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# Recent Genetic Research

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Cover photo: Lime tree in bloom at Fiholm Castle
Photograph: Inger Ekberg

# Preface

*Tilia* is a widely spread genus in America, Asia, and Europe. Several *Tilia* genetics studies were recently published. As in previous reviews of recent genetics studies we have tried to summarize published reports on recent *Tilia* genetics research. A considerable part of the recent genetic research on *Tilia* is concerned with taxonomy. *Tilia* is of limited economic importance, as the wood of *Tilia* species is not used. Trees are frequently planted along the street pavements and avenues of towns but rarely in gardens.

The genus *Tilia* ('lime' in English, 'linden' in German and sometimes in English) consists of about 30 species in temperate regions of the northern hemisphere; for a general discussion see e.g. Clapham et al. (1989) and Pigott (2012). Linnaeus recognized only one European species of lime, which he called *Tilia europaea*. This is now split into 3 species: *T. cordata, T. platyphyllos,* and *T. vulgare.* The natural range of *T. cordata* extends across central, southern, and eastern Europe with its northern limit in southern Scandinavia. *T. platyphyllos* grew over the greater part of northern Europe in the warm postglacial period, but is now common as a native tree only in southern Europe. *T. vulgare* is the most commonly planted tree in Britain and Scandinavia and is thought to be a hybrid between *T. cordata* and *T. platyphyllos.*It appears to have formed frequently in the past and is not completely sterile; the 3 species species all have the same chromosome number. An alternative for *T. vulgare* is *T.* x *europaea.* Linnaeus also recognized only one American species of lime, *T. americana.* Much recent genetic research, often using molecular characters, aims at clarifying the taxonomic relationships, and variation within species and presumptive subspecies, of the genus *Tilia*.x

As usual graphic illustrations are in focus in our review. It should be noted that none of the illustrations were taken from the original papers. As in previous books, papers written in languages that are not understood by the scientific community are not treated. An apology for missing relevant literature in our search for *Tilia* genetic investigations.

Uppsala, May 2024

*Gösta Eriksson and David Clapham*

Contents

## Among-species differentiation

Cai et al. (2015) presented the complete plastid genome for 4 eastern Asian *Tilia* species and presented the species relationships with respect to haploplast genotypes. The following 4 species were included in this study:

*T. amurensis*
*T. mandshurica*
*T. oliveri*
*T. paucicostata*

The plastid genomes of these 4 species were compared with the related species *Gossypium hirsutum* and *Theobroma cacao*.

The base pair size varied in the range 162,653–162,736 for the 4 *Tilia* species, which was slightly larger than for *G. hirsutum* and *T. cacao*: 160,301 and 160,604. The genomic structure was rather similar to the structure of most angiosperms. Among the 130 coding genes 117 were unique and they are collinearly arranged. The number of protein-coding genes was 85 in all species except *G. hirsutum*, which had 84 such genes. There were only minor differences among the 4 *Tilia* species. *T. amurensis* differed most from the 3 other *Tilia* species. It was noted that the evolution of *Tilia* plastid genomes was extremely slow and slower than in the related *Gossypium* genus.

Semirikova et al. (2020) sampled 25 *T. cordata*, 2 *T. sibirica*, and 2 *T. dasystyla* populations for analysis of chloroplast DNA. The latitudinal range of the 25 *T. cordata* populations was 49.8–58.3°N and longitudinal range was 23.8–68.4°E. The 2 *T. sibirica* populations originated from the far east part of Russia, long. 87.1 and 87.2°E, respectively. The 2 *T. dasystyla* populations originated from Crimea at latitudes 44.5 and 44.6°N, respectively. Six different haplotypes were studied.

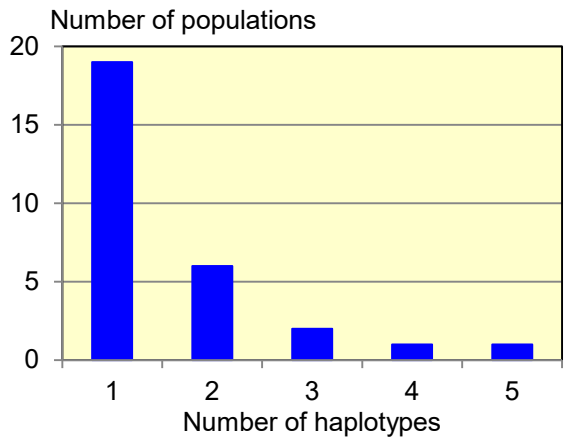


*Figure 1-1. The number of populations with 1–5 haplotypes in 25* T. cordata*, 2* T. dasystyla*, and 2* T. sibirica *populations mainly from Russia studied with 6 haplotypes. Semerikova et al. 2020.*

It was noted that the 6 haplotypes differed considerably from each other. Of the 29 sampled populations, 19 were monomorphic while one population had 4 haplotypes and another had 5 haplotypes (Fig. 1-1). A majority of the populations west of longitude 45°E had 2 or more haplotypes (Fig. 1-2). The population from lat. 59.98°N and long. 30.02°E was the most diverse and contained 5 haplotypes. Only one tree from this population contained the t6 haplotype, which occurs in *T. platyphyllos*. The 2 *T. dasystyla* and the 2 *T. sibirica* populations were both monomorphic. The 2 former populations carried a unique haplotype while the 2 *T. sibirica* populations had the most common haplotype denoted t2. 341 of the 503 trees analyzed carried this haplotype. The second most common haplotype, t1, was represented in 117 trees and monomorphic in 3 eastern populations, longitudes 56.49–57.57°E. These 2 haplotypes are not found in Western Europe. The haplotype diversity observed was attributed to the presence of different refugia during the latest glaciation, with t1 and t2 originating from refugia in southern Siberia and/or the Urals and/or the Russian plain.

Shekhovtsov et al. (2022) sampled material from 13 lime tree populations from Belarus in the west (long. 31.88°E) to Khabarovsk in the east (long. 134.76°E) for clarifying the phylogeny of *Tilia* species via genotyping-by-sequencing analysis. The majority of populations (10) were classified as *T. cordata* or as its subspecies. Two populations originated from the Far East, *T. amurensis* and *T. taquetii*. Finally, one population from the Caucasus classified as *T. begoniifolia* from Armenia was also included in this study.

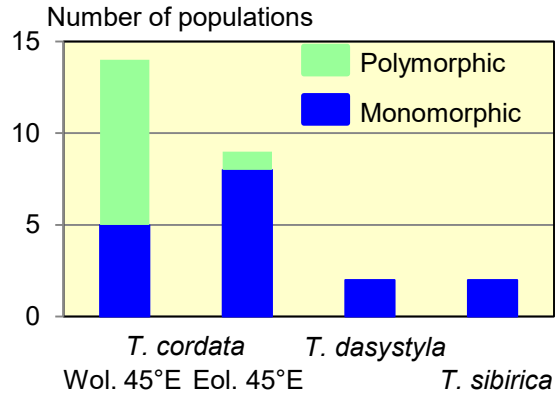


*Figure 1-2. The number of monomorphic and polymorphic populations of* T. cordata *west of longitude 45°E and east of longitude 45°E as well as in 2 populations of each of* T. dasystyla *and* T. sibirica*. Six haplotypes were studied. Two populations containing only 2 trees were excluded. Wol. = west of longitude 45°E, Eol. = East of longitude 45°E. Semerikova et al. 2020.*

The Maximum Likelihood algorithm in RAxMLv.8.2.12 and Bayesian in MrBayes 3.2.7a were used for creation of a phylogenetic tree. Between 1.8 and 23.1 million reads were obtained in the different sampled populations.
Three main clades were identified:

*T. cordata* with its subspecies
*T. amurensis* and *T. taquetii*
*T. begoniifolia*

A further subdivision of *T. cordata*, mainly geographic, was identified:

*T. cordata* subspecies *sibirica* 2 populations
*T. cordata* subspecies *nasczokinii* 2 populations and subspecies *novosibirsk* 1 population
*T. cordata* subspecies *cordata* 3 populations
*T. cordata* subspecies *armenia* and *dagestan* 2 populations

It should be noted that the subspecies *sibirica* and *nasczokinii* by some authors are regarded as full species. They both differ genetically and morphologically from *T. cordata* subspecies *cordata* but the genetic difference is smaller than those between the Caucasian subclade and subspecies *cordata*. Therefore, the authors preferred to regard them as subspecies rather than as separate species. Since no western European populations were included in this investigation there might still be another subclade of *T. cordata*. It is noteworthy that these geographically so widely separated entomophilous populations do not show more pronounced differentiation. A more continuous distribution during earlier times might be a factor contributing to the observed results.

The internal transcribed spacers (ITS) of nuclear ribosomal DNA were used for identification of *Tilia* species from the Hyrcanian forest in northern Iran by Yousefzadeh et al. (2012). Trees from 13 populations were genotyped.
Based on the ITS analysis, 3 main groups A–C were identified (Table 1-1). This table shows that most of the populations belonged to group A with 2 subgroups, of which one was classified as a separate species, *T. hyrcana*. The morphological traits vary in the A1 group. There was a geographic separation of the 3 main groups.
It was concluded that ITS data are useful for taxonomic classification of *Tilia* species.

The complete chloroplast genomes of 5 *Tilia* species were compared with those from 7 previously analyzed *Tilia* species (Yan et al. 2022). Of the 130 genes found in all species, 113 were found to be unique. For the phylogenetic construction, 5 hypervariable region genes were used. The 12 species were grouped into 3 clades.

| | | |
|---|---|---|
| *T. americana* | *T. amurensis* | *T. platyphyllos* |
| *T. miqueliana* | *T. mongolica* | *T. cordata* |
| *T endochrysea* | | *T. mandshurica* |
| | | *T. taishanensis* |
| | | *T. oliveri* |
| | | *T. insularis* |

This grouping of the *Tilia* species was confirmed by analysis of all variable sites in the plastid genome. This grouping is different from the morphological classification of *Tilia* species, which is mainly based on fruit morphology. Thus, as one example *T. insularis* was regarded as a subspecies of *T. amurensis* based on morphological traits, but this investigation put these 2 taxa into different clades. Moreover, *T. insularis* was ranked as a full species based on its plastid genome. It is evident that the genome of chloroplasts is a sensitive means for phylogenetic classification.

In a mitogenome announcement Yang et al. (2018) reported on the complete chloroplast genome of *T. insularis* and the relationship of this species with other *Tilia* species. The UPMGA revealed a close relationship with *T. mandshurica, T. oliveri,* and *T. paucicostata* but less so with *T. amurensis*. In contrast, based on morphological traits Pigott (2008) concluded that *T. insularis* should neither be regarded as a full species nor as a subspecies to *T. amurensis*.

Pigott (2002) presented a review of the chromosome numbers of 25 *Tilia* species. Table 1-1 reveals that the majority of species are diploid. The chromosomes are small, approximately 1µm long, which makes counting in

*Table 1-1. Identification of* Tilia *species in the Hyrcanian forest in northern Iran by use of ITS and morphological characteristics of the taxa. Yousefzadehet al. 2012.*

| Species/subspecies | Number of populations and location in Hyrcanian forest | Leaves | Fruits |
|---|---|---|---|
| A1. *rubra/ caucasica* | 6 central regions | Presence of trichomes, type of serration | Obscurely ribbed – strongly ribbed |
| A2 *hyrcana* | 4 mountain regions | Stellate trichomes on both sides of the leaves | |
| B *T. begoniifolia* | 1 western region | Stellate trichomes of underside of leaves and petioles | Many-flowered inflorescences |
| C *T. dasystyla* | 2 eastern part of central region | Stellate trichomes of underside of leaves and petioles | Stigma pubescent |

*Table 1-2. Ploidy levels of* Tilia *species and species hybrids according to a review by Pigott 2002. Blue are European taxa, red are Asian taxa and black are American taxa. Pigott 2002.*

| Diploid | Teraploid | Octoploid |
|---|---|---|
| *T. cordata* | *T. dasystyla* | *T.* x *euchlora* |
| *T.* x *europaea* | *T. amurensis* | *T. nobilis* |
| *T. platyphyllos* | *T. chinensis* | |
| *T. tomentosa* | *T. henryana* | |
| *T. chingiana* | *T. japonica* | |
| *T. endochrysea* | *T. miquelina* | |
| *T. kiusiana* | *T. mongolica* | |
| *T. mandshurica* | *T. paucicostata* | |
| *T. maximowicziana* | | |
| *T. oliveri* | | |
| *T. tuan* | | |
| *T. americana* | | |
| *T. caroliniana* | | |
| *T. heterophylla* | | |
| *T. mexicana* | | |

pollen mother cells or root tips problematic but it seems that the basic number is 41. Cytometric studies were also used to determine the degree of ploidy.

## Among-population differentiation

Fineschi et al. (2003) sampled 2–10 trees from 17 *T. cordata* populations covering a large part of the distribution in Europe for a study of haplotype variation. Seven universal primer pairs were used for amplification of cpDNA.

As many as 14 haplotypes were detected and labelled by decreasing length of the fragment. Six populations were monomorphic with respect to haplotype, of which 2 populations had only 2 and 4 trees sampled. The number of populations having 1–4 haplotypes in populations with 6 or more trees sampled is illustrated in Fig. 1-3. The 4 monomorphic populations had different haplotypes. The northernmost population from Sweden (No 4) and the Slovakian population (No 16) had 4 haplotypes. Haplotype 1 dominated in France, Germany, and northern Italy, while haplotype 11 dominated in the United Kingdom. The southern Italian population had only one haplotype, No 10, which did not occur anywhere else. Similarly, in the western Russian population 9 of the 10 trees had a unique haplotype, No 4.

The high number of populations with 3–4 haplotypes each contributed to the relatively low $G_{ST}$ estimate of 0.55. Monomorphism with different haplotypes results in high $G_{ST}$ estimates. The total genetic diversity was estimated at 0.88 while the diversity within populations was estimated at 0.40. It was suggested that transfer of seeds and seedlings was responsible for the substantial diversity observed and the relatively low among-population differentiation.

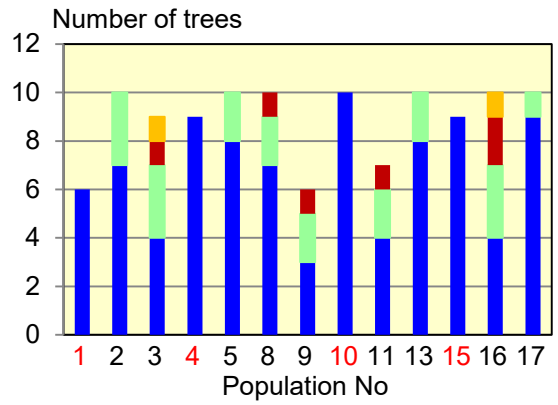


*Figure 1-3. The number of trees with varying numbers of haplotypes, 1–4, in populations with 6 or more trees per population. The colours do not refer to a specific haplotype. Population numbers in red are monomorphic. No 1 = UK, No 4 = Se, No 10 = Fr, No 15 = It. Fineschi et al. 2003.*

An investigation by Mu and Liu (2007), written in Chinese with tables in English, will be presented since it covers a geographic region with no other lime genetics studies. Six *T. amurensis* populations from a latitudinal range of 40–50°N and 6 populations along an elevational range of 600–1090 masl were sampled.

The mean was fairly low, 1.40, and there was a moderately strong relationship between population latitudinal origin and effective number of alleles (Fig. Qiang07-1). The mean expected heterozygosity was fairly low, 0.243. The calculated Shannon information index showed a similar pattern to the expected heterozygosity. The five northernmost populations show a close to linear decline with latitude, $R^2 = 0.89$. It would be interesting to know if the southernmost population is a marginal population and thereby showing less diversity. Unfortunately, no figures for expected heterozygosity and effective number of al-

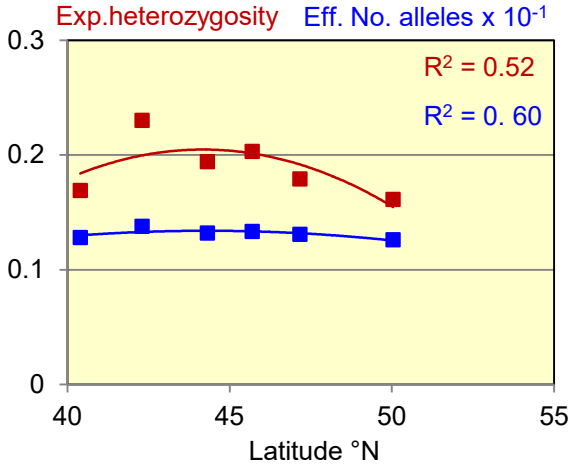


*Figure 1-4. The relationships between* T. amurensis *population latitudinal origin and effective number of alleles or expected heterozygosity. Fourteen microsatellites were analyzed. Mu and Liu 2007.*

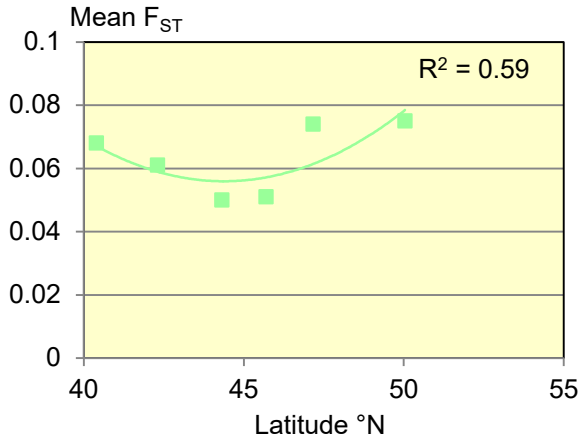


*Figure 1-5. The relationship between latitude at population origin and mean $F_{ST}$. Fourteen microsatellites were used. Mu and Liu 2007.*

leles were presented for the latitudinal range populations. The mean $F_{ST}$s for the 2 categories of populations related to their latitudinal origin are visualized in Figs 1-5 and -6. The 2 northernmost populations had the highest mean $F_{ST}$s. It is hard to interpret this condition without a translation of the Chinese text. The 730 m-elevation population disturbed any relationship between altitude and $F_{ST}$. AMOVAs were calculated for both categories of population with fairly similar results. The among-population variance component was estimated at 23.8 and 27.3%, respectively, and with much larger variance components for within-population variation.

