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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.

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

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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* Hayne], also called common lime or in the past Dutch lime. This hybrid tree is the lime that is often planted in parks and along streets (Wolff et al., 2019). *Tilia* are insect pollinated species, flowering relatively late in the season after the leaves have fully expanded. In Central Europe this is late June for *T. platyphyllos* and for *T. cordata* about 10 days later, early July (Pigott, 2012, 2020). They take at least 20 years to flower and reproduce sexually, but in dense woodland this may be much later (Pigott, 2012). The species are highly outcrossing, with inbreeding coefficients (F_{IS}) close to zero and medium to high genetic diversity (Logan et al., 2015, 2019). In some regions, asexual reproduction through root collar growth, epicormic shoots or rooting of branches that touch the ground (Pigott, 2012; Logan et al., 2018; Erichsen et al., 2019) is common. Generally, clones of the same genotype are only found at short distances from each other (average 5 m for *T. platyphyllos* and 22 m for *T. cordata*, Logan et al., 2019).

The current distribution of *T. cordata* ranges from Italy and Greece in the south to Finland in the north and from Siberia in the east to the United Kingdom in the west. *T. platyphyllos* has a more limited distribution, ranging from Italy and Greece in the south to Denmark in the north, and Romania in the east to the United Kingdom in the west (Logan et al., 2019; www.euforgen.org). *Tilia* is host for many species, such as birds, insects and lichen (Pigott, 2020). Tree stems become hollow after about 200–300 years and form suitable nesting sites for e.g. birds and wild bees (Pigott, 2012) and the hermit beetle (*Osmoderma eremita*), protected as high priority species by the European Union Habitats Directive.

Tilia has played important roles in mixed deciduous forests for a long time. After the last Ice Age it was, along with *Quercus*, the most abundant tree in Western Europe (Huntley and Birks, 1983; Pigott, 2012). The Bavarian Forest National Park (BFNP) is in the central range of both *Tilia* species. Pollen records show that *Tilia* was prominent in the BFNP region from about 10.000 years ago until about 4000 years ago, when its frequency declined in the region (Huntley and Birks, 1983; Van der Knaap et al., 2019). Van der Knaap et al. (2019) were able to distinguish the *Tilia* species in the BFNP 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* (Mylett, 2015).

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 southeast Germany, adjacent to the border with the Czech Republic (48.9595 °N, 13.3949 °E). It covers an area ranging in elevation from 600 m to 1,450 m above sea level (a.s.l.). A buffer zone, situated along the national park border, aims to conserve and protect areas adjacent to the national park from potential damages caused by the non-intervention strategy implemented within the core zone of the national park. No management takes place within the core zone of the park, which comprises about 72% of the total area.

Norway spruce (*Picea abies*), European beech (*Fagus sylvatica*) and Silver fir (*Abies alba*) are the main tree species in the BFNP, mixed with other tree species, such as ash (*Fraxinus excelsior*), sycamore maple (*Acer pseudoplatanus*) and lime (or linden, *Tilia*) depending on the specific location and microclimate (Van der Knaap et al., 2019). Across the elevation gradient, mean annual temperatures vary from 3 °C to 6.5 °C, and mean annual precipitation ranges from 830 mm to 2,230 mm, much of which falls as snow. Snow cover persists for 5–8 months each year depending on elevation (Heurich et al., 2010). This elevation gradient maintains a variety of forest types, which can be split into three broad categories. Above 1,100 m a.s.l., sub-alpine Norway spruce forests dominate, with rowan as a minor component. At 600–1,100 m a.s.l., mixed forests of Norway spruce, silver fir (*Abies alba*) and European beech (*Fagus sylvatica*) dominate with interspersed sycamore (*Acer pseudoplatanus* and *A. platanoides*) and lime (*Tilia cordata* and *T. platyphyllos*). In cold and wet depressions at the bottom of valleys, Norway spruce (*P. abies*), rowan (or Mountain ash *Sorbus aucuparia*) and birch sp. (*Betula pendula* and *Betula pubescens*) dominate (Cailleret et al., 2014).

