Reproductive and population genetic characteristics of leading-edge and central populations of two temperate forest tree species and implications for range expansion

Running Title: Genetics of leading-edge vs central tree populations

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Abstract

It is still a matter of debate how reproductive and genetic characteristics of range-edge populations differ from those central to its range, yet this is important for future changes in species' ranges. Here we use microsatellite markers to assess the genetic diversity, relatedness and clonal reproduction of two lime tree species, *Tilia cordata* and *T. platyphyllos*, from leading range-edge and central locations.

Clonal reproduction was limited in the populations studied, but leading range-edge populations contained more clones than populations sampled from lower latitudes. Although no inbreeding or lower genetic diversity was detected, leading range-edge populations have a higher average relatedness of individuals and a lower effective population size than those populations closer to the centre of the species' distribution. Trees further apart than 26m are unlikely to be clones and those further apart than 61m are not likely to be closely related.

The implications for forest managers are that although leading range-edge populations have a lower effective population size, they are also likely to be better adapted to northern climes. Because locally sourced trees appear to have sufficient genetic diversity and predominantly result from sexual reproduction they are likely to adapt to climate change and be suited for natural migration and a good source for assisted migration. This is a promising prospect for the potential of future natural or managed expansion and increased species abundance.

Keywords: Assisted migration; Climate change; Clonal reproduction; Leading range-edge; Microsatellites; Relatedness; Range shifts; *Tilia*

1. Introduction

Range-edge populations exist in less than optimum conditions, and are often smaller and more isolated than central populations. , The majority of the studies indeed show that range-edge populations are vulnerable to loss of genetic diversity and increased population differentiation through random genetic drift (*e.g.* Arnaud-Haond *et al.,* 2006; Eckert *et al.,* 2008; Gapare & Aitken, 2005). However, a few phylogeographic studies have suggested that current genetic diversity has been influenced by historical range expansions and contractions leading to boundary populations in some species that contain a larger proportion of the species diversity compared to the central populations (Hewitt, 2004; Petit *et al.,* 2003). In addition, the demography and mode of reproduction in range-edge populations may also differ from central populations (Dorken & Eckert, 2001; Vucetich & Waite, 2003), potentially also affecting genetic diversity, population differentiation and the ability to adapt.

Asexual reproduction is a survival mechanism common in plants and is achieved through agamospermy or clonal (vegetative) reproduction *e.g.* root suckering, root collar growth, epicormic shoots, (Bond & Midgley, 2001; Ellstrand & Roose, 1987; Evans & Morris, 2016; Widén *et al.,*1994). Clonality can be seen as an adaptation to persist in adverse conditions (Macaya-Sanz *et al.,* 2016; Meloni *et al.,* 2013) and offers both advantages and disadvantages. For example, being clonal prolongs the lifespan of an individual which may lead to lower genetic diversity among adult stems (De Witte & Stöcklin, 2010). Also, clonal organisms suspend the exchange and recombination of genetic material, and thus fewer novel genotypes are generated. If sexual reproduction is limited to only a few individuals then asexual reproduction is advantageous as otherwise inbreeding and random allele loss may occur (Ennos, 2003), and genetic diversity is maintained (Balloux *et al.,* 2003). In addition, large clones may accumulate somatic mutations, potentially generating diversity and elevating heterozygosity (Balloux *et al.,* 2003; Halkett *et al.,* 2005). It is also possible selection plays a role, i.e. higher survival of more heterozygous genotypes. However,

adaptation, for example to a changing climate, is limited in largely clonally reproducing populations. On the contrary, sexual reproduction would produce an excess of seedlings for natural selection to act on. Therefore, the differences in relative amounts of sexual and asexual reproduction can change the genetic composition, diversity and adaptability of organisms.

In several plant species, strong clonal reproduction, low levels of genetic diversity and high population differentiation in range-edge populations, as compared to those at the centre of the species' range, have been reported. These studies concentrated often on shrubs and weeds (*e.g.* Beatty *et al.,* 2008; Dorken & Eckert, 2001), but some significant work has also been carried out on long-lived organisms, such as forest trees (e.g. Jankowska-Wroblewska *et al*., 2017; Santos-del-Blanco *et al.*, 2013; Stoeckel *et al.,* 2006; Vaughan *et al.,* 2007). Although clonal spread certainly occurs in forest trees, these are generally not considered to be species where clonal propagation is the predominant form of reproduction.

Trees from the genus *Tilia* were important components of woodlands in Europe until a few centuries ago, and the woodlands where they currently exist are largely remnants of their original distribution (Pigott, 2012). As a survival strategy, in sub-optimum conditions (*e.g.* cooler temperatures resulting in shorter flowering period and/or fewer pollinating insects), members of the genus may reproduce asexually (Evans & Morris, 2016; Logan *et al.,* 2018). *Tilia,* may be considered a partially clonal reproducing species, but the extent of asexual reproduction and intra-population relatedness across their range is not known. Therefore, *Tilia* serves as an excellent forest tree model to investigate leading range-edge effects, adding valuable information to the existing literature (*e.g.* Myking, 2002; Siefert *et al.,* 2015).

Species within the genus *Tilia* tend to cope well with cyclic management regimes, such as coppicing (Buckley & Mills, 2015; Pigott, 2012). Coppicing encourages sprouting, and therewith prolongs the lifespan and (clonal) spread of a genotype. Clonal spread is also aided by land erosion, particularly on steep banks and cliffs and individual trees can spread through low hanging branches and fallen trunks (S. A. Logan *pers. obs.*). The ability to

regenerate through vegetative growth and the low rate of regeneration by seed at some locations, *e.g*. range-edge (attributed to the present climate), may explain current distributions (Pigott & Huntley, 1978). Earlier work has shown that populations in the UK clonally reproduce at some sites (Logan *et al.,*, 2015; Mylett, 2015; Phuekvilai, 2014). Likewise, Novák *et al.* (2014) suggest that *T. sibirica* (Siberian lime) shows evidence of clonal growth, which has been confirmed by microsatellite genotyping (Logan *et al.,* 2018). Also, remnant stands of *T. americana* (American basswood) on islands off the coast of Georgia (USA) largely depend on vegetative growth (Evans & Morris, 2016).

Radoglou *et al.* (2009), and references cited therein, suggest that *T. cordata* in south-western Russia has a greater incidence of clonal reproduction than sexual reproduction, claiming that nearly all *Tilia* in the north-east of its European distribution originated from vegetative reproduction. They also claim that 90% of *the Tilia* trees in the Strict Reserve of the Białowieża National Park (BNP) sprout from root collars, and that this phenomenon extends to the range-edge populations in the UK, Finland, and Siberia. These statements, based on morphology, need genetic confirmation. In contrast, it has been noted that *T. cordata* regenerates freely from seedlings in the BNP (Pigott, 1975) and in other areas of Poland (Jaworski *et al.,* 2005). Clonal reproduction was certainly not as high as 90% in sampled UK populations (Logan *et al.,* 2015), and while *T. cordata* trees from western Siberia did show some clonal occurrence, lime trees from BNP in Poland appear not to be clonal but largely a result of sexual reproduction (Logan *et al.,* 2018).

Climate change is altering both the potential and actual distribution, of many animal and plant species (Gibson *et al.*, 2009; Rehm *et al.*, 2015; Walther *et al.*, 2002). Species may need to track their climate induced range shifts (Essl *et al.*, 2015) to higher latitudes, where it is colder - the leading edge, or to lower latitudes, where it is warmer - the trailing edge (Hampe & Petit, 2005; Hewitt, 2004). Here we concentrate on a comparison between leading edge (northerly) populations with those from regions that are central in the species' distribution. Due to the rapidity of climate change, the shift of ranges suitable for habitation

and sexual reproduction of the species may not be synchronized with the actual migration of organisms (Hoegh-Guldberg *et al.*, 2008; McLachlan, *et al.,* 2007; Seddon, 2010; Walther *et al.*, 2002). The migration rates of trees, and in particular of *Tilia*, in the past were much slower than migration rates needed now for some organisms to track potential ranges (McLachlan *et al.,* 2005; Myking, 2002), also due to human habitat use and geography. Therefore, in addition to natural migration, assisted migration, *i.e.* transfer of trees or planting of seeds, may be needed (Aitken & Whitlock, 2013; Seddon, 2010; Whittet *et al*., 2016; Winder *et al.* 2011).