In conclusion, a moderately large genetic diversity was observed in 2 clines, one latitudinal and one altitudinal, of *T. amurensis* populations from northeastern China. Fairly

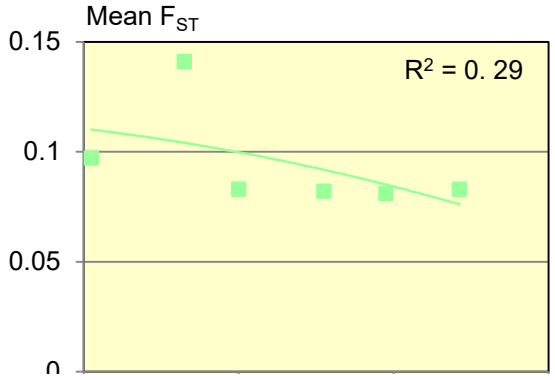


*Figure 1-6. The relationship between altitude at* T. amurensis *population origin and mean $F_{ST}$. Fourteen microsatellites were used. Mu and Liu 2007.*

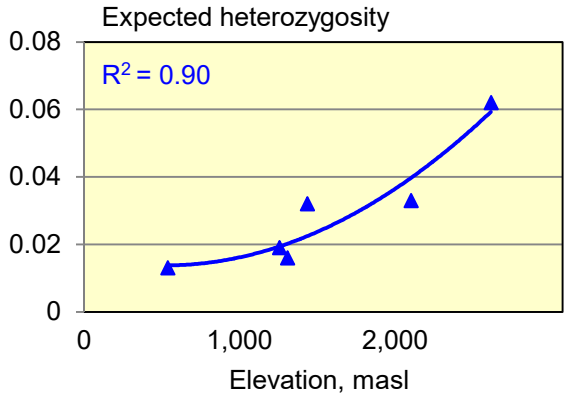


*Figure 1-7. The relationship between population elevation, masl, and expected heterozygosity of 6 Iranian Hyrcanian* T. rubra *populations. Twenty-nine polymorphic RAPD bands were analyzed. Hosseinzadeh Colagaret al. 2013.*

high $F_{ST}$ estimates were noted for individual populations, 0.050–0.141.

The genetic diversity and differentiation of 6 Hyrcanian *T. rubra* populations were estimated using 29 RAPD bands by Hosseinzadeh Colagar et al. (2013). The populations originated from different elevations, 526–2,250 masl, with a maximum distance between the populations being 70 km. Expected heterozygosity, Shannon index and genetic diversity, $G_{ST}$, were estimated. An AMOVA was run to estimate the partitioning of the variance within and among populations.

With such a wide origin of the altitudes of the populations it is *a priori* expected that adaptation to the climatic conditions at the varying altitudes has caused an adaptive variation. Even if RAPDs are neutral markers the adaptive variation would be reflected in the RAPD markers. We have therefore plotted the relationships between elevation at population origin and expected heterozygosity, as well as for mean $G_{ST}$.

Fig. 1-7 shows that the expected heterozygosities were extremely low: all estimates below 0.065. This figure also illustrates that there is a strong relationship between population elevational origin and expected heterozygosity. It has been hypothesized that occurrence of clones in populations should increase the level of heterozygosity. It is likely that the seed maturation is constrained and that asexual propagation is common in the high-elevation populations, which grow at the margin of the distribution of *T. rubra*. The present results support this assumption. Unfortunately, no information on clonality was presented.

Despite the insect pollination in *Tilia*, which *a priori* would lead to gene flow over limited distances, the among-population differentiation was unexpectedly low.

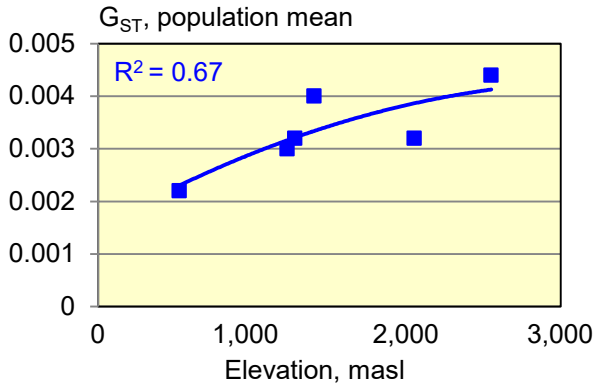


*Figure 1-8. The relationship between population elevation, masl, and mean $G_{ST}$ in 6 Iranian Hyrcanian* T. rubra *populations. Twenty-nine polymorphic RAPD bands were analyzed. Hosseinzadeh Colagar et al. 2013.*

Thus, the AMOVA revealed that only 3% of the variance was attributed to among-population variation and the rest to within-population variation. This suggests that there is a pronounced gene flow among the 6 populations. Moreover, the low $G_{ST}$ estimates (Fig. 1-8) support a strong gene flow among the 6 populations.

The remarkably low heterozygosity in the 6 populations and the low mean $G_{ST}$s had deserved a thorough discussion.

An extremely low expected heterozygosity was noted in the 6 Hyrcanian populations originating from a broad elevational range, 500 – 2,300 masl. However, the AMOVA revealed that 97% of the variance was attributed to within-population variation. Low estimates of $G_{ST}$ were noted. A strong relationship between elevation origin and expected heterozygosity was found.

Logan et al. (2015) studied diversity and differentiation in 16 *Tilia cordata* and 8 *T. platyphyllos* populations. The sampling localities covered most of the United Kingdom. Although efforts were made to include autochthonous

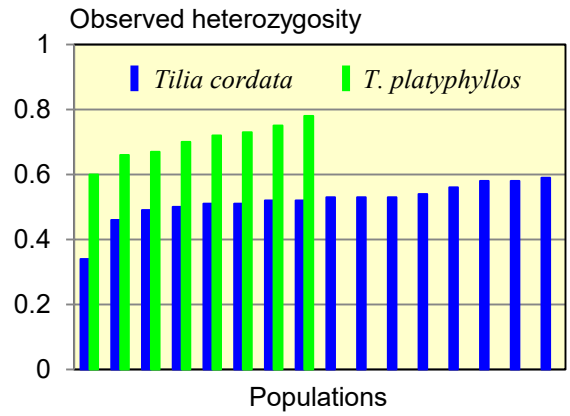


*Figure 1-9. Observed heterozygosity, in 16* Tilia cordata *populations and 8* T. platyphyllos *populations in England. Thirteen microsatellite loci were analyzed. Logan et al. 2015.*

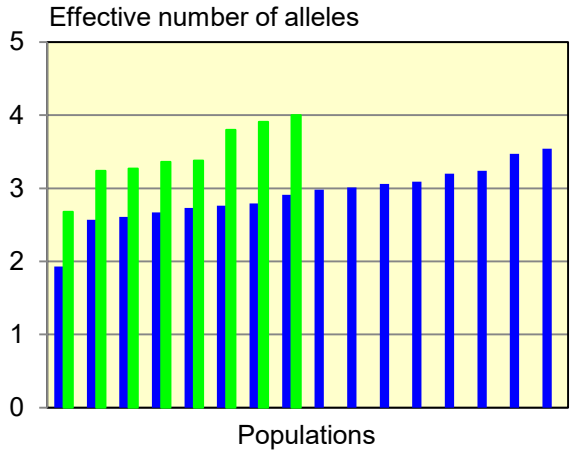


*Figure 1-10. Effective number of alleles, in 16* Tilia cordata *populations (blue) and 8* T. platyphyllos *populations (green) in England. Thirteen microsatellite loci were analyzed. Logan et al. 2015.*

populations only, there was no guarantee that these requirements were fulfilled in all cases. Thirteen microsatellites were used in this investigation. Standard methods were used for estimation of traditional population genetics parameters. Clones occurred frequently (138 of 550 trees); only one individual of these clones was used in the further analyses. Principal Coordinate Analysis (PCoA) based on pairwise genetic distances according to GENAIE.x was used for separation of the 2 *Tilia* species. STRUCTURE:v2.3.4 was also used for this pupose as well as for clustering of populations within the species. The Evano ΔK was used to identify optimal number of clusters. Variance was partitioned among and within populations by AMOVA according to Arlequin v3.5.1.3. A Mantel test according to GENAIE:x was used to test the relationship between genetic and geographic distances.

With one exception, all loci were polymorphic in both species. The deviating locus was monomorphic in *T. cordata*. As regards species separation STRUCTURE:v2.3.4 revealed 2 groups. Two q values were tested, 0.1 and 0.2 (*values of q ranging from 0 to 1 describe how individuals are proportionally assigned to a particular cluster*). The former resulted in 32 species hybrids or introgressed trees and the latter suggested 25 trees.

Except for one locus in *T. cordata,* all other loci showed a large diversity. The diversity was high and larger in *T. platyphyllos* than in *T. cordata* as seen from Figs. 1-9 andd -10. None of the *T. cordata* populations reached the level of any of the *T. platyphyllos* populations. It was noted that sexual regeneration of *T. cordata* in England is less frequent than in *T. platyphyllos,* which contributes to the difference in diversity of these 2 species. The English *Tilia* populations grow close to the limit of distribution of the 2 species. Despite this they both show fairly high diversity. The absence of significant coefficients of inbreeding indicates that genetic drift was of limited importance in the past.

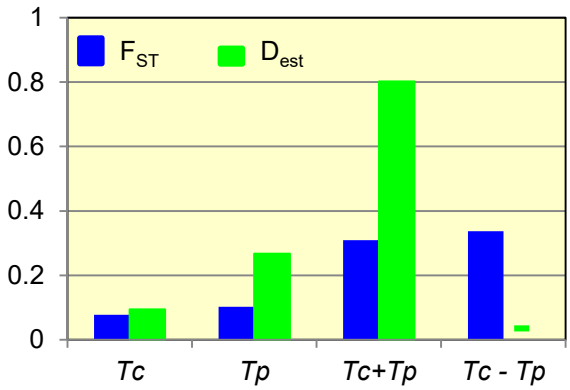


*Figure 1-11. Population differentiation estimated as mean $F_{ST}$ and $D_{est}$ based on 13 microsatellites for* Tilia cordata, *Tc, and* T. platyphyllos, *Tp, separately and pooled, Tc+Tp. Tc–Tp = mean $F_{ST}$ for all pairwise (16 x 8) comparisons between* T. cordata *and* T. platyphyllos *populations. Sixteen* Tilia cordata *and 8* T. platyphyllos *populations in the United Kingdom were studied. Logan et al. 2015.*

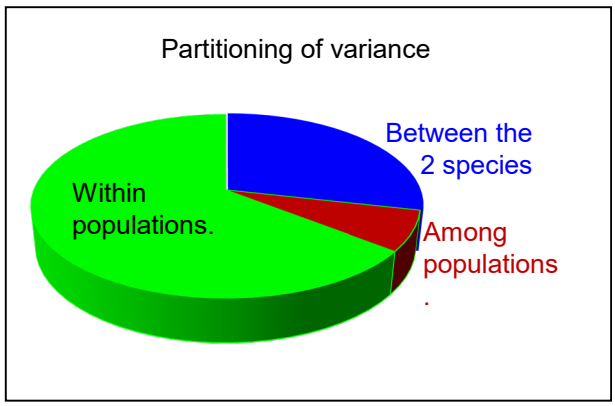


*Figure 1-12. Partitioning of the variance between* Tilia cordata and T. platyphyllos, *among populations within species, and within populations of these 2 species in England. Thirteen microsatellite loci were analyzed. Logan et al. 2015.*

Of the 276 pairwise estimates of $F_{ST}$, 235 were significant. Fig. 1-11 reveals that the interspecific relationships had the highest estimates and the high estimate for the pooled relationships (within and between species) must be attributed to the interspecific $F_{ST}$s. The $D_{est}$ estimates revealed a stronger separation of the 2 species than the $F_{ST}$s. Generally, the $F_{ST}$s for the individual species might be regarded as relatively high for the size of the area studied. The partitioning of the variance according to the AMOVA analysis showed that a little more than 25% was attributed to species and approximately 2/3 was attributed to within-population variation (Fig. 1-12).

The highest $F_{ST}$ estimate, 0.45, was noted for the relationship between the mixed *T. cordata* and *T. platyphyllos* LU population in the southwest and the *T. platyphyllos* population AS in the northeast. Both populations showed high mean estimates for interspecies $F_{ST}$s, 0.40 and 0.39. Neither of these 2 populations was geographically isolated from other populations. It might be remarked that the AS population is the most northwestern *T. platyphyllos* in Europe.

The BH population from southeastern UK had the highest $F_{ST}$ estimates for relationships with other *T. platyphyllos* populations. It is evident that populations AS and BH are the most differentiated from the 6 other *T. platyphyllos* populations. The BH population has been exposed to substantial coppicing in the past. This in combination with its isolated location explains the high $F_{ST}$ for this population. The structure analysis resulted in 3 clusters in both species. Clustering mainly followed the geographic location of the populations (Table 1-3). The 3 clusters in *T. platyphyllos* contained one population each from different geographic regions. The remaining 5 populations contained trees assigned to 2 or 3 clusters. The clustering of the *T. platyphyllos* populations is reflected in the mean $F_{ST}$s (Fig. 1-13).

In conclusion, fairly high genetic diversity in populations of both species and fairly large differentiation among populations within species were noted. The clustering analysis revealed that part of the differentiation was attributed to geographic distance. The 2 species were clearly separated from each other, with 28% of the variance attributed to species differences.

The thesis by Amanda Mylett (2015) dealt with genetic diversity of *T. cordata* and *T. platyphyllos* in Lincolnshire, UK, and tissue culture production. The ultimate objective was to get genetic information for guiding of genetic conservation of the species. According to her: The Limewoods, which contain significant populations of *Tilia cor-*

*Table 1-3. Number of clusters of 16* Tilia cordata *and 8* T. platyphyllos *in United Kingdom. Logan et al. 2015.*

| Clusters | *T. cordata* | *T. platyphyllos* |
|---|---|---|
| 1 | 9 southwestern populations | 1 fairly isolated northeastern population, denoted AS |
| 2 | 2 northwestern populations | 1 fairly isolated southeastern population denoted BH |
| 3 | 5 eastern populations | 1 southwestern population, denoted HW, and 3 populations from the same region have a genetic affinity with the HW population |

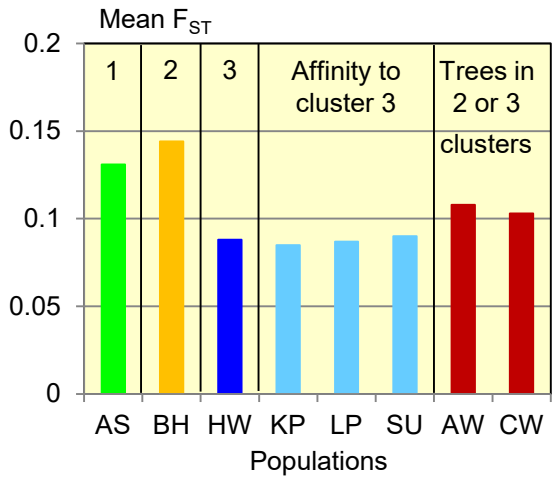


*Figure 1-13. Mean $F_{ST}$ for relationships between* T. platyphyllos *populations in United Kingdom. Clustering analysis suggested 3 clusters. The 4 populations in blue are all located in southwestern UK. Thirteen microsatellite loci were analyzed. Logan et al. 2015.*

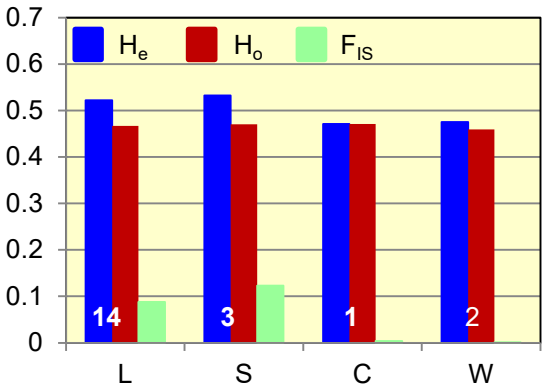


*Figure 1-14. Expected and observed heterozygosity as well as fixation index of T. cordata populations from Lincolnshire (L), Southern (S), central (C) and western (W) England. The number of populations from the different regions are given. Each population was represented by 10 or more trees. Ten microsatellites were used. $F_{IS}$ estimates for Central and Western England are very close to 0.00. Mylett 2015.*

*data* Mill., are highly fragmented and are isolated from each other by farmland. The largest areas of limewoods occur in Lincolnshire and are regarded as ancient semi-natural woodlands. Three main areas of Lincolnshire were included in this investigation, Bardney with 14 populations, Skellingthorpe Old Wood, and Potterhamworth with one population each. In addition, 141 trees were sampled from 25 woodland areas in England, 17 of these woods being within the Forest of Bere, Hampshire. *T. platyphyllos* was represented by 2 populations, one semi-natural and the other planted. Depending on availability, tree samples were mostly growing at a distance of >30m. Ten microsatellites were developed for the diversity and differentiation estimates. Traditional population genetics parameters and pairwise $F_{ST}$s and PCA were estimated.

Estimation of diversity and differentiation should be based on just one individual of each clone. Therefore clonal material had to be identified. Eight clones represented by 2 trees each were identified and 4 clones with 4 trees each. Three genotypes that differed in just one repeat at one allele were also classified as clones. The mean distance between trees belonging to the same clone was estimated at 10.2 meters. This distance was 6 times less than the mean distance among all sampled trees in the Lincolnshire wood.

The presence of clones in some populations was considered in the estimates of diversity and differentiation. The total number of trees was 453, of which 401 were classified as *T. cordata* and 19 as *T. platyphyllos*. Twenty nine trees were classified as interspecific hybrids while 4 were identified as $F_2$. No back crosses were observed.

Separate estimates of diversity were calculated for *T. cordata* alone and for the whole of *Tilia* including *T. platyphyllos* and interspecific hybrids and $F_2$ trees.

In 2 regions the observed heterozygosity was somewhat lower than the expected heterozygosity (Fig. 1-14) for pure *T. cordata*. Generally the estimates are moderately high. As expected, the diversity estimates were slightly higher when *T. platyphyllos* and hybrids were included in the estimations. The number of alleles and number of effective alleles were higher in the populations from Lincolnshire than in the other English populations. The highest estimates of the 2 heterozygosities were noted for a population with 7 of the 20 trees sampled being $F_1$ hybrids, $H_e = 0.73$ and $H_o = 0.69$. The $H_e$s observed for the 2 *T. platyphyllos* populations were high, 0.64 and 0.79, which agrees with other studies of this species. There was virtually no relationship between genetic and geographic distances for any of the diversity parameters.

The fixation index was fairly high in the Lincolnshire populations and southern populations, ≈0.10, while it was close to zero in the central and western populations (Fig. 1-14). The low number of populations in 3 of the regions means that the estimates are imprecise. It was speculated that the high estimates of $F_{IS}$ were attributed to occurrence of null alleles rather than to inbreeding.

