# *Tilia* cultivars in historic lime avenues and parks in the UK, Estonia and other European countries

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**Abstract**

Trees have been planted in cities, towns and notable places for many centuries. Here we investigate which genotypes (cultivars) of *Tilia* have been used in Europe. Accessions of trees from across the UK, Estonia, Denmark, Belgium and the Netherlands were analysed and compared to nursery material, using nuclear microsatellites. The majority of accessions (141 out of 166) appeared to be the hybrid *Tilia* x *europaea,* and remarkably there were two cultivars, named Pallida and Zwarte Linde that were commonly planted across north-western Europe and Estonia. The cultivar Hatfield was only found in the UK. In addition, some trees had genotypes only found at one or two locations. Some of the named commercial cultivars also were the Pallida or Zwarte Linde genotype. For the species *T. cordata* and *T. platyphyllos* there were relatively more unique genotypes among the planted specimens. The three most planted cultivars, Pallida, Zwarte Linde and Hatfield, were not genetically closely related. Pallida and Zwarte Linde showed some genetic similarity to Belgian and Dutch material, but Hatfield did not seem similar to mainland Europe genotypes. The genetic analysis confirmed the limited source of the widely planted *Tilia* trees across many European countries, from the 1600s up till now. When supplementation or replanting is necessary genotyping should be used to confirm the historically correct type and maintain uniformity.

**Keywords**:

Cultivar identification, garden design, landscape history, microsatellites, urban trees

**Introduction:**

There is a long tradition of planting trees along avenues and in towns. The tree shaded promenades or allées has a medieval origin, with trees planted along city walls and constructions, such as ramparts, in Germany, the Netherlands, Belgium and north of France (Lawrence, 1988; Miller, 2004). There were also early tree-lined promenades along canals, e.g. in Amsterdam. Other early tree planting as wood supply and to be aesthetically pleasing was seen in France in the early 16th century (Couch, 1992). This later developed into the avenue as a distinct element of the formal garden. In Italy and France grand avenues, e.g. as approach to palaces, were popular in the Renaissance period and this extended into the Baroque period. For example, Palladio and Scamozzi described tree-lined avenues in Italy (Couch, 1992; drawn from Palladio, 1965 and Scamozzi, 1615). In the UK Baroque influences came from the Netherlands, Germany and France (Hunt and De Jong, 1988), while John Evelyn has also had a major effect on early UK formal gardens through his publication of ’Sylva’ in 1664 (Couch, 1992). Regardless of its origins, the planting of large ornamental trees, either as a sign of status or for aesthetic reasons, appear to have been a common practice in England and throughout much of Europe from the middle of the 17th century (Hunt and De Jong, 1988). In some cases these plantings were also in sections used as hunting grounds (Couch, 1992). In the Swedish provinces of Estonia and Livonia (now the republics of Estonia and Latvia) 17th century improvements took place in the medieval-style ornamental horticulture with the building of urban and manorial gardens. Here, the import of ornamental plants and the publishing of horticultural handbooks intensified, leading to the planting of thousands of trees, primarily fruit trees. Developments in Estonia and Latvia were not much behind the developments elsewhere in Europe, except for the smaller scale and the absence of historical features. Tallinn, which expanded its establishment of summer manors, was not devoid of gardens (Sander et al., 2003).

Responsibility for the restoration of historic and ancient landscapes (e.g. parks, avenues and woodlands) currently falls to the discretion of local and national charities, private landowners, local councils, and government departments, usually with the assistance from landscape historians and ecologists. One of the foremost goals for such landscapes is often historical preservation. In contrast to a diversity in tree species planted in parks, trees that line historic avenues are usually of one type, as was popular at the time of planting in the 17$^{th}$ and 18$^{th}$ century. Maintaining the historic integrity of these avenues means maintaining the phenotype of the trees in the avenue and that ultimately means sourcing trees of the same genotype, *i.e.* the same type or cultivar.

While the tree of choice for such plantings varied depending on the designer, the site in question, and the reason for planting, the common parallel was that they were large and uniform. This is particularly important in the symmetric designs required for gardens in the Baroque style. The first commonly used trees were conifers and sweet chestnut, but later horse chestnut, elm and lime were preferred, particularly for walkways and avenues (Couch, 1992). One tree in particular, commonly known at the time as the ‘Dutch lime’, became a favourite in parks, gardens, and avenues from the 17$^{th}$ century and is still regarded a popular street and park tree. This is due to its ease of propagation, ease of shaping, large size and sweet scent (Pigott, 1992; Bengtsson, 2005a). The Dutch lime, now known as the Common lime *Tilia* x *europaea* L. [*syn: T. vulgaris* Hayne] is the hybrid of *Tilia cordata* (Mill.), small leaved lime, and *T. platyphyllos*, large-leaved lime (Scop.).

*Tilia* also has a long history as a source of fodder for cattle and played a major role in pre-Christian mythology (Miller, 2004; Bengtsson 2005a). In many north western European countries, such as Germany and the Netherlands, lime trees were often planted in villages from prehistoric times onwards, where they may have had, or still have, an important function (Brunner, 2007). For example, justice was spoken (Gerichtslinde) under such trees and it was where young people met (Dorflinde, Tanzlinde). In Tallinn, individual trees have merited attention as early as in the Middle Ages. From the 13$^{th}$ century the Rose Garden (Rosengarten) was publicly accessible. It was owned by the Great Guild of Tallinn and included an old lime tree from the middle of the 15$^{th}$ century (Leimus, et al., 2011).

The uncertain identification of *Tilia* began in the late 18$^{th}$ century when various types were classified as one ‘species’. Today, there are approximately 23 described species and

14 sub-species (Pigott, 2012). Although *T. platyphyllos* has also been used in historic avenues (Pigott, 1992) there was little evidence that *T. cordata* was considered (Couch, 1992). Other *Tilia* species, such as *T. tomentosa,* have not been used in the past but are becoming more popular.

At least two different clones of the common lime have been regularly planted since the 17th and 18th century (Pigott, 1992). However, the clarity among historical and modern cultivars is far from obvious (Jablonski and Plietzsch, 2013). Originating from the Netherlands (Couch, 1992; Pigott, 1992), these two popular clones, and other cultivated species and types from the genus are still widely planted throughout the UK and Europe (Santamour and McArdle, 1985). Pigott in his seminal work (Pigott, 1992) described that, particularly in the UK, late 17th and early 18th century plantings of *Tilia* were mostly clones of two cultivars. These were originally imported from the Netherlands and later also propagated at the estates themselves or in nurseries in England (Couch, 1992). The thousands of limes planted from the 17th century across the Netherlands, Denmark, Sweden, England and Russia were propagated in the Netherlands and hence often referred to as ‘Dutch limes’, or as Linneaus called them *Tilia belgicae* (Bengtsson, 2005b). The ease of production through layering, as compared to raising from seed, allowed rapid propagation and added uniformity to the plant material, needed for the Baroque style.

Pigott (1992, 2012) describes these trees as belonging to two groups, A and B, with significant morphological differences between the two groups. To mention a few characters, Group A has a fluted trunk, with many epicormic sprouts and bosses, conical crown, seven flowers per inflorescence and bright green leaves. While other hybrids do not readily sprout, the amount of sprouting and bosses (burls), even higher up on the stem, in group A is often eye-catching. This group is called ‘Pallida’, but in Europe also called ‘Koningslinde’ or ‘Kaiserlinde’. These last two names originate from the fact that *Tilia* has been, and still is, planted in the Netherlands and Germany for particular occasions, such as royal weddings and commemorations, hence multiple trees indicated with names as ‘Koningslinde’ and ‘Kaiserlinde’ (Hunt and De Jong, 1988). Pallida was imported from the Netherlands in 1623 and planted in one of the earliest avenues in the UK for the Duke of Buckingham at New Hall, Essex (Pigott, 2012, p.362).