Trees from populations nearer to their central ecological/geographical area are unlikely to have the adaptation for optimal reproduction and survival at higher latitudes further north, *e.g.* differences in season and day length or winter and late frost resistance (Kreyling *et al* , 2014; Rehm *et al.* 2015) . Therefore, genetically diverse populations with a diversity of microhabitats outside of the main central range might be key to the survival of some tree species as they are more likely to harbor pre-adaptation to environmental change (Hampe & Petit, 2005; Lobo *et al.*, 2018b). This is particularly important in species such as *Tilia*, where season length is crucial for sexual reproduction. Although there is a view that range-edge populations may not be optimally adapted to their habitat due to persistent gene flow from central populations (*e.g*. Mimura & Aitken, 2007), it is likely that if there is sexual reproduction in a population there will be ample seedlings upon which selection can act. Seed and pollen dispersal in *Tilia* species has not yet been assessed, but is expected to be limited compared to other tree species (Myking, 2002) because *Tilia* is insect pollinated and seeds are heavy and wind-dispersed. Therefore, persistent gene flow from central to range-edge population is unlikely to be the case in *Tilia*, in particular with (currently) fragmented populations. Moreover, there is evidence for adaptation to latitude as spring phenology adaptation has been recorded in *T. cordata* (Lobo *et al.,* 2018a).

Microsatellite markers indicated high levels of polymorphism in *T. cordata* and *T. platyphyllos* (Logan *et al.,* 2015; Phuekvilai & Wolff, 2013) and allowed identification of

clones. Here we investigate clonal architecture, relatedness and genetic diversity at leading range-edge populations in comparison and central populations, using two putative partially clonal *Tilia* tree species. In particular, this study investigates; [1] the level of clonality, [2] the within-population relatedness of individuals and F_{IS}, [3] genetic diversity and structure, and [4] the effective population size of populations. We expect that in regions with hampered sexual reproduction (northern range-edge), asexual reproduction must be more pronounced and that genetic diversity and effective population size will be lower, while population differentiation and relatedness will be higher than in central populations. The study will indicate whether range-edge populations of *Tilia* are potentially suitable for natural and assisted migration, aiding future management of these woodland species.

2. Materials and Methods

2.1 *Study sites and sample collection*

Two species of temperate, long-lived forest tree, *Tilia cordata* Mill. (small-leaved lime) and *T. platyphyllos* Scop. (large-leaved lime) were studied. These two species are mostly found in European parts of the northern hemisphere and their distribution is limited because of poor seed set in the short and cool summers at the northern range-edge (Pigott & Huntley, 1978). The distribution of *T. platyphyllos* extends north to Sweden and south to Greece and Turkey, while its east-west range extends from Wales to Poland, Slovakia and Romania (Fig 1). *Tilia cordata* has a wider distribution, from Finland and Norway in the north to Greece in the south, while its east-west range extends from Wales and Spain to Siberia, Russia. Both species now mainly exist in native or 'ancient' woodlands.

We define leading (northern) range-edge populations as those occurring at >52°N for *T. platyphyllos* and >54°N for *T. cordata* (Table 1, Fig 1). Populations sampled from locations between latitudes 43° - 52°N for *T. platyphyllos* and 43° - 54°N for *T. cordata* are considered central populations. UK samples were from Sites of Special Scientific Interest

and National Nature Reserves. Other collection sites were remnants of ancient woods and not planted, as there is no history of planting *Tilia* in these woodlands. No further details were available about the stands. Only adult trees were included in this study, i.e. trees with a circumference of 10 cm or more. Coordinates were recorded for as many individuals as possible using a Garmin hand-held GPS unit. We calculated geographic distances between trees in GenAlEx v6.502 (Peakall & Smouse, 2006; 2012). Locations could not be systematically sampled with the same distances between trees, due to the terrain and the number and density of trees. Average distances between sampled trees are presented (Table 1). In two populations (SWWC, RUVA) only one coordinate for the population was available and hence mean distances between sampled trees could not be calculated. Also, in POBF locations of nine trees were not available.

2.2 *DNA extraction and microsatellite genotyping*

Leaf samples were dried and stored at -20°C until required for genomic DNA extraction, using the CTAB method (Morgan-Richards & Wolff, 1999). A multiplex Polymerase Chain Reaction (PCR) procedure amplified 12 nuclear microsatellite regions (Tc6, Tc937, Tc920, Tc8, Tc943, Tc4, Tc927, Tc915, Tc963, Tc5, Tc951 and Tc7) following Phuekvilai and Wolff (2013). Microsatellites were genotyped using an ABI 3130XL Genetic Analyser, scored using Genemapper (Applied Biosystems) and binned manually.

2.3 *Clonal structure*

Deviations from Hardy-Weinberg equilibrium (HWE) were tested in GENEPOP on the web v4.2 (Raymond & Rousset, 1995; Rousset, 2008) and the presence of null alleles was tested in MICRO-CHECKER v2.2.3 (Van Oosterhout *et al.*, 2004). The combination of all alleles across all 12 markers together is called a Multi Locus Genotype (MLG). Individuals sharing MLG are likely to be clones. However, mutation rates, in particular for microsatellites,

208 can be high enough for mutations to occur in old clones, making genotypes of members of a
209 clone (ramets) differ from each other for one or more alleles. Although such cases were
210 checked and corrected for genotyping errors this could have contributed to ramets of a single
211 clone being similar but not identical. In such a case ramets might have a MLG that differs by
212 one or few alleles from other ramets of the same clone. In the literature this is sometimes
213 called a Multi Locus Lineage (MLL). Hereafter, when we use the term 'clone' instead of MLL.
214 It is essential to determine the threshold at which MLGs should be considered to be
215 part of a clone (Aizawa *et al.,* 2017; Meloni *et al.,* 2013). Microsatellite mutation rates and an
216 objective criterion are not available. We used GenoDive 2.0b (Meirmans & Van Tienderen,
217 2004) to determine the optimal threshold for assigning samples to clones, by determining the
218 allele difference represented by the smallest number of pairs, using the infinite allele model.
219 For this a matrix of genetic distance between all pairs of trees was calculated. Two trees
220 identical for all alleles are clearly clones. Those differing for a small number of alleles could
221 have arisen due to somatic mutation or due to genotyping errors. It is important to distinguish
222 those that have clearly a different MLG (i.e. at multiple loci) from those that could erroneously
223 be considered different. The threshold indicates the maximum distance (alleles different)
224 allowed between two trees for them to still be considered clone mates. The limit to be set is
225 reached by constructing a histogram of the frequency of the allelic differences. Those to the
226 left of the valley (defined as the number of alleles different with the lowest frequency) will be
227 considered clone members and those to the right of the valley will be considered to be
228 different. This analysis had strong power and showed that trees with a difference of
229 maximally three alleles would be assigned to the same clone (Fig A1). Clonal spread was
230 quantified by averaging the distance between trees belonging to the same clone.
231 Once the clones within each population were determined we used GenClone
232 (Arnaud-Haond & Belkhir, 2007) to provide an unbiased (*i.e.* not affected by genet size)
233 estimate of genotypic richness $R = (G-1)/(N-1)$, where N is the number of samples and G the
234 number of genotypes. *R* is a modified measure of proportion distinguishable genotypes (*Pd*,

Ellstrand & Roose, 1987). The value will be '0' when stands consist of a single clone and '1' when all sampled trees are separate genets (Dorken & Eckert, 2001). To describe clonal heterogeneity, an adapted estimate of the Simpson's complement index (*i.e. D**), independent of sample size (Pielou, 1969), was calculated. The Simpson index (*D*) represents the probability that two randomly sampled plants belong to the same species (Simpson, 1949). The Simpson's complement index (1-*D*) of diversity is commonly reported in clonal studies as *D** (Arnaud-Haond *et al*., 2007) and ranges from '0' when all trees within a population are a single clone to '1' when they are all unique. Additionally, the statistics P_{gen}, the probability of trees having the same genotype (i.e. are part of same clone) by chance following the methods of Parks and Werth (1993) and P_{sex}, the probability that a clonal genotype originates from sexual reproduction at the first reencounter, were estimated. P_{gen} assumes populations are in HWE. An adjusted measure P_{gen} (*f*), taking into account HWE departure can be estimated, providing a more conservative estimate of P_{sex} (Arnaud-Haond *et al*., 2007). These last two estimates indicate the power of the markers to detect clones.

