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Research Article

Keywords:

Posted Date: October 15th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7633213/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Conservation Genetics on April 18th, 2026. See the published version at https://doi.org/10.1007/s10592-026-01772-z.

Museomics reveals genetic swamping over 200 years of a marginal native population of the northern forest tree *Acer campestre* by non-native genotypes introduced as ornamentals

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18 # Abstract

19 Keywords

Introduction

Molecular population genetics focuses on studying the origin, distribution, and extent of genetic variation in natural populations, as well as analysing how this variation evolves over space and time. Consequently, population genetics serves as a critical tool for evaluating changes in genetic diversity and structure, which are increasingly recognized as vital for conservation genetic metrics, estimating resilience to environmental change (Blondel et al., 2021; Klütsch & Laikre, 2021; Rohde et al., 2024).

To date, research has primarily focused on sampling extant populations and utilizing computational or modelling approaches to estimate effective population size, genetic variation, and structure. While such sampling provides a snapshot of genetic variation patterns, it is likely influenced by factors beyond the researcher's control, including seasonal variation, temporal fluctuations, and the Wahlund effect. Many of these snapshot-in-time studies rely on space-for-time substitution (SFTS) models, which use contemporary spatial patterns of biodiversity to infer temporal processes and are often considered an alternative to longer-term studies (Wogan & Wang, 2018). To address or mitigate these limitations, sampling can be repeated over multiple years or applied through a "space-for-time" approach, investigating populations at different stages of successional processes.

Contemporary sampling, however, lacks historical context and cannot capture what was typical for current populations or species prior to recent human-induced impacts on landscapes and ecosystems (Card et al., 2021; Leonard, 2008; Nakahama, 2021). More specifically, space-for-time substitution (SFTS) is applied to genetic diversity by modelling genetic turnover—typically represented by shifts in allele frequencies—in spatial patterns for populations evolving on stationary and non-stationary landscapes. This approach assumes that spatial turnover approximates temporal turnover (Wogan & Wang, 2018). Wogan and Wang emphasize the need for further studies integrating both spatial and temporal patterns of genetic variation, as such research is well positioned to advance numerous areas in ecology and evolution.

DNA extracted from historical specimens (historical DNA or hDNA, Jensen & Leigh (2022)), when available in biological museums or other collections, can add a temporal dimension to the genetic study of endangered species or populations. Such data is pivotal in conservation genetics to assess historical migration patterns, delineation of evolutionary significant (or management) units, as well as to infer changes in population size and structure over time. As estimates of these population parameters can potentially be obtained from the period preceding the extensive human change of the landscape that has taken place over the past decades or centuries, they are essential for informing realistic conservation plans (Leonard 2008; Wandeler *et al.* 2007). Currently, there is a

growing interest in understanding gene flow in a heterogeneous landscape by combining landscape and ecological data with individual genotype data (Wandeler *et al.* 2007). Historical samples prove particularly valuable in this context because they enable direct tests of hypotheses concerning human-induced changes in gene flow patterns and genetic diversity over time (Wandeler *et al.* 2007). Historical DNA is therefore fundamental to unravelling the history of populations and to quantify the amount of admixture between original gene pools (Leonard 2008). Separating native gene pools from introduced ones of the same species is of major concern in a conservation context.

To explore the information content expressed in DNA of historical material, we included 148 historical herbarium samples from southernmost Sweden in a continuous 200-year population genetic study of the broad-leaved forest tree *Acer campestre* L. (field maple). In this region, since the 1980s, seedlings and young individuals originating from cultivated parents have been frequently reported from natural areas, and the species is now relatively common here. However, despite this recent expansion, the only remaining native population is believed to consist of just a few trees, and the species is listed as Critically Endangered in the national Redlist (Artdatabanken 2015, 2020), although with a notion that only the native gene pool is endangered.

The Swedish population of *A. campestre* is located at the northernmost edge of the species range. It is isolated from the closest populations in Denmark at over 100 km over the sea. Such peripheral gene pools may have adapted to their local environment over millennia and may hold specific traits (e.g. phenological or physiological) not replaceable by generic or core population genotypes (Kawecki 2008; Safriel *et al.* 1994). A high level of genetic diversity can be maintained in fragmented tree populations if there are high levels of outcrossing and if populations are sufficiently large; in other words, forest fragmentation should have deleterious effects on genetic diversity only if natural pollen or seed dispersal ranges surpass to the boundaries of forest fragments. Moreover, dispersal can lead to gene flow only if propagules find suitable habitats (for seeds) or conspecific female flowers (for pollen), and this becomes increasingly challenging in fragmented habitats regardless of dispersal mechanisms (Budd *et al.* 2015). All these prerequisites are violated for the native population of *A. campestre* in Sweden and thus long-term survival of the Swedish native population is severely challenged at the national level. Pautasso (2009) points out that habitat fragmentation ultimately affects genetic diversity due to the alteration in landscape features, which leads to reduced gene dispersal. Although peripheral populations often grow in suboptimal environments compared to core populations, they may have pre-adaptations to buffer future adverse climatic or edaphic conditions. Peripheral populations and their unique adaptations are often at risk due to the combination of low effective population size and stronger effects of stochastic factors, calling for stricter protection and

different conservation measures than populations at the core of the range, as proposed by Gapare & Aitken (2005) for another northern forest tree species, *Picea sitchensis*.