Pigott (1992) identifies group B by having a cylindrical trunk, few epicormic buds and bosses, a hemispherical crown and dull-green leaves and 3-5 flowers per inflorescence. Group B is variable in presence and absence of flat stellate hairs underside leaves. The name suggested for this group is ‘Hatfield’ group (from Hatfield House), and before 1750 only known from English parks. Although group B cultivars are similar to the Dutch cultivar ‘Zwarte Linde’, Pigott (1992) considers them not identical, e.g. Zwarte Linde has more flowers (6-8) per inflorescence than Hatfield. Although the early plantings, particular in the UK, are either Pallida or Hatfield, later plantings, e.g. restoration of historical plantings, can be other cultivars, sprouts from the earlier two groups or even seedlings arising from the early plantings (Pigott, 1992).

Bengtsson (2005a) described four more groups, called C, D, E and F, with C being the ‘Zwarte Linde’ and D, E and F being specific to Swedish historical plantings. Santamour and McArdle (1985) mention Pallida (Koningslinde and Kaiserlinde), Longevirens (a German cultivar), Wratislaviensis (a Polish cultivar) and the commonly planted Dutch cultivar Zwarte Linde. Jablonski and Plietzsch (2013) give a more recent listing of 149 valid cultivar names, based on phenotypic observations.

Today, parks and gardens across the UK are home to various ornamental trees. *Tilia* is the most planted urban tree in large Scandinavian cities (Sjöman et al., 2012) and one of the most popular in cities in eastern North America (Raupp et al., 2006) as well as in the Baltic countries. Many different types of *Tilia* and several named cultivars are now available from an array of nurseries, both commercial and non-commercial, across Europe. Especially when specific characteristics of cultivars are described for advising practitioners it is important that the names of those cultivars are correct and traceable. *Tilia* is very variable and plastic, and therefore the morphology can be misleading at the species, but particularly at the cultivar level. While it may be possible to identify some cultivars based on their morphology (Pigott, 1992; Bengtsson, 2005a), a more reliable and robust method using molecular techniques is required.

DNA markers have been useful in cultivar identification. Two regional attempts have been undertaken to identify *Tilia* cultivars. Hansen et al. (2014) genotyped *Tilia* from historical plantings in Denmark, using microsatellites. They concluded that the majority of the historical material belonged to two cultivars, one of which was likely to be 'Zwarte Linde', as did modern planted materials to replace some of the older ones, and the second common genotype was likely to be similar to '*Pallida*'. Vanden Broek et al. (2018) identified lime trees in historical plantings in Belgium and the Netherlands using AFLPs and microsatellites. They concluded that recent plantings and current material is limited to the cultivars Pallida and Zwarte Linde, while historic plantings contained a higher diversity of genotypes. For example, the limes planted around the abbey in Tongerlo were genetically different from those two cultivars. However, both the older morphology based studies (e.g. Pigott, 1992; Bengtsson, 2005a) and recent molecular studies (Vanden Broek et al. 2018; Hansen et al. 2014) need to be brought together through a thorough genotyping effort to understand historical and modern plantings of *Tilia*.

Microsatellites are one of the most commonly used and versatile DNA markers. These markers each have a number of variants called alleles (often 2 -15 per marker) that can easily be recorded for each sample. A combination of a number of microsatellites is genotype specific and can also be used to generate a measure of variability of a group of samples and of genetic distances between samples. These methods are similar to those used for human forensic analyses.

*Tilia* specific microsatellite markers (Phuekvilai and Wolff, 2013; Mylett, 2015) reliably identify wild lime tree individuals from both *T. cordata* and *T. platyphyllos* and their hybrid (Logan et al., 2015). Microsatellite DNA fingerprinting will be used to assess the variation and identify *Tilia* cultivars planted along selected historic avenues and from commercial stocks. The resulting database will aid understanding of historical plantings in North Western Europe. We will test the current idea that the majority of such plantings are Pallida and

Zwarte Linde and that in the UK early plantings have been Pallida and Hatfield. The genetic similarity of the cultivars may enlighten their origin and derivation.

## Materials and methods

Leaf samples were provided from various sources, dried and frozen at -20°C until DNA extraction (Suppl.Table 1, Fig.1). Samples were still extant trees from widely varying historical eras and from a variety of urban and semi-urban types of constructions, such as landscaped gardens, avenues, streets, churches and bastions. Further, the choice is based on various biogeographical regions to which we had access and that were not yet studied in detail. A small number of samples from the two earlier local studies, Denmark and Belgium/the Netherlands, were included to put our samples in a wider and already described perspective. The UK samples were from locations for which we had descriptions of the planting history. In the case of Estonia, Tallinn is particularly suitable with its diverse history of the greenery and nature, and its well described 800 year history, after being conquered by the Danes in 1219. Here, many 250- 300 year old *Tilia* tree are still extant.

Samples from three ancient lime avenues in Oxfordshire (Shipton Court, Cornbury Park and Ascot Park), and from Bushy Park and Walpole Park in London were analysed. Shipton Court Wild Garden has had several phases of replanting, with noticeably different limes. The oldest surviving trees are likely from the early 18$^{th}$ century. Cornbury (Oxfordshire) has avenues laid out with advice from John Evelyn in the 1660s (Couch, 1992), with possibly the first imported Dutch limes in the UK (D. Pigott, pers. comm.). The Cornbury sample included is the only likely original tree remaining in the avenue. Ascot Park (Stadhampton, Oxfordshire) has avenues relating to a house with gate piers built in the 1660s, with earliest trees appearing in the 17$^{th}$ century, and several phases of planting with different types.

Bushy Park in London is one of The Royal Parks owned by the Monarch in right of the Crown. The common lime avenue, known as Chestnut Avenue, was planted in 1689 and lined with horse chestnut in 1699, and is currently one of Europe’s longest lime avenues at

1.5 km. Walpole Park limes were planted in the early 19th century on the Estate of Pitzhanger Manor, possibly from cuttings of trees planted earlier.

Samples from the National Trust (NT) were provided from various historic sites. Their nursery provides the plant material for management and restoration of their numerous sites, much of this is native or historical material. The samples obtained from Westonbirt nursery (Forestry Commission, FC) consisted of several named cultivars. The nursery provides the cultivars grown on their land and are used for forest restoration and maintenance. A UK nursery (Barcham Trees) supplied seven accessions and the National Linde Arboretum (Winterswijk, the Netherlands, NL) 11. Some samples were familiar varieties and known species, others were unknown types. Producing new cultivars is common in nurseries, but confusion and labelling can be misleading.

One of the two most common cultivars encountered in the Danish study and likely to be '*Pallida*' was represented here by a single sample, 'DK Green' (Hansen et al., 2014). The second common genotype in the Danish study, morphologically similar to 'Zwarte Linde', was also exemplified by a single sample, 'DK Blue' (Hansen et al., 2014). Belgian material encompasses material from historic and recent plantings at the Tongerlo Abbey (Belgium, BE) and some material from an arboretum, the National Linden Arboretum in Winterswijk (NL), and from growers (Vanden Broeck et al., 2018). The Belgian and some of the Dutch material used here included 21 samples also used in Vanden Broeck et al. (2018).

Three village trees from Germany were included, namely the 'Boslechlinde' or 'Gerichtslinde' from Schaumburg (Niedersachsen), estimated to be 640 years old (Brunner, 2007) and thought to be *T. cordata*, the Friedhofs linde (*T. platyphyllos)* from Collm (Wermsdorf), thought to be 1000 years old, and the Groβe Linde (also *T. platyphyllos)* from Schmorsdorf, 700 years old. Also, four samples, planted in the 20th century, from street trees in Apeldoorn (NL) were included.

