GENETIC DIVERSITY AND STRUCTURE OF LATVIAN *TILIA CORDATA* POPULATIONS

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*Changing climatic conditions are transforming the ecological and silvicultural roles of broadleaf tree species in northern Europe. Small-leaved lime (*Tilia cordata* Mill.) is distributed throughout most of Europe, and is a common broadleaf species in Latvia. This species can tolerate a broad range of environmental and ecological conditions, including temperature, water availability, and soil types. The aim of this study was to assess the genetic diversity and differentiation of Latvian *T. cordata* populations using nuclear microsatellite markers developed for *Tilia platyphyllos*. After testing of 15 microsatellite markers, Latvian *T. cordata* samples were genotyped at 14 microsatellite loci. Latvian *T. cordata* populations had high genetic diversity, and were not overly isolated from each other, with moderate gene flow between populations. No highly differentiated populations were identified. Vegetative reproduction was identified in most analysed populations, and almost one-third of analysed individuals are of clonal origin. *T. cordata* has high timber production potential under the current climatic and growth conditions in Latvia, and therefore this species has potential for use in forestry, as well as playing a significant role in maintaining biodiversity and other ecosystem services.*

Key words: small-leaved lime, microsatellite markers, clonal reproduction, forestry, climate change.

INTRODUCTION

Changing climatic conditions are also transforming the role of broadleaf tree species in northern European forests. There is also increasing interest in these species from a silvicultural aspect. Small-leaved lime (*Tilia cordata* Mill.) is found throughout most of Europe, except for northern Scandinavia. The distribution of this species in Northern Europe is strongly influenced by temperature, and correlates with the annual isotherm of + 2 °C and July isotherm of + 17 °C (Piggot and Huntley, 1978; Piggot and Huntley, 1980). *T. cordata* is a shade tolerant species, often forming the second storey in forest stands, and is frequently found in the understorey. *T. cordata* successfully regenerates in stands where the dominant species is *T. cordata*, thus ensuring the continuity of these stands. In general, *T. cordata* tolerates short-term water deficit, but summer drought conditions limit the southern distribution of this species in the Mediterranean region (Radoglou *et al.*, 2009). *T. cordata* is tolerant of both high and low temperatures, and temperatures of over 15 °C are required during the growing season to ensure

successful production of fertile seeds (De Jaegere *et al.*, 2016). However, vegetative reproduction is common in this species, with sprouts forming after coppicing and from trunks and branches in contact with the soil (Erichsen *et al.*, 2019). In a study of 27 populations of both *T. cordata* and *T. platyphyllos* in the UK, approximately 25% of individuals were found to be a result of clonal reproduction (Logan *et al.*, 2015).

T. cordata is a common broadleaf species in Latvia, found in all regions. The largest areas of both pure and mixed stands, are found in Eastern Latvia — Augšzeme (730.2 ha, 17.5% of the total stand area), Aiviekste region (623.5 ha, 15.0%), Gaujaszeme (518.4 ha, 12.4%) and South Vidzeme (488.2 ha, 11.7%). Less than 10% of the total area of *T. cordata* stands are located in other regions of Latvia. *T. cordata* stands are mainly found in nutrient-rich or eutrophic dry forest types — Aegopodiosa, Oxalidiosa, Hylocomiosa and Mercurialiosa mel. (91.8% of the linden area). *T. cordata* stands do not occur in nutrient-poor forest growth conditions. Pure and mixed *T. cordata* stands are productive in

good growing conditions, with stand volume up to 100 m³ in age class 1 (1–20 years) and up to 327–502 m³·ha⁻¹ in age classes 4 (61–80 years) and 5 (81–100 years), and while the productivity of *T. cordata* plantation forest stands is highly dependent on the soil type, it was concluded that *T. cordata* is a suitable tree species for plantation forestry in Latvian climatic conditions (Daugaviete *et al.*, 2019).