2.2. Sampling and ecological measurements.

Tilia trees within the Bavarian Forest were exhaustively sampled (Fig. 1). The main source of information was the Natura 2000 survey, which took place in 2004 and 2005 when all habitat types were mapped throughout the park (Nationalparkverwaltung BW, 2008). During this process, all *Tilia* trees were mapped. In addition, all existing databases were searched, and the local forest managers 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 Fischer 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).

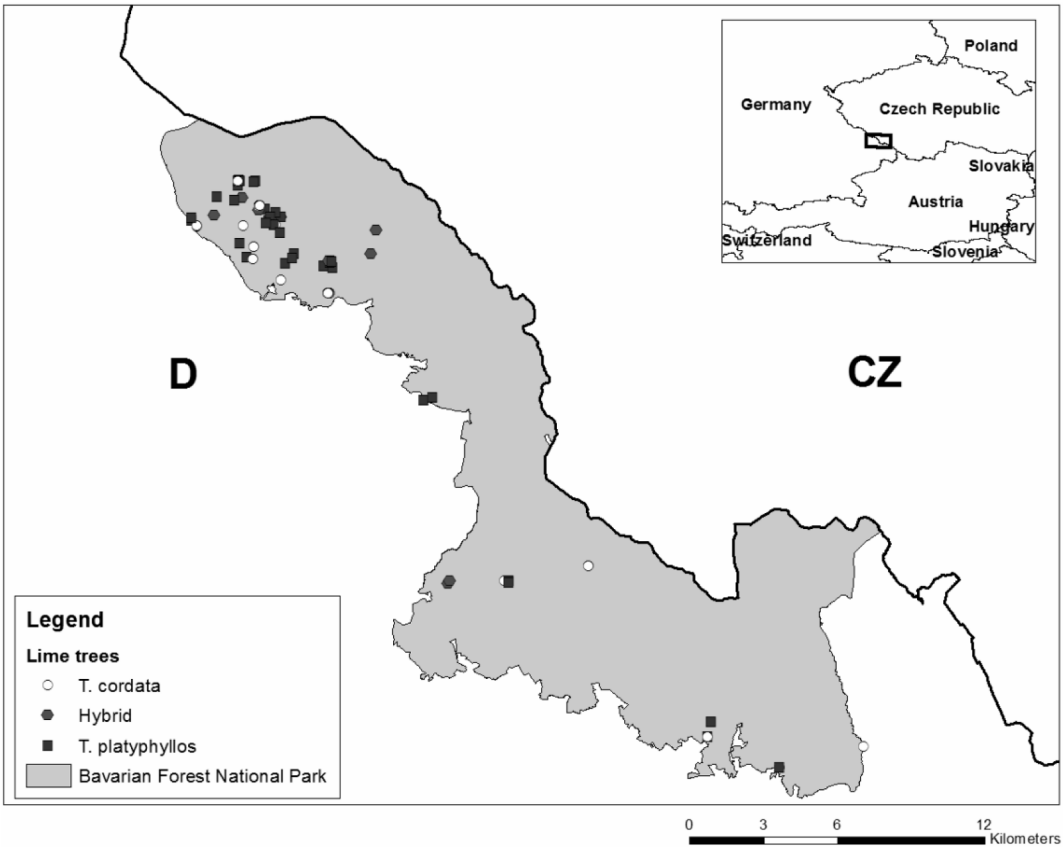

Fig 1. Location of sampled *Tilia* trees in the Bavarian Forest National Park, and the location of the BFNP in Europe. Symbols indicate the *Tilia* species, confirmed by genotyping, with open circles indicating *T. cordata*, filled hexagons are the hybrid *T.* x *europaea* and the filled squares are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

2.3. DNA extraction and microsatellite genotyping

Leaves of 113 adult trees and 41 saplings were dried directly after sampling and then stored at −20 °C. Genomic DNA extraction was performed using the CTAB method (Morgan-Richards and Wolff, 1999). A Polymerase Chain Reaction (PCR) was performed as a multiplex procedure for 20 nuclear microsatellite regions following Phuekvilai and Wolff (2013). Thirteen of those were originally derived from *T. platyphyllos* genomic DNA (Tc6, Tc937, Tc920, Tc8, Tc943, Tc4, Tc927, Tc915, Tc963, Tc11, Tc5, Tc951 and Tc7, Phuekvilai and Wolff, 2013) and seven markers were developed from *T. cordata* (tc1-42, tc2-69, tc2-16, tc3-57, tc1-19, tc3-74 and tc2-86, Mylett, 2015). Microsatellites were genotyped using an ABI 3130XL Genetic Analyser, visualised using Genemapper (Applied Biosystems) and binned and scored manually. Every run a small number of samples were repeated and scored identical across runs. There were no missing data.

