Informed conservation management of rare tree species needs knowledge of species composition, their genetic characteristics and ecological niche

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Abstract

Woodland nature reserves must be scientifically assessed so that subsequent management leads to optimal conservation of biodiversity. This entails knowledge of the species composition, the genetics of the local populations and their ecology. Here we assess *Tilia* species in the Bavarian Forest National Park (BFNP), a large mixed coniferous and deciduous forest in South-Eastern Germany. *Tilia* occurs here at low density, as in many other mixed forests in Central and Northern Europe. Therefore, results are not only relevant to BFNP but also to other areas.

Exhaustive sampling resulted in the collection of 113 mature trees that were genotyped using 20 microsatellite markers, derived from both *T. cordata* and *T. platyphyllos*. For the first time, size and aspect of trees, and their community association were contrasted between the species. Genotyping confirmed that *T. platyphyllos*, *T. cordata* and their hybrid (*T.* x *europaea*) were present in the BFNP and both species deserve conservation. *T. platyphyllos* has a higher genetic diversity for both sets of markers than *T. cordata*, confirming earlier work. Both species showed genetic diversity comparable to other populations in Central Europe, which is likely to be sufficient for the maintenance of the species in the short term. However, increasing the number of trees, ensuring local sources are used, and gene flow from surrounding forests over the next decennia may be crucial for long-term survival.

Further, within the *T. platyphyllos* group there was a set of 11 trees that were distinct from the others: they had a lower genetic diversity and were shorter. We hypothesise that these were planted and should not be used for propagation and augmentation. Most saplings analysed appeared to derive from asexual propagation (36 out of 41), although a few (five out of 41) were novel genotypes. This means that, currently, there is some, but rather limited, regeneration.

T. cordata was found at a lower altitude and less steep terrain than *T. platyphyllos* and the hybrid. The hybrid was taller than the two species, while the diameter at breast height was smallest in *T. cordata*. *T. cordata* shows a preference for mixed and coniferous forests, while *T. platyphyllos* occurs mostly in deciduous forests.

Our results indicate that biodiversity at the species and genetic level as well as species' ecology have to be considered in order to guide informed conservation management. These results form the basis to recommend conservation management improving the long-term viability of *Tilia* in the BFNP and other mixed forests.

Keywords: Forestry, *Tilia*, genetic diversity, habitat preference, protected areas, hybridisation, silviculture

1. Introduction

A broad range of biological and ecological knowledge is needed to understand the functioning and sustainability of forest ecosystem (Aerts and Honnay, 2011). This includes specific knowledge of genetic diversity of forest trees, which aids conservation management at the species and the within-species level and supports forest genetic monitoring (Fussi et al., 2016). Assessment of population genetic diversity and genetic patterns help to determine difficult to distinguish species and their hybrids and to assess neutral diversity of local stock, as compared to other populations. These patterns can highlight whether separate genetic clades exist within a location, for example to find or exclude trees for regeneration or augmentations. For this reason and to track traded timber and wood products genetic markers are developed for many species (Finkeldey et al., 2010). However, differentiation of forest tree populations is often weak and may, in some cases, limit detection of within species genetic patterns (Finkeldey et al., 2010; Phuekvilai, 2014). Assessment of ecological and adaptive diversity and suitability for augmentation cannot be directly determined with neutral markers, instead quantitative genetic studies, e.g. QTL analyses, would be needed (Holderegger et al., 2006).

Forests are among the most diverse ecosystems in the world and harbour a major part of total biodiversity (e.g. FAO, 2015; Fady et al., 2016). Within the forest ecosystem trees are keystone species, providing many ecosystem services and contribute to human wellbeing (Fady et al., 2016). National Parks have been set up in various countries as a measure to protect forest from an unprecedented forest loss and forest degradation worldwide (Curtis et al., 2018) due to overharvesting, land use change and human population growth (Dudley, 2008). This protection may require active management, while considering the natural species and within species diversity.

Under-researched and (currently) non-commercial tree taxa, such as *Tilia* species are lagging behind in knowledge and detailed studies. The two most common and endemic *Tilia* species in North West Europe are *T. cordata* (Mill.) (small leaved lime) and *T. platyphyllos* (Scopp.) (large leaved lime). Where the two species co-occur, they can produce the hybrid called *T. x europaea* L. [*syn: T. vulgaris*

86 Hayne], also called common lime or in the past Dutch lime. This hybrid tree is the lime that is often
87 planted in parks and along streets (Wolff et al., 2019). *Tilia* are insect pollinated species, flowering
88 relatively late in the season after the leaves have fully expanded. In Central Europe this is late June
89 for *T. platyphyllos* and for *T. cordata* about 10 days later, early July (Pigott, 2012, 2020). They take at
90 least 20 years to flower and reproduce sexually, but in dense woodland this may be much later
91 (Pigott, 2012). The species are highly outcrossing, with inbreeding coefficients (F_{IS}) close to zero and
92 medium to high genetic diversity (Logan et al., 2015, 2019). In some regions, asexual reproduction
93 through root collar growth, epicormic shoots or rooting of branches that touch the ground (Pigott,
94 2012; Logan et al., 2018; Erichsen et al., 2019) is common. Generally, clones of the same genotype
95 are only found at short distances from each other (average 5m for *T. platyphyllos* and 22m for *T.*
96 *cordata*, Logan et al., 2019).
97 The current distribution of *T. cordata* ranges from Italy and Greece in the south to Finland in
98 the north and from Siberia in the east to the United Kingdom in the west. *T. platyphyllos* has a more
99 limited distribution, ranging from Italy and Greece in the south to Denmark in the north, and
100 Romania in the east to the United Kingdom in the west (Logan et al., 2019; www.euforgen.org). *Tilia*
101 is host for many species, such as birds, insects and lichen (Pigott, 2020). Tree stems become hollow
102 after about 200 – 300 years and form suitable nesting sites for e.g. birds and wild bees (Pigott, 2012)
103 and the hermit beetle (*Osmoderma eremita*), protected as high priority species by the European
104 Union Habitats Directive.
105 *Tilia* has played important roles in mixed deciduous forests for a long time. After the last Ice
106 Age it was, along with *Quercus*, the most abundant tree in Western Europe (Huntley and Birks, 1983;
107 Pigott, 2012). The Bavarian Forest National Park (BFNP) is in the central range of both *Tilia* species.
108 Pollen records show that *Tilia* was prominent in the BFNP region from about 10.000 years ago until
109 about 4000 years ago, when its frequency declined in the region (Huntley and Birks, 1983; Van der
110 Knaap et al., 2019). Van der Knaap et al. (2019) were able to distinguish the *Tilia* species in the BFNP
111 pollen record: only 7% of pollen was *T. platyphyllos*, while the majority (93%) was *T. cordata*.

However, only *T. platyphyllos* has been reported as currently present in the BFNP (Walentowski et al., 2004; Nationalparkverwaltung BW 2008).

Lime trees have lost their wide abundance since their maximum after the Ice Age due to climatic change, changed landscape use by humans and forestry practices (Huntley and Birks, 1983; Pigott, 2012). *Tilia* is shade tolerant and can survive for a long time in dense forests as shrubs (Belostokov, 1980 as cited in Radoglou et al., 2009). However, dense forests also hamper sexual reproduction as trees generally only flower when their crowns reach sunlight. On the one hand grazers limit the survival of seedlings and saplings (Pigott, 2020), but on the other side they can create the space for saplings to become large trees that can flower. Therefore, we do not know what effect past or current grazing has on *Tilia* abundance.

Cultural and local uses of *Tilia*, as food, fodder, and for rope making and woodcarving, are well known, especially in Central and Eastern Europe. *Tilia* has rarely been planted in woodland and forests (Pigott, 2012; Coello et al., 2013; Hemery et al., 2008). Therefore, those currently present in woodlands across Europe (as opposed to gardens and streets) are considered endemic. In modern silviculture *Tilia* is added as a minority species due to its high environmental and social values (Hemery et al., 2010; Coello et al., 2013), and also because it is shade and drought tolerant (De Jaegere et al, 2016), and its leaf litter improves the soil (Coello et al., 2013; Hommel and de Waal, 2003; Maes and Van Vuure, 1989).

Little is known about the ecology of vulnerable or rare forest tree species, such as *Tilia,* in their natural environment (Myking, 2002). Radoglou et al. (2009) report on the silviculture of three European *Tilia* species, but do not comment on comparisons of the species in their natural environment. Coello et al. (2013) consider that the two species have similar site requirements, with *T. cordata* being better at withstanding stagnant water. There are no studies, as far as we know, that statistically compare *T. cordata* and *T. platyphyllos* in their natural environment for their size, their aspect and communities. This information would aid conservation of genetic resources in the species, in the BFNP as well as in many similar woodlands in Central Europe (Myking, 2002).

It is important for informed conservation management to know what species of *Tilia* trees are represented in a given area, whether they have genetic diversity that is normal for the species and whether they are most likely endemic. We can build on earlier studies of *Tilia* throughout Europe. Logan et al (2015) tested microsatellite markers in mixed woodlands in Britain and showed that the two species are distinctly different and possess some species-specific alleles. They showed that the hybrid is also distinct from the two species. Using these markers Logan et al. (2019) described genetic diversity, clonal reproduction and effective population size in a large number of range-edge as well as Central European populations. To understand genetic diversity and species composition in the BFNP we will use the same markers developed for *T. platyphyllos* (Phuekvilai and Wolff, 2013) and, in addition, markers developed for *T. cordata* (Mylet, 2016).

The main questions from a conservation management perspective are 1) Which *Tilia* species occur in the BFNP and are there hybrids?; 2) Is the genetic diversity in the species as expected and are there genetic structures that have to be considered; 3) are there ecological considerations that can aid the augmentation of the *Tilia species*. We aim to answer these questions through genotypic and ecological analyses of all *Tilia* trees in the national park, revealing their genetic diversity, relatedness, effective population size and reproduction.

2. Materials and methods

2.1 *Study area*

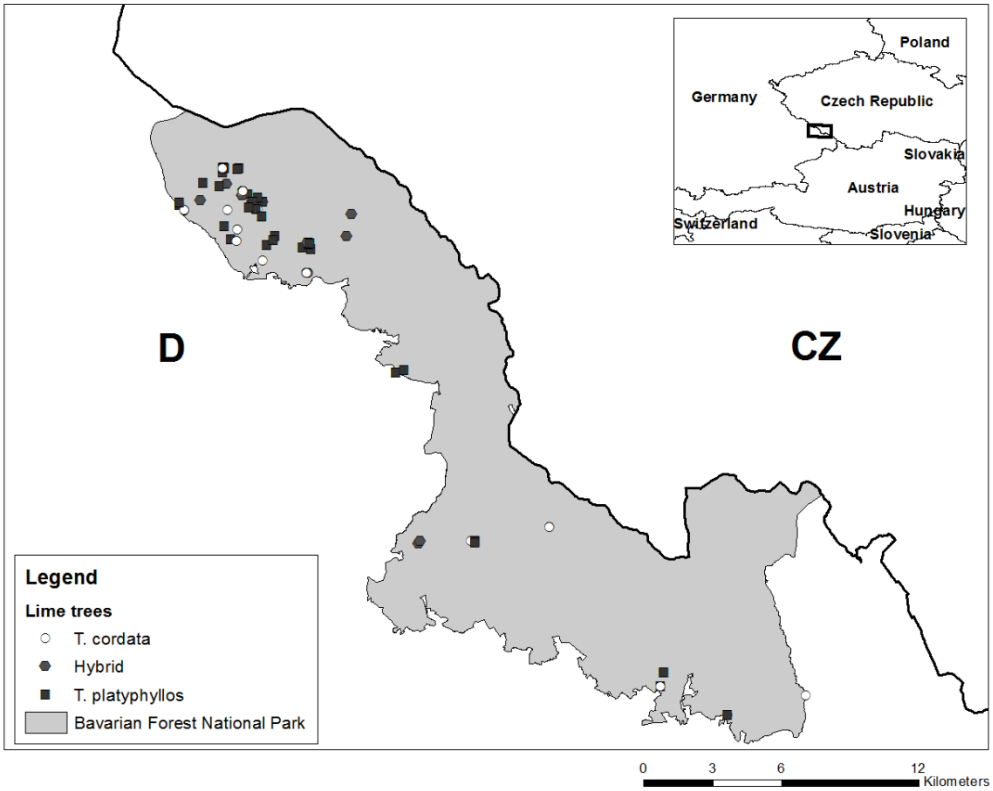
The Bavarian Forest National Park (BFNP) harbours a large diversity of endemic trees, with continuous forest cover since the last Ice Age (Van der Knaap et al., 2019). During times of unregulated forestry, trees were used as firewood for glass production and for the generation of potassium. Since the first half of the 17th century the Royal Bavarian Forest Administration managed the forests in a regulated manner. Also, livestock grazing was common until after WWII (Heurich and Englmaier, 2010). The Bavarian Forest National Park (24,250 ha) is now a strictly protected area in