2.4 *Genetic diversity and differentiation*

In the population genetic analyses we used single representatives of each clone, i.e. at genet level, reflecting genetic characteristics comparable between populations. Genetic diversity, calculated as the expected heterozygosity (H_e), and the fixation index F_{IS} were calculated in GenAlEx 6.502. When testing the difference in F_{IS} between non-clonal and clonal genotypes the fixation index F_{IS} was calculated by hand as $F = (H_e\text{-}H_o)/H_e$, with H_e being the expected heterozygosity over all clonal genotypes in that population. AMOVA analyses were performed to quantify population differentiation of the leading range-edge and of central populations of each species in GenoDive 2.0b using the Weir and Cockerham (1984) method. In addition, GenoDive supplied *G*-statistics G_{ST} (Nei, 1987), adjusted G'_{ST} (correcting for the bias due to small numbers of populations) as well as Jost's D_{est} (Jost, 2008), which is independent of H_e. It also supplied standard deviations through jack-knifing

over loci and 95% confidence intervals (CI) through bootstrapping over loci. These statistics were tested in GenoDive (Compare Groups) for significance of difference between leading range-edge and central populations, using 9999 permutations. We tested Isolation by Distance (IBD) to determine if genetic distance between populations was correlated to geographic distance by performing a Mantel Test in GenAlEx.

2.5 *Relatedness and effective population size*

Relatedness and effective population size were estimated at both genet (with single representative per clone) and ramet level (including all samples), where ramets reflect the actual standing crop. ML-Relate (Kalinowski *et al.,* 2006) uses simulations to determine the most likely relationship (unrelated, half sib, full sib or parent-offspring) and compares this with the likelihood of other relationships for the same pair of trees. We used 100,000 randomisations and counted for each population the number of pairs that have the highest probability of being full sib (50% related), parent-offspring (50% related) and half sibs (25% related). We calculated the number of first degree related pairs as well as the sum of first and second degree related pairs (50% relatedness and 50%+25% relatedness, respectively) as a proportion of the maximum number of pairs within a population ($n * (n-1)/2$, with n being the number of genotypes or ramets). To obtain a quantitative measure of relatedness we averaged the maximum likelihood estimates of relatedness (*r*, ML-Relate) for all pairs of trees in a population. The distance for each pair of genets within a population with a 50% relatedness was averaged to indicate the distance at which two trees can be closely related.

To detect small scale population structure, *i.e*. whether trees that were growing near to one another within a population were genetically more closely related than those at larger distances, the autocorrelation coefficient was calculated in GenAlEx. We tested whether the autocorrelation *r* as a function of the pairwise distance between trees is significantly different from zero and determined the 95% confidence intervals and error bars using 9999 permutations and 9999 bootstraps. In each case we used eight even pairwise distance

289 classes, with sufficient pairwise comparisons per distance class (maximum sample distance
290 in a population divided by eight).
291 We estimated the contemporary (or recent) effective population size (N_e) using the
292 molecular co-ancestry method of Nomura (2008) and the Linkage Disequilibrium (LD)
293 method as implemented in NeEstimator V2.01 (Do *et al,* 2014). The LD method is based on
294 Waples and Do (2008) and includes the Waples (2006) bias correction, which corrects the
295 bias that could be introduced when sample size is lower than the actual effective population
296 size. The 95% CIs are based on Jack-knifing over loci, and in the LD method only alleles with
297 a frequency > 0.05 were used.
298
299 2.6 *Contrasting groups of populations*
300 For testing the fixed-effect categorical variables of region, species and their interaction on
301 clonal reproduction, genetic diversity and relatedness estimates we used averages per
302 population. For most tests we used the average distance between trees (Av Dist) as a
303 covariate to ensure that a difference in a measure was not biased by differences of within
304 population sampling distances between the contrasting groups of populations. For the two
305 populations without individual coordinates we replaced the missing Av Dist with the average
306 for the group. The sample size (number of populations) was 28 for all tests. Three types of
307 statistical analyses were carried out in R (R Core Development Team, 2016). For variables
308 bounded between 0 and 1 (H_e, *r*: ML-Relate, 1st and 2nd degree relatedness, *D** values) beta
309 regression was performed (Ferrari & Cribari-Neto, 2004; betareg R package: Cribari-Neto &
310 Zeileis, 2010). For variables from 0 to infinity (N_e values) log(*n+1*) transformation of the
311 values was used, with infinity set to 999. An infinite N_e estimate is likely caused by an
312 actually large number, but the data do not allow an exact estimate (19 out of 60 estimates).
313 The N_e data were analysed with a GLM model with Gaussian (normal) errors. For variables
314 bounded by -1 and +1 (F_{IS}) we used a logistic quantile regression (Bottai, *et al.,* 2010; lqr R
315 package Galarza, *et al.,* 2016), and we could not use Av Dist as a covariate in this test.

316 Genetic diversity (H_e) at the genet level were compared using a Welch's (unequal variance) *t*-
317 test in R.
318 In addition, differences between range-edge and central populations for the degree of
319 clonal reproduction (D^*), H_e, F_{IS} and population differentiation,were tested using the
320 procedure 'compare groups' in GenoDive 2.0b using 9999 permutations with randomisation
321 of populations over the groups. The difference in H_e and F_{IS} between non-clonal and clonal
322 genotypes in populations was tested using a paired *t*-test in R.
323

324 3. Results

325 Genetic diversity was medium to high in both species for all populations. In *T. platyphyllos*
326 the average number of alleles for the 12 microsatellite loci across all samples was 19.7, with
327 a range of 8-27 across loci. Within *T. platyphyllos* populations this was an average of 7.6
328 alleles per locus (range 3.1-9.8). For *T. cordata* the average number of alleles for the 12
329 microsatellite loci across all samples was 13.7, with a range of 2-35 across loci. Within *T.*
330 *cordata* populations this was an average of 5.8 alleles per locus (range 1.1-14.3). Deviations
331 from HWE were observed at some loci, but were not consistent across populations. No null
332 alleles were detected in the loci included in the analyses.
333

334 3.1 *Clone identification*

335 In total 229 *T. platyphyllos* and 376 *T. cordata* trees were genotyped using microsatellite
336 markers and clones were identified. Using a difference of three alleles as a maximum
337 threshold value, ten trees with a unique MLG were deemed to be part of a clone. In all cases
338 it produced clone members located closely together (average 5m for *T. platyphyllos* and 22m
339 for *T. cordata*). The genotypes of members of a clone that did not have the exact same
340 genotype, but differed for fewer alleles than the threshold value, were compared. Locus
341 Tc963 was the most frequently deviating locus between clone-members, and therefore

seemed to accumulate mutations for members of a single clone (10 out of 17 presumed mutations). A total of 116 trees were not unique genotypes*, i.e.* belonged to a clone (19% of samples). Out of the total of 531 genotypes analysed, 489 were unique and 42 were represented by more than one tree (7.9%). Eight of the 12 populations of *T. platyphyllos* and six of the 16 *T. cordata* populations showed multiple adult trees belonging to a clone. No clones were found between locations. None of the clones were dominating a population, *i.e.* most clones (86%) were presented by two or three trees each (Table A2).