2.4. Genetic data analyses

Deviation from Hardy-Weinberg equilibrium was tested in *T. cordata* and *T. platyphyllos* separately, using Genalex 6.5 (Peakall and Smouse, 2012), with the notion that with small sample sizes and small expected values in some genotypic classes the test is not reliable (Hedrick, 2005). Tests with and without loci that differed substantially for H_o and H_e (Tc11, tc1-42 and tc2-69) and loci with a large number of alleles (>17, namely, Tc915, Tc963 and Tc927) did not yield different results after analyses, so all 20 loci were retained. Further, Genalex 6.5 was used to calculate various measures of genetic diversity (N_a, the number of alleles, N_e, effective number of alleles, H_o, observed heterozygosity, H_e, expected heterozygosity and uH_e, unbiased expected heterozygosity). Genalex was also used to calculate genetic differentiation between groups of samples, with F_{ST}, based on allele frequencies, G''_{ST}, which is corrected for small population size and Dest (Jost, 2008; Meirmans and Hedrick, 2011). FSTAT 2.9.4 (Goudet, 1995) was used to calculate allelic richness (R_s) as the number of alleles per sample group independent of sample size using a rarefaction index. FSTAT was also used to calculate F_{IS} values (per sample group, per locus and for the total) and their significance of being more or less than zero with 1600 randomisations.

Population genetic structure was analysed using two methods, a mathematical visualisation and a population assignment, both without prior specification of population structure. Genetic diversity within and between species was visualised with a Principal Coordinate Analysis (PCoA) in Genalex 6.5, based on individual pairwise genetic distance. Samples were assigned to a set number of clades using a Bayesian clustering method, namely STRUCTURE v2.3.4 (Pritchard et al., 2000; Falush et al., 2003). The PCoA methods excels in visualising diversity, while the Bayesian clustering 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 1–5 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 (https://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}.

3.1. Clones

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

3.2. Recruitment

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

3.3. Species identification and substructure within species

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

Following on from this, a PCoA was performed within each species. Within *T. cordata* there is no substructure to be detected (Fig. 3a). However, in *T. platyphyllos* there is a clear separate and small grouping of eleven individuals (Fig. 3b), from here onward called the 'small group', while the other is called the 'large group' (green and red symbols, respectively in Fig. 3). Some further analyses were performed separately on the 'small' and 'large' group of *T. platyphyllos*.

The Bayesian STRUCTURE analysis confirms the PCoA results. Using all samples the K (data not shown) and ΔK 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.

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* (Figs. 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.

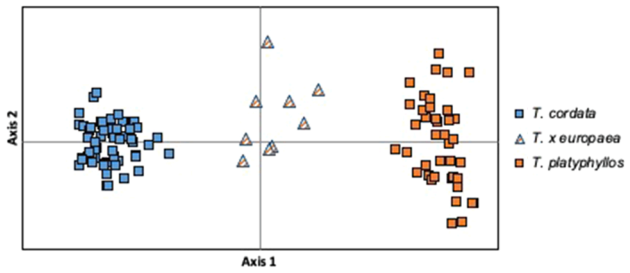

Fig. 2. Principal coordinate analysis of 108 BFNP *Tilia* trees, using 20 microsatellite markers. The first two axes explain 28% and 4%, respectively, of the variation.