There is a paucity of molecular genetic resources for use in study of *T. cordata*. Some previous studies have utilised isozyme markers (Maurer and Tabel 1995; Fromm and Hattermer 2003), and RAPD markers have also been utilised to investigate phylogenetic relationships of *Tilia* species (Liesebach and Sinkó, 2008). However, these marker systems are hampered by technical difficulties, including poor resolution and reproducibility. Microsatellite markers are widely used for population genetic studies due to their high levels of polymorphism and co-dominant inheritance, making it possible to directly assess heterozygosity levels. A major drawback is the effort needed to develop these markers for particular species. However, cross-species amplification enable microsatellite markers developed in one species to be transferred to related species. Fifteen microsatellite markers were developed from *T. platyphyllos*, which were subsequently tested in 23 *Tilia* species (Phuekvilai and Wolff, 2013).

Given the broad range of environmental and ecological conditions that *T. cordata* currently occupies, the ecological importance and silvicultural interest in this species will increase in the future, particularly in Northern Europe. The aim of this study was to assess the genetic diversity and differentiation of Latvian *T. cordata* populations using nuclear microsatellite markers.

MATERIALS AND METHODS

Analysed populations and sample collection. Samples for DNA extraction were collected from 28 *T. cordata* plots located throughout the territory of Latvia (Fig. 1, Table 1). Leaf or twig samples were collected from 24 to 30 individuals from each plot. A total of 784 individuals were collected from 28 populations.

DNA extraction and genotyping. DNA was extracted using a modified CTAB method (Doyle and Doyle, 1990). The 15 microsatellite markers developed specifically for the genus *Tilia* (Tc4, Tc5, Tc6, Tc7, Tc8, Tc11, Tc31, Tc915, Tc918, Tc920, Tc927, Tc937, Tc943, Tc951 and Tc963) (Phuekvilai and Wolff, 2013) were tested on 16 randomly chosen individuals from different populations of *T. cordata*. PCR reactions were performed in a volume of 10 µl containing approximately 50 ng DNA, 2 µl HOT FIREPol® Blend Master Mix (Solis BioDyne) (containing 10 mM MgCl₂), 0.3 µM forward and reverse primers. PCR was carried out in a thermocycler (Eppendorf Mastercycler epgradient) using the following protocol: initial predenaturation step at 95 °C for 5 min, followed by 15 cycles of 95 °C for 15 s, 54 °C for 15 s, and 72 °C for 15 s, and 20 cycles of

Fig. 1. Analysed *T. cordata* populations in Latvia (black dots – site numbering as in Table 1).

89 °C for 20 s, 52 °C for 20 s, 72 °C for 20 s, and a final extension step of 72 °C for 30 min.

All PCR reaction products were diluted 1 : 10 with deionised water, and visualised on an Applied Biosystems ABI Prism 3100xl Genetic Analyser. Genotyping was performed using GeneMapper 4.0 (Applied Biosystems).

After marker testing, Tc8 was discarded as it was monomorphic, and Tc918 was discarded as there was no amplification. Marker Tc7 amplified more than two alleles within individuals, and was subsequently analysed on all individuals, and was genotyped as representing two loci. Therefore, the entire sample set of 784 individuals was genotyped at 14 loci.

Micro-Checker 2.2 (Van Oosterhout *et al.*, 2004) was used to identify potential null alleles. GenAlEx 6.501 (Peakall and Smouse 2012) was used to calculate genetic diversity indices, and AMOVA and pairwise population differentiation (F_{ST}) (using 999 permutations). CERVUS software was used to identify duplicated genotypes (Kalinowski *et al.*, 2007), using a minimum of 12 loci needed for a match, and 1 mismatching locus allowed. STRUCTURE 2.3.4 was used to determine clustering of the populations using a 50000 burnin period, followed by 75000 MCMC steps. K was determined for 1 to 28 with 10 runs for each value of K. The LOCprior model was applied, using the sampling location as default information to assist clustering, which is recommended for use with datasets with weak structure signal (Pritchard *et al.*, 2000). The optimal value of K was determined using the ΔK method (Evanno *et al.*, 2005) using STRUCTURE HARVESTER (Earl, 2012). Coefficients of membership to the most likely K cluster number were visualised using Distruct via the CLUMPAK server (Kopelman *et al.*, 2015).