164 southeast Germany, adjacent to the border with the Czech Republic (48.9595 °N, 13.3949 °E). It
165 covers an area ranging in elevation from 600m to 1,450m above sea level (a.s.l.). A buffer zone,
166 situated along the national park border, aims to conserve and protect areas adjacent to the national
167 park from potential damages caused by the non-intervention strategy implemented within the core
168 zone of the national park. No management takes place within the core zone of the park, which
169 comprises about 72% of the total area.
170 Norway spruce (*Picea abies*), European beech (*Fagus sylvatica*) and Silver fir (*Abies alba*) are
171 the main tree species in the BFNP, mixed with other tree species, such as ash (*Fraxinus excelsior*),
172 sycamore maple (*Acer pseudoplatanus*) and lime (or linden, *Tilia*) depending on the specific location
173 and microclimate (Van der Knaap et al., 2019). Across the elevation gradient, mean annual
174 temperatures vary from 3°C to 6.5°C, and mean annual precipitation ranges from 830mm to
175 2,230mm, much of which falls as snow. Snow cover persists for 5–8 months each year depending on
176 elevation (Heurich et al., 2010). This elevation gradient maintains a variety of forest types, which can
177 be split into three broad categories. Above 1,100m a.s.l., sub-alpine Norway spruce forests
178 dominate, with rowan as a minor component. At 600–1,100m a.s.l., mixed forests of Norway spruce,
179 silver fir (*Abies alba*) and European beech (*Fagus sylvatica*) dominate with interspersed sycamore
180 (*Acer pseudoplatanus* and *A. platanoides*) and lime (*Tilia cordata* and *T. platyphyllos*). In cold and
181 wet depressions at the bottom of valleys, Norway spruce (*P. abies*), rowan (or Mountain ash *Sorbus*
182 *aucuparia*) and birch sp. (*Betula pendula* and *Betula pubescens*) dominate (Cailleret et al., 2014).
183
184 2.2 *Sampling and ecological measurements.*
185 *Tilia* trees within the Bavarian Forest were exhaustively sampled (Fig. 1). The main source of
186 information was the Natura 2000 survey, which took place in 2004 and 2005 when all habitat types
187 were mapped throughout the park (Nationalparkverwaltung BW, 2008). During this process, all *Tilia*
188 trees were mapped. In addition, all existing databases were searched, and the local forest managers
189 and rangers asked for known *Tilia* trees. We also searched for regeneration one tree length around

each tree. This survey resulted in 113 adult trees distributed throughout the park. From each tree, we sampled two leaves with the help of a slingshot (similar to the method used in Ali et al., 2016) and then leaves were dried in paper bags.

Every tree sampled received a unique tree number. Its location was represented by X- and Y-coordinates (GK system) using a Leica Zeno 5 GPS. Height of the trees was measured using a Haglöf Vertex clinometer (Haglöf Sweden AB, Långsele, Sweden) while the diameter at breast height (DBH) was measured with a measuring tape. The wood volume was a calculated value V, calculated as V=G*H*f, with G being basal area and H being height. A diametral quotient (f) is not known for *Tilia*. Therefore, we used the f value for beech, namely f = 0.49 (Kennel, 1969).

The altitude was expressed in meters above sea level (a.s.l.), while slope was expressed in degrees. These were obtained from the survey administration of Bavaria. Vitality was estimated in situ using the system of Roloff (1985). The habitat type was determined following Elling et al. (1987) and the Potential Natural Vegetation (PNV) following Fisher et al. (2013), using the GIS database of the BFNP administration. The PNV is the community of forest plant species you would expect without human interference at that site (Tüxen, 1956).



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206 **Fig 1.** Location of sampled *Tilia* trees in the Bavarian Forest National Park, and the location of the
207 BFNP in Europe. Symbols indicate the *Tilia* species, confirmed by genotyping, with open circles
208 indicating *T. cordata*, filled hexagons are the hybrid *T.* x *europaea* and the filled squares are *T.*
209 *platyphyllos.*

210

211 2.3 *DNA extraction and microsatellite genotyping*

212 Leaves of 113 adult trees and 41 saplings were dried directly after sampling and then stored at -20°C.
213 Genomic DNA extraction was performed using the CTAB method (Morgan-Richards and Wolff,
214 1999). A Polymerase Chain Reaction (PCR) was performed as a multiplex procedure for 20 nuclear
215 microsatellite regions following Phuekvilai and Wolff (2013). Thirteen of those were originally
216 derived from *T. platyphyllos* genomic DNA (Tc6, Tc937, Tc920, Tc8, Tc943, Tc4, Tc927, Tc915, Tc963,
217 Tc11, Tc5, Tc951 and Tc7, Phuekvilai and Wolff, 2013) and seven markers were developed from *T.*
218 *cordata* (tc1-42, tc2-69, tc2-16, tc3-57, tc1-19, tc3-74 and tc2-86, Mylett, 2016). Microsatellites were

219 genotyped using an ABI 3130XL Genetic Analyser, visualised using Genemapper (Applied Biosystems)
220 and binned and scored manually. Every run a small number of samples were repeated and scored
221 identical across runs. There were no missing data.
222
223 2.4 *Genetic data analyses*
224 Deviation from Hardy-Weinberg equilibrium was tested in *T. cordata* and *T. platyphyllos* separately,
225 using Genalex 6.5 (Peakall and Smouse, 2012), with the notion that with small sample sizes and small
226 expected values in some genotypic classes the test is not reliable (Hedrick, 2005). Tests with and
227 without loci that differed substantially for H_o and H_e (Tc11, tc1-42 and tc2-69) and loci with a large
228 number of alleles (>17, namely, Tc915, Tc963 and Tc927) did not yield different results after
229 analyses, so all 20 loci were retained. Further, Genalex 6.5 was used to calculate various measures of
230 genetic diversity (N_a, the number of alleles, N_e, effective number of alleles, H_o, observed
231 heterozygosity, H_e, expected heterozygosity and uH_e, unbiased expected heterozygosity). Genalex
232 was also used to calculate genetic differentiation between groups of samples, with F_{ST}, based on
233 allele frequencies, G''_{ST}, which is corrected for small population size and Dest (Jost, 2008; Meirmans
234 and Hedrick, 2011). FSTAT 2.9.4 (Goudet, 1995) was used to calculate allelic richness (R_s) as the
235 number of alleles per sample group independent of sample size using a rarefaction index. FSTAT was
236 also used to calculate F_{IS} values (per sample group, per locus and for the total) and their significance
237 of being more or less than zero with 1600 randomisations.
238 Population genetic structure was analysed using two methods, a mathematical visualisation
239 and a population assignment, both without prior specification of population structure. Genetic
240 diversity within and between species was visualised with a Principal Coordinate Analysis (PCoA) in
241 Genalex 6.5, based on individual pairwise genetic distance. Samples were assigned to a set number
242 of clades using a Bayesian clustering method, namely STRUCTURE v2.3.4 (Pritchard *et al.*, 2000;
243 Falush *et al.*, 2003). The PCoA methods excels in visualising diversity, while the Bayesian clustering
244 highlights genetic cohesion. This last analysis is thought to be less sensitive with limited genetic

structure than the PCoA (Reeves and Richards, 2009), but enlightens more clearly distinct genetic clusters, e.g. recent genetic mixing or hybridisation.

In the STRUCTURE analysis *K* was set to range from 1 to 6 when analysing all samples together, and 1 to 5 when analysing within species structure. STRUCTURE parameters were kept at the default settings, with a burn-in of 10^4 MCMC iterations, 10^5 runs and 20 replications of each run. Model selection relied on the Evanno *ΔK* statistic (Evanno *et al.*, 2005) estimated in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Assignment probabilities for the optimum *K* were averaged across runs using CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007). To visualise the data we used the program DISTRUCT v1.1 (Rosenberg, 2004). NEWHYBRIDs 1.1 was used to classify samples into genealogical classes, using default settings, a burn-in of 150.000 repetitions and 500.000 MCMC sweeps and no prior allele frequency information (Anderson and Thompson, 2002).

Genotypic richness was calculated as R = (G−1)/(N−1), where N is the number of samples and G the number of genotypes. The value will be '0' when stands consist of a single clone and '1' when all sampled trees are separate genets (Dorken and Eckert, 2001). Unpaired *t*-tests to test for differences in number of alleles, allelic richness, observed and expected heterozygosity and ecological characters between groups within *T. platyphyllos* were performed in Minitab 17.

2.5 *Relatedness of individuals and effective population size*

Individuals that are related will share more alleles at microsatellite loci than randomly. Using the genotype data a quantitative measure of relatedness was obtained using ML Relate (Kalinowski *et al.,* 2006) and calculated as the average maximum likelihood estimate of relatedness for all pairs of trees in the population (*r*, ML-Relate). This programme also presents, using simulations with 10,000 randomisations, whether pairs of individuals were most likely unrelated, half-sib, full sib or parent-offspring. The number of pairs that have a first-degree relationship and those that have a second-degree relationship were counted. Pairs have a first-degree relationship (50% related) if the most likely relationship of the pair is full sib or parent-offspring. Pairs have a second-degree relationship

(25% related) if they are most likely half sibs. The majority of pairs have a relatedness that is significantly less than 25% and are deemed unrelated. The number of first-degree related pairs as well as the sum of the number of first- and second-degree related pairs were then expressed as a proportion of all possible pairs.

To understand the viability of the current population the contemporary (or recent) effective population size (N_e) was calculated using the molecular co-ancestry method of Nomura (2008) and the Linkage Disequilibrium (LD) method as implemented in NeEstimator V2.1 (Do *et al,* 2014). The co-ancestry method is based on frequencies of sibs and half-sibs in the population occurring more often than expected, while the LD method is based on alleles at loci occurring more often together than expected (Wang et al., 2016). The Waples (2006) bias correction was applied in the LD method based on Waples and Do (2008). This corrects for bias that could be introduced when the actual effective population size is larger than the sample size. In the LD method only alleles with a frequency >0.05 were used.

2.6. Ecological and tree traits

Statistical analyses and visualisation were performed in R (Version 3.6.0) (R core Team, 2019). Single variables were tested for normal distribution (shapiro.test) and for variance equality (levene.test). Subsequently an Anova and TukeyHSD were performed. The factorial analyses to understand habitat preferences of the two species were modelled using Generalized Additive Models ("gam" from package mgcv) (Kienast et al., 2012; Dormann et al., 2013). Genetical and ecological data are available through Mendeley (http://dx.doi.org/10.17632/9ztmw296jh.1).

3. **Results**

In total, 113 adult *Tilia* samples were successfully genotyped for 20 microsatellite loci. The discriminatory power of the markers was high, with an average probability of identity of 1×10^{-16}.

297

298 3.1 *Clones*

299 Out of the 113 adult trees, five had identical genotypes to other trees and are considered clones (for
300 details Appendix A). All of the clone pairs were collected close together (same coordinates), i.e.
301 there were no identical genotypes at larger distances. For further analyses, only one of each of the
302 clone sets was maintained in the data set. This left 108 adult trees for analysis.

303

304 3.2 *Recruitment*

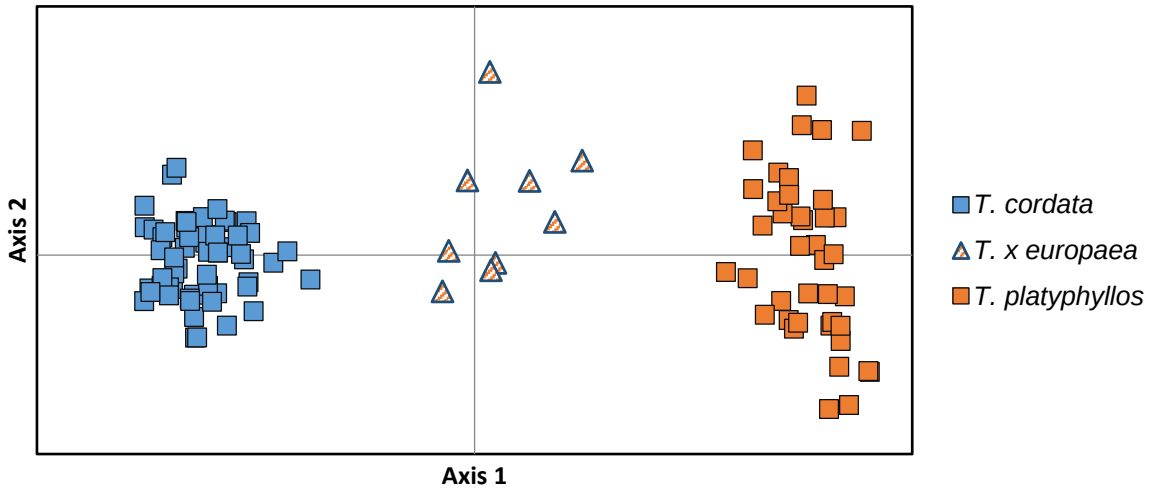
305 Three sets of seemingly newly recruited individuals were genotyped to test whether they were
306 unique genotypes (new recruits through sexual reproduction) or whether they were identical to a
307 nearby tree (clones, asexual reproduction) (for details see Appendix A). One set of six saplings were
308 all clones of a single nearby tree. The second set of 25 saplings appeared all to be clones: 24 were
309 identical to a single mature tree and one sapling was identical to a different mature tree, nearby.
310 Out of the third set of 10 saplings, four were identical to one mature tree, while another one was
311 identical to another tree. The last five of this set of ten were unique, and therefore must have been
312 derived through sexual reproduction. Overall, out of the 41 saplings tested 36 were clones of mature
313 trees and five were derived through sexual reproduction.