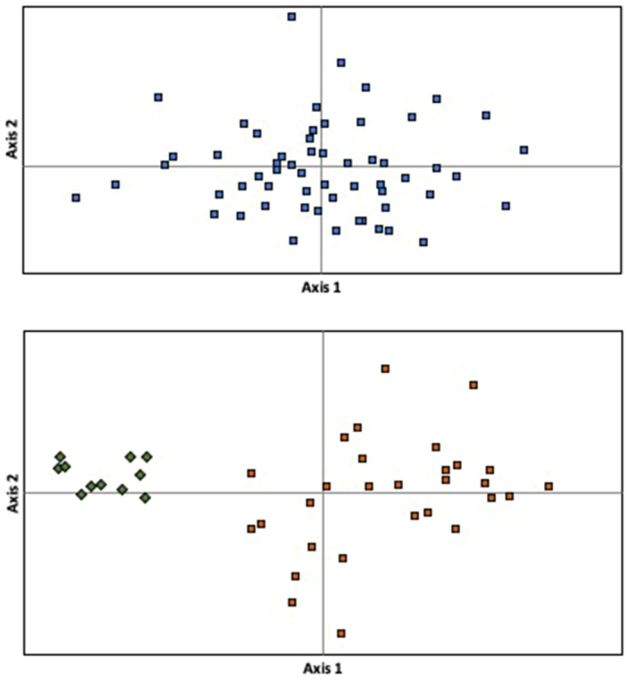

Fig. 3. Principal coordinate analysis of a) Top: *T. cordata* and b) Bottom: *T. platyphyllos* (see text) with red squares being 'large group' and the green diamonds 'small group', using 20 markers. The first two axes explain 11% and 9.7% and 16.2% and 11%, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 to 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 indicated by a large difference between H_e and H_o for these loci. Hence, our analyses were done with and without those loci, 20 and 17 loci, respectively (see Materials and Methods 2.4).

T. cordata has a lower genetic diversity than *T. platyphyllos*, whichever diversity estimate is used (Table 1 and A2). For example, the unbiased expected heterozygosity was lower in *T. cordata* (0.57) than in *T. platyphyllos* (0.74). To test for ascertainment bias, diversity in both species was compared for markers derived from *T. platyphyllos* with those derived from *T. cordata*. Both sets of markers showed that *T. platyphyllos* was more diverse than *T. cordata,* whichever statistic and whichever set of markers was used (Table 1). In addition, in both species the *T. cordata* markers showed less diversity than the *T. platyphyllos* markers.

The inbreeding coefficient (F_{IS} values) indicate that the species are both outcrossing species (Table 1). More specifically, *T. cordata* and the 'large' group *T. platyphyllos* have significantly positive F_{IS} values, for both sets and one set of markers, respectively. However, the 'small' group *T. platyphyllos* has significantly negative F_{IS} values.

Remarkably the group of *T. platyphyllos* trees that formed a separate unit in the PCoA and Bayesian analysis ('small group') has a lower genetic diversity than the 'large group' of *T. platyphyllos* trees, including for the allelic richness corrected for different sample sizes. This is also distinctly lower than for earlier studies (Logan et al., 2019). The number of alleles, effective number of alleles, expected heterozygosity and allelic richness of the 'small group' trees is significantly lower than in the