The number of loci screened was ample to identify MLG according to the resampling procedure in GenClone (data not presented, Arnaud-Haond et al*.,* 2006). The probability of genotypes being identical by chance in a randomly mating population (P_{gen}) as well as the probability that identical genotypes arose through sexual reproduction (P_{sex}) were <0.001 at all locations (Table S1), confirming that repeated genotypes were not due to chance and are in fact clones. Genotypic richness (*R*) was high and rather variable across populations, ranging from 0.278 to 1.00 (Table A1). Clonal heterogeneity or Simpson's index (*D**) was high in most populations, ranging from 0.743 to 1 (Table A1), suggesting a high probability that two randomly chosen trees were genetically different and a low rate of clonal reproduction.

The heterogeneity in clonal reproduction across populations was largely explained by 'region', with range-edge populations having higher clonal reproduction (for *R*: *P <0.0005* and for *D*: P <0.0005*) (Table 2). The average distance between trees, used as covariate in the analysis, was not significant for both measurements (*P* = 0.053 and 0.099, respectively). Species and region x species interaction effects were not significant (*P* >0.05). Distances between trees belonging to a single clone was variable, on average 16.8m and top quintile 26m, albeit smaller in *T. platyphyllos* than in *T. cordata* (average 5.7 and 19.3m, respectively, Fig. 2a and Table A3).

3.2 *Genetic diversity and fixation index*

Genetic diversity is represented by the expected heterozygosity (H_e) and calculated at the genet level (Table A4). H_e was significantly higher in *T. platyphyllos* than in *T. cordata* (*P <0.0005*, Table 3). There was no effect of region, Av Dist or interaction on genetic diversity. Other measures of genetic diversity, such as effective number of alleles per locus, led to the same conclusions and are not presented here. Values of F_{IS} (fixation index) were variable across populations and reflect the outcrossing breeding system, ranging from -0.284 to 0.105. However, there were no significant effects of species or region (species *P = 0.729,* region *P = 0.545*, Table 3, Table A4).

To test whether large clones have accumulated mutations and have the potential to contribute to the maintenance of genetic diversity we contrasted genotypes that consist of single trees with those consisting of more than one tree (*i.e.* non-clonal vs clonal). This was done in the six *T. cordata* populations that had >2 genotypes of both types. In all populations the observed heterozygosity (H_o) was higher and the fixation index (F_{IS}) lower in clonal than in non-clonal genotypes (Table 4). The difference in H_o is significant, both using a paired *t*-test (*t* = -3.73, *P = 0.014*) and using permutations in comparisons of groups in GenoDive (*P = 0.013*). The difference in F_{IS} between clonal and non-clonal genotypes is significant in a paired *t*-test (*t* = 3.60, *P = 0.015*), but is not significant using group comparison in GenoDive (*P = 0.113*).

3.3 *Population differentiation: contrast range-edge to central populations*

F_{ST} values were slightly higher in *T. platyphyllos* as a whole than in *T. cordata*, with 9% and 7% of the variation between populations (F_{ST} 0.090 and 0.070 and F'_{ST} 0.369 and 0.180, respectively). Within each species the differentiation between populations was higher in the range-edge group than in the central group (Table 5 and 6). G_{ST}, G'_{ST} and D_{est} were significantly higher for range-edge than central populations in *T. platyphyllos* (*P*-values 0.014, 0.013 and 0.013, respectively), while this was significant for G'_{ST} and D_{est} in *T. cordata* (*P*-values 0.050, 0.040 and 0.003, respectively. The 95% confidence interval (CI) of F_{ST} for

range-edge and for central was not overlapping for *T. platyphyllos*, with 11.2% and 5.5% of variation between range-edge and central populations, respectively, while in *T. cordata* this is 9.1% and 5.6%, respectively, with a small overlap of the 95% CI. A Mantel Test revealed slight positive correlation between genetic and geographic distances within all four groups: *T. platyphyllos* range-edge (R^2 = 0.208 P < 0.000) and central (R^2 = 0.0811 P < 0.000); *T. cordata* range-edge (R^2 = 0.0571 P < 0.000) and central (R^2 = 0.0101 P = 0.001).

3.4 *Relatedness*

The range-edge populations had a higher relatedness than populations from central regions, expressed as *r* (ML-Relate), but only at the ramet level (*P <0.0005*), and the Av Dist effect was also significant at the ramet level (P = 0.040) (Table 7 and 8). *R* (ML-Relate) was not significantly different between the species (P = 0.079 genotype level and P = 0.279 ramet level).

To test the reliability of indicating the most likely relationships in ML-Relate we combined trees from different locations, expecting no relationships. We found some half-sib relationships across locations, albeit in much smaller numbers than within locations, and no parent-offspring or full sib relationships between locations. The fraction of first degree related pairs ranged from 0 to 0.056 at genet level and 0 to 0.038 at ramet level, while the fraction of first plus second degree relatedness ranged from 0 to 0.097 at genet level and 0.018 to 0.086 at ramet level across populations (Table A4 and A5). Contrasting range-edge and central populations showed more related pairs in range-edge populations, only for the proportion of first degree relationships (*P < 0.003, P < 0.045*, at genotype and ramet, respectively). There was no significant difference between species in fraction of first or first plus second degree relatedness. The co-variate Av Dist and the range x species interaction effects were not significant either. The average distance of parent-offspring and full sib pairs

was 52m across populations, with top quintile 61m (average 79m for *T. platyphyllos*, 34.4m for *T. cordata*, Figure 2b, Table A3).

3.5 *Small scale population structure*

The correlation of the autocorrelation coefficient *r* and geographic distance was tested at the genotype level so that the results were not biased due to ramets of the same clone being close together and having a relatedness of 1. Sixty percent of the populations (12 out of 20) for which we had individual tree locations and enough clones showed a positive correlation of *r* with geographic distance in the first distance class (average 40m). Those populations that were collected with large distances between trees did not show autocorrelation in their first distance class.

3.6 *Effective population size*

Although the estimates for effective population size N_e differ between the estimation methods, coalescent and LD, and 95% CI are large, the values are likely to be comparable within this study. There was an effect of species for three of the four estimates (*P = 0.048* and *P = 0.042*, at the ramet level and $P = 0.009$ and $P = 0.078$ at the genet level, for N_e coalescent and LD, respectively). In all cases the N_e of *T. platyphyllos* was smaller than of *T. cordata*. Also, range-edge populations had lower N_e values than central populations ($P = 0.005$ and $P < 0.001$ at the ramet level and $P = 0.049$ and $P = 0.031$ at the genotype level for N_e coalescent and LD, respectively, Tables A2 and A4). The Av Dist or region x species interaction were not significant for any N_e estimate.