The history of the Tallinn vegetation and the dendrochronologically determined tree ages show that limes have been cultivated in the gardens and church environs of the city for about 500 years (Sander and Elliku, 2002; Sander and Abner, 2009). Accessions from

Tallinn used are 70 – 340 years old, as evidenced by historical sources, the tree-ring and the doubtful bark method (Sander et al. 2003; Läänelaid and Sander, 2004). The earliest trees are likely planted at the end of the 17th and early 18th century, while Estonia was under Swedish rule (Sander et al., 2003*)*. Some were planted at the bastions defending the city Tallinn, e.g. at Skoone (now the park of Rannamägi), a Baroque fortification with a name derived from the Swedish province Skåne. Others had been planted near cemeteries of churches from the 13th century Oleviste (St Olaf's) and Niguliste (St Nicholas) at Old Town, and the Toomkirik (Dome Church or St Mary's Cathedral) at Toompea Hill (Domberg). The oldest lime tree here is known as the lime of Kelch (yard's tree) at the church of Niguliste, probably planted in 1680 and named after the clergyman of this church and chronicler Christian Kelch (1657-1710) who is buried here (Sander and Abner, 2009). It can be expected that some trees were imported from Sweden. However, imports from the Netherlands were likely at the park of Harjumägi (Ingermalnad bastion) and the park of Lindamägi (Swedish bastion) after 1750 (Sander et al. 2003). The material analysed from Tallinn encompasses limes from bastions, church surroundings, avenues, streets and cemeteries. The youngest are samples from avenues of the 1950s.

*DNA extraction, microsatellite genotyping and analyses*

Genomic DNA was extracted using a modified CTAB method (Phuekvilai and Wolff, 2013) The Danish samples were extracted using DNeasy (Qiagen, Hansen et al., 2014), while the Belgian and Estonian samples were extracted using QuickPickTM Plant Kit (Bio-Nobile, Vanden Broeck et al., 2018).

Multiplex Polymerase Chain Reaction (PCR) was carried out as described by Phuekvilai and Wolff (2013). A total of 124 accessions were initially genotyped using 11 markers taken from Phuekvilai and Wolff (2013), namely Tc6, Tc937, Tc8, Tc943, Tc4, Tc927, Tc915, Tc963, Tc5, Tc951 and Tc7. This gave the clear indication that at many sites multiple trees of the same genotype were used. For efficiency, we selected from each site/source a limited sample set (74) and added another six markers (Mylett, 2016) to confirm genotype binning results across sources, namely tc1-42, tc2-69, tc2-16, tc3-57, tc1-19 and tc2-86. Microsatellites were genotyped using an ABI 3130XL Genetic Analyser (Applied Biosystems), and scored using Genemapper (Applied Biosystems). Microsatellites genotype data is made available at Mendeley (doi: …….).

Vanden Broeck et al. (2018) in their genotyping study of 129 Belgian and Dutch samples used 14 microsatellites (Phuekvilai and Wolff, 2013). The 14 microsatellites overlapped with the 11 markers used in our study for all the UK and Danish samples (see above). Genotype data of 42 Estonian samples, genotyped by Vanden Broeck, following Vanden Broeck et al. (2018), were included in the current study. Because 19 of the Belgian and Dutch samples were genotyped with our 17 markers as well as the 14 by Vanden Broeck et al. (2018) this allowed the confirmation of genotypes across the two separate genotyping efforts.

Genalex v6.5 (Peakall and Smouse, 2012) was used to determine identical multi locus genotypes (MLG) using the smaller set of microsatellites (11) and allowed the grouping and naming of the cultivar of each of the samples. The data set for further analyses only contained single representatives of each of the genotypes (28 in total), and was based on the larger set (17) of microsatellites. Genalex was used to calculate the average number of alleles per locus ($N_A$) as a measure of genetic diversity as well as the genetic distance between samples (Peakall and Smouse, 2012). The genetic distance is based on how many alleles are different between two pairs of genotypes across the markers. The distance measure, detailed in Peakall et al. (1995), is considered a relevant measure for co-dominant microsatellites markers (Kosman and Leonard, 2005). Those pairs of accessions differing for fewer alleles than other pairs are likely to share a more similar genetic background, and could potentially be more closely related. MEGA 6.06 (Tamura et al. 2013) was used to construct a Neighbor Joining tree from the distance matrix generated in Genalex.

*Species determination*

Characters based on leaves, stems and especially the hairs on leaves can determine the *Tilia* species (Pigott, 2012). However, often this material is not available, e.g. due to season, or is from a location on the tree that is not suitable for species determination using morphological features, i.e. shaded. These are not barriers to species determination using genetic testing. Logan et al. (2015) were able to clearly separate the species and the hybrid using microsatellites so the species determination was based on their work, using species specific microsatellites markers.

## Results

The markers showed high diversity in the accessions analysed, both using the initial 11 and the total 17 microsatellites, with an average number of alleles ($N_a$) of 11.82 across the 17 loci. Therefore, the markers used have strong discrimination powers between the multi locus genotypes (MLG). There were a large number of accessions with the same MLG: adding the additional six microsatellites did not change the identification of repeated genotypes within or across sources but allowed us more accurate analysis of genetic differences between MLG. Two of the 166 accessions were not included in the analyses as they appeared to be tetraploid (FCWB03 and FCWB08), which causes problems with further genetic analyses.

Out of the total 164 accessions included in the analyses we found 28 MLG (Suppl Table 1, Table 1). Each of the MLG differed from each other for at least nine of the 17 loci. The high discriminatory power of the applied markers means that erroneous conclusions about whether two accessions are different or the same cannot be caused by genotyping error. Of the 28 MLG four were *T. cordata*, 13 *T.* x *europaea* (hybrids), 10 *T. platyphyllos* and one *T. tomentosa*.

MLG shared by multiple samples were named using the standards for Pallida (Pg43, Pg53) and Hatfield (Pg44, Pg47) as collected with Prof Pigott personally, corresponding to his groups A and B (Pigott, 1992). This was combined with knowledge of cultivar names of samples provided by growers and arboretums, which in most cases confirmed their grouping and led to logical naming of the three most common cultivars. Group A is Pallida, group B Hatfield and Zwarte Linde can be considered group C, as indicated by Bengtsson (2005a), and these three groups are indeed the hybrid *T.* x *europaea*. Other repeated genotypes were numbered I to V. Six of the hybrid genotypes were represented by more than one sample in our 164 accessions: Pallida 70 times, Zwarte Linde 50 times, Hatfield six times, type I four times, and types II and III two times each. Repeated genotypes IV and V were *T. platyphyllos*, with two accessions each.

Pallida (43 % accessions) and Zwarte Linde (31 %) are the most commonly planted genotypes over many centuries, in the UK, Denmark, Belgium, Estonia and the Netherlands. From the accessions we analysed we conclude that Hatfield is only planted in the UK (accessions from Wimpole, Hatfield House, Shipton Court and Bushy Park). Most historical UK sites studied have all three types, Pallida, Zwarte Linde and Hatfield planted across their sites, while most sites across mainland Europe include mixtures of Pallida and Zwarte Linde.

At Bushy Park a mixture of MLG was observed: 20 Pallida, two Zwarte Linde trees (one from 1800, Chestnut Av, west side, one from 1950, Circle north-west), one Hatfield (1760, Chestnut Av east side), one type II (from 1740) and three unrelated *T. platyphyllos* trees. Shipton court also has all three most common cultivars, with seven Pallida, three Zwarte Linde and three Hatfield. The National Trust collection originates from various National Trust properties and contains all three common UK hybrid cultivars, with five Pallida, six Zwarte Linde and two Hatfield. Within this collection, the three samples from Wimpole are one representative of each of Pallida, Hatfield and Zwarte Linde. Interestingly, Cornbury has one of the earliest planted cultivars and is a unique hybrid *T.* x *europaea*, confirming observation by Prof Pigott (D. Pigott, pers. comm.). Accessions from Walpole Park contained Pallida and Zwarte Linde.

The Belgian samples show a high diversity of genotypes. The four original trees at the abbey garden in Tongerlo are identical hybrids, but they have a genotype, type I, not seen at other locations in this study. The recent plantings at Tongerlo show one unique *T. platyphyllos*, one Zwarte Linde and two identical hybrids with unique genotype (type III). Some of the Belgian heritage trees are Pallida (in Tellin and Geel, BE). The Danish two most common genotypes, ‘DK blue’ and ‘DK green’ are Zwarte Linde and Pallida, respectively, as hypothesized by Hansen et al. (2014). The three German samples from three different towns were all unique *T. platyphyllos* genotypes.