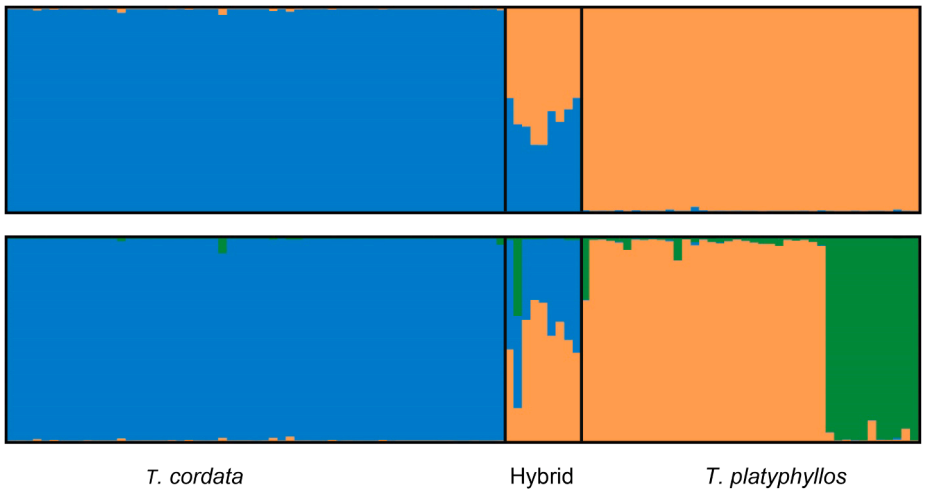

Fig 4. STRUCTURE analysis of all 108 adult Bavarian Forest *Tilia* trees, using 20 markers. Top: K = 2, Blue is *T. cordata*, while orange is *T. platyphyllos*, bottom: K = 3. Blue is *T. cordata*, while orange and green is *T. platyphyllos*, 'large' and 'small' group, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Diversity summary averaged across loci of *T. cordata* and *T. platyphyllos* ('small group' and 'large group'), for the *T. platyphyllos* (Plat, 13 loci) and the *T. cordata* (Cor, 7 loci) separately. N is the number of samples, R is the genotypic richness, an indicator of non-clonal reproduction. N_a is the number of alleles, N_e the effective number of alleles, H_o observed heterozygosity, H_e expected 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

'large group' *T. platyphyllos* ($P = 0.000$, $P = 0.004$, $P = 0.027$ and $P = 0.0019$, respectively, Table 1 and A3).

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

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.

effective population size (Table 2).

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.

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).

4. Discussion

Uniquely, we report here on a combined ecological and genetic survey of an ecologically important broadleaf species, *Tilia,* in a mixed woodland forest in Central Europe. Confirming early presence in the pollen record (Van der Knaap et al., 2019), our findings indicated that both *T. cordata* and *T. platyphyllos* are present in the BFNP, and likely remnants of a once more widespread *Tilia* population within the forests. This is because the genetic diversity for both species is medium to high, in line with other populations central to the distribution of the species. Also, N_e, relatedness, frequency of hybrids and clonal reproduction is comparable to other Central European populations (Logan et al., 2019). Moreover, when analysed within a larger genotype data set the BFNP individuals fit in well with other nearby populations, i.e. they did not form a separate clade in STRUCTURE (data not shown). The diversity observed indicates opportunities for survival of both species, but the current actual census size and isolation of the individuals from one another across the large BFNP are a cause for concern. Several of the forests sampled by Logan et al. (2019) and Phuekvilai (2014) across Europe have larger census size and higher concentrations of *Tilia*.

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

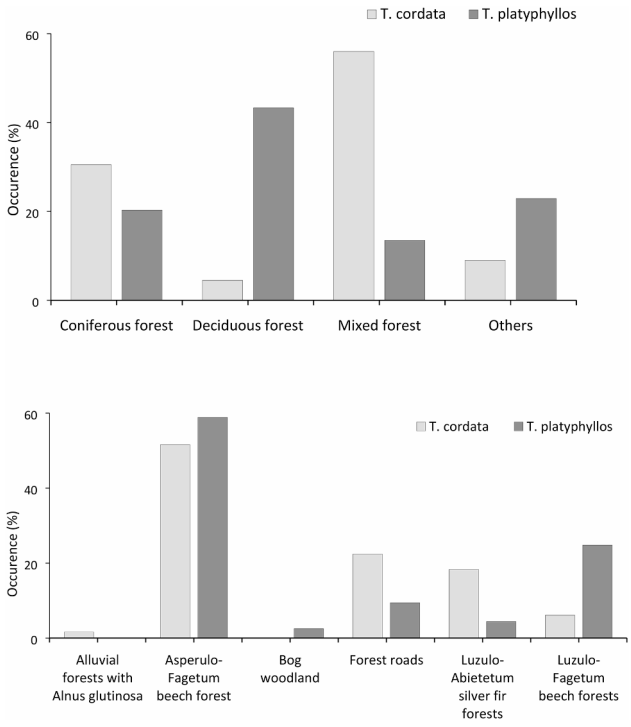

Fig. 5. Habitat (a) Top: forest types (b) Bottom: Potential Natural Vegetation of *T. cordata* (light grey) and 'large group' *T. platyphyllos* (dark grey) in the BFNP.