4. Discussion

Our study shows that leading range-edge populations of two *Tilia* species have more clonal reproduction than populations from central areas of their distribution. From *F*-statistics we

447 can conclude that range-edge populations are not likely to be affected by gene flow from
448 other populations. This is largely concordant with several previous studies showing that
449 range-edge populations, as compared to central range populations, have higher population
450 differentiation (F_{ST}) because of fragmentation and lower long distance gene flow, potentially
451 enabling adaptation to current local conditions (Arnaud-Haond *et al*., 2006; Eckert *et al*.,
452 2008) or due to repeated extinction-founder effects. However, we did not find a significantly
453 lower genetic diversity in range-edge populations, as some studies have indicated for other
454 species (Arnaud-Haond *et al*., 2006). Instead, *Tilia* range-edge populations have medium to
455 high neutral genetic diversity (comparable to other trees species). However, provenance
456 trials would be required to confirm that these range-edge populations also contain sufficient
457 adaptive variation. With a warming climate, gene flow among populations may promote
458 adaptation to novel conditions at northern latitudes.
459
460 4.1 *Clonality in Tilia*
461 Clonal occurrence varies greatly across plant species (Ellstrand & Roose, 1987) and
462 populations (Dorken & Eckert, 2001). Genotypic richness (*R*) quantifies sexual versus
463 asexual reproduction (Silvertown, 2008) and can be broadly compared across taxa. Clonality
464 in *Tilia* at both the range-edge and central populations was generally low compared to other
465 forest trees *e.g. Populus* species, *Prunus avium, P. ssiori, Ulmus minor,* and *Sorbus*
466 *torminalis* (Fuentes-Utrilla *et al.*, 2014; Mock *et al.,* 2008; Nagamitsu *et al.,* 2004; Rasmussen
467 & Kollmann, 2008; Santos-del-Blanco *et al*, 2013; Stoeckel *et al*., 2006; Vaughan *et al*.,
468 2007). This is significant in that even in less optimial conditions, *Tilia* may still mostly
469 reproduce sexually. Therefore, clonality was lower than expected from existing literature,
470 which was based on observations and morphology (*e.g*. Radoglou *et al.*, 2009).
471 As expected, we found more clonal reproduction in range-edge than in central
472 populations. High clonality was expected at Scandinavian, Russian and northerly UK sites
473 due to range-edge effects and limited sexual reproduction (Pigott, 1981; Pigott & Huntley,

1981; Radoglou *et al.*, 2009), while clonality at central range sites, was expected to be lower due to greater sexual regeneration and a greater viability of seed (Pigott, 1981). Various additional factors can be involved in this. For example, *T. platyphyllos* trees at UK sites were large and old and with age comes more opportunity for clonal reproduction. In some populations there is also signs of past coppicing or self-coppicing due to steep terrain (*e.g.* UKBB). Other mechanisms, such as freezing/thawing, especially at northerly locations, or grazing can also have played a role in the tendency for sprouting (Morris *et al*., 2014; Sjölund & Jump, 2014; Wilmking *et al*., 2017).

Production of fertile seeds and regeneration seems to be a relatively recent phenomenon in UK *T. platyphyllos* (Pigott, 2000; 2012). Although references in Pigott (1975) state that fertile fruit are produced in Finland during very warm summers, our study suggests that asexual reproduction is important for maintaining *Tilia* in Finland where it competes with *Picea abies* (Pigott, 2012). Irregular sexual reproduction (of randomly mating individuals) intermittent to clonal reproduction permits the preservation of diversity and that diversity reflects the last time sexual reproduction occurred. Ennos (2003), reported that aspen showed high clonal occurrence and limited flowering but had similar genetic diversity to other outcrossing woody plants and attributes this survival to asexual reproduction of a previously random mating population which flowered regularly when conditions were more suitable. So essentially, genetic diversity was 'frozen in time' (Ennos, 2003).

We expected that other northern-most populations may have a similar ecology and demography to *Tilia* in Finland. However, we found no clones in the Norwegian population, but this is likely because samples were collected with large distances between trees (Table 1) and so additional sampling from a smaller area is needed for a better understanding of the reproductive strategy in Norway. Climatic influences, topography and past management may also play a role in the clonal development *of Tilia* in Russia. Similar to *T. cordata* in our study, clonal occurrence has also been observed in *T. sibirica* (Siberian lime), further east in southern Siberia (Logan *et al.,*2018; Novák *et al.,* 2014). Although, unlike many of the *Tilia*

populations sampled in this present study, *T. sibirica* showed low levels of genetic diversity (Mean H_e = 0.318) and high levels of clonality (Mean R = 0.601). Logan *et al.* (2018) attributed this to population fragmentation and difficulties for regeneration of seedlings due to competition from tall forbs, except in recent canopy gaps where *Tilia* seedlings may emerge (Novák *et al.,* 2014).

The BNP in Poland has had little human management (Miścicki, 2012). A recent study found no clones in the adult trees sampled from three plots within the Special Reserve (Logan *et al.,* 2018), and repeating the analyses here no clones were found. This contradicts Radoglou *et al*. (2009) who reported that *T. cordata* populations in Poland are highly clonal. In contrast rather, there were young trees present in the BNP (S. A. Logan, pers. obs.), aggregated in tight clusters, probably due to establishment of sexually produced seedlings in response to the creation of a recent gap in the canopy (Bobiec, 2007). The young trees from the BNP were not clones of any of the adult trees (Logan, 2016), confirming observations by Pigott (1975) and in other forests of southern Poland by Jaworski *et al.* (2005) that sexual reproduction is taking place there.

The concept that somatic mutations (or genotyping errors) contribute to a clone being composed of similar MLGs is accepted in other studies (*e.g.* James & McDougall, 2014). In an extreme case in *Robinia pseudoacacia* a 'hypervariable' locus was detected (Lian *et al.,* 2004). In *Tilia*, the locus that seemed most mutable (Tc963) also has the largest number of alleles, namely 41. In addition, our finding that genets that are clonal had a higher observed heterozygosity than non-clonal genets (Table 4) follows expectations that large and long lived clones accumulate somatic mutations (Balloux *et al.,* 2003) and therewith potentially contribute to maintenance and generation of genetic diversity. It would be worth repeating this analysis with populations that have larger sample sizes. However, it may also be that trees with higher heterozygosity live longer and/or are more clonal than trees with lower heterozygosity (e.g. Vrankx *et al*, 2014).

While clonal reproduction is an important survival tool for *Tilia* in several parts of its European range, sexual reproduction is still the most important mode of reproduction. This is particularly advantageous for range-edge populations: as the summers become warmer more viable seed will be produced and the exchange and recombination of genetic material will produce genotypes to allow for adaptation to warmer climates.

4.2 *Genetic diversity and differentiation*

Genetic diversity (H_e) in both *Tilia* species was similar to other partially clonal trees (Santos-del-Blanco *et al.,* 2013; Stoeckel *et al.,* 2006; Vaughan *et al.,* 2007). Range-edge populations do not have a significantly lower genetic diversity than those at central locations. This may be because range-edge populations are relicts of a diverse migration front that, through clonal reproduction, have maintained diversity or even increased genetic diversity through somatic mutations (Balloux et al., 2003; Silvertown, 2008). H_e was higher in *T. platyphyllos* than in *T. cordata,* which could potentially be caused by ascertainment bias since the markers used were derived from *T. platyphyllos.* However, preliminary evidence using markers derived from *T. cordata* (Mylett, 2015) also revealed lower genetic diversity in *T. cordata* than in *T. platyphyllos* (Stephenson, Logan & Wolff *unpubl.*).

The near zero fixation index (F_{IS}) reflects the outcrossing breeding system of the two species. There was no significant difference in F_{IS} values between range-edge and central populations, similar to *Acer* (Chybicki *et al.,* 2014). Our low F_{IS} values are in contrast to Ennos (2003), where he reported high F_{IS} values in clonal Aspen, likely due to a shortage of flowering mates. While we have no knowledge of the flowering status of the populations in our study, the F_{IS} values we report suggest that this is not the case in *Tilia.* The overall slightly negative F_{IS} values in the species and the slightly (but not significantly) lower F_{IS} in clonal range-edge populations may well be explained by somatic mutations (Balloux *et al.,* 2003; Meloni *et al.,* 2013).