314

315 3.3 *Species identification and substructure within species*

316 A visualisation using PCoA of all samples showed three loose groups (Fig. 2). Following the species-
317 specific alleles reported in Logan et al. (2015, 2019), 59 individuals (blue squares) were deemed to
318 be *T. cordata*, 40 (orange squares) were *T. platyphyllos* and nine (orange hashed triangles) were *T.* x
319 *europaea* (the hybrid).

320



321

322 **Fig. 2**. Principal coordinate analysis of 108 BFNP *Tilia* trees, using 20 microsatellite

323 markers. The first two axes explain 28% and 4%, respectively, of the variation.

324

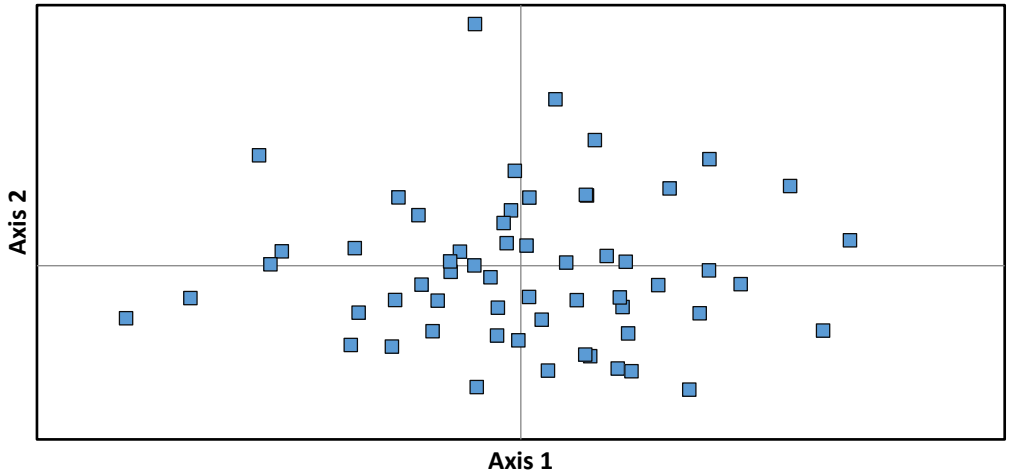
325 Following on from this, a PCoA was performed within each species. Within *T. cordata* there is no

326 substructure to be detected (Fig. 3a). However, in *T. platyphyllos* there is a clear separate and small

327 grouping of eleven individuals (Fig. 3b), from here onward called the 'small group', while the other is

328 called the 'large group' (green and red symbols, respectively in Fig. 3). Some further analyses were

329 performed separately on the 'small' and 'large' group of *T. platyphyllos*.



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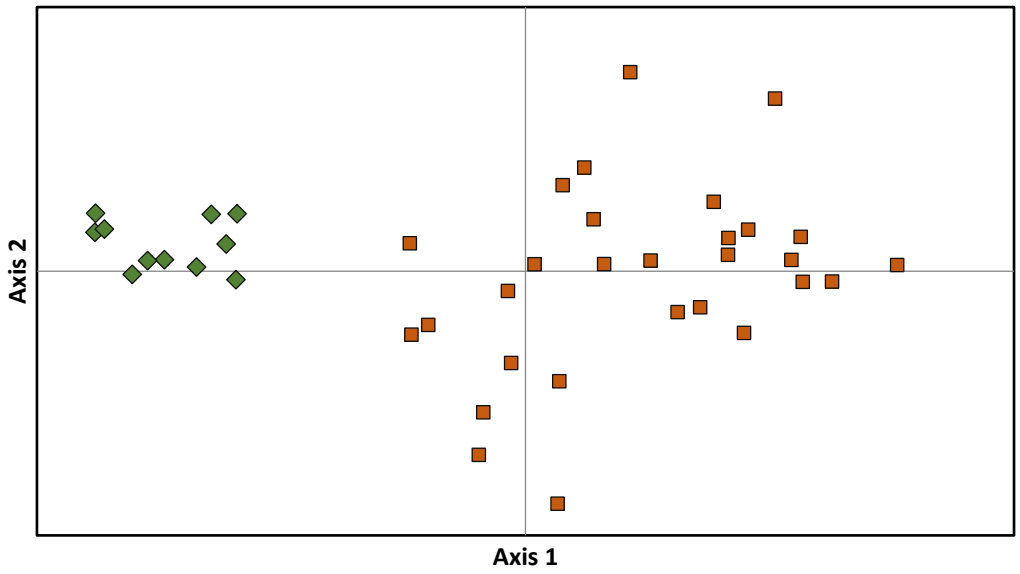
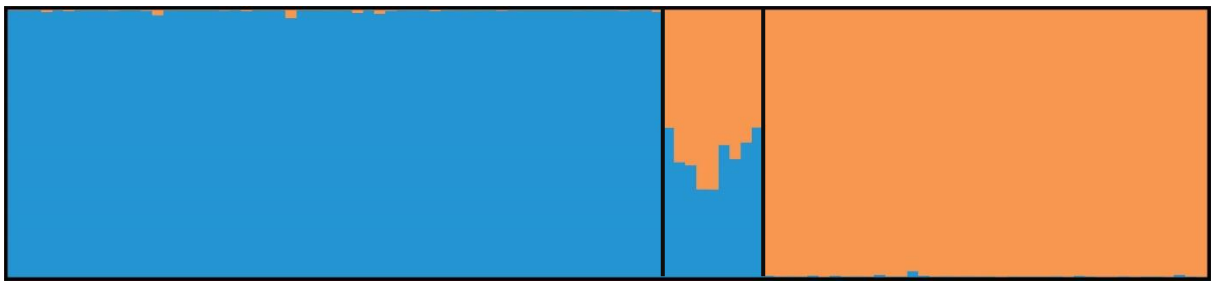


Fig. 3. Principal coordinate analysis of a) Top: *T. cordata* and b) Bottom: *T. platyphyllos* (see text) with red squares being 'large group' and the green diamonds 'small group', using 20 markers. The first two axes explain 11% and 9.7% and 16.2% and 11%, respectively.

The Bayesian STRUCTURE analysis confirms the PCoA results. Using all samples the *K* (data not shown) and Δ*K* values indicate an optimal *K* = 2 or 3 (Fig.4). It clearly separates the two species and indicates that nine samples are hybrids, with roughly equal contributions of both species. The graph showing *K* = 3 suggests that *T. platyphyllos* trees are clearly separated in two clusters, one with 11 and one with 29 individuals, indicating two different genetic units.



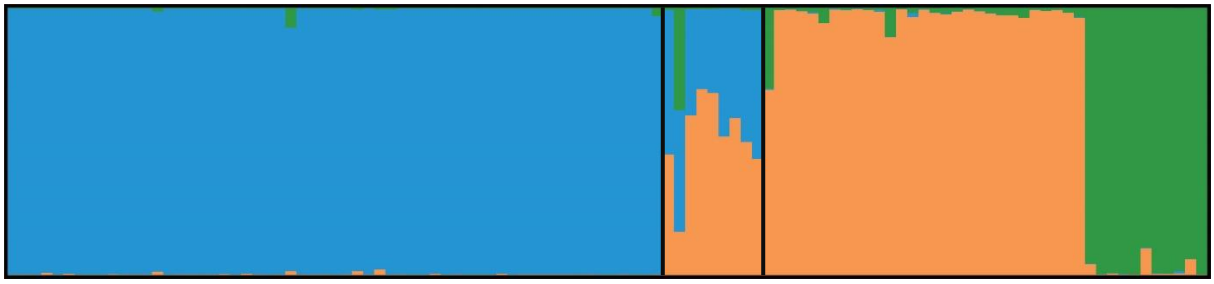


Fig 4. STRUCTURE analysis of all 108 adult Bavarian Forest *Tilia* trees, using 20 markers. Top: K = 2, Blue is *T. cordata*, while orange is *T. platyphyllos*, bottom: K = 3. Blue is *T. cordata*, while orange and green is *T. platyphyllos*, 'large' and 'small' group, respectively.

A STRUCTURE analysis within species revealed no substructure in *T. cordata*, while in *T. platyphyllos* there is substructure, with optimal *K* = 2, separating 11 trees of the 'small' group from the remainder (Suppl. Information Fig. 1A).

Nine hybrids, with close to 50 – 50 contribution of both species were detected across BFNP, which is 8.3% of all 108 trees analysed, and 9.3% when excluding the 11 'small group' *T. platyphyllos* (Fig. 2 and 4). The *T. platyphyllos* contribution to the hybrid was from the 'large group' in eight out of the nine hybrid individuals (Fig. 4). The NEWHYBRID analysis showed similar results (data not shown), with no indication of a hybrid swarm or introgression, albeit that one of the non-pure species individuals could be an F_2 instead of a first-generation hybrid.

3.4 *Genetic diversity*

Genetic diversity analyses were performed separately in the two species. The 11 *T. platyphyllos* 'small group' trees were treated separately. The number of alleles per locus ranged from 1 - 20. Within *T. cordata* locus tc2-16 and in the 'small group' *T. platyphyllos* locus tc1-42 were monomorphic. Several loci showed a significant deviation from Hardy Weinberg (HW) equilibrium (Table A1), although only three were deemed to be caused by the presence of null alleles, as

364 indicated by a large difference between H_e and H_o for these loci. Hence, our analyses were done with
365 and without those loci, 20 and 17 loci, respectively (see Materials and Methods 2.4).
366 *T. cordata* has a lower genetic diversity than *T. platyphyllos*, whichever diversity estimate is
367 used (Table 1 and A2). For example, the unbiased expected heterozygosity was lower in *T. cordata*
368 (0.57) than in *T. platyphyllos* (0.74). To test for ascertainment bias, diversity in both species was
369 compared for markers derived from *T. platyphyllos* with those derived from *T. cordata*. Both sets of
370 markers showed that *T. platyphyllos* was more diverse than *T. cordata,* whichever statistic and
371 whichever set of markers was used (Table 1). In addition, in both species the *T. cordata* markers
372 showed less diversity than the *T. platyphyllos* markers.
373 The inbreeding coefficient (F_{IS} values) indicate that the species are both outcrossing species
374 (Table 1). More specifically, *T. cordata* and the 'large' group *T. platyphyllos* have significantly positive
375 F_{IS} values, for both sets and one set of markers, respectively. However, the 'small' group *T.*
376 *platyphyllos* has significantly negative F_{IS} values.
377 Remarkably the group of *T. platyphyllos* trees that formed a separate unit in the PCoA and
378 Bayesian analysis ('small group') has a lower genetic diversity than the 'large group' of *T. platyphyllos*
379 trees, including for the allelic richness corrected for different sample sizes. This is also distinctly
380 lower than for earlier studies (Logan et al . 2019). The number of alleles, effective number of alleles,
381 expected heterozygosity and allelic richness of the 'small group' trees is significantly lower than in
382 the 'large group' *T. platyphyllos* (P = 0.000, P = 0.004, P = 0.027 and P = 0.0019, respectively, Table 1
383 and A3).
384
385 **Table 1.** Diversity summary averaged across loci of *T. cordata* and *T. platyphyllos* ('small group' and
386 'large group'), for the *T. platyphyllos* (Plat, 13 loci) and the *T. cordata* (Cor, 7 loci) separately. N is the
387 number of samples, R is the genotypic richness, an indicator of non-clonal reproduction. N_a is the
388 number of alleles, N_e the effective number of alleles, H_o observed heterozygosity, H_e expected
389 heterozygosity and uH_e unbiased expected heterozygosity. R_s is the allelic richness, based on sample

size of 11 in each sample group. F_{IS} values indicated with # are significantly larger than 0 and those with ## are significantly smaller than 0. Data indicated with * are from Logan et al (2019) (*T. platyphyllos* loci only) as comparison, 'na' means not assessed.