The 42 Estonian samples, from bastions, around churches and from streets, consisted of 22 Zwarte Linde, 16 Pallida, two unique *T.* x *europaea* and two unique *T. cordata* genotypes. The *T. cordata* trees (EET035 and EET 011) are likely to be at least 300 years old. Some locations, such as Skoone bastion and Niguliste church have a mixture of Zwarte Linde and Pallida, but there is not a clear pattern showing either Zwarte Linde or Pallida trees to be the older trees. The famous old lime of Kelch (EET013) is at least 300 years old and Pallida, while the oldest trees at Skoone (EET006 – EET009 and EET018) are Zwarte Linde.

Commercial named cultivars as well as those kept at arboretums match the supposed MLG in some cases, but not all. The ‘Kozakken linde’ from Deventer (NL) was thought to be Pallida, but it is actually Zwarte Linde. Unique hybrid genotypes are from Gent (BE), Sambeek (NL), Deinze (BE) and Nijenrode (NL). One unique, wild hybrid is from Savelbos. The Zwarte Linde from grower Houtmeyer is indeed Zwarte Linde.  The genotype from Wildenborch (NL) is a unique *T. platyphyllos.* The accession HOU_Konings is erroneously sold as Pallida: it is not a common lime, but a *T. platyphyllos.* It was identified as ‘type IV’, identical to a sample from the Royal Botanical Garden in Edinburgh (RBGE2). The four *Tilia* street trees from Apeldoorn (NL) were Pallida (2x), Zwarte Linde and one unique *T. platyphyllos.* The Zwarte Linde is a high, heavily pollarded tree, as is traditional and common in the Netherlands (Fig. 1).

The FC *T. cordata* ‘Westonbirt Dainty Leaf’ is not a *T. cordata* as it is a tetraploid, instead it could be the tetraploid *T. japonica* (D. Pigott, pers. comm.). Similarly, *T. platyphyllos* ‘rubra’ is not *T. platyphyllos* as it is also tetraploid. Instead, it could be *T. dasystyla* subs *caucasica*, for which *T. rubra* was an old name (D. Pigott, pers. comm.). The FC *T. platyphyllos* ‘Aspenfolia’ and ‘Lacinata’ are identical. The Barcham Trees (CULBA) accessions consisted of one hybrid, four *T. cordata* and two *T. platyphyllos*. The hybrid is indeed Pallida. The *T. cordata* trees all have a different MLG, and so have the *T.*

*platyphyllos*. However, their *T. platyphyllos* Rubra has a different genotype from the same named cultivar from RBGE. The Dutch cultivars from the arboretum in Winterswijk consisted of 11 hybrids with different names, with some matching genotype. ‘Jubilee’, both accessions of ‘Koningslinde’, ‘Wratislaviensis’ and their ‘Pallida’ are all identical to Pallida, group A. Their ‘Hemmen’, ‘Kronen’, ‘Opheusden’, ‘Longevirens’ and indeed their ‘Zwarte Linde’ are all identical to Zwarte Linde, group C.

*Genetic similarity*

To find out whether there is a potential common genetic background to the MLG the genetic distance between all pairs of MLG were analysed (Table 2), noting that genetic similarity can be equated to 1 – genetic distance. The average distance between all MLG (including *T. cordata* and *T. platyphyllos* MLG) was 31.1 (standard error 0.31, with a range from 12 to 47). The average distance between the hybrids only was 25.7 (standard error 0.55, with a range 12 to 36).

Zwarte Linde is least distant from type III, a recent planting at Tongerlo Abbey (Tongerlo AA10 and AA1) with a genetic distance of 12. Two trees from the same location, Tongerlo, also have a small genetic distance (14). The most commonly planted cultivar Pallida is least different from the heritage tree from Deinze (ROE, BE), as well as the Bushy Park TRP27 (a *T. platyphyllos*) and GEN_1555 from Gent (BE) (distance 16, 18 and 19, respectively). The accession from Gent is least distant from Deinze and so are Sambeek from Tongerlo Type I (17 and 18, respectively). There also is low genetic distance between the *T. cordata* cultivars from Barcham Trees (CULBA06 to 09 with 20, 21 and 22).

The genetic distance analysis also showed that the three most common *T.* x *europaea* cultivars, Pallida, Zwarte Linde and Hatfield, have a considerable genetic distance from each other, of 27 or 28, which is higher than the average distance amongst all the hybrid MLG of 25.7 (Table 2). The average distance of Pallida from all other hybrid MLG is 24.4, for Zwarte Linde this is 25.7 and for Hatfield 28.2. This indicates that Hatfield seems to stand out as being rather different from other hybrid accessions, while Pallida and Zwarte Linde have a difference from other hybrid accessions that is average.

The NJ tree depicts the genetic distances between the 28 MLG (Fig 2). The cultivars Pallida and Zwarte Linde have near neighbours from Belgium and the Netherlands while Hatfield and Cornbury are distant from other common limes (Fig 2).

## Discussion

*Tilia* is a remarkably hardy species with ancient trees surviving for many centuries in natural environments, in villages and in avenues, often as long as the urban structures have existed (Bengtsson, 2005a). It is a tree species that has had the greatest impact on the vegetation of cities and manor parks in many countries in terms of the climate, landscape and environmental conditions. Genotyping has shown that at most locations planted avenues and parks trees have had several phases of planting (or replanting) with different *Tilia* genotypes, but that Pallida and Zwarte Linde were used across many centuries. There either has seemingly been little care taken in the past to ensure the historically correct genotypes were used to replace trees, or the identical trees were not available locally. Another likely explanation is that the morphology has been misleading because distinguishing between types solely based on morphology can be difficult and even more so with immature trees. This may have been one of the reasons for the mixed use of Pallida and Zwarte Linde at most locations. In addition, genetics has given clues about a shared genetic background for some genotypes.

*The main cultivars*

Pigott (1992) in his seminal work described the lime trees planted in the UK as group A and B. Group A would contain Pallida and Koningslinde, although he mentions a small difference between them when grown next to each other. Here we found that those named Pallida and Koningslinde from various sources are genetically identical. Although no mutations were observed for the 17 microsatellite markers it is possible that mutations have occurred in genes coding for morphological characters that have since been maintained through clonal propagation. However, a more likely explanation is that plasticity of the morphology gives two genetically identical trees a different appearance, while side by side comparisons of accessions are rare. In practice, a maiden tree will look very different from a tree regularly coppiced. Also, leaves at ground level or in the shade can be totally different from those in the crown, up to the extent that even *T. cordata, T. platyphyllos* and the hybrid can be confused. Nonetheless, a character, such as the bossiness of the Pallida group, remains recognisable for the group.

Pigott (1992) suggested calling Group B Hatfield, and considered Hatfield close, but not identical, to Zwarte Linde. We confirmed that they are in fact genetically quite different. Hatfield is only found in the UK and we have maintained this as group B. Bengtsson (2005a) suggests Zwarte Linde to be placed in group C, which we follow here.

In the UK the most used are Pallida and Zwarte Linde, while Hatfield is used much less frequently. Mainland Europe also has mostly Zwarte Linde and Pallida, in more or less equal frequency, and no Hatfield. Across Europe other *T*. x *europaea* genotypes are also planted, potentially from local sources. This is possible because in natural, mixed *T. cordata* and *T. platyphyllos* woodlands *T.* x *europaea* can be found at low frequency (Phuekvilai, 2014). Various genotypes of *T. platyphyllos* were also planted at historical sites. *T. cordata*, although available from nurseries, was only found in historical plantings in Tallinn. The tree from Schaumburg was described as *T. cordata*. The three historical accessions from towns in Germany (640 – 1000 years old) were genetically different, potentially locally sourced, *T. platyphyllos,* confirming the idea that most German village trees are *T. platyphyllos* (Brunner, 2007).

*Diversity at sites*

It is remarkable that almost all UK sites harbour the three different cultivars, Pallida, Zwarte Linde and Hatfield. At Bushy Park the oldest, and probably original, trees are mostly Pallida (13 out of 16). One was a Zwarte Linde from 1800, in Chestnut Avenue, west side. Also one

Hatfield was found in Chestnut Avenue east side, planted in the 1760s. Remarkably, in 1740 a tree was planted at Bushy Park with the same genotype (type II) as the accession from Sambeek (NL). The 11 more recent plantings (1870 and later) are a mixture, with seven Pallida, one Zwarte Linde (1950) in Circle north-west and three unrelated *T. platyphyllos* trees. Shipton Court also has all three common cultivars. The earliest trees tested, dating to the early to middle 18th century, were split between Pallida and Hatfield. The Zwarte Linde were replacements, from the first half of the 19$^{th}$ century.