RESULTS

The presence of potential null alleles was assessed using Micro-Checker 2.2. Potential null alleles were identified at 9 of the 14 loci, and in most cases, these potential null al-

Table 1. Locations and site descriptions of, and anthropogenic impact on, analysed *T. cordata* populations

Pop. No.	Pop. name	Coordinates, LKS-92		Municipality	Region	Site description	Anthopogenic impact
		X	Y				
1	Nāgelmuiža	527032	6315375	Ropažu	Eastern	Park	High
2	Ziedkalne	470090	6254354	Tērvetes	Western	<i>T. cordata</i> stand	Low
3	Kuprava	711009	6353573	Alūksnes	Eastern	<i>T. cordata</i> stand	Low
4	Irikava	711998	6346607	Viļakas	Eastern	<i>T. cordata</i> stand	Low
5	Mazā Ellīte	587088	6360050	Priekuļu	Eastern	<i>T. cordata</i> stand	Medium
6	Saule	618522	6394155	Valkas	Eastern	Floodplain broadleaf forest	Low
7	Kābeļkalns	627670	6300090	Ikšķiles	Eastern	Castle mound	High
8	Kurmene	551082	6253115	Aizkraukles	Eastern	Park	High
9	Rauda	635957	6200558	Daugavpils	Eastern	Broadleaf forest	Low
10	Lazdukalns	678471	6195196	Daugavpils	Eastern	Broadleaf forest	Low
11	Lupandi	722027	6188724	Krāslavas	Eastern	Planted stand	Medium
12	Jersika	636250	6238950	Līvānu	Eastern	Castle mound	High
13	Dunalka	339455	628357 0	Aizputes	Western	Park	High
14	Salenieki	669018	6285148	Varakļānu	Eastern	Broadleaf forest	Low
15	Pilori	723831	6232751	Dagdas	Eastern	Broadleaf forest	Low
16	Gavieze	335390	6266410	Grobiņas	Western	Broadleaf forest	Low
17	Lukna	339041	6235847	Bārtas	Western	Broadleaf forest	Low
18	Oltūži	524610	6383120	Salacgrīvas	Western	Broadleaf forest	Medium
19	Moricsala	388000	6340600	Ventspils	Western	Broadleaf forest	Low
20	Ciecere	412174	6279352	Brocēnu	Western	Broadleaf forest	Low
21	Lejnieki	518920	6251880	Bauskas	Western	Cemetery	High
22	Krimulda	560440	6335120	Siguldas	Eastern	Pine-broadleaf forest	Medium
23	Aizkraukle	575950	6282570	Aizkraukles	Eastern	Broadleaf forest	Low
24	Skrīveri	564724	6280808	Skrīveru	Eastern	Broadleaf forest	Low
25	Mazsalaca	561480	6413700	Mazsalacas	Eastern	Mixed forest	High
26	Tukums	449432	6318047	Tukuma	Western	Mixed forest	Low
27	Valdemārpils	416137	6360284	Talsu	Western	Park	High
28	Veģi	407735	6327879	Sabiles	Western	Broadleaf forest	Low

leles were found in only one to four populations. Potential null alleles at three loci (Tc4, Tc11 and Tc31) were identified in 14, 11, and 17 populations, respectively. However, as a total of 28 populations were analysed, all loci were retained for further analysis.

The genetic diversity parameters of the analysed loci are summarised in Table 2. The total number of alleles at each locus ranged from two (Tc11) to 31 (Tc963), while the average number of alleles (over all populations) at each locus ranged from two (Tc11) to 13.25 (Tc963). The fixation index or inbreeding coefficient (F) was close to zero for most loci, with the exception of Tc4, Tc11 and Tc31, which were all positive. However, as mentioned previously, this heterozygote excess (indicating the potential presence of null alleles) at these loci was not detected in all populations, and so all loci were retained for analyses.

The genetic diversity parameters of the analysed populations are summarised in Table 3. The average number of alleles (over all loci) ranged from 4.43 (Salenieki) to 7.14 (Nāgelmuiža). The Salenieki population also had the lowest

values for number of effective alleles (Ne) and information index (I). However, the Skrīveri population had the highest Ne and I values, indicating that the allele frequencies were more evenly distributed in this population compared to Nāgelmuiža. The fixation index (F) ranged from -0.07 (Aizkraukle) to 0.20 (Nāgelmuiža). However, most populations had F values close to zero, with only five populations (Saule, Nāgelmuiža, Lupandi, Moricsala and Oltūži) having F values above 0.10.