553 Assuming ideal populations the overall F_{ST} values seem low compared to some other
554 studies and indicate migration rates (Nm values) that cannot be ignored. However, taking
555 into account the long generation time and rare sexual reproduction, migration will have a
556 small effect in these *Tilia* species. Similar to other studies (*e.g.* Arnaud-Haond *et al.,* 2006;
557 Chybicki *et al.,* 2014) we confirmed here that range-edge populations show higher
558 divergence from each other than central populations. It is likely that random genetic drift due
559 to smaller effective population size and higher fragmentation is causing this. This may
560 promote adaptation to current local conditions making them important for range expansion
561 (Arnaud-Haond *et al*., 2006). However, the difference in F_{ST} between range-edge and central
562 populations could be an artefact, namely because range-edge populations are
563 geographically further apart from each other than central populations. The weak but positive
564 relationship between genetic and geographic distance (see section 3.3) hints at this.
565
566 4.3 *Relatedness within populations and effective population size*
567 Range-edge populations have a higher relatedness and a smaller effective population size
568 than those from central areas at ramet, and partially, at genet level. Of course, these
569 parameters are not independent and all contribute to low genetic connectivity between edge
570 populations and indicate the fact that trees in range-edge populations are more closely
571 related and fewer genotypes contribute to the next generation in range-edge population than
572 in central populations. This could be because there is less turnover in range-edge
573 populations; genets may be very old and contributed proportionally more to the standing crop
574 than younger trees, especially if there is a lack of sexual reproduction. In central populations
575 there may be more turnover and more even aged genets, with more equal contribution to the
576 next generation. Also, population fragmentation is likely to be higher at the range-edge, also
577 lowering N_e (Chybicki *et al.,* 2014).
578 The average distance between sampled trees was not identical in all populations and
579 could potentially bias outcomes if, for example, pairwise distances between trees from range-

edge populations were smaller than those from central populations. However, Av Dist only significantly affected relatedness (*r* ML-Relate), and only at the ramet level: this effect is as expected as the average distance between first degree related trees is 52m. Since average distance of trees is not significant as a covariate in other measures of clonal reproduction, diversity and relatedness, we are confident that the main-effect differences between range-edge and central populations are not biased due to sampling scenarios differing between sites. Moreover, the number of ramet pairs within 26m (excluding the top-quintile for clonal pairs) across all populations was similar in range-edge and in central populations in both species (182 vs 165 in *T. platyphyllos* and 594 vs 395 in *T. cordata*). The number of genet pairs within 61m (excluding the top-quintile for first degree related pairs) was also similar in range edge and in central populations (272 vs 310 in *T. platyphyllos* and 652 vs 674 in *T. cordata*). This indicates that there were ample potential candidates for clonal or closely related pairs, i.e. within 'critical distance', in both regions. The conclusion that relatedness between *Tilia* trees is unlikely over distances of more than 61m (top quintile), has important consequences for woodland managers.

4.5 *Population substructure*

Several studies in forest trees have shown that clones or related trees can often only be expected at relatively short distances, *e.g*. only up to 2m in *Quercus crispula* (Aizawa *et al.,* 2017). In *Tilia,* the average distance of full-sibs and parent-offspring related pairs is 52m (median 21m) and the average distance between clones is 16.8m (median 15m), which is similar to the spatial scales revealed in *Fagus grandifolia* and *Prunus avium* (Kitamura *et al*., 2003; Vaughan *et al.,* 2007) and slighlty more than in tanoak (Dodd *et al.,* 2014). This means that close relatedness is only expected to be observed at a rather small scale and in populations with high density of adult trees (Duminil *et al.*, 2016). Indeed this is reflected in some populations with large distances between samples, such as NOSO, not showing any small scale population structure (data not presented).

607

608 4.6 *Conclusion*

609 We can now attempt to answer the questions whether leading range-edge populations are at
610 risk and whether they are suitable for range expansion. Leading range-edge populations of
611 *Tilia* do not have a lower genetic diversity than central populations, making it likely that
612 range-edge populations are remnants of a genetically diverse moving front, with no
613 prolonged bottleneck effects. Although range-edge populations are more clonal than central
614 populations, they contain medium to high genetic diversity (perhaps from an earlier period in
615 time, *e.g.* Ennos, 2003) and show no inbreeding (F_{IS}) so should be able to regenerate,
616 naturally or assisted (Macaya-Sanz *et al.,* 2016). With the climate warming at range-edge
617 locations sexual reproduction may become more prevalent and therefore should promote *in*
618 *situ* adaptation to novel conditions. In addition, current populations may spread: with more
619 continuous populations natural gene flow from more southern populations may occur.
620 However, the effective population size is smaller at range-edge, meaning that if the
621 fragmentation and demographics stay the same, there is a risk that genetic diversity may be
622 lost as few individuals contribute to the next generation, while in central range populations
623 this risk is limited as there is a more even spread of sexual reproduction amongst adult trees.
624 Range-edge populations generally have $N_e < 50$, which is considered low and makes the
625 species vulnerable due to low ability to adapt to changes (Myking, 2002), and this is
626 exacerbated by clonal reproduction, lowering N_e to below 10. For example, the *T.*
627 *platyphyllos* population UKAS, has small N_e and a high rate of clonal reproduction. Those
628 types of populations seem particularly at risk, and maybe at a tipping point, due to the
629 combined effects of strong fragmentation, human activity, and the small number of,
630 sometimes very old, trees, belonging to few clones. Regeneration here may require good
631 management to allow adaptation, *e.g*. to climate change.

632 Woodland managers and foresters wishing to sample trees for planting further north
633 (assisted migration) can use populations that are currently at the northern range-edge of the

634 species because in those populations diversity is not lower than in central regions.
635 Furthermore, northern populations are likely to be better adapted to conditions in the north
636 than those from central regions, *e.g.* to climatic, season length and day light differences
637 (Lobo *et al.,* 2018a). However, further work is required to test whether *Tilia* set seed at these
638 northern locations. An alternative option for replication would be through clonal propagation,
639 natural or assisted. The additional use of other sources, e.g. from central ranges with diverse
640 microclimates, may generate the optimal genetic composition and diversity for adaptation. In
641 the wake of a warming climate and a shifting natural range, successful sexual regeneration
642 will greatly contribute to adaptation to local and novel environmental conditions (Thomas *et*
643 *al*., 2014).

644 Forest managers need to take into account sufficient distance between donor trees
645 (be it seed or clonal material), *i.e.* 26m between sampling to avoid two identical genotypes,
646 and 61m to avoid two trees that are related to the first degree (avoiding all but top quintile).
647 Adding diverse tree species to forests has become popular, and *Tilia* in particular has shown
648 to increase the herb layer (Normann *et al.*, 2016) and the decomposition of leaf litter (Muys *et*
649 *al.,*1992). Furthermore, *Tilia* is known to be a strong tree with specific associated
650 communities performing important ecosystem services (Hommel *et al.,* 2007).

651 Here we have compared northern range-edge with central populations, but we
652 recognise that a similar study should be performed on 'trailing edge' or 'stable rear edge' *Tilia*
653 populations to ensure conservation of the diversity in these species (Hampe & Petit, 2005).
654 Pinpointing the existence of such populations and obtaining samples is particularly difficult.
655 Nonetheless, our findings may also be of importance for trailing edge populations because
656 *Tilia* used to be one of the dominant tree species in Europe and current populations are
657 remnants of these woodlands (*e.g.* UK and Denmark), and are, similarly to leading edge
658 populations, now fragmented and limited due to human interference and climate change.
659

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Author contributions:

SL, PP and KW collected the genotype data, SL and KW designed the experiment, collected most of the samples, performed the genetic data analyses and wrote the manuscript, RS performed the GLM statistical analyses. All authors read and commented on the ms.