Hansen et al. (2014) concluded from their study of 200 accessions from Royal Danish Gardens in North Zealand (Denmark) that group A (Pallida) and group C (Zwarte Linde) were by far the most used in this region – and that unique MLGs had primarily/only been used as replacements at later times, when single trees had died in the plantings. Our results confirm this.

The historical and notable trees planted in Estonia are mostly Pallida or Zwarte Linde, with no clear pattern regarding age or sources. The oldest tree is the 'Lime of Kelch', planted in 1680, at a time similar to the earliest landscape plantings in the UK and Scandinavia. This tree is Pallida as are most of the trees at this location, between 250 and 70 years old. However, there is also a Zwarte Linde in the 160 year old avenue and a 160 year old unique *T.* x *europaea*. The other old church in Tallinn, Toomkirik, has a Zwarte Linde and a *T. cordata* presumably planted after the big fire of 1684. The old cemetery of Mõigu has one Zwarte Linde and one *T. cordata* tree. There was strong Dutch and German influence in the 18$^{th}$ and 19$^{th}$ centuries, suggesting these countries as the potential source of Pallida and Zwarte Linde. The bastions are other important historic sites in Tallinn. Skoone was certainly influenced by Sweden and the Netherlands, and here we see again both Zwarte Linde and Pallida, with Zwarte Linde being the older of the plantings. The parks of Harjumägi and Lindamägi are thought to have been planted with trees originating from the Netherlands, all being Zwarte Linde, except for one unique *T.* x *europaea* genotype. Most of the trees imported from Sweden have not survived, but even if they did their initial origin would be the Netherlands.

Current planting across Europe is still mainly of the two main cultivar groups (groups A and C), which is remarkable. Group A is well known for its frequent sprouting and epicormics bosses. Although this is notable and interesting, it is not always aesthetically pleasing and causes high maintenance costs. Group B and C are well known for its susceptibility to aphid infestations, causing honey-dew problems for cars parked below the trees (Pigott, 1992).

*Origin of planted Tilia*

There was a lively trade of plants when the ‘royal houses’ and established elites were building large landscaped gardens in the 17$^{th}$ and 18$^{th}$ century and in particular the design of symmetric gardens and long avenues were popular. *Tilia* reproduction from seed was very difficult in northern European countries, while reproduction through layering or other types of cloning, were easily accomplished (Pigott, 2012). The improving transport made the import across a broad area of Europe of large numbers of limes easier. There are additional cultivars with single or few representatives across sites. It is not clear why these genotypes were shared, but at a limited scale. In addition there are trees, such as from the German locations, Nijenrode, Deinze, Wildenborch and Apeldoorn that were found in only one location. It seems that at the time, in the 17$^{th}$ and 18$^{th}$ centuries, locally available wild trees have been used occasionally in the ‘low countries’, without going through the process of propagation.

From limited historical and contemporary records the geographic origin of common lime, Pallida and Zwarte Linde, has been assumed to be in the Netherlands (Bengtsson, 2005a; Hansen et al. 2014). The Swedish limes are thought to be similar to the very old tree from Deventer (NL), which is indeed confirmed to be Zwarte Linde (Bengtsson, 2005a). However, Bengtsson (2005a) also claimed similarity to the *Tilia* from Sambeek (NL), which is erroneous as this is an unrelated type II hybrid. For Hatfield the original source is unclear, but since it is only found in the UK, a UK origin could be hypothesized.

Genetic distance of trees can potentially shed light on the origin of MLG. We note that care must be taken to interpret this as there may be a bias due to the accessions included in the analysis. The three most common *T.* x *europaea* cultivars, Pallida, Zwarte Linde and Hatfield, have a genetic distance from each other that is similar to or higher than the average distance amongst hybrid MLG and therefore these three cultivars are unlikely to be related or have arisen from the same genetic background (Table 2 and Suppl. Table 1). On the other hand, Zwarte Linde is least distant from type III, a recent planting at Tongerlo Abbey (Tongerlo AA10 and AA1), Pallida is least distant from the heritage tree from Deinze (ROE, BE) and Bushy Park TRP27 (a *T. platyphyllos*, UK) from GEN_1555 from Gent (BE). The accession from Gent (BE) is most similar to Deinze (BE) and Sambeek (NL) to Tongerlo Type I (BE). These similarities could indicate these pairs could come from the same region or, more likely, have shared ancestry.

There is also little genetic distance between the *T. cordata* cultivars from Barcham Trees (CULBA06 to 09); they could, for example, have been grown from a collection of seeds from a single mother, with multiple fathers. Two MLG (A15 and Type I) from the location Tongerlo, also show little genetic difference, and as Tongerlo A15 is a *T. platyphyllos* the hybrid type I could theoretically be an offspring of this tree. However, the *T. platyphyllos* is a recent planting and type I is considered original, making that explanation less likely as only the *T. platyphyllos* could be the parent and not the other way round. The similarity of Pallida and Zwarte Linde with European cultivars in general, and with some more specifically, confirms historical evidence of a Dutch or Belgian origin for Pallida and Zwarte Linde. For Hatfield there is least similarity with other cultivars, either in general or specifically, confirming a UK origin for Hatfield, fitting in with its current UK distribution and historical sources.

In the literature (Bengtsson, 2005b) it has been mentioned that production of common lime, e.g. Pallida and Zwarte Linde, was through both seed and layering, while others claim that *T.* x *europaea* is infertile, allowing propagation only through layering. We can confirm that layering is the only option for producing trees with the same genotype and phenotype as the parental individual. *T.* x *europaea* is infertile, as evidenced by the absence of second generation trees (introgression) in our extensive studies of natural mixed populations (Wolff, pers. obs.).

Others claim that seeds from some cultivars ‘breed true’, meaning that offspring are very similar in phenotype, due to having the same, or almost the same genotype, as the original (parental) tree. However, this would only be possible in highly inbred individuals or those that use complete asexual reproduction. *Tilia* instead is outcrossing and highly heterozygous and therefore offspring will always have a recombined genome that is dissimilar from the parental trees or siblings and hence a different morphology. The clones of Pallida and Zwarte Linde have shown their hardiness over the last 500 years and likely usefulness in the future.

*Cultivar supply and MLG database*

Similar to Vanden Broeck et al. (2018) our results show that the majority of the cultivar names are correct, but some are not. Occasional mislabelling is possible, but also a mutation in a gene affecting morphology may give a distinct appearance and yet may go undetected in a genotyping assay. Naming of cultivars, accessions, types and groups is confusing and various groupings have been suggested over the last three decades. For example, Santamour and McArdle (1985) and Jablonski and Plietzsch (2013) mention ‘Longevirens’ and ‘Wratislaviensis’ (from Poland) as separate cultivars, but we show they were identical to Zwarte Linde and Pallida, respectively. Jablonski and Plietzsch (2013) suggest naming the three main *T.* x *europaea* cultivar groups the Kaiserlinde group (our group A), which we called Pallida, Hatfield (group B) and Zwarte Linde (group C).Hatfield is found in most UK stately parks and should therefore be available from a collection in case historically correct replacements are needed. From the accessions analysed here this seems currently not to be the case. Similarly, the unique Cornbury genotype may deserve preservation.

Our MLG database is not exhaustive as inclusion of certain samples, and not others, was largely based on availability. It could be extended for further use. For example, it would be interesting to include the groups D - F as described by Bengtsson (2005a), such as the Malmvik lime, brought from the Netherlands to Sweden in 1618, and planted in the courtyard of Malmvik manor.

The 17 markers we have used here are sufficient to identify the different MLG. This is indicated by the fact that all MLG differ by nine loci or more. Since this is not a randomly mating population we cannot give the exact level of power for our 28 MLG set. Instead we can glean the discriminating power from a large wild population from the Bavarian forest, with a similar level of diversity (Wolff, data unpublished). Here, the probability of obtaining a MLG by chance is $6.8 \times 10^{-24}$. Such a high diversity means that it is unlikely that MLG can be obtained by chance alone.