4.1. Species identification

We are confident that the molecular markers have clearly identified the presence of both *Tilia* species (Logan et al., 2015, 2019; Phuekvilai, 2014). Moreover, we confirm the pollen representation data of Van der Knaap et al. (2019) that *T. platyphyllos* is less common in the BFNP than *T. cordata*, which is a contradiction to the Natura 2000 monitoring in the national park that did not mention the occurrence of *T. cordata* in the park (Nationalparkverwaltung BW, 2008). Pollen analysis (van der Knaap et al., 2019) and the study presented here indicate that *T. platyphyllos* is less numerous than *T. cordata*, both in the past and at present. It is common that surveys do not correctly identify the species in the genus *Tilia,* in particular for these two closely related species that also generate hybrids with intermediate characteristics. This is because in *Tilia,* it is relatively easy to distinguish the two species and its hybrid if flowers or seeds are available at ground height, but to accurately identify species from leaves benefits from an 'expert eye' (Pigott, 2012, 2020). The leaf characteristics of the species show overlap, and, combined with considerable plasticity in leaf shape, this means that they are often mixed up (Phuekvilai, 2014). In addition, the species only flower for a short period (two weeks) and many trees, especially in the understory, do not flower at all. Surveys that do not include the flowers will therefore struggle to identify the species and the hybrid correctly. In these cases, molecular markers are needed for accurate species determination.

4.2. Planted trees

Often the most substantial proportion of genetic variation in outcrossing species is within populations and not between, making it hard to use markers, such as microsatellites, for forensic applications, e.g.

finding the source of trees, timber or wood products. Earlier work showed that using chloroplast markers in *Tilia* would not improve the ability to detect population differences due to the large proportion of shared haplotypes between the two species and limited geographic structure within species (Fineschi et al., 2003; Phuekvilai, 2014).

Similarly, it is challenging to detect planted trees from those that are endemic, especially if planted trees are from local and diverse sources. Phuekvilai (2014) reports low differentiation between populations across Europe, for *T. cordata* F_{ST} values range from 0.021 to 0.181 and for *T. platyphyllos* from 0.020 to 0.171. Therefore, even if some trees were planted in the past, this may often go undetected in a genetic survey if they originated from another natural and nearby stock and no records of planting exist. There are no specific records of planting *Tilia* in the BFNP. Despite that here we detected a small set of trees ('small group') in *T. platyphyllos*, genetically dissimilar to the 'large group' both in the PCoA and STRUCTURE analysis. They have a genetic distance (F_{ST}) with 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 and they most likely migrated more or less simultaneously after the Ice Age (Pigott, 2012). Here the effective population size (N_e) is higher in *T. cordata* than in *T. platyphyllos*, similar to a Europe wide study by Logan et al (2019). Both census size and geographic range would predict a lower genetic diversity in *T. platyphyllos* (being lower and more restricted, respectively), not higher as observed. Hamrick et al. (1992) reviewed genetic diversity in trees and conclude that within species genetic diversity differs between species and often remains unexplained.

4.4. Hybrids

The frequency of the hybrids (approximately 9%) is similar to that found in other mixed forests (Phuekvilai, 2014; Logan et al., 2015; unpublished data K Wolff). No introgression has been observed in BFNP or in other mixed forests studied so far and there is no conservation concern if *T.* x *europaea* is simply maintained in the park (Phuekvilai, 2014; Logan et al., 2015; unpublished data K Wolff).

For the first time the size of hybrids and their parental species was measured in their natural environment. Because the hybrids are taller and had a larger DBH than the parental species it indicates that hybrids did not originate recently and *T. cordata* and *T. platyphyllos* must have shared their location in the BFNP for several centuries. The reason for the significantly greater height and DBH of the hybrids is likely to be 'hybrid vigour', a term used for the phenotypic superiority of hybrids as compared to its parental species. Zanewich et al. (2018) found that in poplar (*Populus* sp.) the combined (small) effects of multiple traits and metabolic diversity gave hybrids an advantage in several environments. Here the height and DBH superiority of the hybrids could be because they live longer than the parental species, allowing them to grow bigger before the main stem deteriorates, and/or they have a higher growth rate. Whether it is higher longevity or faster growth remains to be tested, e.g. in a growth trial or by counting rings in a core, with the proviso this only indicates the age of the stem, not the genotype.