	Marker source	N	R	N_a	N_e	H_o	H_e	uH_e	R_s	F_{IS}
T. cordata	Plat	59	1.0	7.8	3.94	0.52	0.59	0.60	5.20	0.19#
	Cor	59		6.1	3.23	0.43	0.51	0.51	4.12	0.12#
T. platyphyllos	Plat	29	0.848	12.2	6.31	0.77	0.79	0.80	8.34	0.05
'large group'	Cor	29		7.0	4.12	0.55	0.63	0.64	5.36	0.14#
T. platyphyllos	Plat	11	na	5.1	3.54	0.81	0.65	0.68	5.08	-0.13##
'small group'	Cor	11		3.3	2.06	0.45	0.38	0.40	3.29	-0.11##
T. cordata *	Plat	23	1.0	na	na	na	0.573	na	na	0.013
*T. platyphyllos**	Plat	21	0.981	na	na	na	0.745	na	na	-0.024

3.5 *Genetic divergence between genetic units*

Genetic divergence, expressed as $F_{ST,}$ G''_{ST} and Dest between the 'large group' and 'small group' *T. platyphyllos* was moderate (0.092, 0.296 and 0.218, respectively) and significantly different from zero for all three measures. The hybrid had a smaller genetic difference from the 'large group' *T. platyphyllos* than from the 'small group, with $F_{ST,}$ G''_{ST} and Dest being, 0.078 vs. 0.183, 0.319 vs 0.557 and 0.263 vs 0.482, respectively.

3.6 *Relatedness and effective population size.*

The average relatedness (r – ML) of trees within species was low and shows that on average pairs of trees only share between 2.2% and 4.7% of their alleles (Table 2). R – ML within *T. cordata* was higher (0.045) than within the 'large group' of *T. platyphyllos* (0.022), but the 'small group' of *T. platyphyllos* trees had the highest relatedness (0.047), particularly larger than the trees in the 'large

group' *T. platyphyllos*. The proportion of pairs with a first-degree and the first- plus second-degree relatedness was smallest in the 'large group' of *T. platyphyllos* (0.042 and 0.002, respectively). The proportion of first-degree related pairs was highest in the 'small group' of *T. platyphyllos* (0.054 and 0.073, respectively). The effective population sizes (N_e) of *T. cordata* was larger than the 'large group' *T. platyphyllos* for both estimation methods (Table 2). However, the 'small group' *T. platyphyllos* had the lowest effective population size (Table 2).

Table 2. Relatedness, expressed as the average relatedness r (r – ML) and the proportion of first- plus second-degree and first-degree related pairs of individuals, as well as the effective population size (N_e) and number of samples (n), based on Co-ancestry and Linkage Disequilibrium (LD). Data presented here were based on the set of 17 markers.

	r – ML Relate	1st and 2nd degree relatedness	1st degree relatedness	N_e Co-ancestry	N_e LD	n
T. cordata	0.045	0.103	0.005	87.2	1639.3	59
T. platyphyllos 'large group'	0.022	0.042	0.002	28.7	254.1	29
T. platyphyllos 'small group'	0.047	0.073	0.054	11.1	68.4	9
T. cordata, Central*)	0.046	0.048	0.009	Infinite	494.5	-
T. platyphyllos, Central*)	0.038	0.042	0.013	48.1	44.5	-

* From Logan et al. 2019: average for populations from Central Europe.

3.7 Traits and habitat

We compared growth and ecological characteristics between *T. cordata*, the hybrid and the 'large group' *T. platyphyllos*. Hybrids were significantly taller and had a significantly larger DBH than both *T. cordata* and 'large group' *T. platyphyllos* and *T. cordata* has a significantly smaller DBH than *T. platyphyllos* (Table 3). Analysing their location within the BFNP, both the hybrid and 'large group' *T.*

platyphyllos were located at significantly higher altitudes and steeper slopes than *T. cordata*. There is no significant difference in altitude or slope aspect between *T. platyphyllos* and the hybrid.

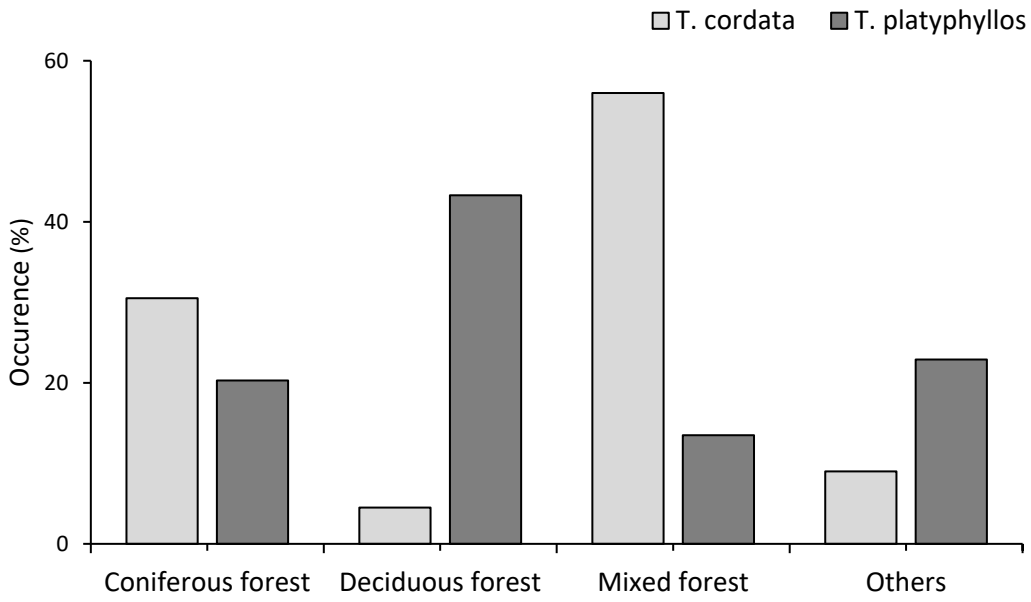
Table 3. Averages of ecological and size measurements of *T. cordata*, 'large group' *T. platyphyllos* and their hybrid, with number of individuals (n), standard deviation in brackets, and *P* values representing the results of differences between the species (Tukey test)

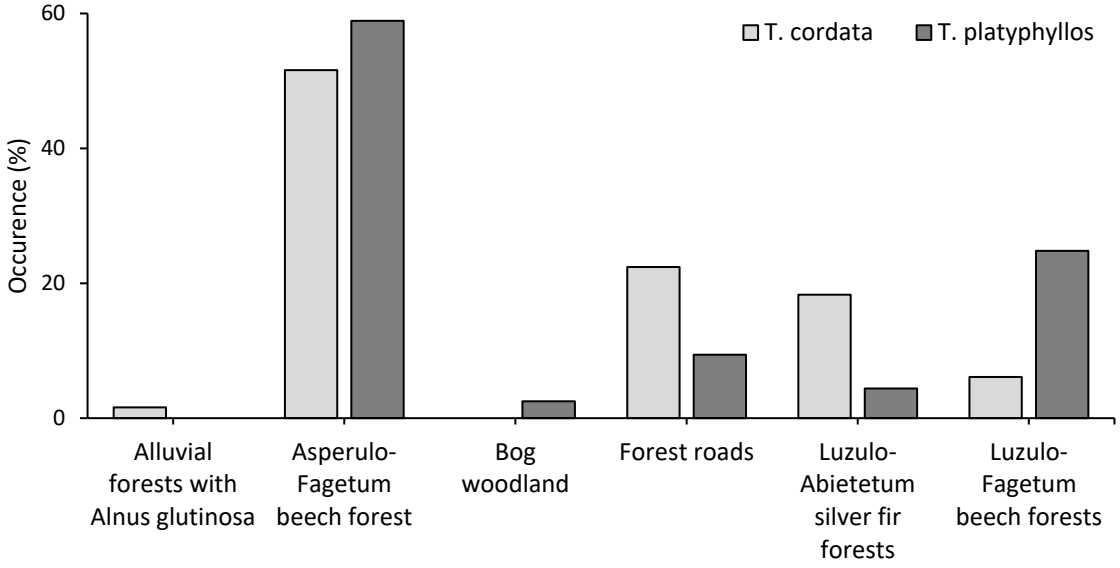
	n	Height (m)	DBH (cm)	Altitude (m a.s.l.)	Slope (degrees)
T. cordata	59	20.8 (6.21)	30.8 (15.2)	701.0 (56.4)	4.1 (3.6)
T. platyphyllos	29	21.6 (6.84)	56.2 (28.2)	746.5 (74.4)	9.6 (3.9)
Hybrid	9	30.3 (4.79)	75.1 (28.2)	774.2 (100.5)	8.6 (7.0)
P- values					
T. cordata vs hybrid		0.0003	0.0000	0.0072	0.0076
T. platyphyllos vs *T. cordata*		0.9084	0.0001	0.0009	0.0000
T. platyphyllos vs hybrid		0.0002	0.0138	0.7148	0.6359

In a separate comparison of 'large group' and 'small group' *T. platyphyllos, t*he 'small group' have less stem volume, lower vitality and DBH than the 'large group' *T. platyphyllos*, while the height was not significantly different ($P = 0.00$, $P = 0.014$, $P = 0.001$ and $P = 0.232$, respectively, see also Table A3).

The species occur in different forest types and in different Potential Natural Vegetation (PNV) types. Hybrids and 'small group' *T. platyphyllos* were not included in this analysis as data were limited. *T. cordata* is mostly found in mixed and coniferous forests, while the 'large group' *T. platyphyllos* is

found across all forest types, although having the highest preference for deciduous forests (Fig. 5 and Table A4). Analysing their PNV both *T. cordata* and *T. platyphyllos* were most often found in nutrient rich Asperulo-Fagetum beech forest, with *T. cordata* also present in mixed coniferous and 'other' areas in the forest, and *T. platyphyllos* additionally in nutrient poor Luzulo-Fagetum beech forest. For *T. cordata* the model explained 50.3% of the variation, while for *T. platyphyllos* 37.3% was explained (Table A4).





448 **Fig. 5.** Habitat (a) Top: forest types (b) Bottom: Potential Natural Vegetation of *T. cordata*
449 (light grey) and 'large group' *T. platyphyllos* (dark grey) in the BFNP.
450
451 4. **Discussion**
452 Uniquely, we report here on a combined ecological and genetic survey of an ecologically important
453 broadleaf species, *Tilia,* in a mixed woodland forest in Central Europe. Confirming early presence in
454 the pollen record (Van der Knaap et al., 2019), our findings indicated that both *T. cordata* and *T.*
455 *platyphyllos* are present in the BFNP, and likely remnants of a once more widespread *Tilia*
456 population within the forests. This is because the genetic diversity for both species is medium to
457 high, in line with other populations central to the distribution of the species. Also, N_e, relatedness,
458 frequency of hybrids and clonal reproduction is comparable to other Central European populations
459 (Logan et al., 2019). Moreover, when analysed within a larger genotype data set the BFNP
460 individuals fit in well with other nearby populations, i.e. they did not form a separate clade in
461 STRUCTURE (data not shown). The diversity observed indicates opportunities for survival of both
462 species, but the current actual census size and isolation of the individuals from one another across
463 the large BFNP are a cause for concern. Several of the forests sampled by Logan et al. (2019) and
464 Phuekvilai (2014) across Europe have larger census size and higher concentrations of *Tilia*.
465
466 4.1 *Species identification*
467 We are confident that the molecular markers have clearly identified the presence of both *Tilia*
468 species (Logan et al., 2015, 2019; Phuekvilai, 2014). Moreover, we confirm the pollen representation
469 data of Van der Knaap et al. (2019) that *T. platyphyllos* is less common in the BFNP than *T. cordata*,
470 which is a contradiction to the Natura 2000 monitoring in the national park that did not mention the
471 occurrence of *T. cordata* in the park (Nationalparkverwaltung BW, 2008). Pollen analysis (van der
472 Knaap et al., 2019) and the study presented here indicate that *T. platyphyllos* is less numerous than
473 *T. cordata*, both in the past and at present. It is common that surveys do not correctly identify the