*Cultivar choice: past, present and future*

One can wonder why only such a limited number of cultivars has been planted. As described above, this may be inferred from $17^{th}$- and $18^{th}$-century notions, horticultural habits and tree trade at the time. Dutch and Belgian tree growers may have attempted to clonally reproduce many types of *Tilia* trees, and Pallida and Zwarte Linde may have been the easiest to generate the large numbers necessary for avenues. Combined with the habit of international trade and travel of the Dutch, and possibly aggressive sales techniques, made Pallida and Zwarte Linde easily transported across middle and northern Europe. At that time in Russia Peter the Great had grand ambitions and he wished to put the Russian state on the global map, on a par with Western Europe, particularly to rein in the influence of Sweden in this region. Similarly, the Russian elite needed to put their palaces and parks on a par with Western Europe. Peter the Great went to study in the Netherlands and France and this is where he was influenced by palaces and parks in those areas. In addition, Estonia was exposed to influences from Sweden and Germany, increasing the cultural diversity.

The popularity of the cultivars Pallida and Zwarte Linde, in the past and now, was likely based on availability and fashion. Obtaining *Tilia* from traders was the only option in northern European countries where the cold and short summers, particularly a few centuries ago, made natural, sexual reproduction unlikely. In middle and southern European countries the option of locally sourcing material was an option and especially used for planting individual trees, e.g. German, Austrian and French village trees, as opposed to avenue plantings. Similarly, in Tallinn we identified an individual *T. cordata* tree used amongst a larger cohort of *T.*x *europaea*, potentially from local wild sources. Near the cloister church in Stams (Austria) an individual *T. cordata* tree was genotyped as the offspring of a tree in the nearby oak-lime forest (Wolff, unpublished results), also indicating use of local genotypes where available. Therefore, there was little reproduction and trade of each of the local genotypes, keeping their distribution restricted (i.e. some Belgian genotypes).

In the 17th and 18th century considerations regarding a tree's suitability to habitat conditions and restrictions were not considered, but, as described above, more likely to depend on fashion and availability. With *Tilia* being able to withstand low temperatures in winter, the landscape gardeners in cool climates continued to plant them. In urban areas they were, and still are, liked because they seem to adapt well and can grow in many different conditions, including in polluted and drought stricken cities. Furthermore, *Tilia* is considered excellent for increasing biodiversity in forests and urban areas due to its broad ecological needs and excellent ecosystem services (De Jaegere et al., 2016; Morgenroth et al., 2016). It is not invasive and very few diseases or insect threats have been recorded (De Jaegere et al., 2016). Middle to southern Europe has the benefit of a larger array of locally available species to use in urban areas, from the wild or from nurseries, (Morgenroth et al., 2016), including *T. tomentosa*.

Development of future, optimised cultivars, better suited to the changing climate and more extreme urban conditions, has been extremely limited in most broadleaved trees, including *Tilia*, due to the time and scale that such work requires. It seems commercial cultivars have come about by chance, from sowings or cuttings. A strategy using locally sourced material, when possible, may be a good starting point to obtain adapted genotypes. However, due to trees maturing very slowly, selecting ‘better’ or specific cultivars for certain situations is inevitably a very long and difficult process (Sjöman and Nielsen, 2010). Main criteria are likely to be resistance to pests and diseases, drought and pollution resistance, flexibility in terms of roots and crown, pruning and management opportunities, their shape (e.g. pyramid), density and colour of foliage, susceptibility to aphid infestation and suitable adult height. Furthermore, trees with improved ecosystem services, e.g. availability of food for pollinator insects, would further enhance the benefits of trees in urban settings (Morgenroth et al., 2016). Setting up trials of large numbers of cultivars, likely obtained from diverse wild sources, at different locations that are large enough for reliable results may not be realistic, and therefore reporting of successes and failure may be the best way forward. Eventually a database, such as ‘Citree’ may be able to contain reliable information of various commercial cultivars (Sjöman and Nielsen, 2010; Vogt et al., 2017). This would require the use of an associated genotype database, such as developed here, to ensure names and descriptions fit the correct cultivar.

## Conclusion

Naming of types and cultivars is confusing and, if based on morphology, often misleading. A genotype database, using microsatellite markers, is a valuable aid in determining cultivars and a recommended tool to confirm the replacement of historically correct trees and to maintain uniformity of avenues.

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**References**

Bengtsson, R., 2005a. Variation in common lime (*Tilia* x *europaea* L.) in Swedish Gardens of the17th and 18th centuries. PhD thesis. Swedish University of Agricultural Sciences, Alnarp.

Bengtsson, R., 2005b. Propagation of trees of common lime (*Tilia* x *europaea* L.) planted in Swedish gardens in the 17th and 18th centuries. Arboricultural Journal 28, 243-251.

Brunner, M., 2007. Bedeutende Linden, 400 Baumriesen Deutschlands. Haupt Verlag, Bern, Switzerland.

Couch, S.M., 1992. The practice of avenue planting in the Seventeenth and Eighteen centuries. Garden History 20, 173-200.

De Jaegere, T., Hein, S., Claessens, H., 2016. A review of the characteristics of small-leaved lime (*Tilia cordata* Mill.) and their implications for silviculture in a changing climate. Forests 7, 56.

Hansen, O.K., Thomsen, P., Waage Rasmussen, C., 2014. DNA markers provide insight about common lime in historical plantings –An example from the Royal Danish Gardens. Urban Forestry & Urban Greening 13, 543–552.

Hunt, J.D., De Jong, E., 1988. The Anglo-Dutch garden in the age of William and Mary. Journal of Garden History 8 (2,3).Taylor & Francis, London.

Jablonski, E., Plietzsch, A., 2013. Kultivierte Linden, II: Sorten von *Tilia cordata* Mill., *T. platyphyllos* Scop. und *T.* x *europaea* L. Mitteilungen der Deutschen Dendrologischen Gesellschaft 98, 89-110.

Kosman, E., Leonard, K.J., 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. Molecular Ecology 14, 415-424.

Lawrence, A.H., 1988. Origins of the tree-lined boulevard. Geographical Review 78 (4), 355-374.

Läänelaid, A, Sander, H., 2004. History and age of old limes (*Tilia* spp.) in Tallinn, Estonia. In: Forestry Serving Urbanised Societies. Ed. Konijnendijk, C.C., Schipperijn, J., Hoyer, K.K., IUFRO World Series Vol 14, Vienna. pp 267-280.

Leimus, I, Loodus, R., Mänd, A., Männisalu, M., Raisma, M., 2011. Tallinna Suurgild ja gildimaja [The Great Guild of Tallinn and the Guild Hall]. Eesti Ajaloomuuseum, Tallinn, Printon.

Logan, S.A., Phuekvilai, P., Wolff, K., 2015. Ancient woodlands in the limelight: delineation and genetic structure of ancient woodland species *Tilia cordata* and *Tilia platyphyllos* (Tiliaceae) in the UK. Tree Genetics and Genomes 11, 1-12. doi:10.1007/s11295-015-0872-z.

Miller, R.W., 2004. Urban Forestry: History and introduction. In: Forestry Serving Urbanised Societies. Eds. Konijnendijk, C.C., Schipperijn, J., Hoyer, K.K., IUFRO World Series Vol 14, Vienna. pp: 17-23.

Morgenroth, J., Östberg, J., Konijnendijk van den Bosch, C., Nielsen, A.B., Hauer, R., Sjöman, H., Chen, W., Jansson, M., 2016. Urban tree diversity – taking stock and looking ahead. Urban Forestry & Urban Greening 15: 1-5.

Mylett, A.J., 2015. Genetic diversity and regenerative potential of *Tilia cordata* Miller in the Lincolnshire Limewoods. PhD thesis, University of Lincoln.

Palladio, A., 1965. The Four Books of Architecture, Isaac Ware, 1738, facsimile, New York: Dover Publications.Peakall, R., Smouse, P.U., Huff, D.R., 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalo grass *Buchloeë dactyloides*. Molecular Ecology 4, 135-147.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics28, 2537-2539.