A total of 244 individuals with identical matching multilocus genotypes (MMGs) were found in all analysed populations except for Ciecere and Lukna. All individuals with MMGs were identified within populations, indicating that this was a result of vegetative reproduction, rather than a lack of discriminating power of the utilised markers. This indicates that 31% of the analysed individuals were propagated vegetatively. A total of 106 groups of individuals with MMGs were identified, with the number of individuals in each group ranging from 2 to 21 (average 9.4). The populations with the highest number of MMG groups were Aizkraukle and Tukums, which had a total of ten groups

Table 2. Genetic diversity parameters of the analysed loci

	Tc4	Tc5	Tc6	Tc7	Tc11	Tc937	Tc7_1	Tc920	Tc951	Tc943	Tc31	Tc963	Tc915	Tc927
Ntot	11	17	15	11	2	10	3	12	4	4	9	31	16	3
Na	7.04 (0.25)	6.50 (0.30)	7.57 (0.32)	6.54 (0.18)	2.00 (0.00)	4.89 (0.21)	2.50 (0.10)	7.96 (0.18)	3.36 (0.11)	2.64 (0.12)	4.32 (0.22)	13.25 (0.66)	8.61 (0.36)	2.43 (0.11)
Ne	4.47 (0.21)	3.22 (0.14)	4.17 (0.20)	3.79 (0.15)	1.74 (0.04)	2.18 (0.12)	1.74 (0.04)	4.63 (0.21)	2.19 (0.06)	1.95 (0.06)	2.00 (0.10)	7.40 (0.50)	4.03 (0.21)	1.23 (0.04)
I	1.64 (0.04)	1.37 (0.04)	1.62 (0.05)	1.53 (0.03)	0.60 (0.02)	0.99 (0.05)	0.65 (0.02)	1.72 (0.03)	0.89 (0.03)	0.73 (0.03)	0.87 (0.05)	2.15 (0.10)	1.64 (0.05)	0.32 (0.04)
Ho	0.63 (0.03)	0.69 (0.02)	0.79 (0.02)	0.75 (0.02)	0.26 (0.03)	0.54 (0.03)	0.42 (0.02)	0.78 (0.02)	0.50 (0.02)	0.53 (0.03)	0.28 (0.02)	0.82 (0.04)	0.72 (0.02)	0.16 (0.02)
uHe	0.78 (0.01)	0.68 (0.02)	0.76 (0.01)	0.74 (0.01)	0.42 (0.02)	0.52 (0.02)	0.42 (0.02)	0.79 (0.01)	0.54 (0.01)	0.48 (0.02)	0.48 (0.03)	0.83 (0.03)	0.75 (0.02)	0.17 (0.02)
F	0.17 (0.04)	-0.03 (0.03)	-0.07 (0.02)	-0.04 (0.02)	0.36 (0.06)	-0.07 (0.03)	-0.02 (0.03)	-0.01 (0.02)	0.05 (0.04)	-0.1 (0.05)	0.38 (0.05)	-0.01 (0.02)	0.02 (0.02)	0.02 (0.04)

Ntot, total number of alleles; Na, average number of alleles over all loci; Ne, average number of effective alleles over all loci; I, average information index over all loci; Ho, average observed heterozygosity over all loci; uHe, average unbiased expected heterozygosity over all loci; F, average inbreeding coefficient over all loci

Table 3. Genetic diversity parameters of the analysed populations (average values over all analysed loci)