474 species in the genus *Tilia,* in particular for these two closely related species that also generate
475 hybrids with intermediate characteristics. This is because in *Tilia,* it is relatively easy to distinguish
476 the two species and its hybrid if flowers or seeds are available at ground height, but to accurately
477 identify species from leaves benefits from an 'expert eye' (Pigott, 2012, 2020). The leaf
478 characteristics of the species show overlap, and, combined with considerable plasticity in leaf shape,
479 this means that the they are often mixed up (Phuekvilai, 2014). In addition, the species only flower
480 for a short period (two weeks) and many trees, especially in the understory, do not flower at all.
481 Surveys that do not include the flowers will therefore struggle to identify the species and the hybrid
482 correctly. In these cases, molecular markers are needed for accurate species determination.
483
484 4.2 *Planted trees*
485 Often the most substantial proportion of genetic variation in outcrossing species is within
486 populations and not between, making it hard to use markers, such as microsatellites, for forensic
487 applications, e.g. finding the source of trees, timber or wood products. Earlier work showed that
488 using chloroplast markers in *Tilia* would not improve the ability to detect population differences due
489 to the large proportion of shared haplotypes between the two species and limited geographic
490 structure within species (Fineschi et al., 2003; Phuekvilai, 2014).
491 Similarly, it is challenging to detect planted trees from those that are endemic, especially if
492 planted trees are from local and diverse sources. Phuekvilai (2014) reports low differentiation
493 between populations across Europe, for *T. cordata* F_{ST} values range from 0.021 to 0.181 and for *T.*
494 *platyphyllos* from 0.020 to 0.171. Therefore, even if some trees were planted in the past, this may
495 often go undetected in a genetic survey if they originated from another natural and nearby stock
496 and no records of planting exist. There are no specific records of planting *Tilia* in the BFNP. Despite
497 that here we detected a small set of trees ('small group') in *T. platyphyllos*, genetically dissimilar to
498 the 'large group' both in the PCoA and STRUCTURE analysis. They have a genetic distance (F_{ST}) with
499 the 'large group' (0.092), that is greater than the distance observed between geographically more

distant central *T. platyphyllos* populations (0.055, Logan et al., 2019). However, we note that an F_{ST} estimation between two populations with different sample sizes and different diversity may be misleading. The hybrids have a smaller genetic distance (F_{ST}) from the 'large group' (0.078) than from the 'small group' (0.183), indicating that the hybrids have an ancestry similar to the 'large group' and not to the 'small group'. The 'small group' also have a remarkably low genetic diversity, and a higher relatedness amongst themselves than the endemic trees of the same species (Logan et al., 2019) and the 'large group' of trees in the BFNP. The leaves of 'the small group' of trees do not stand out with regard to their leaf morphology, i.e. they are clearly *T. platyphyllos* (personal observation, Wolff). This 'small group' of trees must have been planted, and the uniqueness of each individual tree in this group leads to the hypothesis that they were derived from seed from a limited number of parental trees, which in turn were genetically distant to the local trees. The negative F_{IS} in this group, unusual for *Tilia*, may indicate that by chance the seed source(s) of the small group happen to be rather heterozygous trees. From here on the 'large group' of trees will be considered wild and endemic and the 'small group' as being planted. In addition, the presumably planted trees in the 'small group' have a significantly lower volume, vitality and DBH than the endemic *T. platyphyllos* trees. This may be due to relatively recent planting (e.g. 50 – 100 years ago), unfavourable location or maladaptation to the BFNP.

4.3 *Genetic diversity and differentiation*

In addition to analyses with 20 markers, we also did analyses on a set of 17 markers, excluding the three loci with a large difference between H_o and H_e. Loci with a large number of alleles have a high number of genotypes per locus, and even with reasonable sample sizes, this can cause deviation between expected and observed genotype numbers for those loci, explaining deviations from HW for the other markers. To ensure the outcome was robust we also analysed a set of 14 markers, additionally excluding three markers with many alleles (Tc915, Tc963 and Tc927) because genotyping errors are more likely to occur with many alleles. This is particularly important for

relatedness and N_e analyses as small differences in allele size can change the outcome of the analysis. We note that conclusions from the set of 20, 17 or 14 markers were very similar.

Both species have a moderate to high genetic diversity, despite their low current abundance. This is likely to be because of their high abundance during warmer periods of the post-glacial warming, with the outcrossing mating system and high longevity retaining diversity for a long time (Myking, 2002). Whichever diversity statistic is used, *T. cordata* is less diverse than *T. platyphyllos.* There is always a concern that ascertainment bias due to the source of the markers (being target or non-target species) skews results. It is sometimes found that applying markers across species leads to higher diversity in the species from which the markers are derived than in non-source species (e.g. Li and Kimmel, 2013). In earlier *Tilia* studies Logan et al. (2015, 2019) only used markers developed for *T. platyphyllos* in both *T. platyphyllos* and in *T. cordata* and discussed the fact that *T. cordata* had a lower genetic diversity, potentially caused by ascertainment bias. Here we tested, for the first time in *Tilia*, for presence of ascertainment bias by using two sets of markers derived from contrasting focal species, namely of *T. cordata* and *T. platyphyllos*. In both species the *T. cordata* markers showed less diversity than the *T. platyphyllos* markers. It is unclear why this is the case. More importantly, both sets of markers show the same difference between the two species, *T. platyphyllos* always being more diverse than *T. cordata,* whichever statistics and whichever set of markers is used. Therefore, we conclude that ascertainment bias can be discounted as a reason for lower diversity in *T. cordata*, as was suggested as a potential reason in earlier papers.

It is difficult to explain why *T. platyphyllos* is more genetically diverse than *T. cordata* in the BFNP, but also across all European populations (Logan et al., 2015 & 2019; Phuekvilai, 2014). Past demographic and life history can have a substantial effect on genetic diversity for microsatellites (Li and Kimmel, 2013). In general, species with larger effective population size, shorter generation times (more turnover) and higher gene flow have a higher genetic diversity (Hague and Routman, 2016). Geographic range, breeding system and mode of seed dispersal also partly determine diversity in species (Hamrick et al., 1992). However, the biology and life history of the two species is very similar

552 and they most likely migrated more or less simultaneously after the Ice Age (Pigott, 2012). Here the
553 effective population size (N_e) is higher in *T. cordata* than in *T. platyphyllos*, similar to a Europe wide
554 study by Logan et al (2019). Both census size and geographic range would predict a lower genetic
555 diversity in *T. platyphyllos* (being lower and more restricted, respectively), not higher as observed.
556 Hamrick et al. (1992) reviewed genetic diversity in trees and conclude that within species genetic
557 diversity differs between species and often remains unexplained.
558
559 4.4 *Hybrids*
560 The frequency of the hybrids (approximately 9%) is similar to that found in other mixed forests
561 (Phuekvilai, 2014; Logan et al. 2015; unpublished data K Wolff). No introgression has been observed
562 in BFNP or in other mixed forests studied so far and there is no conservation concern if *T.* x *europaea*
563 is simply maintained in the park (Phuekvilai, 2014; Logan et al., 2015; unpublished data K Wolff).
564 For the first time the size of hybrids and their parental species was measured in their natural
565 environment. Because the hybrids are taller and had a larger DBH than the parental species it
566 indicates that hybrids did not originate recently and *T. cordata* and *T. platyphyllos* must have shared
567 their location in the BFNP for several centuries. The reason for the significantly greater height and
568 DBH of the hybrids is likely to be 'hybrid vigour', a term used for the phenotypic superiority of
569 hybrids as compared to its parental species. Zanewich et al. (2018) found that in poplar (*Populus* sp.)
570 the combined (small) effects of multiple traits and metabolic diversity gave hybrids an advantage in
571 several environments. Here the height and DBH superiority of the hybrids could be because they live
572 longer than the parental species, allowing them to grow bigger before the main stem deteriorates,
573 and/or they have a higher growth rate. Whether it is higher longevity or faster growth remains to be
574 tested, e.g. in a growth trial or by counting rings in a core, with the proviso this only indicates the
575 age of the stem, not the genotype.
576
577 4.5 *Relatedness and effective population size.*

578 It is important to understand whether a population is large and diverse enough, to allow its
579 conservation as well as adaptation to new environments and changing climate. This cannot be
580 determined directly from genetic diversity for neutral markers, such as microsatellites, yet these can
581 help us understand population size. Population size can be expressed in how related random pairs of
582 individuals are or as the effective population size. Although both cannot be regarded as hard and
583 accurate measures, comparable studies can be used to draw conclusions. Data presented here are
584 based on the set of 17 markers, avoiding those with potential null alleles as this would particularly
585 affect outcomes of these types of analyses. However, we note that outcomes using 20, 17 or 14
586 markers led to very similar results and the same conclusions. Here we can compare this with an
587 earlier study (Logan et al., 2019). In line with their Central European populations, the relatedness
588 and effective population size (N_e) of *T. cordata* is higher than that of *T. platyphyllos*. Analogue to the
589 lower genetic diversity in the presumably planted *T. platyphyllos* trees they seem to be closer
590 related to each other than to the endemic *T. platyphyllos* trees, as also reflected in the smaller
591 effective population size in the presumably planted trees.
592 The estimates for N_e at BFNP were as high as or higher than the actual census size of the two
593 species. It is well known that the genetic diversity generally reflects 'recent' population sizes, not
594 current population sizes (Hare et al., 2011). Due to high longevity of the trees 'recent' may be a
595 period of tens or several hundred years. Therefore, it is likely the current situation is the result of an
596 ongoing bottleneck, e.g. over the last several 100 years.
597 Rules have been suggested for the minimum number of individuals and source populations
598 to conserve. However, there are no agreements on this and it seems impossible to have a hard
599 standard rule for species with very diverse life history characteristics. Some have discussed the
600 minimum viable population (MVP) size as being the number of individuals to conserve to ensure that
601 95% of the variation is maintained and random genetic drift minimised. A 50/500 rule was
602 suggested, with 50 ensuring inbreeding was avoided in the short term, and 500 individuals needed
603 to avoid random genetic drift and opportunities for adaptation and evolution in the longer term

604 (LehmKuhl, 1984; Jamieson and Allendorf, 2012). Flather et al. (2011) and Jamieson and Allendorf
605 (2012) remark that it is impossible to give exact MVP numbers that would guarantee survival of
606 species and therefore conservation decisions cannot be made whether a species has reached certain
607 threshold or not.
608 Myking (2002) discusses the concept of Multiple Population Breeding Strategy (MPBS), first
609 coined by Namkoong (1984) designed for vulnerable tree species with large between population
610 diversity. The most economic way is *in situ* conservation combined with silvicultural management
611 (Myking, 2002). It asks for an effective population size (N_e) of 50 individuals from 20 populations,
612 ensuring low loss of diversity through genetic drift. In *Tilia*, the majority of the variation is within
613 populations, and therefore fewer populations probably suffice.
614 Here N_e for *T. cordata* is estimated as 87 – 1639, with a census size of 59 and for *T.*
615 *platyphyllos* N_e is between 29 and 254, with a census size of 29. With $N_e > 50$ short-term risks of
616 inbreeding are limited. However, for *T. platyphyllos* current numbers are smaller than the minimum
617 of 50, albeit that genetic diversity in the 29 individuals is not diminished as compared to other
618 populations. Also, 29 is the minimum census size as it is possible that saplings of the species have
619 been missed in the collection and survey. However, long-term risks must be addressed, especially
620 since the remaining trees are spread over a large area, limiting gene flow between trees, and gene
621 flow with surrounding forests is limited due to limited pollen transport by its pollinators in a patchy
622 environment (Osborne et al., 2008).
623
624 4.6 *Ecology*
625 While a limited number of previous studies have focused on ecological traits such as DBH and height
626 of *Tilia* stands, the two species are often treated as one taxon (e.g. Jacob et al., 2010) and rarely do
627 any consider the hybrid (*T. x europaea*) in natural *Tilia* populations. Here we have investigated the
628 growth and site preference of these two closely related *Tilia* species along with (where applicable)
629 their hybrid, growing intermixed in their natural environment. Most results seem to confirm