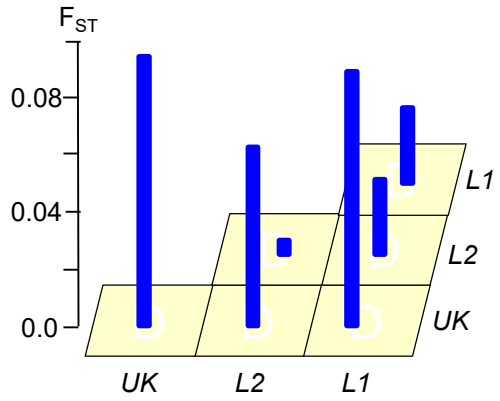


*Figure 1-15. Mean $F_{ST}$ for 3 groups of* T. cordata *populations. L1 = 12 populations from Lincolnshire growing adjacent to each other, L2 = 2 populations from Lincolnshire but at some distance from the L1 populations, UK = 6 populations from all over England. Ten microsatellites were used. Mylett 2015.*

We have visualized the mean $F_{ST}$s for the 4 regions sampled in Fig. 1-15, which shows that there is limited differentiation in the Lincolnshire region but a considerable differentiation between Lincolnshire and the pooled data from other parts of England. There was also a large differentiation among the non-Lincolnshire populations. In spite of insect pollination and fragmented distribution of the Lincolnshire populations the differentiation is rather modest, which is contrary to the *a priori* expectation. The results of the AMOVA indicate a limited among-population differentiation since only 4% of the variance was attributed to among-population differentiation. The PCA analysis supported the AMOVA results. The analysis comprising all *Tilia* data, pure species and interspecific hybrids, resulted in a clear separation of the 2 species and the interspecific hybrids. As regards genetic conservation of *T. cordata* in Lincolnshire it was stated that it would be enough to conserve one population since the differentiation was so limited.

Summary. A comprehensive study of genetic diversity and genetic differentiation of 14 *T. cordata* populations from Lincolnshire was presented. The populations occurred in a fragmented landscape with woods and farmland in a mixture. The expected and observed heterozygosities were estimated at around 0.50. Most of the fixation indices were positive, which was interpreted as a consequence of null alleles rather than inbreeding. A limited number of *T. platyphyllos* trees as well as some interspecific trees were found but no backcrosses to the parental populations. As a comparison, 2 *T. platyphyllos* populations were studied. They had higher diversity than found in *T. cordata*. The mean $F_{ST}$s for the 14 populations were fairly low: 0.023. This suggested that the gene flow is larger than expected among the scattered populations. The $F_{ST}$ for the Lincolnshire populations and *T. cordata* populations from other parts of England was fairly high, 0.080. No strong relationship between genetic diversity and geographic diversity was detected.

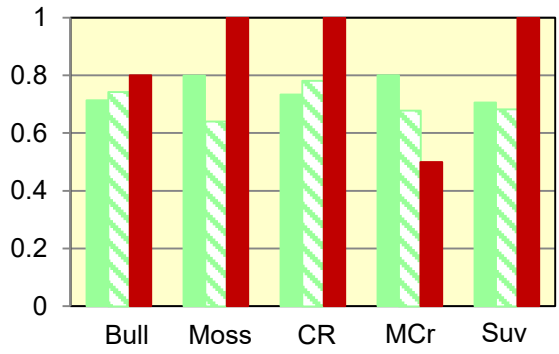


*Figure 1-16. Filled green columns = observed heterozygosity, Striped columns = expected heterozygosity and brown columns = clonal richness in 5* T. americana *var.* caroliniana *populations from Georgia and Florida, USA. Eight microsatellites were analyzed. Evans and Morris 2016.*

Evans and Morris (2016) studied genetic diversity and differentiation in 5 *T. americana* var. *caroliniana* populations from south-eastern USA. The focus was on 2 populations, Bull and Moss, at the trailing edge of the species. They are growing on 2 coastal islands in Georgia, USA. The 3 other populations were regarded as reference populations. The demography of the trailing edge populations was followed over a 10-year period. Eight microsatellite loci were used in this investigation. Expected heterozygosity, observed heterozygosity, clonal richness, fixation index and population differentiation were estimated.

In both the Bull and Moss populations there was a decline of genets over time: 31 to 22 in Bull and 23 to 18 in Moss. An adult genet is defined as an individual with a main stem + its associated basal sprouts. Over this 10-year period flowering occurred only once. This suggests that asexual regeneration is considerable in these 2 populations. In the Bull populations 17 genotypes were found among the 21 individuals while all 18 individuals in the Moss population were of different genotypes. No genotype was shared among the 5 populations.

Fairly high observed and expected heterozygosities were noted in the 5 populations (Fig. 1-16). Lower heterozygosities were *a priori* expected in such marginal populations that would suffer from genetic drift. It might be speculated that the predominating vegetative propagation preserved the diversity. The lack of clonal individuals in 3 of the populations was a conspicuous observation. The low number of individuals means that the imprecision of the estimates is high.

The $F_{ST}$ estimate of 0.055 for the difference of these 2 populations located only 900 m from each other must be regarded as high. Again the mainly vegetative propagation in these 2 populations might be responsible for such a differentiation. The mean $F_{ST}$s varied in the range 0.048–0.076. The partitioning of the variance among and within populations according to the AMOVA revealed that 8% of the variance was attributed to among-population differentiation.

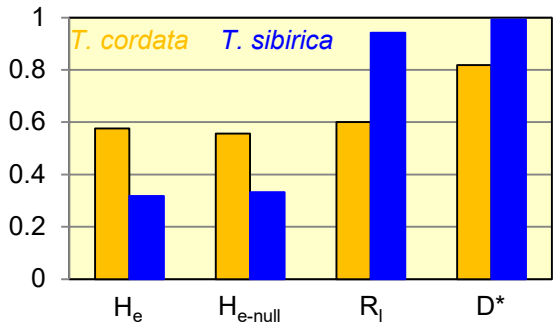


*Figure 1-17. Mean expected heterozygosity ($H_e$), mean expected heterozygosity adjusted for null alleles ($H_{e\text{-}null}$), genotypic richness ($R_I$), and Simpson's index for genotypic diversity (D*) in 6* T. cordata *(1 Austrian, 3 Polish, and 2 western Siberian) and 5* T. sibirica *populations. Twelve microsatellite loci were used. Logan et al. 2018.*

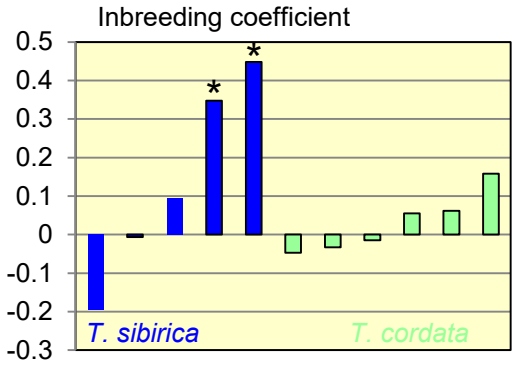


*Figure 1-18. Mean inbreeding coefficients in 5* T. sibirica *and 6 eastern European* T. cordata *populations. Twelve microsatellite loci were used. Logan et al. 2018..*

It was concluded that there was a long-term persistence through sprouting in the 2 edge-trail populations and that gene flow is probably strongly limited.

Summary. Unexpectedly high diversity was noted for 2 isolated edge-trail populations from 2 coastal islands in Georgia, USA. Regeneration takes place mainly through sprouting. The genetic distance of these 2 populations was fairly high considering the limited geographic distance between them. Clones were not shared among the 5 populations analyzed.

Whether *T. cordata* and *T. sibirica* are 2 distinct species was one of the objectives of an investigation by Logan et al. (2018). Furthermore, it was hypothesized that the genetic diversity of the fragmented *T. sibirica* is lower than in *T. cordata* and asexual reproduction is more common in *T. sibirica* than in *T. cordata*. In addition, the history of these 2 species and especially the number of generations since the split occurred was another focus in this investigation. Sampling of 6 *T. sibiric*a populations from the Kemerovo region in southern Siberia (Lat. 53.30–53.33°N, 87.24–87.28°E).was carried out. For comparison, one Austrian, 3 Polish and 2 western Siberian populations of *T. cordata* were sampled. Twelve microsatellites were analyzed. Clonality was estimated as genetic richness: R = (G − 1)/(n − 1), where n is the number of sampled ramets and G the number of genets (Dorken & Eckert 2001). For the estimation of the time for split between the 2 *Tilia* species, 16 summary statistics (different estimates of diversity, differentiation, allele characteristics, and variances) were recorded to build a Simple Split Model reference table.

The principle coordinates analysis revealed a clear distinction between *T. sibirica* and *T. cordata*. The 2 ways of estimation of expected heterozygosity both revealed a clear difference between the 2 species (Fig. 1-17) with almost twice as large expected heterozygosity in *T. cordata* populations. Low estimates of the coefficient of inbreeding were noted for *T. cordata* while wide variation of the $F_{IS}$ was noted for *T. sibirica* (Fig. 1-18). Generally, less isolated populations were noted for *T. cordata*. The fragmented distribution leading to isolation of the *T. sibirica* populations would lead to impacts of genetic drift. The finding that more than 40% of the *T. sibirica* trees actually were clones, suggests that vegetative reproduction was frequent in *T. sibirica*. The clonality and Simson's index for genotypic diversity both showed large differences between the two species (Fig. 1-19). Except for one of the Russian *T. cordata* populations R and D* were 1.0 and thus not showing any clonal trees.

We have preferred to illustrate $F_{ST}$ estimates separately for the 2 species, partly for intra-specific crosses and interspecific crosses in Fig. 1-19. For the majority of populations there is a dramatic difference between intra- and

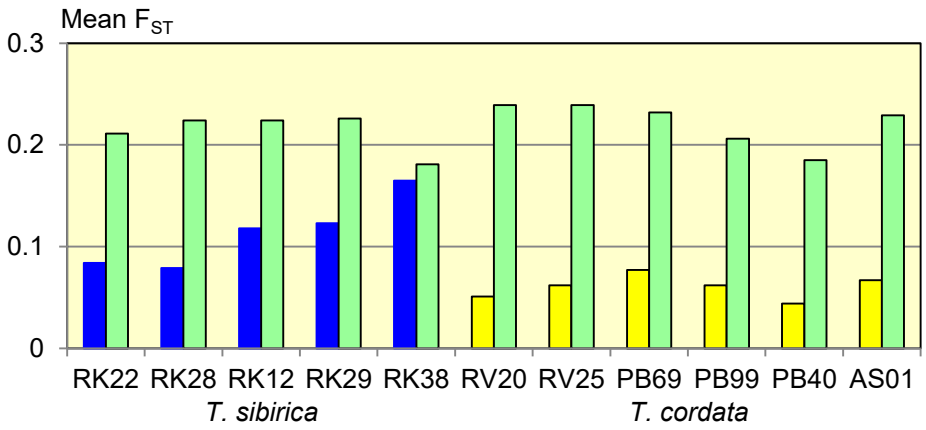


*Figure 1-19. Mean estimates of $F_{ST}$ for 5* T. sibirica *and 6* T. cordata *populations in intra-specific (blue and yellow) and interspecific crosses (green). Twelve microsatellites were used in this investigation. Logan et al. 2018.*

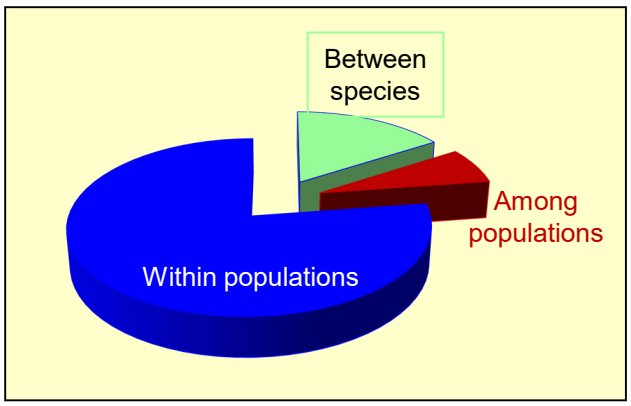


*Figure 1-20. Partitioning of the variance between* T. cordata *and* T. sibirica *as well as among populations, and within populations of the 2 species. Logan et al. 2018.*

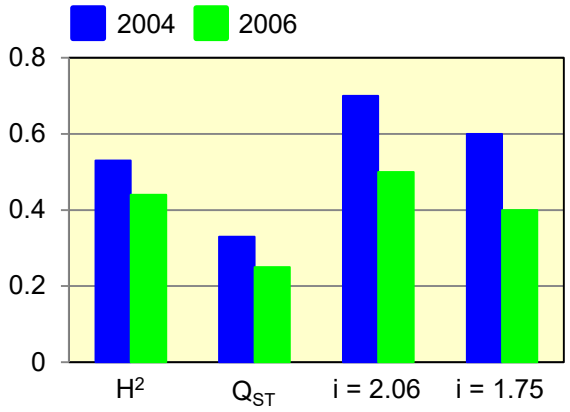


*Figure 1-21. Broad-sense heritability, $H^2$, and $Q_{ST}$ for bud flushing, as well as response to selection with 2 selection criteria 5% and 10%, i = selection intensity. Blue = recordings in 2004, green recordings in 2006. Lobo et al. 2018.*

interspecific crosses. Generally, the intraspecific $F_{ST}$s are larger among the *T. sibirica* populations than among the *T. cordata* populations. In spite of the geographically wide origin of the *T. cordata* populations their mean $F_{ST}$s were lower than for the corresponding *T. sibirica* populations. The AMOVA revealed that the largest differentiation was noted for individuals within populations (77%) followed by the difference between the 2 species (16%) and the lowest for variation among populations within species (7% Fig. 1-20).

The time of split of the 2 species was estimated at 4,760 generations ago. With an estimated generation time of 100 years, the split should have taken place approximately 475,000 years ago. It should be remarked that the 95% confidence interval around 4,470 was extremely wide; 1,770–10,900 generations.

It was stated that 3 *T. sibirica* populations, RK12, RK28, and RK29, would be suitable for seed production as well as inclusion in genetic conservation of the species. It was also recommended to establish *ex situ* genetic resource populations for seed production. Thus obtained seeds should be used for replantation at their original localities. It was also suggested that plantation outside the present range of distribution would be sensible to mitigate the effect of global warming. Further it was strongly argued that some protection of the still remaining *T. sibirica* is required.

Summary. The hypotheses that *T. cordata* and *T. sibirica* are 2 different species and that the latter has lower diversity than *T. cordata* were supported by the results in this investigation.

Lobo et al. (2018) tested the hypothesis that the existing adaptedness of bud flushing in 12 fragmented Danish *T. cordata* populations from 4 ecoregions in western Denmark was caused by disruptive selection. Two southern ecoregions had 2 populations each, while the northern and central regions had 4 populations each. Flushing in 6 classes was studied in one clone trial at one occasion in 2004 and 2006 at ages 6 and 8 after establishment of the trial. Each clone was represented by one graft in each of 6 blocks. The material in the clone trial was genotyped by 9 microsatellites. Broad sense heritability was estimated as $V_G/V_P$, in which $V_G$ is the clonal variance and $V_P$ is the total phenotypic variance ($V_G$ + environmental variance $V_E$). The response (R) to selection of 5% or 10 % of the clones was estimated as $R = iH^2(\sqrt{V_P})$. The genetic differentiation in bud flushing was estimated by $Q_{ST}$; $Q_{ST} = V_{POP}/ (V_{POP} + V_E)$, in which $V_{POP}$ is the variance between populations and $V_G$ is the variance between clones.

The bud flushing stages varied among the populations between 1.3 and 2.8 in 2004 and between 2.1 and 3.3 in 2006. There were strongly significant differences

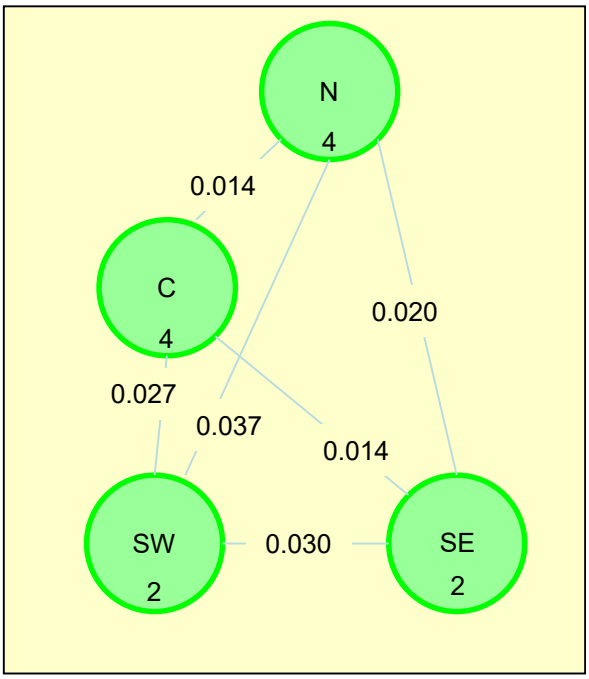


*Figure 1-22. Mean $F_{ST}$s for* T. cordata *populations in 4 ecoregions in western Denmark based on 9 microsatellite loci. The number of populations in each ecoregion are shown. Lobo et al. 2018.*

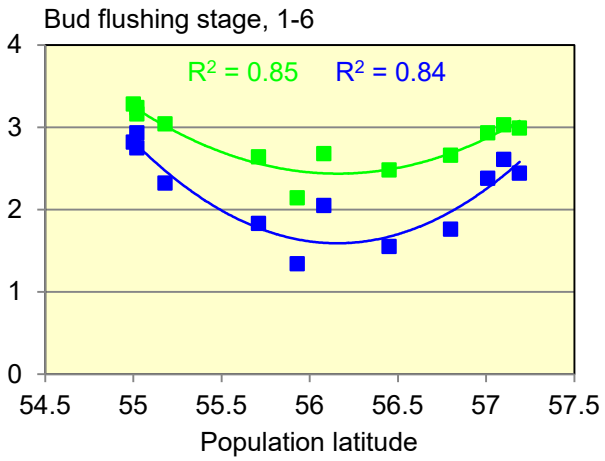


*Figure 1-23. The relationship between latitude at population locality and bud flushing stage in 2004 (blue) and 2006 (green). Lobo et al. 2018.*

between the clones within populations. As a corollary of this, high broad-sense heritabilities were noted both years, 0.53±0.05 and 0.44±0.06 (Fig. 1-21). The $Q_{ST}$s were somewhat lower, 0.33±0.12 and 0.25±0.11. $F_{ST}$s were somewhat lower than the $Q_{ST}$ estimates (Fig. 1-22). However, all of them were significant except for the relationship between Central and Southeast.