4.5. Relatedness and effective population size

It is important to understand whether a population is large and diverse enough, to allow its conservation as well as adaptation to new environments and changing climate. This cannot be determined directly from genetic diversity for neutral markers, such as microsatellites, yet these can help us understand population size. Population size can be

expressed in how related random pairs of individuals are or as the effective population size. Although both cannot be regarded as hard and accurate measures, comparable studies can be used to draw conclusions. Data presented here are based on the set of 17 markers, avoiding those with potential null alleles as this would particularly affect outcomes of these types of analyses. However, we note that outcomes using 20, 17 or 14 markers led to very similar results and the same conclusions. Here we can compare this with an earlier study (Logan et al., 2019). In line with their Central European populations, the relatedness and effective population size (N_e) of *T. cordata* is higher than that of *T. platyphyllos*. Analogue to the lower genetic diversity in the presumably planted *T. platyphyllos* trees they seem to be closer related to each other than to the endemic *T. platyphyllos* trees, as also reflected in the smaller effective population size in the presumably planted trees.

The estimates for N_e at BFNP were as high as or higher than the actual census size of the two species. It is well known that the genetic diversity generally reflects 'recent' population sizes, not current population sizes (Hare et al., 2011). Due to high longevity of the trees 'recent' may be a period of tens or several hundred years. Therefore, it is likely the current situation is the result of an ongoing bottleneck, e.g. over the last several 100 years.

Rules have been suggested for the minimum number of individuals and source populations to conserve. However, there are no agreements on this and it seems impossible to have a hard standard rule for species with very diverse life history characteristics. Some have discussed the minimum viable population (MVP) size as being the number of individuals to conserve to ensure that 95% of the variation is maintained and random genetic drift minimised. A 50/500 rule was suggested, with 50 ensuring inbreeding was avoided in the short term, and 500 individuals needed to avoid random genetic drift and opportunities for adaptation and evolution in the longer term (LehmKuhl, 1984; Jamieson and Allendorf, 2012). Flather et al. (2011) and Jamieson and Allendorf (2012) remark that it is impossible to give exact MVP numbers that would guarantee survival of species and therefore conservation decisions cannot be made whether a species has reached certain threshold or not.

Myking (2002) discusses the concept of Multiple Population Breeding Strategy (MPBS), first coined by Namkoong (1984) designed for vulnerable tree species with large between population diversity. The most economic way is in situ conservation combined with silvicultural management (Myking, 2002). It asks for an effective population size (N_e) of 50 individuals from 20 populations, ensuring low loss of diversity through genetic drift. In *Tilia*, the majority of the variation is within populations, and therefore fewer populations probably suffice.

Here N_e for *T. cordata* is estimated as 87 – 1639, with a census size of 59 and for *T. platyphyllos* N_e is between 29 and 254, with a census size of 29. With $N_e > 50$ short-term risks of inbreeding are limited. However, for *T. platyphyllos* current numbers are smaller than the minimum of 50, albeit that genetic diversity in the 29 individuals is not diminished as compared to other populations. Also, 29 is the minimum census size as it is possible that saplings of the species have been missed in the collection and survey. However, long-term risks must be addressed, especially since the remaining trees are spread over a large area, limiting gene flow between trees, and gene flow with surrounding forests is limited due to limited pollen transport by its pollinators in a patchy environment (Osborne et al., 2008).

4.6. Ecology

While a limited number of previous studies have focused on ecological traits such as DBH and height of *Tilia* stands, the two species are often treated as one taxon (e.g. Jacob et al., 2010) and rarely do any consider the hybrid (*T.* x *europaea*) in natural *Tilia* populations. Here we have investigated the growth and site preference of these two closely related *Tilia* species along with (where applicable) their hybrid, growing intermixed in their natural environment. Most results seem to confirm descriptions of earlier work, allowing quantification in the current study. For growth results of the hybrid we refer to Section 4.4. In *T. cordata* the DBH was much smaller than in *T. platyphyllos*, which confirms Pigott (2012, 2020) who indicated that DBH of 1.5 m can be reached at 200 years in *T. platyphyllos*, while this would take 400 years in *T. cordata*. In the BFNP the two species reach similar heights, while Pigott (2012, 2020) describes that *T. cordata* grows up to 20 m and *T. platyphyllos* up to 40 m. The reason why we find similar heights for both species in BFNP may be partially explained by the fact that *T. platyphyllos* in BFNP occurs here at higher altitude than *T. cordata* and the colder climate at higher elevations may hamper length growth in trees in general or in *T. platyphyllos* specifically.