630 descriptions of earlier work, allowing quantification in the current study. For growth results of the
631 hybrid we refer to section 4.4. In *T. cordata* the DBH was much smaller than in *T. platyphyllos*, which
632 confirms Pigott (2012, 2020) who indicated that DBH of 1.5m can be reached at 200 years in *T.*
633 *platyphyllos*, while this would take 400 years in *T. cordata*. In the BFNP the two species reach similar
634 heights, while Pigott (2012, 2020) describes that *T. cordata* grows up to 20m and *T. platyphyllos* up
635 to 40m. The reason why we find similar heights for both species in BFNP may be partially explained
636 by the fact that *T. platyphyllos* in BFNP occurs here at higher altitude than *T. cordata* and the colder
637 climate at higher elevations may hamper length growth in trees in general or in *T. platyphyllos*
638 specifically.
639 Both the hybrid and 'large group' *T. platyphyllos* were located at higher altitude than *T.*
640 *cordata*. Pigott (2012) describes that *T. cordata* occurs on flat areas, but also on steep slopes, while
641 *T. platyphyllos* is additionally found on unstable screes. This confirms our finding that *T. platyphyllos*
642 occurs on steeper slopes. Personal observation (K. Wolff) similarly showed *T. cordata* at the foot of
643 mountains/hills and *T. platyphyllos* at steep and unstable screes higher up the mountain/hill at
644 several locations in Europe, e.g. at Leopolds berg in Vienna, Austria.
645 In the BFNP *T. cordata* is mostly found in mixed and coniferous forests, while *T. platyphyllos*
646 was found across all forest types, although having the highest preference for deciduous forests (Fig.
647 5 and Table A4). The slightly higher light intensity in deciduous forest may allow *T. platyphyllos* to
648 have more radial growth (higher DBH, Table 4) than *T. cordata*. Pigott (2012) describes that both
649 species mostly occur in broadleaved forests, but also writes that *T. platyphyllos* is not found in *F.*
650 *sylvatica* with *Abies alba* communities, confirming our low frequency of this species in mixed and
651 coniferous forests. Radaglou et al. (2009) also describes that both occur in deciduous broadleaved
652 forests, but that *T. cordata* can also occur mixed in coniferous forests, confirming our result.
653 Analysing their Potential Natural Vegetation communities both *T. cordata* and *T. platyphyllos*
654 were most often found in Asperulo-Fagetum beech forests with *T. cordata* also present in Luzulo-
655 Abietum silver fir forests and along forest roads. *T. platyphyllos* can additionally be found in Luzulo-

Fagetum beech forests. It has been suggested by others that *Tilia* prefers nutrient rich alkaline soils with higher calcium content (Jaworski, 1995; Pigott, 2012). Here we have shown that in the BFNP, *Tilia* prefer mixed beech stands of Asperulo-Fagetum type, generally indicating alkaline soils with calcium, with more than half of all BFNP *Tilia* trees (approximately 50% of *T. cordata* and 60% of *T. platyphyllos*), while this forest type just covered 5% of the park and Luzulo fagetum covered 41 % (Fig. 5b).

4.7 *Conservation management*

Before human intervention *Tilia* played a dominant role in natural forests and is well known for its beneficial effects on biodiversity and its ecosystem services. *Tilia* still present are minor remnants of once vast forests. Nowadays, climate is warming and species, such as *Tilia*, can potentially increase their range and density (Myking, 2002; Hemery et al., 2010). However, *Tilia* generally struggles to increase its numbers and to re-establish once it has disappeared and may not have the ability to follow the potential range shift due to slow migration rates and low current contribution to mixed forests (Myking, 2002; Logan et al., 2019). Therefore, to maintain *Tilia* in reserves and national parks they may need active management to maintain and increase their presence, i.e. assisted migration, augmentation and protection of seedlings and saplings. In the BFNP with its non-intervention core zone this mostly needs to take place in the surrounding buffer zone.

Considerations for management are:

(1) We have to conserve the two *Tilia* species, but do not have to consider genetic structure within the BFNP. For biodiversity management it is important to have accurate species inventories, and in some cases also to detect hybrids. We showed that in this case molecular markers were needed for accurate species determination. The abundance of both species in the BFNP is low compared to other species in the NP, which is a matter of concern. Therefore, management may be needed to ensure the two endemic species are maintained in the reserve. Vulnerable or rare species, such as

682 *Tilia,* could benefit from clone archives from populations (Myking, 2002) representing the various
683 habitats that the species occupy.
684
685 (2) Hybrids in general can pose problems if substantial introgression leads to the loss of one or both
686 species and the hybrid swarm could be more invasive than the parental species (Gaskin, 2017).
687 However, no introgression has been observed in *Tilia* in BFNP or other mixed forests studied so far
688 (Phuekvilai, 2014; Logan et al. 2015). This means that the hybrids will not contribute to the next
689 generation but can simply be left in the park.
690
691 (3) Trees that are planted from unknown origin need to be removed to prevent contamination of the
692 gene pool. The trees that appear planted from sources outside the BFNP and that appear from a
693 rather limited seed source should be avoided for augmentation and potentially should be felled. If
694 more detailed genetic knowledge, e.g. SNP or QTL analyses, of *Tilia* was available the 'planted' trees
695 should be further analysed in that framework to assess whether they are indeed foreign and do not
696 hold valuable genetic variants. In future care must be taken to use cuttings or seeds of local endemic
697 trees or from a source suited to the habitat and latitude to ensure the success of the active
698 management (Fady et al 2016; Lobo et al. 2018).
699
700 (4) Genetic diversity needs to be maintained for survival and future adaptation.
701 This means management could consist of protection of those present and potentially planting of
702 trees. As discussed in section 4.5 the N_e of the two *Tilia* species in BFNP seems sufficient for the
703 requirement of 50, but current census size points in the direction of a lower N_e in future. To reach a
704 future N_e of 500 gene flow with forests from outside the BFNP also may need improving. Therefore,
705 augmentation, using local or other suitable trees (see (3) above), in currently low-density areas to
706 ensure improved sexual reproduction, i.e. planting *Tilia* in the vicinity of existing trees, as well as in
707 the buffer zone of the BFNP.

For future augmentation local trees should be tested with molecular tools to see whether they are likely to be endemic, i.e. whether they represent the local diversity. Trees can be obtained through clonal reproduction (cuttings and layering) or through sexual reproduction, by harvesting and germinating seeds from local trees. Both strategies have problems and advantages. Ingvarson and Dahlberg (2019) suggest that clones can be used, as long as a reasonable number of trees (representing a reasonable proportion of the total diversity) are used. One could suggest, for example, to make clones of 20 genotypically different individuals from the local environment. This would combine capturing a large proportion of local diversity and speed up the process of obtaining saplings. From a genetic perspective a better option would be to collect seed from a similar number of trees (20, bulked seeds), and after germination saplings could be planted in the buffer zone (Ingvarson and Dahlberg, 2019). This would capture more of the genetic diversity of the population, but although germinating *Tilia* seed is possible, it is time consuming and not always successful (pers. experience Wolff). Therefore, generating clonal saplings seems more secure and faster.

(5) Regeneration can potentially be improved through various silvicultural measures. Mixed stands are ideal, giving shade for *Tilia* saplings while they are young (Coello et al., 2013). Young trees can also be used to fill in gaps, e.g. when another tree dies. For forest diversification, in pure stands, small numbers of *Tilia* could be planted in the understory of woodlands to improve diversity and environmental value of the forest. Once saplings reach a certain height, for example four meters, removal of competing trees in the neighbourhood may expedite further growth and flowering. Protection against wildlife browsing, e.g. through fencing or measures to protect single seedlings, may limit the loss of seedlings and saplings. For example, *T. cordata* could benefit from less browsing in *Picea abies* ecosystems (Cailleret et al., 2014).

Further work is needed, at both the genetic and the ecological level. In economically important species there has been much advance in understanding functional genes and their variation across

734 species range, understanding climate change adaptation, insect and disease resistance. For many
735 less common and less commercially important species this is not yet within reach. Large field trials at
736 multiple locations are essential to understand to what extend there are adaptive traits and how they
737 can benefit silviculture and biodiversity conservation in forest ecosystems.
738
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746 **7. References**
747 Aerts R, Honnay O. 2011. Forest restoration, biodiversity and ecosystem functioning. BMC Ecology
748 11(1): 29.
749 Ali AM, Darvishzadeh R, Skidmore AK, van Duren I, Heiden U, Heurich M. 2016. Estimating leaf
750 functional traits by inversion of PROSPECT: Assessing leaf dry matter content and specific leaf
751 area in mixed mountainous forest. International Journal of Applied Earth Observation and
752 Geoinformation, 45: 66-76.
753 Anderson EC, Thompson EA. 2002. A model-based method for identifying species hybrids using
754 multilocus data. Genetics 160: 1217-1229.
755 Belostokov GP. 1980. Morphogenesis of *Tilia cordata* Mill.; bush-shaped regrowth. Lesoved 6: 53–59.
756 Cailleret M, Heurich M, Bugmann H. 2014. Reduction in browsing intensity may not compensate
757 climate change effects on tree species composition in the Bavarian Forest National Park.
758 Forest Ecology and Management 328: 179-192.

759 Coello J, Becquey J, Ortisset J-P, Gonin P, Baiges T, Piqué M. 2013. Lime (*Tilia platyphyllos* and *T.*
760 *cordata)* for high quality timber. Centre de la Propietat Forestal: Santa Perpètua de Mogoda,
761 Spain, 2013: 54–61.
762 Curtis PG, Slay CM, Harris NL, Tyukavina A, Hansen MC. 2018. Classifying drivers of global forest loss.
763 Science 361(6407): 1108-1111.
764 De Jaegere T, Hein, S, Claessens H. 2016. A review of the characteristics of Small-Leaved lime (*Tilia*
765 *cordata*) and their implications for the silviculture in a changing climate. Forests 7: 56.
766 Doi:10.3390/f7030056.
767 Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. 2014. NeEstimator v2: re-
768 implementation of software for the estimation of contemporary effective population size (*Ne*)
769 from genetic data. Molecular Ecology Resources 14(1): 209-214.
770 Dorken ME, Eckert CG. 2001. Severely reduced sexual reproduction in northern populations of a clonal
771 plant, *Decodon verticillatus* (Lythraceae). J. Ecol. 89: 339–350.
772 Dormann CF, Ellith J, Bacher S, Buchmann C, Carl G, Carre G. 2013. Collinearity: a review of methods to
773 deal with it and a simulation study evaluating their performance. Ecography 36: 27–46.
774 Dudley, N., 2008. Guidelines for applying protected area management categories. IUCN.
775 https://doi.org/10.2305/IUCN.CH.2008.PAPS.2.en.
776 Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing
777 STRUCTURE output and implementing the Evanno method. Conservation Genetic
778 Resources 4: 359–361. doi.org/10.1007/s12686-011-9548-7.
779 Elling W, Bauer E, Klemm G, Koch H. 1987: Nationalpark Bayerischer Wald – Klima und Böden,
780 Waldstandorte. 2. Aufl. – Nationalpark Bayerischer Wald 1, München.
781 Erichsen EO, Wolff K, Hansen OK. 2019. Genetic and clonal structures of the tree species *Tilia cordata*
782 Mill. in remnants of ancient forests in Denmark. Population Ecology. 61(3): 243-255. doi-
783 org.eres.qnl.qa/10.1002/1438-390X.12002.

784 Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the
785 software structure: a simulation study. Molecular Ecology 14 (8): 2611-2620. Doi:
786 10.1111/j.1365-294X.2005.02553.x.
787 Fady B, Cottrell J, Ackzell L, Alia R, MuysB, Prada A, Gonzalez-Martinez SC. 2016. Forests and global
788 change: what can genetics contribute to the major forest management and policy challenges
789 of the twenty-first century? Reg Environ Change 16: 927-939. DOI 10.1007/s10113-015-0843-9
790 Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype
791 data: linked loci and correlated allele frequencies. Genetics 164: 1567–1587.
792 FAO (Food and Agriculture Organization of the United Nations). 2015. Global Forest Resources
793 Assessment 2015—How are the world's forests changing. http://www.fao.org/3/a-i4793e.pdf.
794 Fineschi S, Salvini D, Taurchini D, Carnevale S, Vendramin GG. 2003. Chloroplast DNA variation of *Tilia*
795 *cordata* (Tiliaceae) Canadian Journal of Forest Research 33(12): 2503-2508
796 https://doi.org/10.1139/x03-179.
797 Finkeldey R, Leinemann L, Gailing O. 2010. Molecular genetic tools to infer the origin of forest plants
798 and woods. Appl Microbiol Biotechnol 85: 1251-1258 https://doi.org/10.1007/s00253-009-
799 2328-6.
800 Fischer HS, Winter S, Lohberger E, Jehl H, Fischer A. 2013. Improving transboundary maps of potential
801 natural vegetation using statistical modelling based on environmental predictors. Folia
802 Geobotanica 48(2): 115-135.
803 Flather CH, Hayward GD, Beissinger SR, Stephens PA. 2011. Minimum viable populations: is there a
804 'magic number' for conservation practitioners. Trends in Ecology and Evolution 26: 307-316.
805 doi.org/10.1016/j.tree.2011.023.001.
806 Fussi B, Westergren M, Aravanopoulos F, Baier R, Kavaliauskas D, Finzgar D, Alizoti P, Bozic G,
807 Avramidou E, Konnert M, Kraigher H. 2016. Forest genetic monitoring: an overview of
808 concepts and definitions. Environ Monit Assess 188: 493. DOI 10.1007/s10661-016-5489-7.