A significant relationship between minimum temperature in May and bud flushing was reported, r = 0.75. Since species at high latitudes frequently show strong relationships between latitudinal origin and phenology traits, we tested such relationships for the 2 years of observations (Fig. 1-23). The 2 second degree polynomials showed strong fits to the observed data ($R^2$ = 0.84 and 0.85) and a pattern opposite to many other investigations with latest flushing in central populations. It is obvious that latitude does not describe temperature climate well in western Denmark.

The finding that $Q_{ST}$ was larger than $F_{ST}$ suggests that disruptive selection had taken place in past generations of *T. cordata* in this part of Denmark. This was further supported by the relationship between minimum temperatures in May and bud flushing. As a conclusion of the phenology study it was stated that differentiation of the populations was attributed to the environmental conditions rather than to isolation by distance. Genetic drift can probably be ruled out as responsible for the differentiation of the populations since the inbreeding coefficients were very close to zero in the 4 ecoregions. Fragmentation of the populations might have a greater impact on population differentiation in coming generations.

The response to selection was not spectacular but it showed that there were potentials for adaptation to changed ambient conditions.

Summary. High broad-sense heritabilities were estimated for bud flushing in an investigation with 12 populations from 4 ecogeographical regions in western Denmark. Based on 9 microsatellite loci, 5 of the 6 pairwise $F_{ST}$ estimates were significant. The $Q_{ST}$ for bud flushing was larger than the $F_{ST}$. There was a relationship between minimum temperature in May at population origin and bud flushing. It was concluded that adaptation to the ambient conditions rather than isolation by distance was responsible for the observed differences among the ecogeographical regions.

Erichsen et al. (2019) studied genetic diversity, genetic differentiation, and clonality in 774 trees in 9 Danish *T. cordata* populations. In 2 populations subpopulations were identified. Selection in the individual populations or subpopulations was described as exhaustive or stratified. Traditional population genetics parameters were estimated. Both $F_{ST}$ and $G_{ST}$ were estimated to reveal relationships between populations. STRUCTURE 2.2 was applied to detect any clustering of the 9 populations. One focus in this investigation was occurrence of clones and their impact on genetic diversity. Clones were identified by Cervus according to Kalinovski et al. (2005). Since it is hypothesized that asexual reproduction preserves genetic variation by counteracting the effects of genetic drift it becomes important to study the occurrence of clones in populations. Sexual reproduction in clones probably varies with the growth pattern of the clones in a population. Intermixed (guerrilla-based) pattern as opposed to tightly packed occurrence (phalanax) will promote sexual reproduction. Therefore, clone characteristics were one focus in this investigation. The parameter clonality was derived in the following way: *clonality in each population was characterized by a measure of genotypic richness R = (G − 1)/(n − 1), where n is the number of sampled ramets and G the number of genets (Dorken & Eckert,2001). Genotypic richness = 0.0 means that all trees have the same genotype and 1.0 means that all trees have different genotypes. The mingling index is a calculation of the probability that two neighbors have the same genotype by taking spatial information into account (Pommerening 2002).* Thus, a mingling index of 1.0 means that all ramets are tightly packed in a stand.

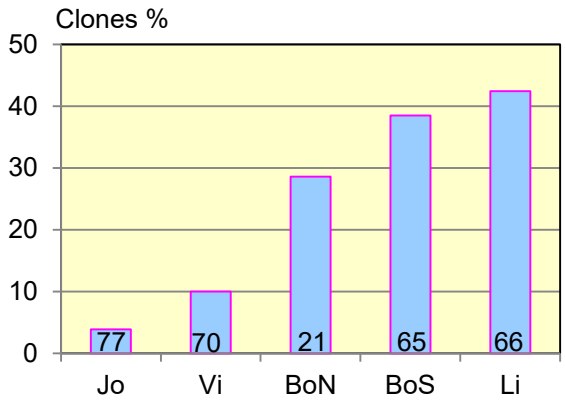


*Figure 1-24. Percentage of genotypes with 2 or more ramets in samples from 5 Danish* T. cordata *populations. Total number of genotypes are shown. Erichsen et al. (2019).*

Among the 774 trees analyzed, 730 proved to be *T. cordata* and the rest were either *T. platyphyllos* or its hybrid with *T. cordata*. Only 4 of the populations had only *T. cordata* trees. No less than 104 clone groups were noted among the 484 genotypes. The largest clone group, 27 trees, was detected in the subpopulation Bo north. Most clone groups consisted of just 2 trees; 46 of the 104 clone groups. A wide variation in the percentage of clones was noted for the 5 populations investigated (Fig. 1-24). The genotypic richness and mingling index were estimated in 5 and 3 populations, respectively (Fig. 1-25). Not surprisingly there was a strong relationship between percentage of clonal genotypes in the populations and genotypic richness, $R^2 = 0.91$. The clonality drops with increasing percentage of clones in the population. Based on this relationship the genetic diversity in a population is well reflected in just the percentage of clones in a population. The average distances from clone centers of the ramets of individual clones were rather limited, 3.8–7.1 meters. Despite this, the mingling indices illustrated in Fig. 1-25 were moderately high. The obtained mingling indices indicate that the ramets of the individual clones did not occur clustered. This means that there are good possibilities for outcrossing in the analyzed populations. Four of the 9 populations are no longer actively managed. For these populations there was a strong negative relationship between breast height diameter and genotypic richness, $R^2 = 0.988$. This means that populations consisting of many large trees are characterized by low clonal propagation.
As seen from Fig. 1-26 the differences among populations with respect to rarefacted number of alleles and effective number of alleles are moderate. Similarly, there were no dramatic differences among the populations with respect to observed or expected heterozygosity. Both of them had the same mean value, 0.62. This indicates that *T. cordata* is an outcrossing species. Generally, estimates of the coefficient of inbreeding were low, with the range –0.09 to +0.03. Thus, inbreeding did not play any great role in the past history of the 9 Danish populations.

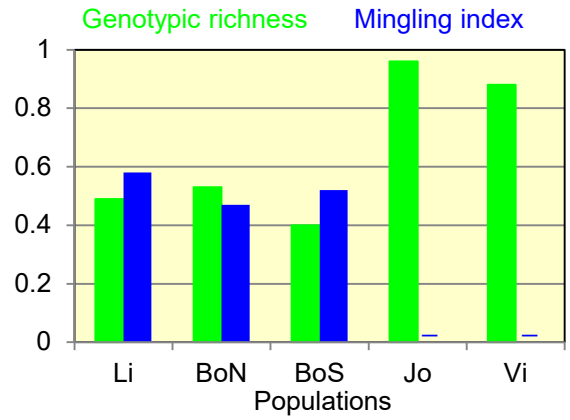


*Figure 1-25. Genotypic richness in 5 Danish* T. cordata *populations and mingling index in 3 of these populations. Nine microsatellite loci were analyzed. Erichsen et al. 2019.*

In 2 populations genetic diversity was tested separately for clonal and non-clonal genotypes. There were only marginal differences for $N_{a\text{-}rare}$, $N_e$. $H_o$, $H_e$, or $F_{IS}$ within each of the populations. The effect of clonal structure on genetic diversity is thus marginal in this material.
The fragmentation of the Danish *T. cordata* populations has taken place for some time, which would have caused a pronounced genetic differentiation. This is not observed in the mean $F_{ST}$ and $G_{ST}$ estimates visualized in Fig. 1-27. The reason for this might be a late fragmentation, which would be reflected only in coming generations of lime populations. The non-significance of the $F_{ST}$ and $G_{ST}$ mantel tests also suggested limited geographic isolation. The Dr population is a fairly isolated population growing on an island outside the southernmost part of Jutland. This population had the highest estimates of $F_{ST}$ and $G_{ST}$. The Asb population grows in northern Jutland, has no geographi-

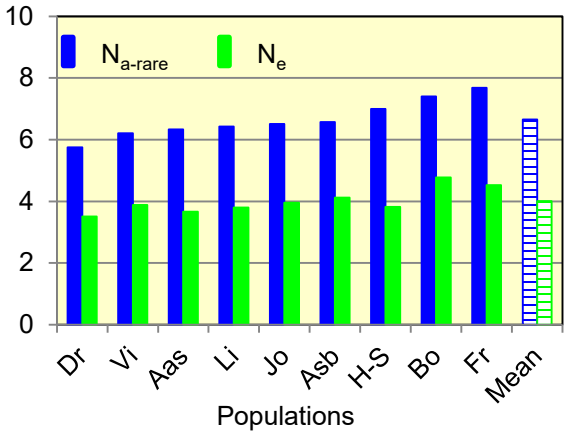


*Figure 1-26. Rarefacted number of alleles, $N_{a\text{-}rare}$ and effective number of alleles, $N_e$, in 9 Danish* T. cordata *populations based on 9 microsatellites. Population means are also shown. Populations Vi and Bo with 2 and 4 subpopulations were exhaustively sampled. Erichsen et al. (2019).*

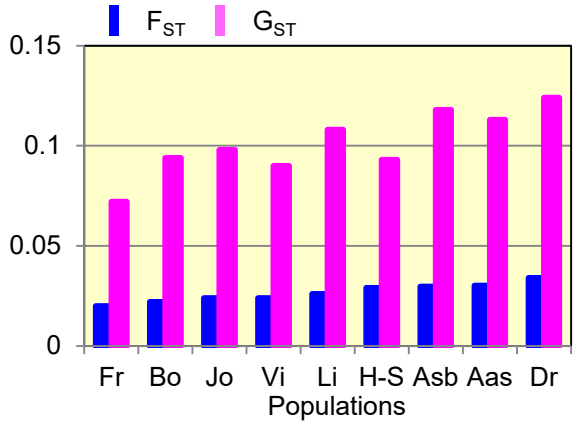


*Figure 1-27. $F_{ST}$ and $G_{ST}$ in 9 Danish* T. cordata *populations based on 9 microsatelliet loci.. Erichsen et al. 2019.*

cally close neighbours in this investigation and was one of the most differentiated populations.

Logan et al. (2019) tested the hypothesis that range-edge populations are more differentiated and have lower diversity than central populations. Moreover, range-edge populations have higher clonality than central populations. Genotypic richness R = (G − 1)/ (n − 1) was used as an estimate of clonality. Sixteen *T. cordata* and 12 *T. platyphyllos* populations from Central and Northern Europe were genotyped using 12 microsatellites. The number of trees per population varied between 11 and 40. Populations north of latitude 54 and 52°N for *T. cordata* and *T. platyphyllos* were regarded as range-edge populations. We have tried to synthesize the major findings as regards genetic diversity and differentiation between range-edge and central populations in Fig. 1-28. The differences in heterozygosity were minor even if one of the comparisons was significant. These limited differences among the populations with respect to diversity suggest that the range-edge populations are remnants of ancient populations, which have not passed any bottlenecks in their past history. Transfer of populations further north was suggested as a means to mitigate the effects of global warming (cf. Eriksson et al. 2013). Since the northern range-edge populations in this investigation have a satisfactory diversity, this would be feasible. Thus, populations so to say can move together with the climate.

The differences for the estimates of population differentiation were much larger in both species and in many cases significant. The partitioning of the variance according to the AMOVA showed that the among-population variances of range-edge populations were 9.1 and 11.2% in *T. cordata* and *T. platyphyllos*. The corresponding figures for central populations were 5.6 and 5.5%. *A priori* it is expected that the range-edge populations are more isolated than the central populations and for this reason might be more exposed to genetic drift. There were no signs of

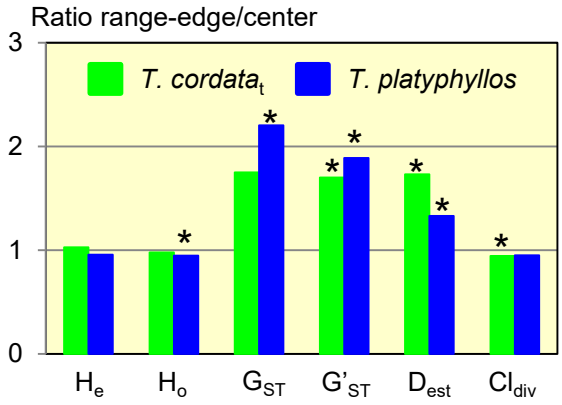


*Figure 1-28. The ratio between estimates for range-edge populations over center populations for expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), fixation index ($G_{ST}$), fixation index corrected for bias due to limited number of population differentiation independent of $H_o$ ($G_{ST}$) and clonal diversity as measure of amount of sexual reproduction ($Cl_{div}$). Significances are indicated. Logan et al. 2019.*

genetic drift since the mean inbreeding coefficients were negative in 3 cases of 4 and only positive and low for central populations of *T. cordata* (0.013). (Fig. 1-29). It was remarked that the range-edge populations were growing further apart than the central populations, which might cause an exaggeration of the estimates for differentiation of the 2 types of species.

There were significant relationships between genetic and geographic distances but the degree of explanation was lower than 25% in all 4 relationships. The highest estimate, 20.8%, was noted for the range-edge populations of T. *platyphyllos* with a geographically wide distance of 7 degrees of latitude. For all other estimates the degree of explanation was less than 10%.

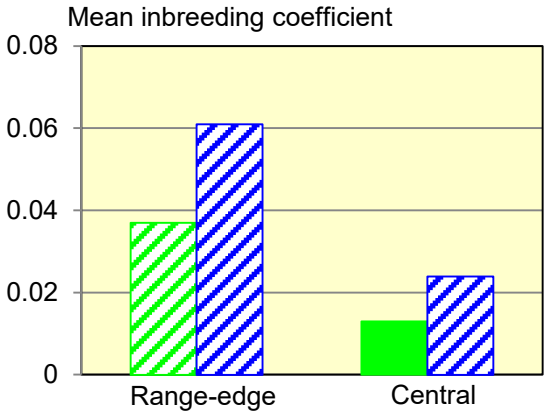


*Figure 1-29. Mean inbreeding coefficient in range-edge and central populations of* T. cordata (*green*) *and* T. platyphyllos *(blue). Striped columns = negative estimates. Twelve microsatellite loci were used. Logan et al. 2019.*

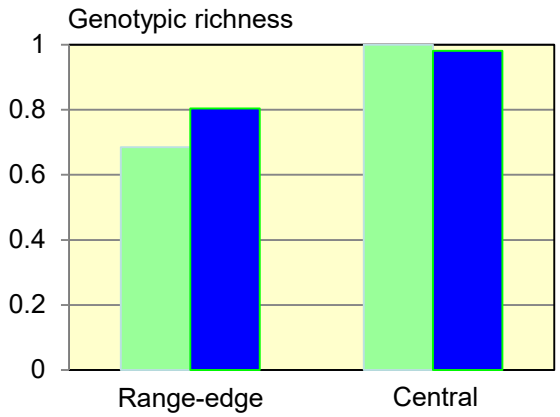


*Figure 1-30 Genotypic richness in range-edge and central populations of* T. cordata *(green) and* T. platyphyllos *(blue). Twelve microsatellite loci were used. Logan et al. 2019.*

High estimates of genotypic richness were noted for the central populations of both species (Fig. 1-30) indicating limited occurrence of clones in these populations. In contrast, clones occurred frequently in range-edge populations.

Dramatic differences in effective population sizes were obtained for the different populations with the highest number, 494.5, for the central *T. cordata* populations (Fig. 1-31). The coancestry method resulted in low effective population sizes for the 2 types of *T. platyphyllos* populations, $N_e$ = 8.7 and 16.2, which are far below the desired $N_e$ = 50 for genetic conservation. One reason for the lower $N_e$ of range-edge populations might be that these populations consist of old trees. Besides, a low sexual reproduction owing to harsh condition will lead to lower $N_e$ in range-edge populations.

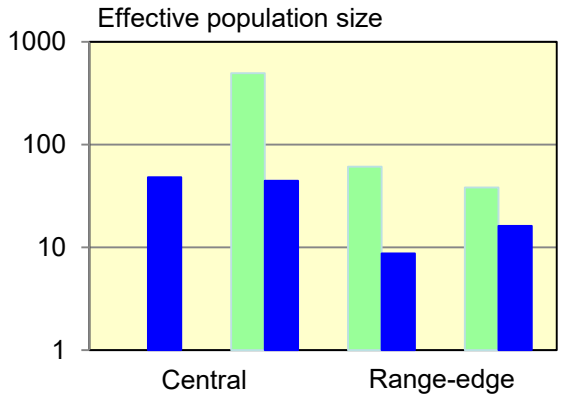


*Figure 1-31 Median effective population size of central and range-edge populations of* T. cordata *(green) and* T. platyphyllos *(blue) populations from Europe. Columns 2 and 5–6 refer to estimates based on co-ancestry. Column 3–4 and 7–8 are estimates based on linkage equilibrium. Logan et al. 2019.*

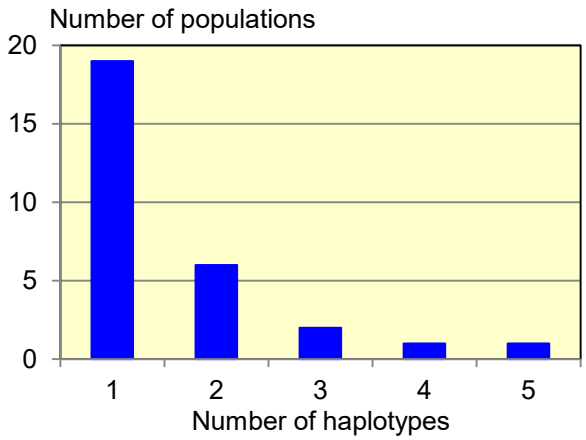


*Figure 1-32 The number of populations with 1–5 haplotypes in 25* T. cordata*, 2* T. dasystyla*, and 2* T. sibirica *populations mainly from Russia studied with 6 haplotypes. Semerikova et al. 2020.*

Semirikova et al. (2020) sampled 25 *T. cordata,* 2 *T. sibirica*, and 2 *T. dasystyla* populations for analysis of chloroplast DNA. The latitudinal range of the 25 *T. cordata* populations was 49.8–58.3°N and longitudinal range was 23.8–68.4°E. The 2 *T. sibirica* populations originated from the far eastern part of Russia, long. 87.1 and 87.2°E, respectively. The 2 *T. dasystyla* populations originated from Crimea at latitudes 44.5 and 44.6°N, respectively. Six different haplotypes were studied.