Pigott, C.D., 1992. The clones of common lime (*Tília* x *vulgaris* Hayne) planted in England during the seventeenth and eighteenth centuries. New Phytologist 121, 487–493.

Pigott, C.D., 2012. Lime-trees and Basswoods. A Biological Monograph of the Genus *Tilia*. Cambridge University Press, Cambridge, UK, 395pp.

Phuekvilai, P., Wolff, K., 2013. Characterization of microsatellite loci in *Tilia platyphyllos* (Malvaceae) and cross-amplification in related species. Applied Plant Science 1, 1200386

Phuekvilai, P., 2014. Relicts, refugia, and reticulation: a study of population history, hybrids and phylogeny in the long-lived flowering tree genus *Tilia*. Ph.D. Thesis, Newcastle University.  Raupp, M.J., Cunning, A.B., Raupp, E.C., 2006. Street tree diversity in eastern North America and its potential for tree loss to exotic borers. Arboriculture & Urban Forestry 32: 297-304.

Sander, H., Abner, O. 2009. The Stateliest Parks in Tallinn. Laur, M.,Marvet, A. (eds.). The Nature of Tallinn. MTÜ Loodusajakiri. Printed by Baltic Print & Banners OÜ, Tallinn, 34-57.

Sander, H., Elliku, J. 2002. The Diversity of Woody Vegetation in Tallinn, Estonia. – Baltic Forestry. Vol. 8, N0 2, 78-84.

Sander, H., Elliku, J., Läänelaid, A., Reisner, V., Reisner, Ü., Rohta, M., Šestakov, M., 2003. Urban trees of Tallinn, Estonia. Proceedings of the Estonian Academy of Science Biology and Ecology 52, 437-452.

Santamour, F.S., McArdie, A.J., 1985. Checklist of cultivars of linden (*Tilia*) species. Journal of Arboriculture 11, 157-164.

Scamozzi, V., 1615. Dell Idea Della Architettura Universale, Venice.

Sjöman, H., Nielsen, A.B., 2010. Selecting trees for urban paved sites in Scandinavia – a review of information on stress tolerance and its relation to the requirements of tree planner. Urban Forestry & Urban Greening 9, 281-293.

Sjöman, H., Östberg, J., Bühler, O., 2012. Diversity and distribution of the urban tree population in ten major Nordic cities. Urban Forestry & Urban Greening 11, 31-39.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30, 2725-2729.

Vanden Broeck, A., Cox, K., Melosik, I., Maes, B., Smets, K., 2018. Genetic diversity loss and homogenization in urban trees: the case of *Tilia* x *europaea* in Belgium and the Netherlands. Biodiversity and Conservation 27, 3777–3792.

Vogt J., Gillner, S., Hofmann, M., Tharang, A., Dettmann, S., Gerstenberg, T., Schmidt, C., Gebauer, H., Van de Riet, K., Berger, U., Roloff, A., 2017. Citree: a database supporting tree selection for urban areas in temperate climate. Landscape nad Urban Planning 157, 14-25.

**Figure legends:**

**Fig. 1.** Images of some of the trees used; a) Zwarte Linde, Walpole Park tree 618; b) Zwarte Linde, Bushy Park TRP tree 10981; c) Zwarte Linde park of Lindamägi; d) Zwarte Linde, NLAP01, pollarded street tree; e) Hatfield, Pg44; f) Hatfield, Bushy park TRP10389; g) Pallida, Pg43; h) Pallida, UKAL, Alnwick Castle; i) Pallida, Ascott avenue; j) *T. platyphyllos*, GESC, Schaumburg, Germany; k) *T. platyphyllos,* GECL, Collm, Germany.

**Fig 2**. Neighbour Joining tree based on genetic distance of the 28 *Tilia* MLG. Accessions are *T.* x *europaea*, except those indicated with Tp (*T. platyphyllos)*, Tc (*T. cordata*) and Tt (*T. tomentosa*).

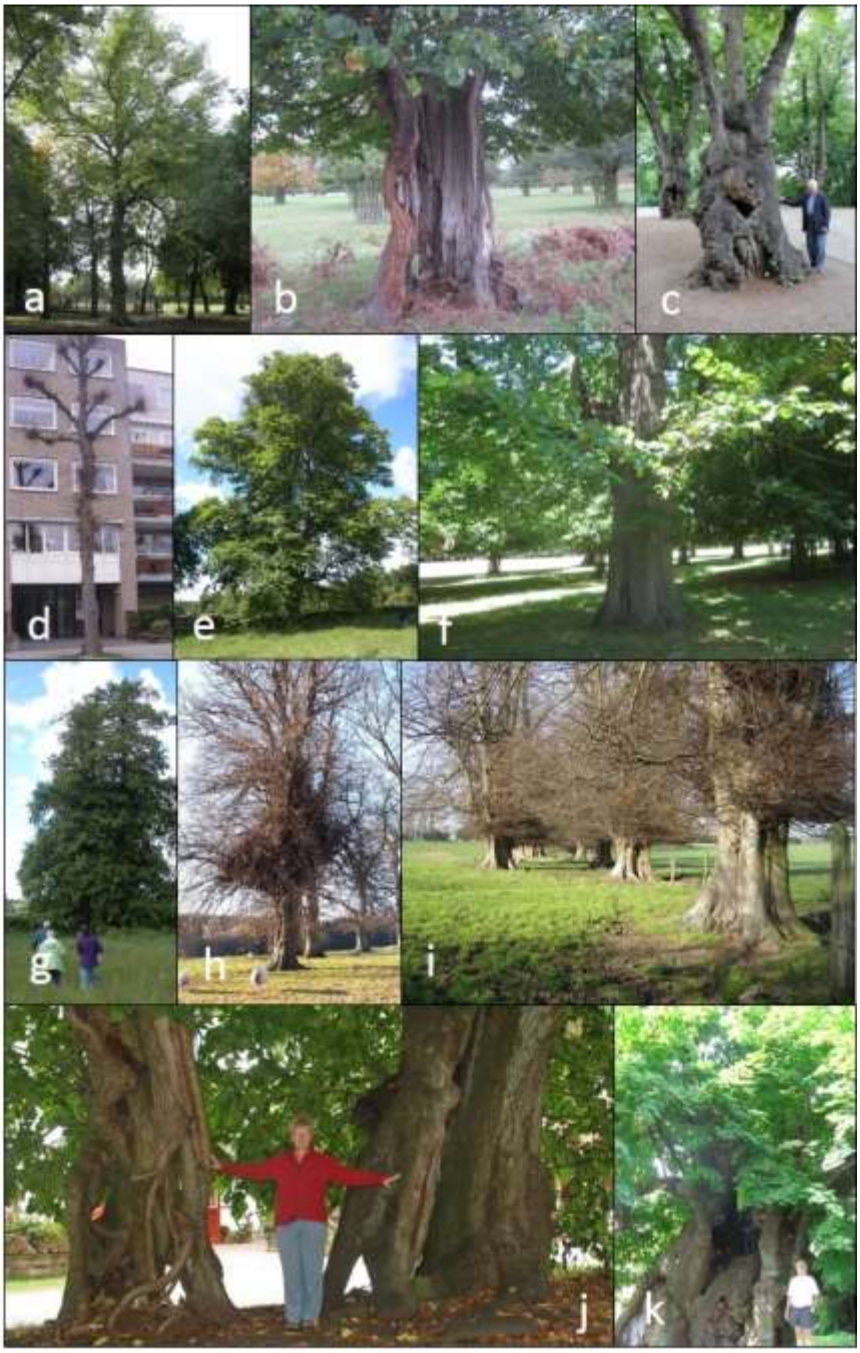


Fig. 1.