809 Gaskin JF. 2017. The role of hybridisation in facilitating tree invasion. AoB Plants 9: plw079. Doi:
810 10.1093/aobpla/plw079.
811 Goudet J, 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of
812 Heredity 86: 485-486.
813 Hague MT, Routman EJ. 2016. Does population size affect genetic diversity? A test with sympatric
814 lizard species. Heredity (Edinb*).* 116(1): 92–98. doi:10.1038/hdy.2015.76.
815 Hamrick JL, Godt MJW, Sherman-Broyles SL. 1992. Factors influencing levels of genetic diversity in
816 woody plant species. New Forest. 6: 95–124.
817 Hare MP, Nunney L, Schwartz MK, Ruzzante DE, Burford J, Waples RS, Ruegg K, Palstra F. 2011.
818 Understanding and estimating effective population size for practical application in marine
819 species management. Conservation Biology 25(3): 438-49. DOI: 10.1111/j.1523-
820 1739.2010.01637.x
821 Hedrick PW. 2005. Genetics of Populations. Third Edition. Sudbury, Massachusetts: Jones and Bartlett
822 Publishers.
823 Hemery G, Spiecker H, Aldinger E, Kerr G, Collet C, Bell S. 2008. COST Action E42. Growing
824 valuable broadleaved tree species. Final Report. 40 pp.
825 Hemery GE, Clark JR, Aldinger E, Claessens H, Malvolti ME, O'Connor E, Raftoyannis Y, Savill PS, Brus R.
826 2010. Growing scattered broadleaved tree species in Europe in a changing climate: a review of
827 risks and opportunities. Forest 83: 65-81. DOI: 10.1093/forestry/cpp034.
828 Heurich M, Beudert B, Rall H, Křenová Z. 2010. National parks as model regions for interdisciplinary
829 long-term ecological research: the Bavarian Forest and Šumavá National Parks underway to
830 transboundary ecosystem research. In Long-Term Ecological Research (pp. 327-344). Springer,
831 Dordrecht.
832 Heurich M, Englmaier KH. 2010. The development of tree species composition in the Rachel–Lusen
833 region of the Bavarian Forest National Park. Silva Gabreta 16(3): 165-186.

834 Holderegger R, Kamm U, Gugerli F. 2006. Adaptive vs. neutral genetic diversity: implications for
835 landscape genetics. Landscape Ecology **21:** 797–807. DOI: 10.1007/s10980-005-5245-9.
836 Hommel PWFM, De Waal RW. 2003. Rijke bossen op arme bodems. Alternatieve boomsoortenkeuze
837 verhoogt soortenrijkdom ondergroei op verzuringgevoelige gronden. Landschap 20 (4): 193-
838 204.
839 Huntley B, Birks HJB. 1983. An Atlas of past and present pollen maps for Europe: 0 – 13000 years ago.
840 University Press, Cambridge.
841 Ingvarsson PK, Dahlberg H. 2019. The effects of clonal forestry on genetic diversity in wild and
842 domesticated stands of forest trees, Scandinavian Journal of Forest Research, 34:5, 370-379,
843 DOI: 10.1080/02827581.2018.1469665.
844 Jacob M, Leuschner C, Thomas FM. 2010. Productivity of temperate broad-leaved forest stands
845 differing in tree species diversity. Annals of Forest Science 67 (5): 503. doi:
846 10.1051/forest/2010005. hal-00883624.
847 Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing
848 with label switching and multimodality in analysis of population structure. Bioinformatics 23:
849 1801–1806.
850 Jamieson IG, Allendorf FW. 2012. How does the 50/500 rule apply to MVPs? Trends in Ecology and
851 Evolution 27: 578-584. Doi:10.1016/j. tree.2012.07.001.
852 Jaworski A. 1995. Silviculture characteristics of forest trees. Kraków, Gutenberg: 237.
853 Jost L. 2008. GST and its relatives do not measure differentiation. Molecular Ecology 17: 4015-4026.
854 Kalinowski ST, Wagner AP, Taper ML. 2006. ML-RELATE: a computer program for maximum likelihood
855 estimation of relatedness and relationship. Molecular Ecology Notes *6*(2): 576-579.
856 Kennel R. 1969: Formzahl- und Volumentafeln für Buche und Fichte. Institut für Ertragskunde der
857 Forstlichen Forschungsanstalt München.
858 Kienast F, Bollinger J, Zimmermann NE. 2012. Species Distribution Modeling (SDM) with GLM, GAM
859 and CART. Ftp://ftp.wsl.ch/pub/zimmermann/teaching/ETHZ_ALE/

860 Lehmkuhl JF. 1984. Determining size and dispersion of minimum viable populations for land
861 management planning and species conservation. Environmental Management 8: 167–176.
862 https://doi.org/10.1007/BF01866938.
863 Li B, Kimmel M. 2013*;* Factors Influencing Ascertainment Bias of Microsatellite Allele Sizes: Impact on
864 Estimates of Mutation Rates Genetics 195(2): 563-572. Doi: 10.1534/genetics.113.154161.
865 Lobo A, Hansen OK, Hansen JK, Erichsen EO, Jacobsen B, Kjær ED. 2018. Local adaptation through
866 genetic differentiation in highly fragmented *Tilia cordata* populations. Ecology and Evolution 8
867 (12): 5968-5976. Doi.org/10.1002/ece3.4131.
868 Logan SA, Phuekvilai P, Wolff K. 2015. Ancient woodlands in the limelight: delineation and genetic
869 structure of ancient woodland species *Tilia cordata* and *Tilia platyphyllos* (Tiliaceae) in the UK.
870 Tree Genetics and Genomes 11: 1-12. doi:10.1007/s11295-015-0872-z.
871 Logan SA, Chytrý M, Wolff K. 2018. Genetic diversity and demographic history of the Siberian lime
872 (*Tilia sibirica*). Perspectives in Plant Ecology, Evolution and Systematics 33: 9-17.
873 Logan SA, Phuekvilai P, Sanderson R, Wolff K. 2019. Reproductive and population genetic
874 characteristics of leading-edge and central populations of two temperate forest tree species
875 and implications for range expansion. Forest Ecology and Management 433: 475-486.
876 https://doi.org/10.1016/j.foreco.2018.11.024.
877 Maes N, Van Vuure T. 1989. De linde in Nederland: verspreiding, ecologie en toekomstmogelijkheden
878 van de lindesoorten in Nederland en aangrenzende gebieden. Stichting Kritisch Bosbeheer.
879 Meirmans PG, Hedrick PW. 2011. Assessing population structure: FST and related measures*.* Molecular
880 Ecology Resources 11: 5-18.
881 Morgan-Richards M, Wolff K. 1999. Genetic structure and differentiation of *Plantago major* reveals a
882 pair of sympatric sister species. Molecular Ecology 8(6):1027-1036. doi:10.1046/j.1365-
883 294x.1999.00665.x
884 Myking T. 2002. Evaluating genetic resources of forest trees by means of life history traits – a
885 Norwegian example. Biodiversity and Conservation 11: 1681-1696.

886 Mylett AJ. 2015. Genetic diversity and regenerative potential of *Tilia cordata* Miller in the Lincolnshire
887 Limewoods. PhD thesis, University of Lincoln.
888 Namkoong G. 1984. A control concept of gene conservation. Silvae Genetica 33: 160-163.
889 Nomura, T. (2008). Estimation of effective number of breeders from molecular coancestry of single
890 cohort sample. Evolutionary applications *1*(3): 462-474.
891 Nationalparkverwaltung Bayerischer Wald (2008): Natura 2000 Management im Nationalpark
892 Bayerischer Wald.
893 Osborne JL, Martin AP, Carreck NL, Swain J, Knight ME, Goulson D, Hale RJ, Sanderson RA. 2008.
894 Bumblebee flight distances in relation to the forage landscape. Journal of Animal Ecology 77:
895 406-415. doi:10.1111/j.1365-2656.2007.01333.x.
896 Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for
897 teaching and research--an update. Bioinformatics 28: 2537-2539.
898 Phuekvilai P, Wolff K. 2013. Characterisation of microsatellite loci in *Tilia platyphyllos* (Malvaceae) and
899 cross-amplification in related species. Applied Plant Science 1: 1200386.
900 Phuekvilai P. 2014. Relicts, refugia, and reticulation: a study of population history, hybrids and
901 phylogeny in the long-lived flowering tree genus *Tilia*. Ph.D. Thesis, Newcastle University
902 Pigott CD. 2012. Lime-trees and Basswoods. A Biological Monograph of the Genus *Tilia*. Cambridge
903 University Press, Cambridge, UK, 395pp.
904 Pigott CD. 2020. Biological flora of the British Isles: *Tilia platyphyllos*. Journal of Ecology 108: 2638-
905 2676. Doi: 10.1111/1365-2745.13490.
906 Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus
907 genotype data. Genetics 155: 945–959.
908 Radoglou K, Dobrowolska D, Spyroglou G, Nicolescu V-N. 2009. A review on the ecology and
909 silviculture of limes (*Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia tomentosa* Moench.)
910 in Europe. Die Bodenkult. 60: 9-20.

911 R Core Team. 2019. R: A language and environment for statistical computing. RFoundation for
912 Statistical Computing, Vienna, Austria.
913 Reeves PA, Richards CM. 2009. Accurate inference of subtle population structure (and other genetic
914 discontinuities) using Principal Coordinates. PLoS ONE 4(1): e4269. Doi:
915 10.1371/journal.pone.0004269.
916 Roloff A. 1985. Morphologie der Kronenentwicklung von *Fagus silvatica* L. (Rothuche) unter
917 besonderer Berücksichtigung möglicherweise neuartiger Veränderungen. Diss. G.A. Univ.
918 Göttingen, 178 S.
919 Rosenberg NA. 2004. Distruct: a program for the graphical display of population structure. Molecular
920 Ecology Notes 4: 137–138.
921 Tüxen R. 1956. Die heutige potentielle natürliche Vegetation als Gegenstand der
922 Vegetationskartierung. Angew. Pflanzensoz. 13: S 5-42.
923 Van der Knaap WO, van Leeuwen JF, Fahse L, Szidat S, Studer T, Baumann J, Heurich M, Tinner W.
924 2019. Vegetation and disturbance history of the Bavarian Forest National Park, Germany.
925 Vegetation History and Archaeobotany: 1-19.
926 Walentowski H, Ewald J, Fischer A, Kölling C, Türk W. 2004. Handbuch der natürlichen
927 Waldgesellschaften Bayerns: Ein auf geobotanischer Grundlage entwickelter Leitfaden für die
928 Praxis in Forstwirtschaft und Naturschutz. Verlag Geobotanika, Freising.
929 Wang J, Santiago E, Caballero A. 2016. Prediction and estimation of effective population
930 size. Heredity 117(4): 193–206. Doi:10.1038/hdy.2016.43.
931 Waples RS. 2006. A bias correction for estimates of effective population size based on linkage
932 disequilibrium at unlinked gene loci. Conservation Genetics *7*(2): 167-184.
933 Waples RS, Do C. 2008. LDNE: a program for estimating effective population size from data on linkage
934 disequilibrium. Molecular Ecology Resources 8(4): 753-756.

935 Wolff K, Hansen OK, Couch S, Moore L, Sander H, Logan SA. 2019. *Tilia* cultivars in historic lime
936 avenues and parks in the UK and Estonia, with reference to other European countries. Urban
937 Forestry & Urban Greening 43: 126346 doi.org/10.1016/j.ufug.2019.05.008.
938 Zanewich KP, Pearce DW, Rood SB. 2018. Heterosis in poplar involves phenotypic stability:
939 cottonwood hybrids outperform their parental species at suboptimal temperatures. Tree
940 Physiology 38: 789-800. Doi: 10.1093/treephys/tpy019.
941

Appendix:

A. Details regarding clonal growth and sexual reproduction

Numbers of trees starting with GEBW are numbers as used in the genetic analysis, while numbers with four or five digits are codes used in the BFNP.

Clones:

Three genotypes were present with multiple copies: two pairs (GEBW005=GEBW006 (9501=9502) (*T. platyphyllos*) and GEBW096=GEBW097 (1537=1538) (*T. cordata*) and one with four copies (GEBW034 to GEBW037 (5503-5506) (*T. platyphyllos*).

