

The plasmid pCGUA0 resulted in the highest level of transient GUS in the florescent MUG assay (2mM MUG in GUS extraction buffer with 40% methanol) while the lowest level was obtained with pGUSINT. The luciferase firefly gene was also tested for transient gene expression. One vector pPCV8145 yielded hig levels of transient gene expression.

In spite of microprojectile bombardment of several hundreds of somatic embryos only two of them showed GUS activity. The vector used in this case was pB1426 while three other vectors did not yield any GUS activity in the somatic embryos. One of them gave rise to plants with uniform expression of the GUS gene in hypocotyl, cotyledons and roots but without any further growth. The other line gave rise to plants with random expression of the GUS gene.

In conclusion, transformation was achieved but it failed to give rise to any growing transformed plants, which must be distressing for the amount of work invested in this project.
3.8 Summary

Ambitious reciprocal recurrent selection programs were developed for production of *L. decidua* x *L. kaempferi* seeds. Some with a detailed presentation of numbers for breeding populations and for seed orchards. The inclusions of supportive research for the programs were also outlined. One so called cathedral tunnel seed orchard was developed and implemented for production of hybrid larch seeds. The consequences of the unique pattern of meiosis in larch pollen mother cells for location of seed orchards were touched upon. Especially, location of *L. sibirica* seed orchards under maritime climatic conditions might be problematic. A series of investigations were carried out to clarify flowering biology after open pollination and artificial crosses. In one investigation the percentage of full seeds was highest in the middle part the tree crown. Opposite results were also reported. As expected, selfing resulted in much lower percentage of full seeds than after open pollination. Embryo degeneration was the dominating cause for this observation. Lack of pollination is another important contributing factor. Receptivity was found to last for short time only.

Most studies on flowering stimulation comprised *L. decidua*, *L. kaempferi*, and *L. laricina*. Gibberelline GA$_4/7$ alone or in combination with root pruning or girdling stimulated flowering. Gibberellin combined with root pruning or girdling showed the strongest effects. The point of time for promotion treatment is critical. For *L. laricina* the effect on young trees, < 10 years, were much stronger than on old trees, ≈ 50 years. Flowering stimulation of 1-2 years old plants by GA$_4/7$ was observed. Reduction of flowering was noted in some investigations. Heat treatment caused increased flowering. Mulching promoted female and male flowering. This was attributed to drought caused by mulching. Techniques for collection of pollen and for minimizing pollen quantities used in artificial pollinations were successfully developed.

Great success in production of hybrid European x Japanese larch was reported for three German seed orchards with one female clone of *L. decidua* and several *L. kaempferi* clones. Alarming selfing, > 60%, was reported for a Finnish seed orchard with one *L. sibirica* clone and six European larch clones. High percentages, > 80%, of hybrids *L. gmelinii* x *L. kaempferi* was noted for a Japanese seed orchard with one row of female clones surrounded by four rows of male clones. The percentage of larch hybrids may be improved by the hybrid seed orchard design such as one clone of one species as female and several clones of the other species as males. Generally rooting success and root quality of cuttings are influenced by:

1. Treatment conditions
2. Position in the tree crown for cutting collection
3. Age of ortet with a negative relationship with age
4. Date of cutting collection
5. Genetic factors

Broad sense heritability for number of major roots was highest in the first date of cutting collection in *L. laricina*. The ratio male/female strobili per graft with scions from mature trees varied strongly among four families of *L. laricina*. Seedlings of *L. decidua* x *L. kaempferi* and reciprocal families showed superior growth to their corresponding cuttings. $F_1$ trees of *L. decidua* x *L. kaempferi* showed a better growth performance than $F_2$. High multiplication factors for scions production were noted for several treatments.

Mainly during the 1990ties, great efforts were devoted to production of somatic embryos. For the maturation of somatic embryos ABA, PEG, and sucrose concentrations as well as drought treatments were tested. Different duration of treatments were also tested. Large numbers of somatic embryos were obtained following treatment with 20 and 40 µM ABA of immature embryos. The collection date (= the stage of the immature embryo) had a great impact on the production of somatic embryos. Transformations by aid of microprojectile bombardment were tested. The GUS gene was used to assess the success of the bombardment. Different taxa responded differently on the bombardment. No fully transformed plants were reported.
4. References


Fedorkov, A. 2014. Vitality and height growth of two Larix species and provenances in a field trial located in north-west Russia. Silva Fenn. 48 article id. 1053. 7pp.