976 **Table 1** *Tilia* populations, ordered by location codes within groups, country, average distance
977 (Av Dist) between trees in m, number (N) and coordinates of trees: non-shaded blocks are
978 leading range-edge populations, while shaded blocks are central range populations (na not
979 assessed)

Species/Code	Location	Country	Av Dist	N	Latitude (°N)	Longitude (°E)
T. platyphyllos						
DEBOp	Bolderslev	Denmark	38	11	54.9978	9.3512
SWWC	West Coast	Sweden	na	15	58.8967	11.0419
UKAS	Aplegarth	UK	67	13	54.4093	-1.8165
UKAW	Anston Wd	UK	54	15	53.3402	-1.1984
UKHD	Hudswell	UK	269	25	54.4012	-1.7573
UKHW	Halesend Wd	UK	52	24	52.1407	-2.3815
AUDOp	Dobra	Austria	27	14	48.5907	15.3974
AULE	Leopoldsberg	Austria	91	25	48.2774	16.3522
AUSOp	Sommerein	Austria	58	14	47.9848	16.6947
FRML	Mont Lure	France	1250	20	44.1252	5.8705
FRVE	Ventoux	France	374	20	44.1869	5.2382
GELC	Lichtenstein	Germany	362	31	48.4071	9.2627
T. cordata						
DEBOc	Bolderslev	Denmark	55	30	54.9978	9.3516
FIIS	Iso Kirvessaari	Finland	49	20	62.5548	27.7626
FINI	Niinisaar	Finland	36	20	61.8171	29.3895
NOSO	Sogn Fjordane	Norway	854	16	61.8505	6.1375
RUVA	Vagay area	Russia	na	20	57.5096	69.1954
SWOL	Øland	Sweden	71	27	56.6070	16.4503
UKBB	Brignal Banks	UK	36	19	54.4968	-1.9114
UKRO	Roudsea	UK	47	40	54.2337	-3.0260
AUDOc	Dobra	Austria	50	21	48.5909	15.3975
AUSOc	Sommerein	Austria	83	23	47.9848	16.6947
AUST	Stams	Austria	106	21	47.2757	10.9772
AUTH	Thayatal	Austria	725	17	48.8448	15.8878
CZVO	Velky Osek	Czech Repub	67	20	50.1012	15.1788
FRMO	Mouthiers	France	69	20	48.9120	4.9138
GECO	Colbitz	Germany	270	20	52.3310	11.5559
POBF	Białowieża	Poland	930	40	52.7272	23.8351

980

Table 2 Means of estimates of clonal occurrence in *Tilia* and significance of fixed and covariate effects.

Species/region/*P*-values	*N*	*G*	*R*	*D**
T. platyphyllos				
Range-edge	17.2	14.7	0.804	0.947
Central	21.0	20.7	0.981	0.998
T. cordata				
Range-edge	24.0	17.6	0.685	0.937
Central	23.0	23.0	1.000	1.000
P-values				
Region			**< 0.0005**	**< 0.0005**
Species			0.143	0.186
Av Dist			0.053	0.099
Region x species interaction			0.291	0.425

Footnote: Number of samples (N), number of genotypes (G) and clonal occurrence presented as genotypic richness *R* = (G-1)/(N-1) and Simpson's complement index for genotypic diversity *D**, from range-edge and central locations, *P values* for significance of fixed-effect of region and species, co-variate effect of the average distance between samples (Av Dist), interaction of species and region effect, significant effects are indicated in bold

990 **Table 3** Genetic characteristics of *Tilia* as means per group of populations at the genet level
991 and outcomes of statistical tests (*P* values)

Species/region	H_e	F_{IS}
T. platyphyllos		
Range-edge	0.690	-0.061
Central	0.745	-0.024
T. cordata		
Range-edge	0.553	-0.037
Central	0.573	0.013
P-values		
Effect region	0.319	0.545
Effect species	**< 0.0005**	0.729
Av Dist	0.373	NA
Region x species interaction	0.161	0.874

992 **Footnote:** Expected heterozygosity *(H_e)* and fixation index (F_{IS}) and *P*-values for fixed effects
993 of region, species and interaction effects, as well as significance of the average distance
994 between samples (Av Dist) within a population, used as a covariate, significant effects are
995 indicated in bold.
996

Table 4. Expected heterozygosity H_e in all genotypes of a population, observed heterozygosity H_o and fixation index F_{IS} of non-clonal and clonal MLL in six populations of *T. cordata*, with number of samples in a group indicated as n.

Population	H_e over all (n)	H_o non-clonal (n)	H_o clonal (n)	F_{IS} non-clonal genets	F_{IS} clonal genets
DEBOc	0.575 (18)	0.545 (13)	0.550 (5)	0.052	0.043
FIIS	0.505 (12)	0.552 (8)	0.583 (4)	-0.093	-0.155
FINI	0.499 (13)	0.481 (9)	0.583 (4)	0.035	-0.169
RUVA	0.576 (14)	0.483 (10)	0.563 (4)	0.161	0.024
UKBB	0.520 (6)	0.556 (3)	0.639 (3)	-0.069	-0.229
UKRO	0.566 (29)	0.561 (19)	0.600 (10)	0.008	-0.060
Average	**0.540**	**0.530**	**0.586**	**0.016**	**-0.091**

Table 5. Diversity and G-statistics of leading range-edge and central populations of *Tilia* species and *P*-values for comparisons between the regions (GenoDive 2.0b) with 95% confidence intervals (bootstrapping) between brackets at the genet level, significant effects are indicated in bold

Species/region	H_o	H_s	G_{is}	G_{st}	G'_{st}	D_{est}	Clonal div
T. platyphyllos							
Range-edge	0.732 (0.651-0.805)	0.72 (0.652-0.772)	-0.017 (-0.064-0.032)	0.108 (0.092-0.124)	0.442 (0.325-0.564)	0.375 (0.252-0.507)	0.951
Central	0.764 (0.649-0.861)	0.760 (0.648-0.850)	-0.005 (-0.042-0.034)	0.049 (0.041-0.060)	0.234 (0.166-0.350)	0.195 (0.123-0.320)	0.998
P-value	0.054	**0.035**	0.657	**0.014**	**0.013**	**0.013**	0.068
T. cordata							
Range-edge	0.570 (0.420-0.699)	0.575 (0.424-0.705)	0.008 (-0.050-0.069)	0.084 (0.064-0.104)	0.214 (0.135-0.331)	0.142 0.070-0.261)	0.946
Central	0.558 (0.406-0.694)	0.587 (0.425-0.731)	0.050 (-0.015-0.126)	0.048 (0.030-0.070)	0.126 (0.069-0.206)	0.082 (0.036-0.158)	1
P-value	0.644	0.525	0.189	0.064	**0.050**	**0.040**	**0.003**

H_o observed heterozygosity and H_S expected heterozygosity within populations, G_{IS} inbreeding coefficient and G_{ST} fixation index, G'_{ST} is the fixation index corrected for bias due to limited number of populations, and Jost's D_{est} population differentiation independent from H_s, and clonal diversity as measure of amount of sexual reproduction, analogous to Simpsons D*

Table 6. Distribution of genetic diversity (AMOVA) within and among groups of populations in *Tilia* species at the genet level (GenoDive 2.0b). Standard deviations (St dev) of F-statistics were obtained through jack-knifing over loci and 95% confidence intervals of F-statistics were obtained through bootstrapping over loci. P values indicate significance of F-value > 0.

	%var	F-statistic	F-value	St dev	P
T. platyphyllos					
Range edge					
Within individuals	0.886	F_{it}	0.114 (0.063-0.167)	0.028	-
Among individuals	0.002	F_{is}	0.002 (-0.050-0.059)	0.029	0.486
Among populations	0.112	F_{ST}	0.112 (0.098-0.127)	0.008	0.001
Central					
Within individuals	0.940	F_{it}	0.053 (0.016-0.096)	0.210	-
Among individuals	0.003	F_{is}	0.003 (-0.038-0.034)	0.019	0.631
Among populations	0.055	F_{ST}	0.055 (0.046-0.068)	0.006	0.001
T. cordata					
Range edge					
Within individuals	0.900	F_{it}	0.099 (0.044-0.148)	0.027	-
Among individuals	0.008	F_{is}	0.099 (-0.048-0.065)	0.031	0.306
Among population	0.091	F_{ST}	0.091 (0.066-0.115)	0.013	0.001
Central					
Within individuals	0.903	F_{it}	0.097 (0.027-0.176)	0.041	-
Among individuals	0.041	F_{is}	0.043 (-0.022-0.117)	0.038	0.001
Among population	0.056	F_{ST}	0.056 (0.038-0.079)	0.011	0.001

Table 7 Means or medians of relatedness characteristics of *Tilia*, at the genet level and outcomes of statistical tests for region, species and interaction effects (*P* values, significant effects are indicated in bold).

	r ML-Relate mean	1^{st}+2^{nd} degr rel mean	1^{st} degr rel mean	N_e Co-an median	N_e LD median
T. platyphyllos					
Range-edge	0.060	0.060	0.027	8.7	16.2
Central	0.038	0.042	0.013	48.1	44.5
T. cordata					
Range-edge	0.054	0.060	0.023	60.9	38.2
Central	0.046	0.048	0.009	∞	494.5
P-values					
Effect region	0.236	0.120	**0.003**	**0.049**	**0.031**
Effect species	0.079	0.269	0.674	**0.009**	0.078
Av.Dist.	0.381	0.697	0.518	0.578	0.889
Region x species interaction	0.134	0.215	0.180	0.885	0.292

Footnote: Shown are the average relatedness of individuals (*r*, ML-Relate), proportion of first and second degree related individuals, proportion of first degree related individuals and effective population size (N_e) calculated based on Co-ancestry and on Linkage Disequilibrium (LD).