It was noted that the deduced 6 haplotypes differed considerably from each other. Of the 29 sampled populations, 19 were monomorphic, while one population had 4 haplotypes and another had 5 haplotypes (Fig. 1-32). A majority of the populations west of longitude 45°E had

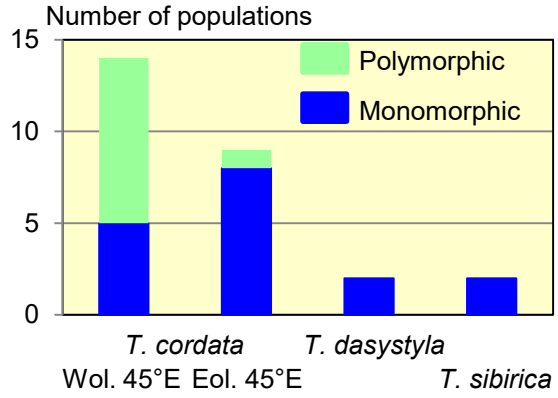


*Figure 1-33 The number of monomorphic and polymorphic populations of* T. cordata *west of longitude 45°E and east of longitude 45°E as well as in 2 populations of each of* T. dasystyla *and* T. sibirica*. Six haplotypes were studied. Two populations containing 2 trees only were excluded. Wol. = west of longitude 45°E, Eol. = East of longitude 45°E. Semerikova et al. 2020.*

2 or more haplotypes (Fig. 1-33). The population from lat. 59.98°N and long. 30.02°E was the most diverse and contained 5 haplotypes. The only *t6* haplotype found in this study occurred in one tree from this population. This haplotype is common in *T. platyphyllos*. The 2 *T. dasystyla* and the 2 *T. sibirica* populations were both monomorphic. The 2 former populations carried a unique haplotype while the 2 *L. sibirica* populations had the most common haplotype denoted *t2*; 341 of the 503 analyzed trees carried this haplotype. The second most common haplotype, *t1*, was represented in 117 trees and monomorphic in 3 eastern populations, longitudes 56.49–57.57°E. These 2 haplotypes are not found in Western Europe. The haplotype diversity observed was attributed to the presence of different refugia during the latest glaciation with *t1* and *t2* originating from refugia in Southern Siberia and/or Urals and/or Russian plain.

Danusevičius et al. (2021) used 12 microsatellites to describe the genetic structure of *T. cordata* in Lithuania. Twenty-three populations and 543 trees from 3 main regions, western, central, and eastern, were included in this investigation. The number of sampled trees per population was 17–-26 trees. The sampled trees grow at least 20 metres from each other. Care was taken to avoid sampling of hybrids with the non-native *T. platyphyllos,* which is cultivated especially at big estates.

Standard population genetics parameters were estimated such as number of alleles, effective number of alleles, observed and expected heterozygosity, rarified allelic richness, inbreeding coefficients, and effective population size. Population differentiation was tested by $G_{ST}$, $D_{est}$, and AMOVA. STRUCTURE software ver. 2.2.3 was used to test the structuring of *T. cordata* in Lithuania. They used the MIGRATE_N method to estimate the reciprocal number of migrants per generation at the population level for the 3 regions, with a subdivision of the western and eastern regions into one northern and one southern subregion. Bottleneck effects at the regional level were tested with the software BOTTLENECK 1.2.02 (Cornuet & Luikart, 1997) and the Wilcoxon rank test (Luikart, 1997).

Results were presented partly for 3 regions, Western, Central, and Eastern, and partly for 5 regions after a separation of Western and Central regions into 2 subregions, southern and northern.

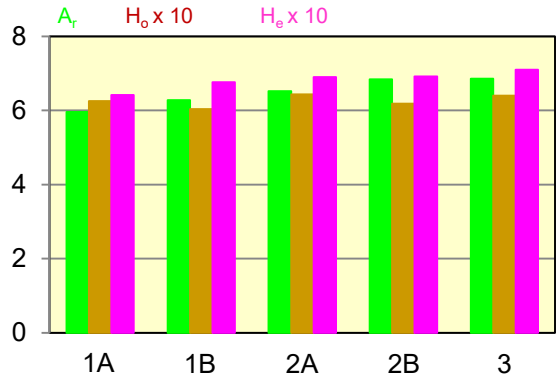


*Figure 1-34. Mean allelic richness $A_r$ observed ($H_o$) and expected ($H_e$) heterozygosity, in 23* T. cordata *populations from 5 regions in Lithuania, 1 = Western, 2 = Central, 3 = Eastern; A = northern part, B = southern part. Twelve microsatellite loci were analyzed. Danusevičius et al. 2021.*

Fig. 1-34 reveals that observed and expected heterozygosities at 0.60–0.70 were relatively high for an insect-pollinated species and somewhat higher than in *T. cordata* from other countries. The expected heterozygosity was significantly lower in the western region than in the 2 other regions (Fig. 1-34). Similarly, the allelic richness was significantly lower in the western region. It is likely that gene flow among populations compensated for probable losses of diversity owing to genetic drift and stabilizing natural selection. The gene flow among the populations was substantial as seen from Table 1-4. The crosses Western South x Central North and Eastern x Central North showed much higher numbers than the reciprocal crosses: 195 vs 95 and 208 vs 41. It was also noted that lowland *T. cordata* populations growing in rich riparian conditions were not cut to any large extent during the great loss of forest land after the Second World War in Lithuania. The absence of clones and thus asexual reproduction could also have contributed to the relatively high diversity observed in this investigation. A substantial human impact on the genetic diversity was assumed; mainly via pollination from planted *T. cordata* at estates mainly in western Lithuania. Reforestation by seedlings originating from seed collections in urban plantations might also have contributed to the large diversity observed. It had also

*Table 1-4. Above the diagonal the numbers refer to confirmed pollinations Western North x Western south – Central South x Eastern. The reciprocal transfers are shown below. Twelve microsatellite loci were analyzed Danusevičius et al. 2021.*

| | Western North | Western South | Central North | Central South | Eastern |
|---|---|---|---|---|---|
| Western North | | 62 | 195 | | |
| Western South | 113 | | 81 | 80 | |
| Central North | 95 | 63 | | 66 | 41 |
| Central south | | 122 | 74 | | 107 |
| Eastern | | | 208 | 84 | |

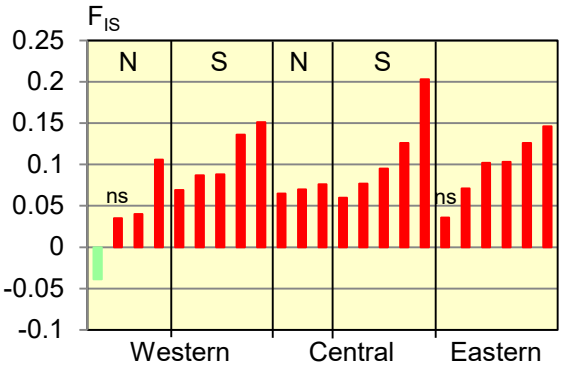


*Figure 1-35. Inbreeding coefficients, $F_{IS}$, in 22* T. cordata *populations from 5 regions in Lithuania. Twelve microsatellites were analyzed. Estimates non-significantly different from 0 are indicated. Danusevičius et al. 2021.*

resulted in smaller populations, which in turn had led to increased genetic drift in this region.

There was a large variation in the inbreeding coefficients, $F_{IS}$, ranging from –0.039 to +0.203 (Fig. 1-35), and 18 of them were significantly different from 0. These results suggest that small cohorts had caused genetic drift during the past history of these populations. Even bottleneck effects might have occurred in the past, although it was only proven for the Eastern region.

The effective population size, $N_e$, varied considerably in the northern part of the Western region, 3.0 – 24.6. Generally, $N_e$ was lower in the 2 western regions than in the other regions (Fig. 1-36). However, the difference among regions was insignificant. It was noted that the human impact was largest in the Eastern region.

The genetic differentiation estimated in 3 different ways showed rather limited differentiation (Fig. 1-37). Especially, the low differentiation among the 3 regions as obtained in the AMOVA analysis is striking. However, Lithuania is a small country with limited difference in climatic conditions from the lowland in the west to the more hilly country in the east. The exchange of migrants among the

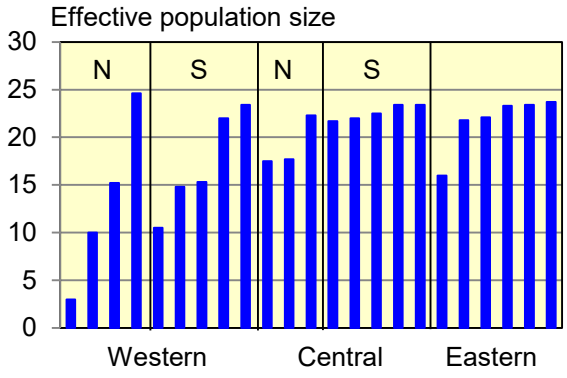


*Figure 1-36. Effective population size in 23* T. cordata *populations from 5 regions in Lithuania. Twelve microsatellite loci were analyzed. Danusevičius et al. 2021.*

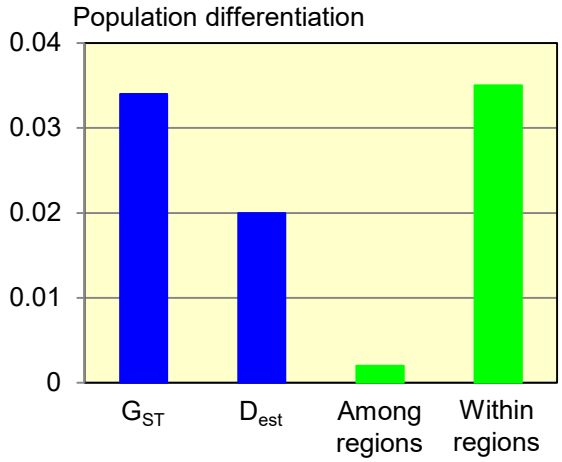


*Figure 1-37. Various estimates of population differentiation among 23* T. cordata *populations from 3 regions in Lithuania. Twelve microsatellite loci were analyzed. The 2 green columns refer to an AMOVA analysis separating the variation among the 3 regions and the variation within regions. Twelve microsatellite loci were analyzed. Danusevičius et al. 2021.*

5 regions summarized in Table 1-4 reveals a considerable gene flow among the regions, which is a great constraint to divergence among the 23 populations. There was a significant relationship between geographic and genetic distance but the degree of explanation of this relationship was low.

Summary: The authors found high genetic diversity in all regions in Lithuania and strongly varying estimates of inbreeding coefficients, with 22 of the 23 being positive. The effective population size was lowest in the Western region. The population differentiation was limited, $G_{ST}$ = 0.034. There was a significant relationship between geographic and genetic data but the degree of explanation of this relationship was low.

Rungis and Krivmane (2021) used 14 microsatellites to describe the genetic structure of *Tilia cordata* in Latvia. In all, 28 populations were sampled including *T. cordata* stands, mixed stands (broadleaf and broadleaf + pine), parks and one cemetery. The 28 populations covered the area of Latvia. Standard population genetics parameters were estimated.

No fewer than 224 of the 748 analyzed trees had identical matching multilocus genotypes (MMG), which indicate that they were asexually propagated. The 224 trees were grouped into 106 multilocus genotypes with 2–21 trees in the individual groups. Contrary to expectation 2 natural populations had the highest number of MMGs. The genetic diversity was moderately high. The range of observed heterozygosity did not differ much between natural and planted populations, 0.46–0.64 versus 0.51–0.63 (Fig. 1-38). As regards the range for inbreeding coefficients it was larger in the natural populations than in the planted populations (Fig. 1-39). One planted and 4 natural po-

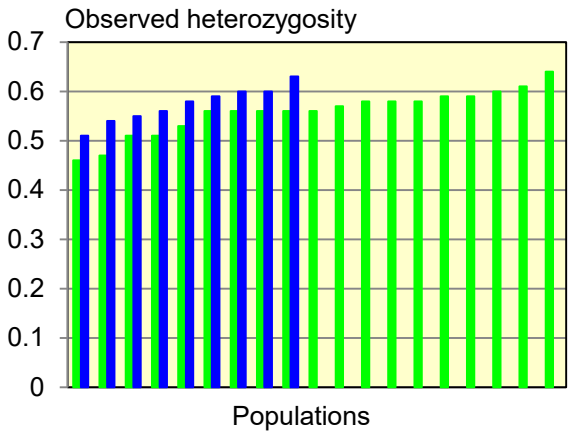


*Figure 1-38. Observed heterozygosity in 19 natural populations and 9 planted Latvian* T. cordata *populations. Green = natural populations, blue = planted populations. Fourteen microsatellite loci were analyzed. Rungis and Krivmane 2021.*

pulations had $F_{IS}$ higher than 0.10. One of the planted populations and 4 natural populations had an excess of heterozygotes.

The AMOVA revealed that 6.4% of the variance was attributed to among-population variation. The range of mean $F_{ST}$s for individual populations was 0.013–0.129, the lowest being a park population and the highest being a natural population with low human impact in the past. Unique alleles occurred in relatively high frequencies in 10 populations, range 0.02–0.14. At least for the populations with highest frequencies of unique alleles it indicates some isolation of these populations. A weak relationship between genetic and geographic position was noted. There was no clear difference between natural and planted populations. A Bayesian analysis suggested that

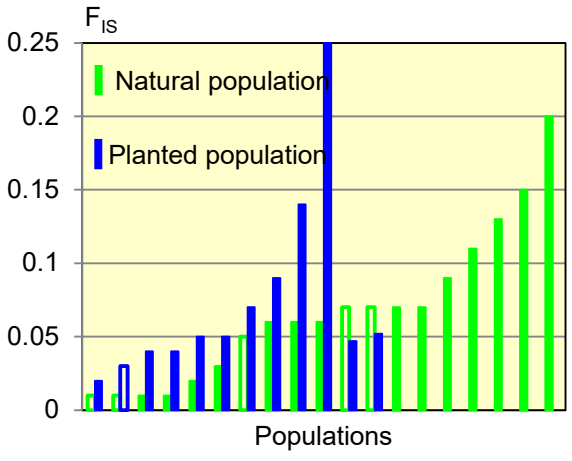


*Figure 1-39 Inbreeding coefficients in 19 natural populations and 9 planted Latvian* T. cordata *populations. Open columns show negative $F_{IS}$ estimates. Fourteen microsatellite loci were analyzed. Rungis and Krivmane 2022.*

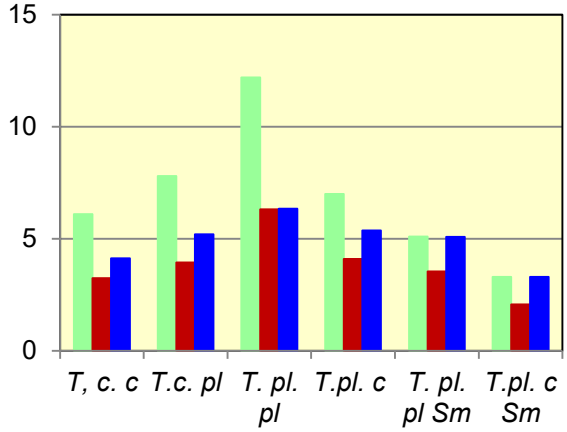


*Figure 1-40. Number of alleles (green), effective number of alleles (brown) and allelic richness (blue) of* T. cordata *and* T. platyphyllos *in the Bavarian Forest National Park. The estimations were based on 7 microsatellites developed for* T. cordata *(c) or 13 microsatellites developed for* T. platyphyllos *(pl). Sm stands for small group of trees. Wolff et al. 2021.*

there were 16 clusters. It was concluded that gene flow in *T. cordata* was not *substantially restricted* within Latvia.

All adult *Tilia* trees in the Bavarian Forest National Park were sampled for an analysis of genetic diversity and differentiation (Wolff et al. 2021). This park has an area of 24,250 hectares and is a strictly protected area since World War II. Recommendation for conservation of *Tilia* in the park was an additional objective of this study. Thirteen microsatellites developed for *T. platyphyllos* and 7 microsatellites developed for *T. cordata* were analyzed. Analyses were separated for these 2 groups of microsatellites. Seedlings within the radius of tree height were searched around the adult trees. In all, 59 *T. cordata*, 40 *T. platyphyllos* and 9 *T.* x *europaea* were found and included in this investigation. A principle coordinate analysis (PCoA) revealed two groups among the *T. platyphyllos* trees, of 29 and 11 trees. They were denoted as large and small groups. The 2 groups were separately analyzed. No grouping was found in *T. cordata*. Traditional population genetics parameters were estimated. Genalex 6.5 was used to estimate observed and expected heterozygosity and unbiased expected heterozygosity ($uH_e$), effective population size, $F_{ST}$, $G_{ST}$ and $D_{est}$.

The number of alleles was much higher in the large *T. platyphyllos* group analyzed with *platyphyllos* microsatellites than in the other analyzed groups. Similarly, the number of effective alleles was highest in this group. The small group of *T. platyphyllos* in contrast has the lowest estimates of all parameters illustrated in Fig. 1-40. It was speculated that the trees in the small group were planted and that they originated from a limited number of parents.

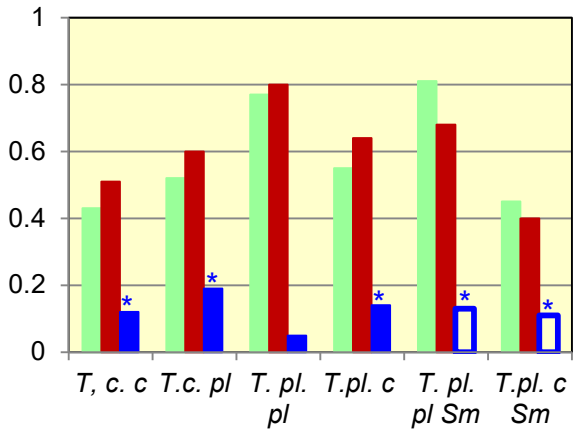


*Figure 1-41. Observed heterozygosity (green), unbiased expected heterozygosity (brown) and fixation index (blue) of* T. cordata *and* T. platyphyllos *in the Bavarian Forest National Park. The estimates were based on 7 microsatellites developed for* T. cordata *(c) or 13 microsatellites developed for* T. platyphyllos *(pl). Sm stands for small group of trees. Empty columns = negative estimates. $F_{IS}$ estimates significantly different from zero are indicated. Wolff et al. 2021.*

Fig. 1-41 reveals that heterozygosity was fairly high in *T. platyphyllos* and higher than in *T. cordata*. Considering the much larger population size of *T. cordata* one would *a priori* expect larger diversity in *T. cordata* than in *T. platyphyllos*. The ecological characteristics of the 2 species are fairly similar and they cannot be responsible for the difference between them. It was speculated that the relatively high diversity for such small and scattered populations might be attributed to an earlier warmer period with much larger populations than those present now. Especially in *T. platyphyllos* there was a large difference in the 2 estimates of heterozygosity depending on the microsatellites analyzed. In both species the *T. platyphyllos* markers had higher estimates of genetic diversity. Possible causes of this were thoroughly discussed, but without finding any plausible explanation.