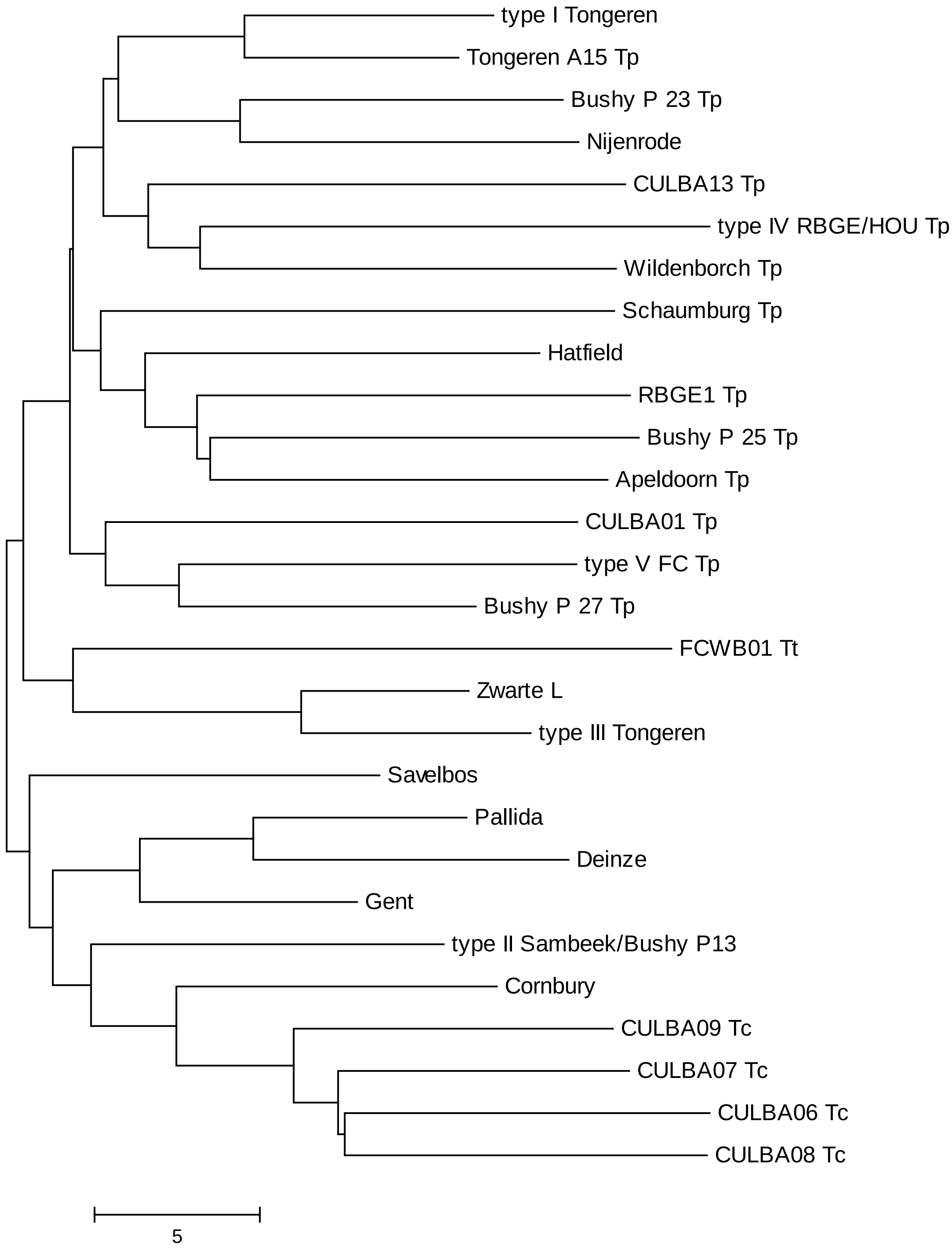
type I Tongeren
Tongeren A15 Tp
Bushy P 23 Tp
Nijenrode
CULBA13 Tp
type IV RBGE/HOU Tp
Wildenborch Tp
Schaumburg Tp
Hatfield
RBGE1 Tp
Bushy P 25 Tp
Apeldoorn Tp
CULBA01 Tp
type V FC Tp
Bushy P 27 Tp
FCWB01 Tt
Zwarte L
type III Tongeren
Savelbos
Pallida
Deinze
Gent
type II Sambeek/Bushy P13
Cornbury
CULBA09 Tc
CULBA07 Tc
CULBA06 Tc
CULBA08 Tc
5

Fig 2.

**Table 1.** Multi locus genotypes, their species, the number of accessions of that MLG and origin. Txe is *T.* x *europaea*, Tp is *T. platyphyllos*, Tc is *T. cordata* and Tt is *T. tomentosa*.

| MLG | Species | Nr of accessions | Locations |
|---|---|---|---|
| Pallida (group A) | *Txe* | 70 | All countries |
| Zwarte Linde (group C) | *Txe* | 50 | All countries |
| Hatfield (group B) | *Txe* | 6 | UK |
| Type I | *Txe* | 4 | Tongerlo (BE) |
| Type II | *Txe* | 2 | Sambeek (NL), Bushy Park (UK) |
| Type III | *Txe* | 2 | Tongerlo (BE) |
| Type IV | *Tp* | 2 | RBGE (UK),'Konigslinde' (BE) |
| Type V | *Tp* | 2 | 'Laciniata', 'Rubra' (FC) |
| FCWB01 | *Tt* | 1 | FC (UK) |
| CO001 | *Txe* | 1 | Cornbury (UK) |
| CULBA01 | *Tp* | 1 | Barcham Trees |
| CULBA06 | *Tc* | 1 | Barcham Trees |
| CULBA07 | *Tc* | 1 | Barcham Trees |
| CULBA08 | *Tc* | 1 | Barcham Trees |
| CULBA09 | *Tc* | 1 | Barcham Trees |
| CULBA13 | *Tp* | 1 | Barcham Trees |
| TRP23 | *Tp* | 1 | Bushy Park (UK) |
| TRP25 | *Tp* | 1 | Bushy Park (UK) |
| TRP27 | *Tp* | 1 | Bushy Park (UK) |
| GESCB | *Tp* | 1 | Germany |
| TO_TAD_A15 | *Tp* | 1 | Tongerlo (BE) |
| GEN_1555 | *Txe* | 1 | Gent (BE) |
| WIL_Teur138 | *Tp* | 1 | Wildenborch (NL) |

| | | | |
|---|---|---|---|
| SCH_Teur66 | *Txe* | 1 | Savelbos (NL) |
| ROE_438-798-B49 | *Txe* | 1 | Deinze (BE) |
| NIJ_Nijenrode | *Txe* | 1 | Nijenrode (NL) |
| NLAP2 | *Tp* | 1 | Apeldoorn (NL) |
| RBGE1 | *Tp* | 1 | RBGE (UK) |

**Table 2.** Genetic distance of MLG pairs with the smallest genetic distance and the distances between the three most common cultivars. All are *T.* x *europaea* accessions, except those indicated Tp (*T. platyphyllos*) and Tc (*T. cordata*).

| MLG 1 | MLG 2 | Genetic distance |
|---|---|---|
| Type III Tongerlo | Zwarte L | 12 |
| Tongerlo A15 (Tp) | Type I, Tongerlo | 14 |
| Deinze | Pallida | 16 |
| Gent | Deinze | 17 |
| Sambeek | Type I, Tongerlo | 18 |
| Bushy Park 27 (Tp) | Pallida | 18 |
| GEN_1555 | Pallida | 19 |
| CULBA07 (Tc) | CULBA06 (Tc) | 20 |
| CULBA09 (Tc) | CULBA07 (Tc) | 20 |
| CULBA08 (Tc) | CULBA07 (Tc) | 20 |
| Nijenrode | Bushy Park 23 (Tp) | 20 |
| CULBA09 (Tc) | CULBA08 (Tc) | 21 |
| CULBA09 (Tc) | CO001 | 21 |
| Bushy Park 27 (Tp) | FCWB07 (Tp) | 21 |
| Gent | Savelse bos | 21 |
| CULBA08 (Tc) | CULBA06 (Tc) | 22 |
| CULBA13 (Tp) | Tongerlo 15 (Tp) | 22 |
| Gent | Tongerlo 15 (Tp) | 22 |
| Zwarte Linde | Pallida | 27 |
| Hatfield | Zwarte Linde | 28 |
| Hatfield | Pallida | 28 |