	Na	Ne	I	Ho	uHe	F
Saule	5.86 (0.94)	3.13 (0.40)	1.22 (0.15)	0.51 (0.07)	0.61 (0.06)	0.14 (0.08)
Ziedkalne	5.93 (1.01)	3.45 (0.64)	1.21 (0.18)	0.58 (0.07)	0.58 (0.07)	-0.01 (0.05)
Nāgelmuiža	7.14 (1.16)	3.33 (0.45)	1.33 (0.17)	0.51 (0.07)	0.62 (0.06)	0.20 (0.05)
Mazā Ellīte	5.36 (0.92)	3.30 (0.58)	1.15 (0.18)	0.57 (0.08)	0.58 (0.07)	0.01 (0.07)
Kuprava	4.71 (0.59)	2.82 (0.35)	1.10 (0.13)	0.61 (0.07)	0.58 (0.05)	-0.07 (0.06)
Irikava	5.71 (0.85)	3.35 (0.50)	1.24 (0.15)	0.56 (0.06)	0.63 (0.06)	0.07 (0.06)
Kābeļkalns	5.00 (0.72)	2.96 (0.45)	1.11 (0.14)	0.54 (0.08)	0.58 (0.05)	0.09 (0.09)
Lazdukalns	6.14 (0.99)	3.35 (0.62)	1.23 (0.17)	0.56 (0.06)	0.60 (0.06)	0.04 (0.07)
Jersika	4.93 (0.73)	2.65 (0.39)	1.05 (0.13)	0.53 (0.07)	0.55 (0.06)	0.02 (0.06)
Lupandi	5.00 (0.66)	2.58 (0.33)	1.03 (0.13)	0.46 (0.07)	0.54 (0.06)	0.13 (0.07)
Rauda	5.57 (0.84)	3.11 (0.46)	1.19 (0.15)	0.60 (0.06)	0.61 (0.05)	0.02 (0.08)
Kurmene	6.07 (0.91)	3.40 (0.48)	1.29 (0.16)	0.60 (0.07)	0.63 (0.05)	0.05 (0.05)
Pilori	5.57 (1.04)	3.15 (0.64)	1.16 (0.16)	0.55 (0.06)	0.58 (0.06)	0.04 (0.06)
Salenieki	4.43 (0.58)	2.52 (0.26)	1.02 (0.13)	0.56 (0.07)	0.55 (0.06)	-0.05 (0.08)
Krimulda	6.07 (0.98)	3.51 (0.66)	1.27 (0.16)	0.59 (0.06)	0.62 (0.06)	0.03 (0.03)
Skrīveri	6.21 (1.03)	4.05 (0.78)	1.34 (0.18)	0.64 (0.06)	0.65 (0.06)	-0.01 (0.05)
Veģi	5.43 (0.86)	3.00 (0.38)	1.16 (0.14)	0.60 (0.07)	0.59 (0.05)	0.01 (0.06)
Ciecere	5.79 (0.87)	3.24 (0.45)	1.25 (0.14)	0.58 (0.06)	0.63 (0.04)	0.06 (0.06)
Moricsala	6.21 (1.16)	3.32 (0.54)	1.22 (0.18)	0.51 (0.08)	0.59 (0.06)	0.15 (0.08)
Mazsalaca	5.57 (0.98)	3.28 (0.56)	1.19 (0.17)	0.56 (0.07)	0.60 (0.06)	0.06 (0.07)
Lejnieki	4.71 (0.79)	2.59 (0.29)	1.04 (0.12)	0.58 (0.06)	0.57 (0.04)	-0.03 (0.07)
Tukums	6.14 (1.01)	3.36 (0.54)	1.23 (0.17)	0.56 (0.06)	0.60 (0.06)	0.06 (0.03)
Aizkraukle	5.79 (0.92)	2.98 (0.44)	1.14 (0.17)	0.59 (0.07)	0.56 (0.07)	-0.07 (0.05)
Valdemārpils	6.93 (1.00)	3.77 (0.69)	1.34 (0.18)	0.56 (0.07)	0.62 (0.06)	0.07 (0.05)
Gavieze	6.64 (1.05)	3.55 (0.61)	1.30 (0.17)	0.58 (0.07)	0.63 (0.06)	0.09 (0.08)
Dunalka	5.57 (0.86)	3.53 (0.52)	1.30 (0.14)	0.63 (0.06)	0.66 (0.04)	0.05 (0.05)
Lukna	6.07 (1.03)	3.58 (0.61)	1.27 (0.16)	0.59 (0.06)	0.64 (0.05)	0.07 (0.07)
Oltūži	4.64 (0.63)	2.62 (0.30)	1.04 (0.14)	0.47 (0.06)	0.54 (0.06)	0.11 (0.06)

Na, number of alleles; Ne, number of effective alleles; I, information index; Ho, observed heterozygosity; uHe, unbiased expected heterozygosity; F, inbreeding coefficient

Fig. 2. PCoA of pairwise Nei genetic distances between analysed *T. cordata* populations. Percentage of variation explained by axis 1 – 37.62, axis 2 – 13.74.

each, comprising of a total 18 and 19 individuals, respectively. Nine MMGs were identified in both Kuprava and Saule (comprising of a total of 21 and 17 individuals, respectively). Between one and seven MMG groups were identified in the remaining populations.