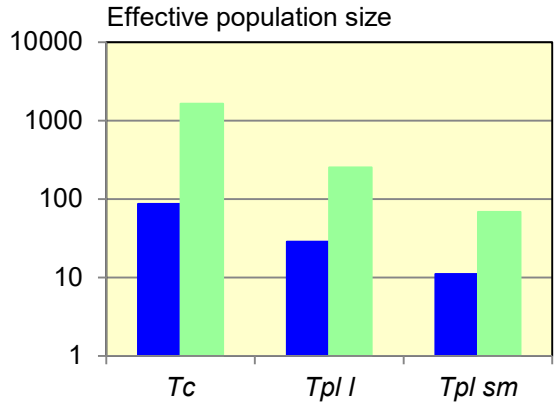


*Figure 1-42. The effective population size estimated by 2 methods, co-ancestry (blue) and linkage disequilibrium (green), for* T. cordata *and* T. platyphyllos *in the Bavarian Forest National Park. The estimations were based on 7 microsatellites developed for* T. cordata *(c) or 13 microsatellites developed for* T. platyphyllos *(p). Sm stands for small group of trees. Wolff et al. 2021.*

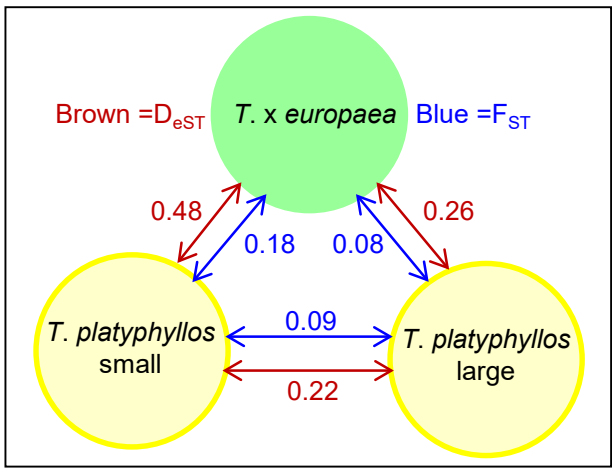


*Figure 1-43. The differentiation between a small and a large* T. platyphyllos *group as well as differentiation between them and the hybrid* T. x europaea. *Wolff et al. 2021.*

No clones were found in *T. cordata* and the frequency of vegetatively propagated trees in *T. platyphyllos* was low: genotypic richness was 0.85. Moreover, the relatedness was low in both species: <5%. This means that vegetative propagation had not contributed much to the existing populations.

The inbreeding coefficients, $F_{IS,}$, were in 5 tests significantly different from zero (Fig. 1-41). Surprisingly, the small group of *T. platyphyllos* had negative and significant estimates of $F_{IS}$. With its low census number it was expected that genetic drift might have played a great role in this population. However, it is the past history of this population that is responsible for the current $F_{IS}$, which suggests that a much larger census number existed earlier. Both methods for estimation of the effective population size gave the same results with respect to relations between the 3 groups of trees (Fig. 1-42). The methods differed considerably as regards the census numbers, which were several times higher in *T. cordata* than in the 2 other groups.

The $F_{ST}$ and $D_{est}$ estimates for the difference between the hybrid and the 2 groups of *T. platyphyllos* trees differed with higher estimates for the relationship with the small group (Fig. 1-43). The $G_{ST}$s showed the same pattern. No estimates were given for the difference with the *T. cordata* group of trees. The PCoA analysis showed a clear separation between the 3 taxa in this investigation.

The hybrids were taller and had larger DBH than both of the parental species, which was interpreted as hybrid vigour (Fig. 1-44). It was speculated that the hybrids grow to a higher age than the parental species, which could explain their superiority. Since the age of the trees are not known it is hard to know the reason for the hybrid superiority. Noteworthy is the comparatively poor growth of the *T. cordata* trees.

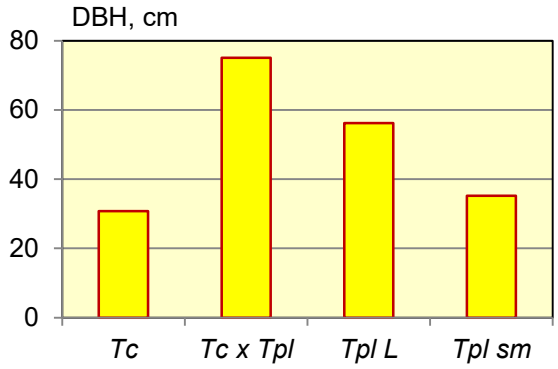


*Figure 1-44. The breast height diameter of 4 entries. The estimates were based on 7 microsatellites developed for* T. cordata *(c) or 13 microsatellites developed for* T. platyphyllos *(pl). Sm stands for small group of trees. Wolff et al. 2021.*

Summary. Twenty microsatellites were analyzed. There was a clear genetic separation of the 2 parental species and their interspecific hybrid. The genetic diversity was larger in *T. platyphyllos* than in *T. cordata*. The effective population size was much larger in *T. cordata* than in *T. platyphyllos*. The small group of *T. platyphyllos* trees had lower genetic diversity and differed most from the interspecific hybrids. The interspecific hybrids were taller and had larger DBH than the parental species.

The objective of an investigation by Ekart et al (2021) was to estimate the genetic diversity of 2 *T. nasczokinii* populations growing near Krasnoyarsk and their differentiation from one *T. sibirica* and 6 *T. cordata* populations, all from Russia. The number of trees per population varied in the range 19–29 trees. Eleven microsatellite loci were analyzed with a range of 4–17 alleles per loci. Standard population genetics estimates were calculated. In addition to $F_{ST}$, D according to Nei (1972) was estimated. The number of alleles was much higher in *T. cordata* (4.8) than in the other 2 species (2.7 and 2.2). As a corollary of this, the observed heterozygosity was much higher in *T.*

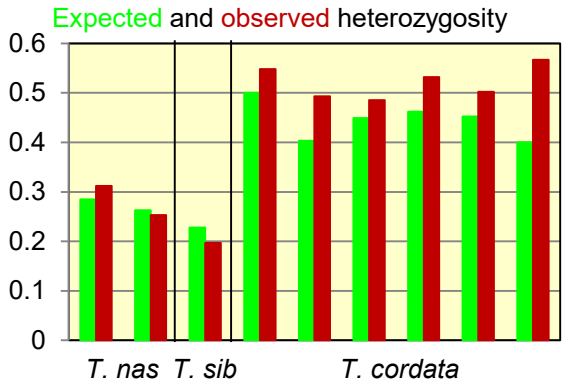


*Figure 1-45. Observed (green) and expected (brown) heterozygosity in 2* T. nasczokinii, *one* T. sibirica, *and 6* T. cordata *populations. Nine microsatellites were analyzed. Ekart et al. 2021.*

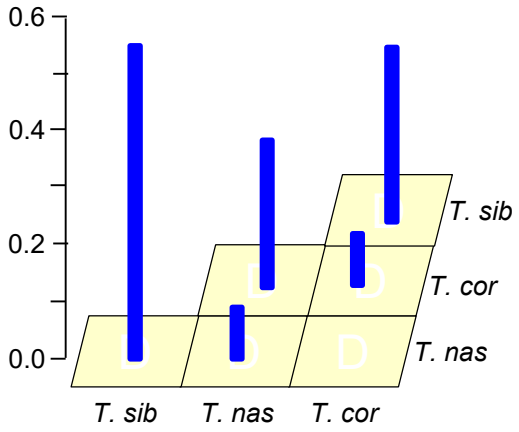


*Figure 1-46. Mean $F_{ST}$ among taxa (6* T. cordata, *2* T. nasczokinii, *one* T. sibirica *populations) based on 9 microsatellite loci. Ekart et al. 2021.*

*cordata* than in *T. nasczokinii* and *T. sibirica* with low estimates (Fig. 1-45). The $F_{IS}$ estimates of the 2 *T. nasczokinii* populations were 0.08 and –0.003, which indicates that inbreeding had not played a great role in these 2 populations. In contrast, 4 of the *T. cordata* populations had $F_{IS}$ estimates >0.10 with a maximum for the Kaliningrad population of 0.32, which suggests considerable inbreeding in this population. It ought to be added that there was a significant deviation from the Hardy-Weinberg equilibrium in six of the loci in this population. The reason for the high $F_{IS}$ estimate was not discussed

Large estimates for the inter-taxa differentiation were noted (Fig. 1-46). The $F_{ST}$ for the difference between the 2 Siberian species *T. sibirica* and *T. nasczokinii* was unexpected large, $F_{ST} = 0.55$. This suggests that *T. nasczokinii* is a full species, which was hypothesized by the authors. Further support for the separation of *T. nasczokinii* as a full species was obtained from the principal component analysis and the portioning of the variance based on the AMOVA run (Fig. 1-47). The authors stated that a larger number of populations of the 2 Siberian species has to be analyzed to get a final answer whether or not these 2 tentative species should be classified as 2 separate species.

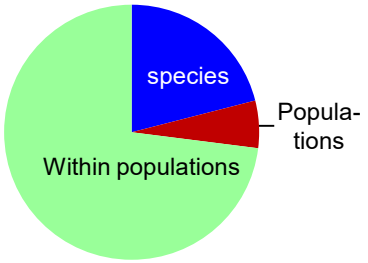


*Figure 1-47. The partitioning of the variance attributed to species differences, difference between populations within species, and differences within populations. Ekart et al. 2021.*

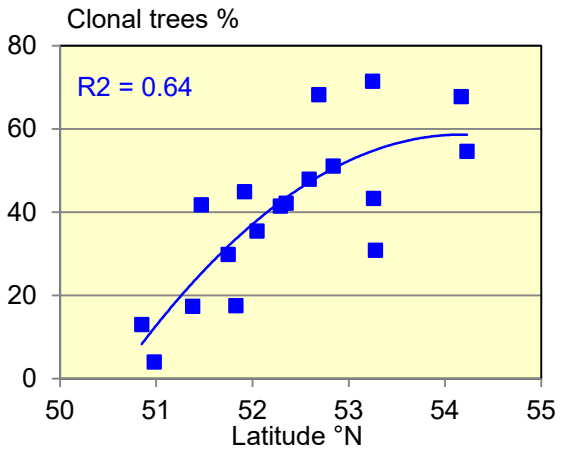


*Figure 1-48. The relationship between latitudinal origin and genotypic richness in 18* T. cordata *populations from UK. Barker et al. 2022.*

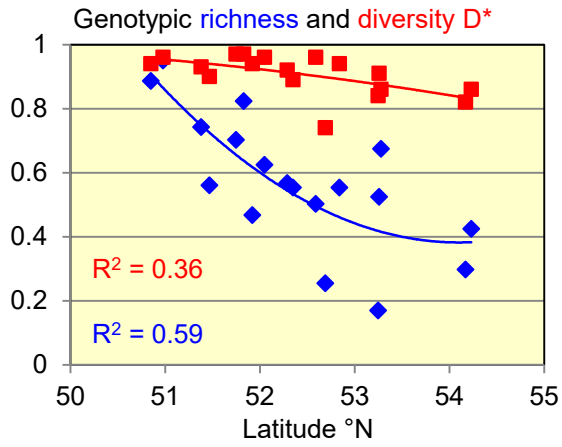


*Figure 1-49. The relationship between latitudinal origin and genotypic richness or genotypic diversity in 18* T. cordata *populations from UK. Barker et al. 2022.*

The hypothesis that vegetative propagation increases with decreasing ambient temperature was tested by Barker et al. (2022). Eighteen English semi-natural *T. cordata* populations from the latitudinal range 50.8–54.3°N and longitudinal range from 3.0° W to 1.1°E were included in this study. At each location the numbers of adult and juvenile individuals in a square of 30 x 30 meters were counted. Ten microsatellite loci were analyzed. The following parameters were estimated:

Number of alleles, allelic richness R

Observed and expected heterozygosity, $H_o$ and $H_e$, respectively

Inbreeding coefficients, $F_{IS}$

Genotypic richness (R), genotypic diversity (complement to Simpson's index, 1-D, denoted as D*)

Clonal dominance index $D_i = (N_S - 1)/(N_T - 1)$ , in which $N_S$ is number of ramets and $N_T$ is total number of individuals.

The relationship between latitude and percentage of clonal trees showed an increase with latitude (Fig. 1-48). The fit to the 2nd degree polynomial was fairly strong, $R^2 = 0.64$. It is assumed that the temperature climate decreases with increasing latitude.This means that the results obtained support the hypothesis presented above. It should be noted that the relationship between latitude and temperature climate is not absolute. Local conditions cause deviations from this relationship.

Other estimates give further support to the hypothesis. The means of the observed and expected heterozygosities were fairly similar, 0.61 and 0.58 respectively. $F_{IS}$ was negative in 11 of the 18 populations, 3 of them being significantly different from 0. None of the positive $F_{IS}$s showed significance. Neither $F_{IS}$ nor $H_o$ and $H_e$ were correlated with latitudinal origin of the populations.

Vegetative reproduction had occurred in all populations with a mean of genotypic richness at 0.57. Generally, asexual reproduction increased with latitude. Thus, there was a fairly strong relationship between population latitudinal origin and genotypic richness ($R^2 = 0.62$, Fig. 1-49). Population Ivy Wood at latitude 53.25°N deviated most from this relationship. This population had the highest percentage of juvenile individuals of all populations. It might be speculated that such a high percentage might have contributed to a low genotypic richness. On the other hand, this population did not deviate much from the relationship between latitudinal origin and genotypic diversity. The population Hockering Wood at latitude 52.69°N deviated strongly from that relationship. Exclusion of this population from this relationship improved the $R^2$ from 0.36 to 0.59.

It was observed that clones occur together in small cohorts with a mean for maximum distances within cohorts of 3.9 meters. Usually, no other genotypes were observed within the clonal cohorts. In case of changing environmental conditions it might be an advantage with the aggregated pattern of clones since one or a few of the ramets might remain after adverse conditions had hit the population. In this way the genotypic diversity will be more resistant to environmental changes.

Several factors were tested to identify predictors of the proportion of clonality. Only 2 of them were significant: the density of juvenile stems and the mean daily maximum temperature in July, $R^2 = 0.66$. In contrast, wind speed and solar radiation during flowering did not predict the proportion of clonality.

In conclusion the results supported the hypothesis that clonal reproduction is more prevalent under cooler than under warmer climatic conditions. Clones tended to be aggregated together.

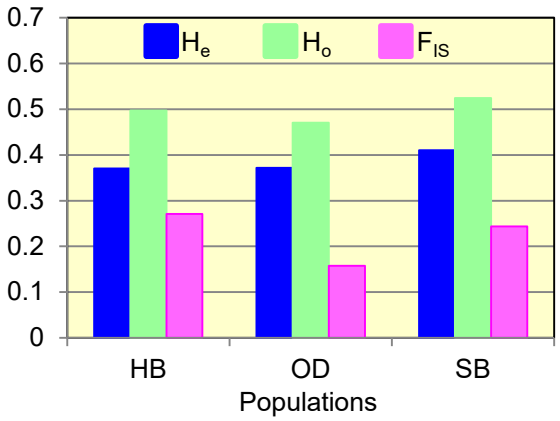


*Figure 1-50. Mean expected and observed heterozygosities as well as fixation indices in 3 South Korean populations of* T. amurensis. *The mean $F_{IS}$ estimates were all negative. Fifteen microsatellites were analyzed. Chun et al 2022.*

Chun et al. (2022) used next-generation sequencing to develop new microsatellites for *T. amurensis*. From a total of 4,774 microsatellite regions, 15 reproducible polymorphic primers were selected. Three South Korean populations of *T. amurensis* were studied. The number of trees sampled was 108.

The number of alleles per locus varied in the range 2–9 with mean values in the range 3.5–3.8. As is evident from Fig. 1-50 the observed heterozygosity was larger than the expected heterozygosity in all 3 populations. The $F_{IS}$ estimates varied dramatically among the loci from +0.105 to –0.792. It would have been useful to have a discussion on the observed genetic diversity parameters. Especially the high nominal values for $F_{IS}$ deserve a thorough discussion.

Tal (2011) carried out a phenological study of flowering over 3 years in 30 *T. cordata* trees in a German stand near Leipzig. Unfortunately, no data from individual trees were presented. Female and male flowering overlapped more or less during the entire flowering period. There was a significant negative relationship between the point of time and the ratio of male/female flowering.

Phuekvilai and Wolff (2013) developed 15 microsatellite loci for *T. platyphyllos* relating to 1–15 alleles. The 15 microsatellites were tested in 2 French *T. platyphyllos* populations. The mean observed heterozygosities were 0.77 and 0.70. A test for amplification of these 15 markers in 22 other *Tilia* species was carried out. It was found that 12 of them could be amplified in all 22 species while one marker was amplified in 5 species only.

It is concluded that there is no morphological basis for separating *T. insularis* from *T. amurensis,* either as one of a closely related group of species, or as a subspecies.

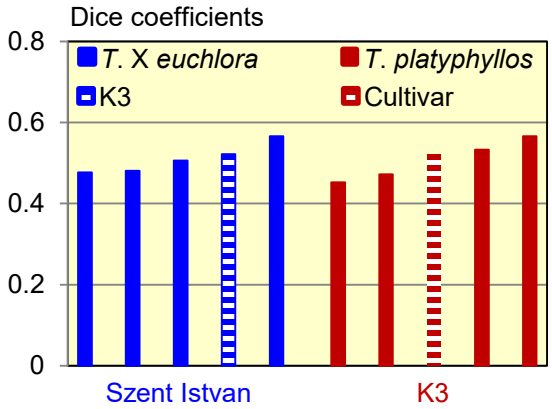


*Figure 1-51. The 5 lowest éstimates of minimum distances between 2 avenue cultivars, Szent Istvan and K3, and 35 other genetic entries. The matching entries are shown. Seventeen RAPD primers were tested. Liesebach and Sinkó 2008.*

## Urban trees

Determination of the taxonomic status of 2 avenue cultivars, 'Szent Istvan' and 'K3', was the objective of a study by Liesebach and Sinkó (2008). RAPD analysis was carried out with 17 primers that had reproducible and suitable banding patterns. As reference material 34 trees with known taxonomic status were included in the investigation. DICE coefficients (DC) estimating similarity between 2 genetic entries were calculated according to:

DC = 2**a**/(2**a** + **b** +**c**),

in which **a** is the number of matches while **b** and **c** are the number of mismatches

In addition, representatives for European species and their hybrids, and exotic species were included. The UPGMA clustering and the neighbour joining methods were used to identify possible relationships to the 2 avenue cultivars.