Both the hybrid and 'large group' *T. platyphyllos* were located at higher altitude than *T. cordata*. Pigott (2012) describes that *T. cordata* occurs on flat areas, but also on steep slopes, while *T. platyphyllos* is additionally found on unstable screes. This confirms our finding that *T. platyphyllos* occurs on steeper slopes. Personal observation (K. Wolff) similarly showed *T. cordata* at the foot of mountains/hills and *T. platyphyllos* at steep and unstable screes higher up the mountain/hill at several locations in Europe, e.g. at Leopolds berg in Vienna, Austria.

In the BFNP *T. cordata* is mostly found in mixed and coniferous forests, while *T. platyphyllos* was found across all forest types, although having the highest preference for deciduous forests (Fig. 5 and Table A4). The slightly higher light intensity in deciduous forest may allow *T. platyphyllos* to have more radial growth (higher DBH, Table 3) than *T. cordata*. Pigott (2012) describes that both species mostly occur in broadleaved forests, but also writes that *T. platyphyllos* is not found in *F. sylvatica* with *Abies alba* communities, confirming our low frequency of this species in mixed and coniferous forests. Radoglou et al. (2009) also describes that both occur in deciduous broadleaved forests, but that *T. cordata* can also occur mixed in coniferous forests, confirming our result.

Analysing their Potential Natural Vegetation communities both *T. cordata* and *T. platyphyllos* were most often found in Asperulo-Fagetum beech forests with *T. cordata* also present in Luzulo-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 *Tilia,* could benefit from clone archives from populations (Myking, 2002) representing the various habitats that the species occupy.

(2) Hybrids in general can pose problems if substantial introgression leads to the loss of one or both species and the hybrid swarm could be more invasive than the parental species (Gaskin, 2017).

However, no introgression has been observed in *Tilia* in BFNP or other mixed forests studied so far (Phuekvilai, 2014; Logan et al., 2015). This means that the hybrids will not contribute to the next generation but can simply be left in the park.

(3) Trees that are planted from unknown origin need to be removed to prevent contamination of the gene pool. The trees that appear planted from sources outside the BFNP and that appear from a rather limited seed source should be avoided for augmentation and potentially should be felled. If more detailed genetic knowledge, e.g. SNP or QTL analyses, of *Tilia* was available the 'planted' trees should be further analysed in that framework to assess whether they are indeed foreign and do not hold valuable genetic variants. In future care must be taken to use cuttings or seeds of local endemic trees or from a source suited to the habitat and latitude to ensure the success of the active management (Fady et al, 2016; Lobo et al., 2018).

(4) Genetic diversity needs to be maintained for survival and future adaptation.

This means management could consist of protection of those present and potentially planting of trees. As discussed in Section 4.5 the N_e of the two *Tilia* species in BFNP seems sufficient for the requirement of 50, but current census size points in the direction of a lower N_e in future. To reach a future N_e of 500 gene flow with forests from outside the BFNP also may need improving. Therefore, augmentation, using local or other suitable trees (see (3) above), in currently low-density areas to ensure improved sexual reproduction, i.e. planting *Tilia* in the vicinity of existing trees, as well as in 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. Ingvarsson 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 (Ingvarsson 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 species range, understanding climate change adaptation, insect and disease resistance. For many less common and less commercially important species this is not yet within reach. Large field trials at multiple locations are essential to understand to what extend there are adaptive traits and how they can benefit silviculture and biodiversity conservation in forest ecosystems.

CRediT authorship contribution statement

Kirsten Wolff: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Software, Visualization, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Bernhard Depner:** Data curation, Investigation, Formal analysis, Methodology. **Samuel A Logan:** Investigation, Formal analysis, Visualization, Writing - review & editing. **Marco Heurich:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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