Unique alleles were found in ten populations (Mazā Ellīte, Lazdukalns, Jersika, Pilori, Krimulda, Veģi, Valdemārpils, Gavieze, Dunalka, Oltūži), with frequencies ranging from 0.019 to 0.143 (average 0.063). Analysis of molecular variance (AMOVA) indicated that 6% of the genetic variation was between populations, and 94% within populations ($F_{st} = 0.064$, $p < 0.01$). Pairwise F_{st} values ranged from 0.013 (Nāgelmuiža-Moricsala) to 0.129 (Salenieki-Oltūži). Pairwise Nei genetic distances were calculated, and were used to generate a PCoA plot (Fig. 2). Most of the samples representing analysed populations clustered together, with Kābeļkalns, Irikava, Jersika and Salenieki being the most distinct. There was no evidence of higher order structure, either by region (Eastern and Western), or anthropogenic impact (high, medium, low), with AMOVA indicating that differentiation between these groups was low – $F_{st} = 0.01$ ($p < 0.01$) in both cases. Mantel analysis of the pairwise genetic and geographic distance matrices showed a slightly positive correlation both at a population level ($R_{xy} = 0.092$), which was not significant ($p > 0.05$), and at an individual level ($R_{xy} = 0.077$), which was significant ($p < 0.01$).

Bayesian clustering was performed to indicate the population of origin of the analysed individuals. This assists clustering in sample sets with low differentiation. Using the ΔK method (Evanno *et al.*, 2005), the most likely number of clusters was 16, but there were several additional peaks in the $K/\Delta K$ graph (Fig. 3) The coefficients of membership for $K=16$, indicating the probability that an individual is assigned to a particular cluster, are presented in Figure 4.

Fig. 3. DeltaK and mean L(K) plots for K values 1–28.

DISCUSSION

The analysed Latvian *T. cordata* populations had low to moderate levels of genetic differentiation. The populations were sampled from throughout the territory of Latvia, and

Fig. 4. Distract plots of coefficients of membership for $K = 16$, the most likely number of clusters as determined by the ΔK method, indicating the probability that each analysed individual is attributed to the corresponding population inferred from STRUCTURE analysis.

had differing levels of anthropogenic impact, ranging from strictly protected areas (e.g. Moricsala) to populations from parks, cemeteries and planted stands. However, these differing conditions did not have a significant impact on the genetic clustering of the populations, or on other genetic diversity parameters. Populations with the highest or lowest diversity parameters were not differentiated by anthropogenic impact. There was no differentiation identified between the eastern and western regions, but there was a slightly positive correlation between genetic and geographic distances, indicating that isolation by distance may have some impact on the genetic structuring of Latvian *T. cordata* populations. However, no highly differentiated or inbred population was identified, indicating that there was sufficient gene flow, with no significant barriers identified. Vegetative reproduction was identified in most analysed populations, and almost $1/3^{\text{rd}}$ of analysed individuals were considered as being of clonal origin. This is a similar level of clonal reproduction as reported by other studies utilising DNA markers for the analysis of *Tilia* populations — in the UK, 25% of individuals were reported to be clonal (Logan *et al.*, 2015). In Denmark, 48% of *T. cordata* individuals were clonal (Erichsen *et al.*, 2019), but in that study, nine populations were sampled, and in some of the analysed populations, all individuals in a stand or section were sampled.