We have illustrated the 5 entries with the lowest DICE coefficients for the 2 avenue cultivars in Fig. 1-51. It is evident that matching of 'Szent Istvan' with *T.* x *euchlora* entries is dominating. Similarly, *T. platyphyllos* entries match best with 'K3'. The UPMGA and neighbor-joining analysis dendrograms supported the DICE results. It might be added that DICE coefficients for the relationship with the exotic species, *T. americana* and *T. henryana*, were estimated at 0.75 and 0.88, respectively. In contrast, low estimates were noted among three *T.* x *euchlora* entries, 0.01–0.09.

It was suggested that future breeding for avenue trees should focus on crosses between *T. dasystyla* as one parent and *T. cordata* and *T. platyphyllos* as the other parent. *T. dasystyla* is believed to be one of the parents in *T.* x *euchlora*.

The objective of this investigation was determination of the taxonomic status of 2 avenue cultivars. One showed most relationship with *T.* x *euchlora* and the other with *T. platyphyllos*.

*Table 1-5. Number of trees in different materials and their planting years as well as the number of clones denoted as `blue` and `green'. Hansen et al. 2014.*

| | No. of trees | Blue | Green |
|---|---|---|---|
| Fredenborgs slottshave planted in 1760s | 115 | 106 | 5 |
| Dybe allé planted 1720–1725 Fredrik Madsen's allé planted 1720–1725 | 16 | | 16 |
| and Bernstorff slottshave planted in 1765 | 9 | 6 | 2 |
| Kongens have planted in 1664 | 19 | 1 | 17 |
| Frederiksbergs have planted in 1708 | 16 | 14 | 2 |
| Substitutions with 20-year-old trees Fredenborgs slottshave planted 2013 | 20 | 20 | |
| Roots from 9 trees in Brede allé | 9 | | |
| *T. cordata* trees from a natural forest | Among the 10 trees sampled, 2 belonged to one clone | | |

Genotyping with 12 microsatellite markers was used to identify material in old avenues of lime trees in Denmark (Hansen et al 2014). The materials studied and year of plantation are listed in Table 1-5.
It is evident from Table 1-5 that the old plantations mostly consist of single clones and that the same clones are still in use centuries after the first plantations. The genotyping of the roots resulted in 9 out of 10 trees being one clone. This suggests that trees are not grafts, rather they were reproduced by layering. It is surprising that the same clones as used 300 years ago still are performing well. Moreover, they are produced in commercial nurseries today.
Four trees from Fredenborg's castle garden had unique genotypes and were believed to be *T. cordata* trees in contrast to the blue and green clones, which are interspecific hybrids *T. cordata* x *T. platyphyllos*.

Twenty-five planted *T. tomentosa* trees from the city of Duzce in western Turkey as well as 3 trees from the neighbouring forest (≈15 km apart) were genotyped by RAPDs by Filiz et al. (2015). Nei's genetic diversity and Shannon's information index were calculated. The result of a PCA was also presented. The unweighted pair group method with arithmetic mean, UPGMA, was also presented. Of 25 primers tested, 8 produced a suitable banding pattern. Of the 53 bands obtained, 48 were polymorphic. Nei's genetic diversity and Shannon's information index were estimated at 0.34 and 0.50, which must be regarded as high estimates for such a limited area. The PCA indicated 3 distinct groups with 4, 6, and 18 trees. The largest group contained the 3 forest trees. The latter 3 were closely related according to the UPGMA analysis. It was concluded that there was no dramatic difference between the urban and forest trees. However, this is somewhat premature based on just 3 trees from the forest.

The concern for limited genetic variation in urban plantations and nurseries was the reason for a study of lime trees in Belgium and the Netherlands (van den Broek et al. 2018). They sampled 21 *T.* x *europaea* trees (interspecific hybrids between *T. cordata* and *T. platyphyllos*) from a Belgian abbey avenue established in 1676–1677. Eleven replacement trees (6 *T.* x *europaea* and 5 *T. platyphyllos*) were also sampled from this avenue. In addition, they sampled 18 *T. platyphyllos*, 35 *T.* x *europaea* and 3 *T.* x *euchlora* (unclear origin) from different plantations, as well as samples of different lime taxa from commercial nurseries. Reference samples of the 4 taxa were also analyzed. In all, 14 microsatellite and 150 polymorphic AFLP loci were analyzed.
The software GenoDive 2.Ob23 was used to identify occurrence of clones among the sampled trees. An UPGMA dendrogram was calculated to visualize the differentiation among the sampled trees. Different banding structures were noted for *T. cordata* and *T. platyphyllos*, which suggests that species-specific alleles occur in these 2 species. However, it was stressed that more populations ought to be studied to verify the existence of species-specific alleles.
The trees planted in 1676–1677 all belonged to one clone, which does not exist in present-day nurseries. Only 3 clones were found in the nurseries included in this investigation while 12 clones were found among the "old trees". It was stressed that the limited genetic material available from commercial nurseries ought to be addressed. In this connection it might be noticed that it is important to distinguish between demands for genetic diversity in domestic populations (= all kinds of plantations) and breeding populations. The latter type of population requires a large genetic diversity to enable genetic progress, while domestic populations require lesser diversity. A good example of this is the 350-year-old single-clone avenue included in this investigation.
The urban *Tilia* populations analyzed showed that most of the trees in existing plantations originate from vegetatively propagated material. In all, 12 clones were identified. The genetic variation in commercial nurseries was still more limited, 3 clones.

Wolff et al. (2019) stated that *naming of types and cultivars is confusing and, if based on morphology, often misleading*. This was the background to their genotyping

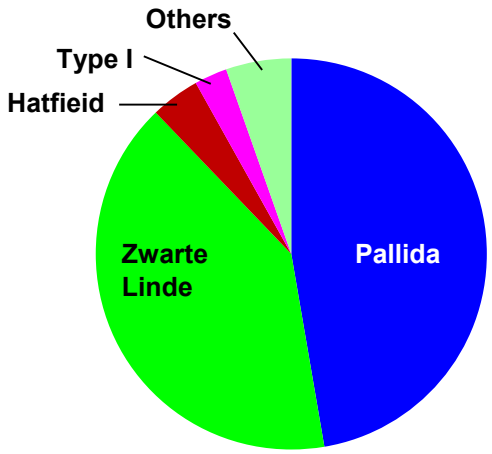


*Figure 1-52. The proportiom of cultivars among 102 genotypes, mainly T.* x *europaea.24 clones of* T. platyphyllos *were included in Others. Wolff et al. 2019.*

of 166 *Tilia* trees from Belgium, Denmark, Estonia, Germany, the Netherlands, and the United Kingdom with 17 microsatellites.
The mean number of alleles per marker was 11.82, which means that the discrimination among the multilocus genotypes is strong. In all, 28 genotypes were found among the 166 trees. 13 were *T.* x *europaea*, 10 were *T. platyphyllos*, 4 were *T. cordata*, and one was *T. tomentosa*. There were 20 genotypes with just one tree. The number of genotypes with 2 or more trees is illustrated in Fig. 1-52, which shows that the cultivars Pallida and Zwarte Linde are dominating. The cultivar Hatfield was only found in UK. The neighbour-joining analysis revealed that the 3 most commonly occurring cultivars were not closely related.
As in other urban tree investigations, cultivars used during the 17th century are still in use.
The constraints involved in breeding lime trees for future changed environmental conditions were discussed. The main constraints are the long time for evaluation and the cost involved in breeding. It was pointed out that the cultivars used for centuries had probably appeared by chance. It was suggested that growing local material might be an option for finding new superior clones for urban cultivation.

Six genetic entries of *Tilia* from Romania and one sample from Germany were genotyped by RAPDs (Gabur et al. 2019). One of the Romanian materials was *T. cordata,* the rest being *T. tomentosa* clones. The German *T. euchlora* cultivar Krumlinde was included as a reference material. Nine primers could be used for the genetic analyses.
In all, 91 fragments could be analyzed, of which 70 were polymorphic. The UPGMA diagram revealed that the *T. cordata* and *T. euchlora* grouped together and the *T. tomentosa* clones constituted another group. In contrast the PCA separated the *T. euchlora* from the rest of the tested materials.

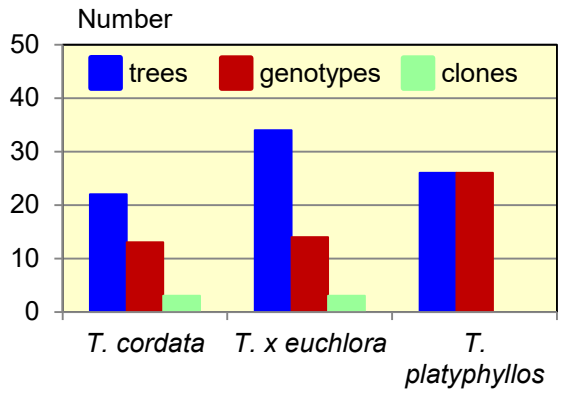


*Figure 1-53. The number of trees genotyped, the number of genotypes and the number of clones in 3* Tilia *taxa. Andrianjara et al. 2021.*

In all, Andrianjara et al. (2021) selected 80 lime trees from various localities in Paris for morphological characterization and genotyping by 9 microsatellites as a platform for improvement of future management in urban areas. They analyzed both sides of the leaves, the exterior of twigs, buds, bracts and fruits and identified 5 taxa: *T. cordata, T. dasystyla, T.* x *euchlora, T.* x *europaea,* and *T. platyphyllos.* The number of trees morphologically classified gave the following result:

| | |
|---|---|
| *T.* x *euchlora* | 36 |
| *T. cordata* | 19 |
| *T. platyphyllos* | 18 |
| *T. dasystyla* | 6 |
| *T.* x *europaea* | 3 |
| one unidentified tree | |

The principle component analysis according to Structure Harvester program suggested 2 clusters: one containing *T.* x *euchlora* and the other containing the other 4 taxa. A separation into 3 taxa based on the PCA resulted in the following groups: *T. cordata*, *T.* x *euchlora,* and *T. platyphyllos*. The total number of trees in these 3 groups, the number of different genotypes and the number of clones are visualized in Fig. 1-53. No clonal groups were found among the 18 *T. platyphyllos* trees. The 2 other taxa contained 3 clones each with the highest mean number of trees in each clone, 6.7.
It was noted that there was a fairly good agreement between the phenotypic classification of the trees and the genotyping of them. There was an agreement for 73 of the 80 trees.
Also in this paper the need for genetic diversity of urban tree species was stressed.
The good agreement between the detailed morphological classification and the classification by microsatellite analysis is an important result in this investigation. No clones of *T. platyphyllos* were detected.

With the objective of getting information on growth traits Yûcedaĝ et al. (2019) studied 3 trees from 47 locations in 2 regions in Turkey, Marmara and Western Black Sea. In all, 8 traits were included: tree height, breast height diameter, crown length and width, leaf length and width, fruit length, number of flowers per $m^2$. ANOVAs were run for all traits. Pairwise correlations between traits were also estimated.
Significant differences for locations within regions as well as for regions were noted for all traits. Except for number of flowers, strong pairwise correlations were noted for all other traits. Correlations with altitude, temperature, and precipitation were tested but the degrees of explanation of these relationships were all less than 20%. No genetic conclusions can be drawn from this investigation since there are no replications of the materials. The ambient conditions influenced the performance of the trees and the large differences in age probably influenced the growth of the tested trees.

Bilous et al. (2022) used 6 expressed sequence tag-single repeats (EST-SSR) for genotyping of six 200–600-year-old *T. cordata* trees from Ukraine. In addition to the genetic characterization of the 6 trees, the relationship between genetic and geographic distance was also aimed at. In all, 14 alleles were detected with allele frequencies in the range 0.08–0.67. In 26 out of the 36 possible cases only one allele was detected. There was no relationship be-tween genetic and geographic distance. This is not surprising with limited geographic distances among the trees. Moreover, it is not known whether the trees analyzed are representative for their present location.

## Summary

### Among species differentiation

As regards the plastid genomes there were only minor differences among *T. amurensis, T. mandshurica, T. oliveri,* and *T. paucicostata* with the largest difference between *T. amurica* and the other 3 species.
The haplotypes of 25 *T. cordata*, 2, *T. sibirica,* and 2 *T. dasystyla* populations were compared in one investigation, which detected 6 different haplotypes. Two of the haplotypes occurred exclusively in eastern populations. The 2 *T. dasystyla* and *T. sibirica* populations were monomorphic. *T. dasystyla* contained a unique haplotype.
Sampling of 13 lime tree populations from Belarus to Khabarovsk in the east was carried out to clarify the phylogeny of lime tree species. Genotyping-by-sequencing analysis was performed. Three main clades were identified: *T. cordata* and its subspecies, *T. amurensis* + *T. taquetii,* and *T. begoniifolia* from the Caucasus. *T. cordata* could be further divided into 4 subspecies. Two of them are regarded as separate species by some authors, *T. sibirica* and *T. nasczokinii*. Despite being an entomophilous genus it does not show large species differentiation.
Another investigation using the entire chloroplast genome also resulted in 3 clades, the first being *T. americana,* and 2 Chinese species *T. miqueliana* and *T. endochrysea*. The second clade consisted of 2 East Asian species, *T. amurensis* and *T. mongolica*. The third clade consisted of 7 Eurasian species, among them *T. cordata* and *T. platyphyllos*. This classification is different from the morphological grouping of *Tilia* species.
The chromosomes of *Tilia* are tiny, which makes counting chromosome numbers laborious. Three ploidy levels were reported for *Tilia*, diploid, tetraploid and octoploid. The basic number is 41, and 15 species are diploids, 8 are tetraploids, and 2 are octoploids, *T. nobilis* and the hybrid *T.* x *euchlora*.

### Population differentiation

In Figs 1-54 we have summarized the data for observed heterozygosity, fixation index, and $F_{ST}$ in various publications. For investigations in which observed heterozygosity was not reported, expected heterozygosities are presented; indicated by e in Fig 1-54. It should be noted that the design of the studies varies, which excludes a direct comparison across studies, but trends might be revealed.
Except for the investigation on *T. rubra*, all other investigations on genetic diversity among populations within species were run with microsatellites. The expected heterozygosity was extremely low in *T. rubra*. Substantial clonality in these marginal populations might be a contributing factor to the low heterozygosity. Fairly low heterozygosities were also noted for *T. nasczokinii, T.*

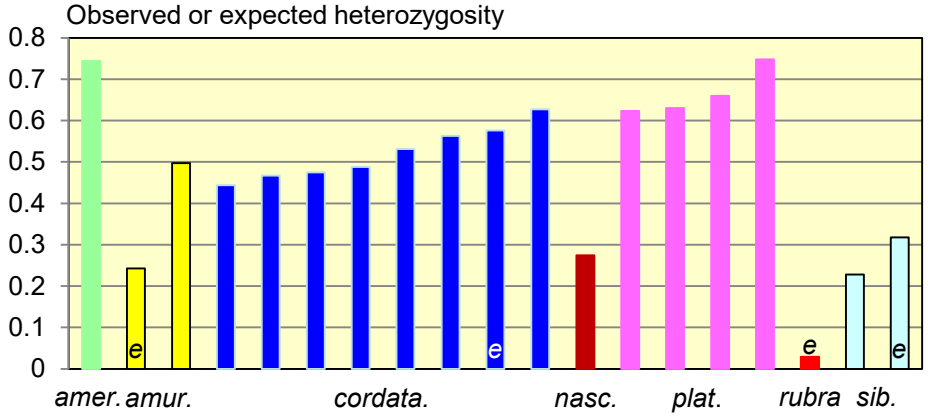


*Figure 1-54. Mean observed or expected (e) heterozygosities in individual populations. Except for* T. rubra *the other estimates were based on microsatellites. amer* = T. americana, *amur* = T. amurensis, *nasc*= T. nasczokinii, *plat* = T. platyphyllos, *sib* = T. sibirica.

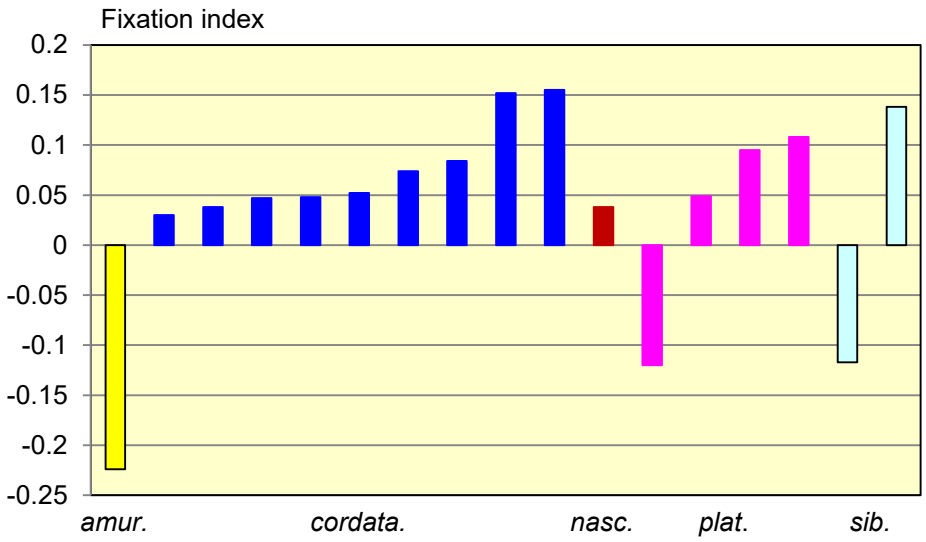


*Figure 1-55. Mean fixation index for populations included in various studies.* *amur* = T. amurensis, *nasc*= T. nasczokinii, *plat* = T. platyphyllos, *sib* = T. sibirica.

*sibirica,* and one investigation dealing with *T. amurensis*, which all had isolated populations. The heterozygosities were higher in *T. platyphyllos* than in *T. cordata,* in which the 2 species were studied in the same investigation. It is obvious that these 2 species contain a fairly high heterozygosity in spite of their scattered distribution. This means that the gene flow in these 2 insect pollinated species is higher than anticipated. The observed heterozygosity was higher in the 3 *T. amurensis* populations from South Korea than in the 10 Chinese *T. amurensis* populations. Unfortunately, no detailed information on the 13 populations studied was given.