Recruitment:

Six saplings found near GEBW014 (8506) (a hybrid) were identical to each other, but not to GEBW014. However, they were all identical to GEBW020 = 5606, a nearby tree with the same coordinates. Twenty -ive saplings near GEBW007 (10501) (*T. cordata*) were analysed. All bar one were identical (i.e. clones) and indeed identical to GEBW007. The other genotype (a single individual) in this offspring set was identical to GEBW020 (5606, *T. cordata*). Near GEBW118 (1565) (*T. cordata*) ten samples of young trees analysed. This revealed a total of six genotypes. Four of the young saplings are identical and identical to GEBW091=1532 (*T. cordata*) at 38m distance to GEBW118 (asexual reproduction) (*T. cordata*), while another one was identical to GEBW108=1555 (*T. cordata*) with the same coordinates as GEBW118 (also asexual reproduction). However, the other five genotypes were unique, and, therefore, resulted from sexual reproduction. Using MLRelate we analysed potential parents of these genotypically unique saplings. All potential parents appeared to be close to the location of the sapling. One sapling was potentially the offspring of GEBW098 (1539) (at 24m distance), GEBW103 (1544) (at 24 m distance) or GEBW108 (1555) (same coordinates) (all *T. cordata*). One sapling was potential offspring of GEBW109 (1556) (same coordinates) and GEBW115 (1562) (same coordinates), and one sapling of GEBW103 (1544) (at 24m distance) and GEBW108

966 (1555) (same coordinates) (all *T. cordata*). Two juvenile offspring genotypes were not related to any

967 tree analysed in our set but may derive from a tree no longer alive.

968

969 ***Table A1:*** Results of tests for Hardy Weinberg equilibrium for each locus and the three *Tilia* clades
970 detected in the BFNP, showing the Chi square values, the P-values indicating probability of the
971 presence of HW equilibrium and the level of significance (ns=not significant, * P<0.05, ** P<0.01 and
972 *** P<0.001).

	T. cordata			*T. platyphyllos* 'large group'			*T. platyphyllos* 'small group'		
Locus	**ChiSq**	**Prob**	**Signif**	**ChiSq**	**Prob**	**Signif**	**ChiSq**	**Prob**	**Signif**
Tc6	14.935	0.826	ns	51.620	0.903	ns	19.556	0.550	ns
Tc937	2.331	0.993	ns	69.928	0.731	ns	7.586	0.669	ns
Tc920	50.460	0.006	**	73.856	0.004	**	7.639	0.937	ns
Tc8	59.000	0.000	***	62.036	0.240	ns	11.770	0.301	ns
Tc943	8.204	0.609	ns	0.616	0.893	ns	13.020	0.005	**
Tc4	96.576	0.000	***	103.535	0.522	ns	9.503	0.850	ns
Tc927	0.179	0.981	ns	193.434	0.417	ns	20.900	0.829	ns
Tc915	98.963	0.648	ns	169.848	0.026	*	1.873	0.599	ns
Tc963	214.450	0.108	ns	202.583	0.000	***	21.169	0.449	ns
Tc11	77.139	0.000	***	27.647	0.483	ns	7.639	0.054	ns
Tc5	90.254	0.025	*	135.423	0.002	**	17.539	0.678	ns
Tc951	2.112	0.909	ns	27.188	0.508	ns	11.000	0.001	***
Tc7	7.781	0.650	ns	31.842	0.930	ns	3.592	0.732	ns
tc1-42	15.227	0.811	ns	25.307	0.000	***	monomorphic		

tc2-69	129.501	0.000	***	138.717	0.015	*	10.993	0.753	ns
tc2-16	monomorphic			5.134	0.882	ns	0.543	0.909	ns
tc3-57	70.095	0.083	ns	60.869	0.924	ns	10.321	0.112	ns
tc1_19	2.790	0.425	ns	1.496	0.683	ns	0.274	0.601	ns
tc3-74	0.235	0.628	ns	0.009	0.925	ns	0.025	0.875	ns
tc2-86	19.248	0.203	ns	44.391	0.002	**	16.701	0.081	ns

973

Table A2 Diversity expressed as the number of alleles (N_a) effective number of allele (N_e), observed, expected and unbiased expected heterozygosity heterozygosity (H_o, H_e and uH_e), allelic richness (R_S) as well as the inbreeding coefficient F_{IS}. *T. cordata* (n = 59) is indicated as Tcor, *T. platyphyllos* 'large group' (n = 29) is indicated as Tp_l and the 'small group' *T. platyphyllos* (n = 11) as Tp_s. Na indicates not assessed. Loci derived from *T. platyphyllos* are indicated with 'Plat', and those from *T. cordata* with 'Cor'. Also indicated are means for the 'Plat' and the 'Cor' loci separately, as well as across all loci.

Locus		**N_a**			**N_e**			**H_o**			**H_e**			R_S			**F**	
Plat	Tcor	Tp_l	Tp_s	Tcor	Tp_l	Tp_s	Tcor	Tp_l	Tp_s	Tcor	Tp_l	Tp_s	Tcor	Tp_l	Tp_s	Tcor	Tp_l	Tp_s
Tc6	7	12	7	3.84	7.86	5.26	0.69	0.90	1.00	0.74	0.87	0.81	5.06	8.89	7	0.07	-0.01	-0.219
Tc937	5	13	5	1.80	6.98	3.27	0.49	0.83	0.82	0.44	0.86	0.69	3.37	9.19	5	-0.10	0.05	-0.13
Tc920	8	10	6	4.64	3.57	2.88	0.66	0.76	0.91	0.78	0.72	0.65	6.244	6.89	6	0.17	-0.04	-0.35
Tc8	2	11	5	1.07	6.65	4.57	0.00	0.93	1.00	0.07	0.85	0.78	1.57	8.28	5	1.00	-0.08	-0.24
Tc943	5	3	3	2.59	1.94	2.05	0.54	0.48	0.55	0.61	0.48	0.51	3.82	2.38	3	0.12	0.02	-0.02
Tc4	11	15	6	5.96	6.65	4.17	0.59	0.83	0.91	0.83	0.85	0.76	8.05	9.35	6	0.29	0.04	-0.15
Tc927	3	20	8	1.33	12.65	5.50	0.25	0.97	1.00	0.25	0.92	0.82	2.16	12.79	8	-0.01	-0.03	-0.18
Tc915	15	17	3	7.72	7.51	2.26	0.81	0.90	0.73	0.87	0.87	0.56	9.50	10.69	3	0.07	-0.02	-0.26

Tc963	20	17	7	12.77	11.14	4.94	0.83	0.69	1.00	0.92	0.91	0.80	11.84	11.90	7	0.11	0.26	-0.21
Tc11	4	8	3	1.64	3.92	2.47	0.17	0.66	0.91	0.39	0.74	0.60	2.75	5.76	3	0.57	0.14	-0.49
Tc5	12	14	7	3.58	6.65	5.26	0.68	0.86	1.00	0.72	0.85	0.81	6.71	9.81	7	0.07	-0.00	-0.19
Tc951	4	8	2	2.35	2.02	1.20	0.61	0.38	0.00	0.57	0.50	0.17	3.42	5.36	2	-0.06	0.26	1.00
Tc7	5	10	4	1.96	4.49	2.20	0.39	0.79	0.73	0.49	0.78	0.55	3.15	7.1	4	0.21	-0.00	-0.29
Mean Plat	**7.8**	**12.2**	**5.1**	**3.94**	**6.31**	**3.54**	**0.52**	**0.77**	**0.81**	**0.59**	**0.79**	**0.65**	**5.20**	**8.34**	**5.08**	**0.19**	**0.05**	**-0.13**
Cor																		
tc1-42	7	4	1	1.73	2.36	1.00	0.42	0.34	0.00	0.42	0.58	0.00	3.87	3.24	1.00	0.00	0.42	na
tc2-69	13	15	6	5.25	10.32	3.78	0.44	0.97	0.91	0.81	0.90	0.74	6.94	10.87	6	0.46	-0.05	-019
tc2-16	1	5	3	1.00	4.61	1.45	0.00	0.83	0.36	0.00	0.78	0.31	1	4.98	3	na	-0.04	-0.13
tc3-57	11	13	4	7.05	4.34	3.06	0.81	0.72	0.91	0.86	0.77	0.67	7.96	8.53	4	0.06	0.08	-0.31
tc1_19	3	3	2	2.83	2.60	1.31	0.61	0.52	0.27	0.65	0.62	0.24	3.00	3.00	2.00	0.06	0.18	-0.11
tc3-74	2	2	2	1.13	1.04	1.10	0.12	0.03	0.09	0.11	0.03	0.09	1.77	1.38	2	-0.06	-0.02	-0.00

tc2-86	6	7	5	3.59	3.57	2.75	0.61	0.45	0.64	0.72	0.72	0.64	4.26	5.51	5	0.16	0.39	0.05
Mean Cor	**6**	**7**	**3**	**3.23**	**4.12**	**2.06**	**0.43**	**0.55**	**0.45**	**0.51**	**0.63**	**0.38**	**4.12**	**5.36**	**3.29**	**0.12**	**0.14**	**-0.11**
Mean all loci	7.2	10.4	4.5	3.69	5.54	3.02	0.49	0.69	0.69	0.56	0.73	0.56	4.82	7.30	4.45	0.14	0.07	-0.18
SE	1.118	1.175	0.456	0.655	0.722	0.338	0.058	0.056	0.079	0.063	0.047	0.058	na	na	na	0.059	0.033	0.069
		N_a			**N_e**			**H_o**			**H_e**			R_S			**F**	
Mean all loci and all individuals		7.24			4.24			0.66			0.65			7.58			0.00	
SE		0.498			0.302			0.034			0.027			na			0.036	

Table A3. Means of genetic and ecological characters of 'large group' (n=29) and 'small group' (n=11) *T. platyphyllos*, with outcome of t-test

	'Large group' *T. platyphyllos*	'Small group' *T.platyphyllos*	t-value	P Value
Number of alleles, N_a	10.35	4.45	4.68	0.000
Effective nr of alleles, N_e	5.54	3.02	3.16	0.004
Observed heterozygosity, H_o	0.69	0.69	0.05	0.959 ns
Expected heterozygosity, H_e	0.73	0.56	2.30	0.027
Allelic richness, R_s	7.30	4.45	3.30	0.002
Volume	3.67	1.10	3.73	0.001
Vitality	2.00	0.91	2.73	0.014
Height	21.56	19.40	1.22	0.232 ns
DBH	56.2	35.2	3.47	0.001

ns means non-significant

Table A4. Parametric regression analysis of habitat and Potential Natural Vegetation characteristics of *T. cordata* (n=59) and *T. platyphyllos* (n=29). An estimate of each effect of actual habitat as well as the Potential Natural Vegetation (PNV), with t-value and probability, are indicated.

	T. cordata				*T. platyphyllos*			
Forest/PNV	Estimate	Std. Error	t-value	Pr(>|t|)	Estimate	Std. Error	t-value	Pr(>|t|)
Intercept	0.990	1.130	0.876	0.383	-1.560	1.126	-1.387	0.169
Deciduous forest	-0.144	1.678	-0.086	0.932	0.989	1.274	0.774	0.441
Mixed forest	4.656	1.734	2.685	0.009 **	-2.552	1.266	-2.015	0.047 *
Coniferous forest	1.73	1.710	1.012	0.314	-0.524	1.256	-0.417	0.678
PNV Asperulo-Fagetum beech forests	-4.123	1.387	-2.973	0.004 **	2.192	1.008	2.175	0.032 *
PNV Alluvial forests with Alnus glutinosa	16.720	4145.175	0.004	0.997	-16.992	4145.175	-0.004	0.997
PNV Tilio-Acerion forests of slopes, screes and ravines	-18.412	4145.175	-0.004	0.0996	-16.992	4145.175	-0.004	0.997

PNV Luzulo-Fagetum beech forests	-4.847	1.604	-3.022	0.003 **	3.135	1.239	2.53	0.0131 *
PNV Luzulo-Abietetum silver fir forests	14.846	1196.610	0.012	0.990	-15.482	1196.609	-0.013	0.990
R- squared (Adjusted)	0.542				0.362			

Note: ** means P< 0.001, * means P < 0.01

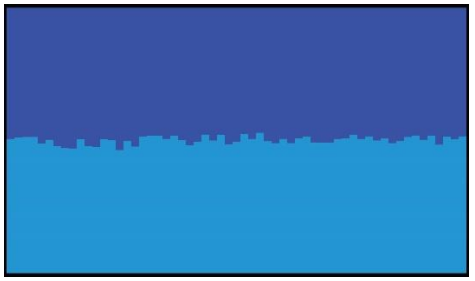

Fig 1A. Substructure within species, STRUCTURE result using 20 markers and K = 2, a) Top: *T. cordata* with no substructure; b) Bottom: *T. platyphyllos*, with green representing the ‘small group’ and orange the ‘large group’.