Table 8 Means or medians of relatedness characteristics of *Tilia*, at the ramet (sample) level and outcomes of statistical tests for region, species and interaction effects (*P* values, significant effects are indicated in bold).

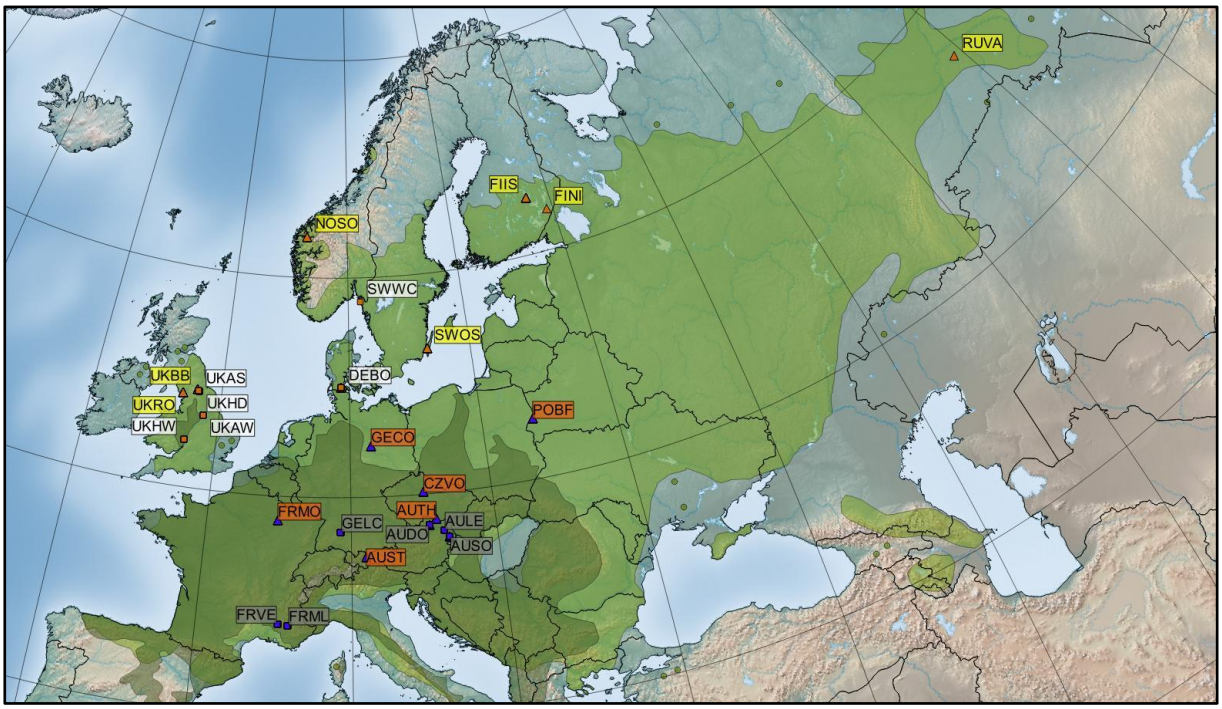
	r ML- Relate mean	1^{st}+2^{nd} degr rel mean	1^{st} degr rel mean	N_e Co-an median	N_e LD median
T. platyphyllos					
Range-edge	0.109	0.060	0.027	9.4	5.85
Central	0.040	0.042	0.013	48.2	37.1
T. cordata					
Range-edge	0.108	0.060	0.023	8.8	3.0
Central	0.046	0.048	0.009	∞	494.5
P-values					
Effect region	**<0.0005**	0.519	**0.045**	**0.005**	**< 0.0005**
Effect species	0.279	0.154	0.453	**0.048**	**0.042**
Av.Dist.	**0.040**	0.942	0.474	0.335	0.352
Region x species interaction	0.688	0.349	0.132	0.425	0.079

Footnote: Shown are the average relatedness of individuals (*r*, ML-Relate), proportion of first and second degree related individuals, proportion of first degree related individuals and effective population size (N_e) calculated based on Co-ancestry and on Linkage Disequilibrium (LD)

Figure legends:

Fig 1. Distribution of *Tilia cordata* (light green area and dots) and *T. platyphyllos* (dark green area and dots). Distribution data from EUFORGEN (www.euforgen.org). Map constructed using QGIS v2.16.2 (www.qgis.org). Triangles are *T. cordata* populations sampled while circles are *T. platyphyllos.* Orange icons show leading range-edge populations, for *T. cordata* (>54°N) with yellow background and for *T. platyphyllos* (>52°N) with white background. Blue icons are populations from central range of the two species (>43°N), *T. cordata* with orange background and *T. platyphyllos* with grey background.

Figure 2 Counts of numbers of a) clone pairs, in *T. platyphyllos* (green) and *T. cordata* (blue) across distance classes b) first degree related pairs of individuals (full sib and parent-offspring), three pairs with distance of 483, 684 and 809m are combined into >300m class



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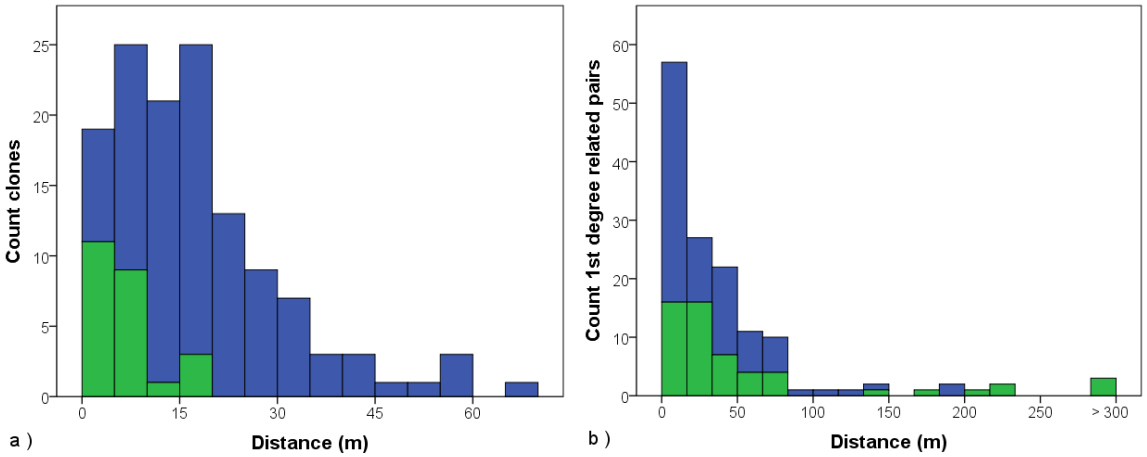

Figure 2 Counts of numbers of a) clone pairs b) first degree related pairs of individuals (full sib and parent-offspring), in *T. platyphyllos* (green) and *T. cordata* (blue) across distance classes. Three related pairs with distances of 483, 684 and 809m are combined into the >300m class