Genetic diversity parameters of Latvian *T. cordata* populations were similar to those reported in the UK (Logan *et al.*, 2015), Denmark (Erichsen *et al.*, 2019) and across Europe (Logan *et al.*, 2019). Genetic differentiation of Latvian *T. cordata* populations was moderate (overall F_{st} was 0.064), which is close to that of *Tilia* populations in the UK (0.071) (Logan *et al.*, 2015), but higher than reported for Danish populations (0.034) (Erichsen *et al.*, 2019). Unique alleles were found in ten of the 28 Latvian populations, and while only a total of 13 unique alleles were identified, they had relatively high frequencies, ranging from 0.019 to 0.143 (average 0.063). This indicates some degree of isolation between populations; however gene flow does not seem to be substantially restricted within Latvia. Gene flow is expected to be lower to that of many other forest tree species due to *Tilia* being insect pollinated and with heavy and wind-dispersed seeds (Logan *et al.*, 2019). Bayesian analysis indicated that the most probable number of clusters was 16,

supporting the hypothesis that the Latvian *T. cordata* populations are somewhat isolated from each other.

It was reported that clonality increased in range-edge *T. cordata* populations compared to central populations (Logan *et al.*, 2019). Leading (northern) range-edge *T. cordata* populations were defined as occurring North of 54°N , which includes the entire territory of Latvia (55°N to 58°N). The relatively low levels of clonality found in Latvian populations could be related with the sampling method, as the represented populations were not sampled exhaustively, and individuals collected were spatially separated, which would reduce the number of clonal individuals sampled. *T. cordata* stands are not actively managed in Latvia (including coppicing), which also reduces flowering and thus increases the proportion of clonal reproduction (Waller *et al.*, 2012). Previous reports that have not utilised DNA analyses to identify clones, have reported high levels of clonality (80%) (Sakss, 1960; Chistyakova, 1982; Radoglou *et al.*, 2009), which is probably a reflection of the lower discrimination and informativeness of morphological and observation data (Logan *et al.*, 2019). Previous studies have reported that while clonal reproduction is important in *T. cordata*, sexual reproduction is still the most important mode of reproduction in the major part of the distribution range (Logan *et al.*, 2019). Based on the results of this study, this also is applicable to Latvian *T. cordata* populations, and with an expected increase in temperature associated with climate change, seed production and sexual reproduction could be expected to increase.

In conclusion, the analysed Latvian *T. cordata* populations had high genetic diversity, and were not overly isolated from each other, with moderate gene flow between populations. No highly differentiated populations were identified. The results of this study can be used to identify stands with the highest genetic diversity, including rare or unique alleles, as well as to determine the most genetically differentiated populations. This information is crucial for potential future breeding efforts, as well as to effectively designate *T. cordata* genetic resource stands. *T. cordata* is productive under the current climatic and growth conditions in Latvia, and therefore this species has potential for use in forestry, as well as playing a significant role in maintaining biodiversity and other ecosystem services.

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LATVIJAS *TILIA CORDATA* POPULĀCIJU ĢENĒTISKĀ DAUDZVEIDĪBA UN STRUKTŪRA

Mainīgie klimatiskie apstākļi maina platlapju koku sugu ekoloģisko un mežkopības nozīmi Ziemeļeiropā. Parastā liepa (*Tilia cordata* Mill.) ir sastopama lielākajā daļā Eiropas, un ir bieži sastopama platlapju koku sugu Latvijā. *T. cordata* spēj pielāgoties dažādiem vides un ekoloģiskajiem apstākļiem, tādiem kā temperatūra, ūdens pieejamība, augšnes veidi. Šī pētījuma mērķis bija novērtēt *Tilia cordata* ģenētisko daudzveidību un diferenciāciju Latvijas populācijās, izmantojot kodola mikrosatelīta marķierus. *T. cordata* Latvijas populācijās novērota augsta ģenētiskā daudzveidība, un tās nebija īpaši izolētas viena no otras, ar mērenu gēnu plūsmu starp populācijām. Augsti diferencētas populācijas netika identificētas. Veģetatīvā reprodukcija tika identificēta lielākajā daļā analizēto populāciju, un gandrīz 1/3 no analizētajiem indivīdiem bija klonālas izcelsmes. *T. cordata* ir produktīva pašreizējos Latvijas klimatiskajos un augšanas apstākļos, un tāpēc šai sugai ir potenciāls tikt izmantotai mežsaimniecībā, kā arī tai ir nozīmīga loma bioloģiskās daudzveidības un citu ekosistēmas pakalpojumu uzturēšanā.