With a few exceptions, the fixation indices were positive, which suggests that genetic drift had played a role in the previous history of these populations. Most of the $F_{IS}$ estimates for *T. cordata* were positive but they did not deviate much from 0. An extremely high excess of heterozygotes was noted in all 3 South Korean populations of *T. amurensis* (Fig. 1-55). It should be noted that the $F_{IS}$ estimates varied dramatically among the 15 markers analyzed. Limited information was given about the populations in this investigation. Similarly, no information was given on the *T. sibirica* population with the strong negative $F_{IS}$ estimate. The strong negative $F_{IS}$ estimate for *T. platyphyllos* was based on a fairly low number of trees (11) in a natural park in Bavaria, Germany.

## Population differentiation

In Fig. 1-56 we have compiled $F_{ST}$ estimates on population differentiation in *T. cordata*. The 4 lowest $F_{ST}$s for *T. cordata* originate from Lincolnshire in England, Denmark, Latvia, and Lithuania and reflect fairly homogeneous ambient conditions within each of these regions. Population No. 9 concerns populations all over England and reflects the limited gene flow in this country. The hypothesis that the differentiation is larger among range-edge populations than among central populations was tested in one investigation. Column 5 refers to central populations and column 11 refers to range-edge populations. The mean $F_{ST}$ for the range-edge populations (column 11) was almost twice as large as the $F_{ST}$ for the central populations (column 5), which supports the hypothesis that the differentiation is larger in range-edge populations than among central populations.

No strong relationships with geographic data were observed.

A Chinese study of 6 *T. amurensis* populations along 2

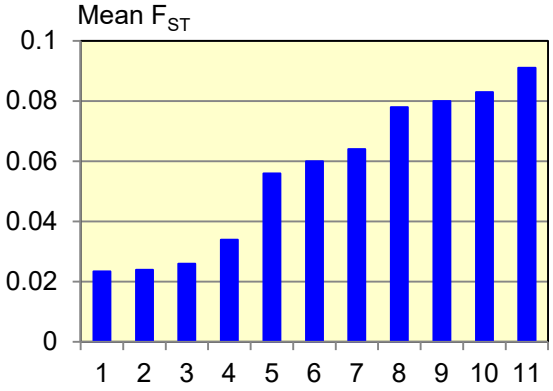


*Figure 1-56. Mean $F_{ST}$ for population differentiation in various studies of* T. cordata*. The estimates were based on microsatellites.*

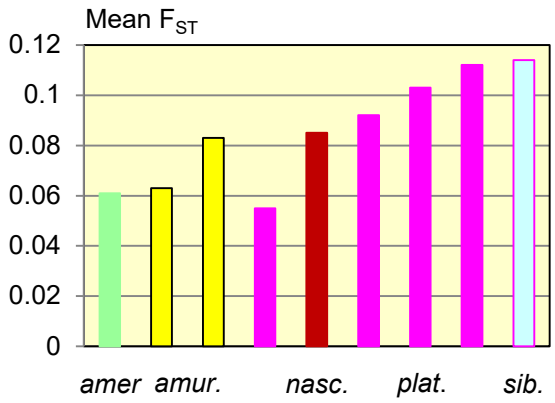


*Figure 1-57. Mean $F_{ST}$ for population differentiation in various studies of* Tilia *species. Except for* T. rubra *the other estimates were based on microsatellites amer* = T. americana*, amur* = T. amurensis*, nasc*= T. nasczokinii*, plat* = T. platyphyllos*, sib* = T. sibirica*.*

geographic transects, one latitudinal 40–50°N and one altitudinal 600–1,100 masl, resulted in mean $F_{ST}$s of 0.063 and 0.083 respectively (Fig. 1-57). These estimates reflect the different ambient conditions along these transects.

One of the *T. platyphyllos* studies comprised 8 evenly distributed populations in the UK. A fairly high mean $F_{ST}$ was noted, 0.103. Also for *T. platyphyllos,* $F_{ST}$s of central and range-edge populations were compared. In this case the $F_{ST}$ of range-edge populations was twice as large as that for central populations (columns 8 and 5). Another investigation concerned a Bavarian natural park, in which one large group of trees and one small group were included. A fairly high $F_{ST}$ was noted, 0.092, for the differentiation of the 2 groups in the natural park. For both investigations, limited gene flow among populations was attributed to these results.

The $F_{ST}$ for the 5 *T. sibirica* populations from a limited area in Siberia was high, which was attributed to limited gene flow among these 5 scattered populations. The high mean $F_{IS}$ of these populations supports this interpretation. A fairly high $F_{ST}$, 0.055, was noted for the difference between 2 *T. americana* populations growing only 900 meters apart. However, the mean $F_{ST}$ for all 5 populations from a much wider origin was only slightly higher, 0.061, which suggest that gene flow occurs in a haphazard way among these 5 populations from southeastern USA.

Only 2 populations of *T. nasczokinii* were studied. Their $F_{ST}$ estimate (0.083) is high for populations growing geographically not far from each other.

Except for the investigation including *T. nasczokinii*, most among-population estimates from AMOVAs supported the $F_{ST}$ estimates. For that species, around 25% of the variance was attributed to among-population variation, which is larger than expected from $F_{ST}$ estimates.

Generally, the other genetic diversity parameters supported the information from observed heterozygosities and fixation index.

The hypothesis that vegetative propagation increases with decreasing temperature climate was tested in 18 *T. cordata* populations from varying climatic conditions in UK. The results supported the hypothesis that clonal reproduction is more prevalent under cooler than under warmer climatic conditions. It was also noted that clonal trees tended to be aggregated together.

### Urban trees

Several reports on genotyping of urban trees were published with somewhat shifting objectives. One commonly occurring objective is the identification of avenue trees to enable identical substitution when there is need for that. Another objective was to study genetic diversity and differentiation. However, in most cases this is not possible owing to the low number of trees analyzed. Identification of needs for broadening of the genetic diversity in urban plantations was another objective of urban trees genetics. One striking observation was that the cultivars planted centuries ago still are produced in commercial nurseries. The need for broadening the genetic base for urban tree plantations was remarked on in some reports.

Inheritance of isozyme markers in *Tilia cordata* was studied in 138 trees from central Germany by Fromm and Hattemer (2003). In addition 67 *T. platyphyllos* trees and 7 *T. cordata* x *T. platyphyllos* trees were analyzed.
The inheritance was studied in open-pollinated offspring, which complicates the analysis of the inheritance. The following conditions must be fulfilled:

- progenies from a homozygous parent must possess the allele of the parent
- progenies from a heterozygous tree must contain one of the parental alleles
- the number of heterozygotes from a heterozygotic parent, $a_ia_j$, is expected to be equal to the number of the 2 homozygotes, thus the number of $a_ia_j$, = the number of $a_ia_i$ + $aja_j$
- the number of $a_ia_k$ progenies is expected to be equal to the number of $a_ja_k$

These conditions build on the assumption of regular meiotic segregation and fertilization as well as absence of any selection.
No significant deviations from the expected segregations were noted for the following isozyme systems:
AP – aminopeptidase
MDH – malate dehydrogenase
MNR – menadione reductase
FDH – formate dehydrogenase
PGM – phosphoglucomutase
PGI – phosphoglucose isomerase
SKDH – shikimate dehydrogenase
IDH – isocitrate dehydrogenase
ACO – aconitase

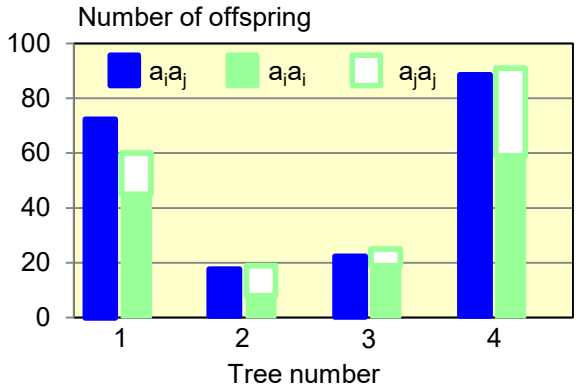


*Figure 2-1. Number of heterozygotes and number of homozygotes at Mnr-A locus in progenies from 4 German* T. cordata *trees. Fromm and Hattemer 2003.*

Although *T. cordata* was assumed to be a hexaploid the inheritance was in all cases disomic.
One example of the observed results for Mnr-A-locus is illustrated in Fig. 2-1, which shows a good agreement between the number of heterozygotes and the total number of homozygotes for all 4 trees tested. The difference in offspring number of the 2 homozygotes is attributed to the allele frequencies of the alleles $a_i$ and $a_j$ in the pollen cloud.
It was noted that different banding patterns occurred in the 2 *Tilia* species studied, suggesting species-specific alleles in the 2 species. It was stated that more populations had to be analyzed to verify if there are species-specific alleles in these 2 species. Isocitrate dehydrogenase showed variation in *T. platyphyllos* but not in *T. cordata*.

Table 2-1. The questions asked and the results of 6 different experiments with 2 selected clones denoted K1 and K3. Jámbor-Benczúr et al. 2001.

| Questions | Results |
|---|---|
| Flushing the year of budding of a selected clone denoted K1 | The undesired flushing at the end of the growth season following budding was significantly lower in K1 than in the *T. platyphyllos* and *T. tomentosa* clones (Fig. 2-2). |
| One-year tree height of K1 and comparison trees | The height growth was much larger in the K1 and the *T. americana* clones than in the other tested clones (Fig. 2-3). |
| Two-year heights and circumference of K1 and of a *T. cordata* clone | The 2-year heights and circumference of K1 were much larger than the corresponding traits of *T. cordata* clone. |
| The effect of rootstock after budding on *T. cordata* and *T. tomentosa* | Much better growth of the K1 clone on *T. tomentosa* than on *T. cordata* rootstock. |
| Annual growth of a selected *T. platyphyllos* clone denoted K3 and K1 and a *T. euchlora* clone following budding on *T. platyphyllos* | The K3 clone grew a little better than the K1 clone and significantly better than the *T, euchlora* clone. |

Lime trees were selected in an avenue surrounded by heavily polluting traffic in Budapest (Jambor-Benzcúr et al. 2001). Most lime trees in the avenue suffered severely from the pollution. The 70 trees of the alley were scored for 3 years with a focus on the following characteristics:

Crown shape
Circumference
Damage-free
Green leaves until autumn coloring

Six experiments with different objectives were carried out and their results are compiled in Table 2-1.

In conclusion the selected clones K1 and K3 can be recommended for cultivation under stressful conditions in Hungary.


## Summary

The inheritance of isozyme markers in *T. cordata* was studied. No significant deviation from disomic inheritance was noted for 8 isozyme systems. The analysis of isozymes in *T. platyphyllos* suggested that species-specific isozyme markers occurred in *T. cordata* and *T. platyphyllos.* Trees in a heavily polluted avenue in Budapest were selected and 6 clonal trials were established, in which bud flushing and tree growth were recorded. It was noted that one of the selected clones had superior characteristics for avenue plantations.


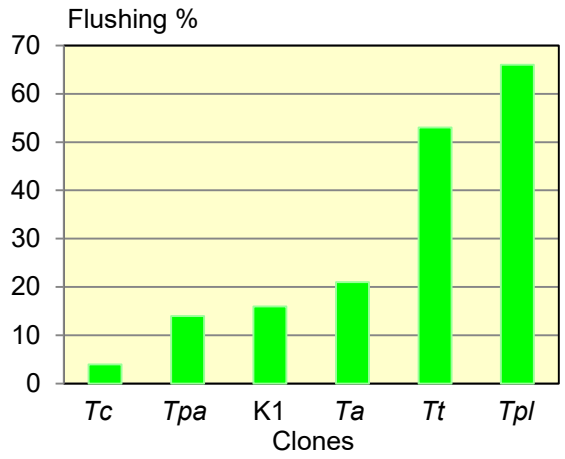


*Figure 2-2. The flushing during the year of budding in the selected K1 clone and Tpl* = T. platyphyllos, *Tt = 2* T. tomentosa, *Ta = 2* T. americana. *Tc = 2* T. cordata. *Tpa* = T. pallida *clones in a Hungarian clone trial. Jambor-Benzúr et al. 2001.*

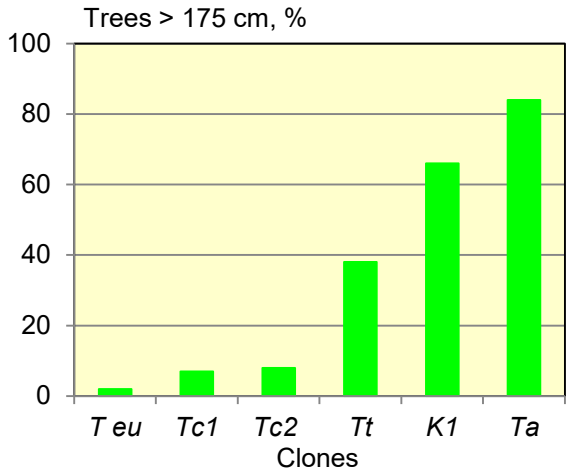


*Figure 2-3. Percentage of trees taller than 175 cm at age 1 after budding of the selected K1 clone and Teu* = T. euchlora, *Tt* = T. tomentosa, *Ta* = T. americana, *Tc* = T. cordata *clones in a Hungarian clone trial. Jambor-Benzúr et al. 2001.*

# 3. Breeding

A method for selection was presented by Lee et al. (2023) to mitigate the effect of overexploitation and degradation of *T. amurensis* in South Korea. This species occurs on 3 main types of habitats in South Korea:

Type A, piedmont or gently hilly area near a valley
Type B, mountainside
Type C, ridge with adverse growth conditions

In all, 20 populations were appointed for selection of plus trees. Four criteria were used for selection of trees from these 20 populations and the weights put on the individual criteria:

| | |
|---|---|
| Tree volume | 0.30 |
| Tree stem form | 0.40 |
| Damage caused by abiotic and biotic stress | 0.15 |
| Vitality under the existing site conditions | 0.15 |

Sophisticated calculations considering site conditions and age were used in order to standardize the performance of 176 candidate trees. Fig. 3-1 reveals that there was a slight increase of plus trees from site A and a corresponding decrease of plus trees from type C sites.

It should be stressed that however careful the selection of plus trees may be, it is still a phenotypic selection. The genetic value of the plus trees cannot be estimated until data from progeny trials are available.

In conclusion an extremely detailed and careful selection of plus trees of *T. amurensis* in different site conditions in South Korea was presented.

Owing to the poor seed maturation of *T. cordata* in northern marginal populations, Amanda Mylett in her thesis from 2015 studied the possibility of using tissue culture to obtain regeneration material. It is beyond the scope of this review to go into details of her efforts. She tackled her task in a systematic way via careful analysis of the following steps of tissue culture:

Explant selection
Aseptic sterilization
Tissue culture media
Plant growth regulators

Contamination by bacteria and fungi turned out to be a great problem. The most successful explants were obtained from newly emerged axial buds. She stated that: *The most effective subsequent sterilisation comprised a pre-treatment of a 15 minute, 0.05% Teepol® wash and a 1 minute dip in 70% ethanol followed by sterilisation of the tissue for 20 minutes with a 20% Domestos® solution and three 20 minute rinses in sterile water.* It was concluded that more work is needed to find cost efficient tissue culture techniques. Propagation by layering might be an alternative to propagation by tissue culture.

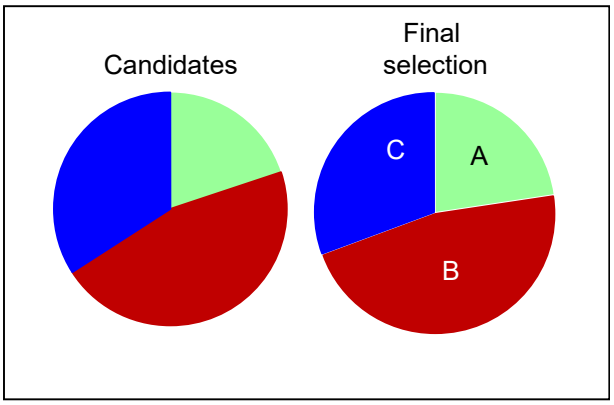


*Figure 3-1. The percentages of candiate trees and finally selected plus trees of* T. amurensis *in different site conditions in South Korea. A = piedmont or gently hilly area near a valley, B = mountainside, C = ridge with adverse growth conditions. Lee et al. 2023.*

Summary. This was a methodic study of the possibilities of using tissue culture for plant production. The results were more the identification of critical conditions for tissue culture production than presentation of successful data from this technique.

Efforts to induce somatic embryos in *T. amurensis* and *T. cordata* were presented by Kim et al. (2007) and Kärkönen (2000). In one case hormonal treatment with 2,4-D was used to induce somatic embryos in glandular trichomes on zygotic embryos in *T. amurensis*. Treatment with abscisic acid and polyethylene glycol to stimulate formation of cotyledonary somatic embryos in *T. cordata* was carried out. No development into plantlets was reported.

## Summary

An extremely detailed and careful selection of plus trees of *T. amurensis* in different site conditions in South Korea was described. Percentage weights were given to 4 traits: stem form 40, tree volume 30, damage caused by abiotic and biotic stress 15, and vitality under the existing site conditions 15.

To overcome the problem with poor seed maturation in *Tilia* a methodic study of the possibilities of using tissue culture for plant production was carried out. The results were more the identification of critical conditions for tissue culture production than presentation of successful results of this technique.

A general inventory of *Tilia* genetic resources in Bulgaria was presented by Zhelev et al. (2020). Conservation is accomplished by selection of seed production stands. Management is focused on promotion of flowering and seed production. The number and size of such seed production areas are presented in Table 4-1. In addition, 3 provenance trials and 3 seed orchards of *T. tomentosa* may be regarded as *ex situ* genetic conservation units. Within EUFORGEN, technical guidelines for genetic conservation of lime trees were presented by Jensen (2003). In principle the MPBS (Multiple Population Breeding System) concept originally presented by Namkoong (1984) for genetic conservation was suggested with a series of *in situ* populations denoted as genetic resource populations. Selection should be based on ecogeographic conditions. At the time of publishing these technical guidelines, *Tilia* genetic knowledge was very limited. It was stated that such *in situ* populations might not be satisfactory owing to poor regeneration or pollen contamination from planted lime trees. In such cases *ex situ* establishment of plantations with material from the same climatic region might be a remedy. Combined breeding and conservation is desired. Moreover, it was stated that: Combining conservation and use is especially necessary for species of low economic interest.

*Table 4-1. The number and size of* Tilia *seed production areas in Bulgaria. Zhelev et al. 2020.*

| Species | Number | Area, ha |
|---|---|---|
| *T. cordata* | 2 | 2.5 |
| *T. platyphyllos* | 22 | 38.7 |
| *T. tomentosa* | 121 | 1,433.8 |

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One of Sweden's longest lime tree avenues, which is approximately 1 km. It was established between Sparreholm Castle and Hyltinge church. Photograph: Gösta Eriksson.

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