In this study of the historical genetic diversity of *Acer campestre,* spanning the last 200 years, we aim to test several hypotheses: (1) Due to low recruitment, genetic drift and diminishing population size, the genetic diversity of *Acer campestre* in Sweden has probably decreased over time, but today the process might have been reversed due to recent introduction and massive cultivation of material brought in from the Continent which probably carries different genotypes; (2) If this initial hypothesis could be supported, we also test whether this trend may recently have inversed due to a high inflow of cultivated plants from the continent, flooding the genetic landscape with new alleles, resulting in a higher number of and heterozygotes and the dilution of specific native alleles of high adaptive importance . As a result of such a process, if so, we expect the number of alleles and private alleles to first decrease slowly and then increase rapidly in recent decades. In addition, (3) a sudden shift in allele frequencies is expected with a decrease of native alleles private to the historical landscape and a simultaneous emergence of new alleles introduced from the Continent. Ultimately, the genetic structure may change from a few dominating, native gene pools to a new, largely non-native gene pool. These hypotheses are expressed by the questions: How has the genetic landscape changed over the last two centuries? Which trends of genetic diversity, change of allelic composition and change of dominating gene pool can be documented? To what extent can this transition be assessed through studies of old specimens in museums?

Methods

Study species

Acer campestre, commonly known as field maple, is a medium-sized tree that typically reaches a height of 15 meters. It can grow either as a tree or as a shrub in the understorey. Its leaves are arranged in opposite pairs, emerging bright green and darkening with age. In autumn, the foliage transitions to a rich gold colour, occasionally tinged with red, and persisting for an extended period. The small, yellow-green flowers appear in clusters of about ten, typically blooming in late April, either simultaneously with or just before bud burst. Field maple is a monoecious species, producing hermaphroditic flowers, with individuals often displaying complex temporal patterns of sex expression throughout the flowering season. While pollination is primarily entomophilous, some pollen dispersal via wind is also possible (Zecchin et al., 2016). Wahlsteen et al. (2023) demonstrated that *Acer campestre* exhibits genetic structuring across the European continent, although a high degree of admixture exists between the identified genetic clusters.

The natural distribution of field maple spans most of Europe, from central and southern England, southern Sweden, and Denmark to the Pyrenees, Sicily, Greece, and northern Turkey, with isolated populations in Spain and North Africa. Although not extensively planted outside its natural range, it is occasionally cultivated as an ornamental tree. Field maple thrives in a wide range of ecological conditions but is more common in mesophilic stands, particularly deciduous oak forests, from sea level to altitudes of 1600 meters. It prefers warmer climates but is winter-hardy and tolerates the temperature extremes of continental environments.

Due to its low commercial value, field maple is rarely managed silviculturally and often occurs in spontaneously established, semi-natural populations. On the continent, it is frequently found in mixed broad-leaved woodlands, particularly alongside species of *Quercus*, *Tilia*, *Ulmus*, and *Castanea* (Zecchin et al., 2016). While its maximum lifespan is not definitively established, it is estimated to range from 100 to 200 years, with a generation time of approximately 10 years under favorable conditions.

Herbarium samples

Samples were extracted from whole leaves or parts of leaves detached from herbarium specimens. Samples were obtained from the university herbaria at Lund (LD), Uppsala (UPS), Gothenburg (GB) and the Oskarshamn Biological Museum (OHN). Focus for the sampling was southern Sweden (county Scania, sampling area 5 700 km2) represented by major sampling localities of Brännemölla, Helsingborg, Lund, Malmö, Silversborg, and Svedala.

In addition to sampling historical specimens, data from two contemporary datasets were incorporated in the subsequent analysis. A continental data set, comprising 460 samples from 42 populations all over the species range, earlier generated for Wahlsteen *et al.* (2023), and a set of 71 samples from southern Sweden earlier generated for Wahlsteen (2021).

DNA extraction

Historical DNA was prepared using DNeasy Plant Pro kits (Qiagen, Manchester, UK) following the manufacturer's instructions. Degradation and fragmentation of DNA was mitigated using a combination of Restorase™ (Merck UK, Gillingham, UK), and an in-house developed pre-PCR thermal cycling process. Briefly, 500 ng genomic DNA was added to 20µl total volume 1X Restorase buffer, and pre-incubated for 15m at 37°C, following the manufacturer's protocol. Incubated template was pre-amplified via 20 PCR cycles comprising 30s at 94°C, 30s at 52°C (ramp rate 0.5°C s-1), and 2m at 72°C.

Nuclear microsatellite genotyping

Twelve nuclear microsatellite loci previously described for other closely related *Acer* species were cross-amplified in *Acer campestre*: MAP9, MAP40, MAP46 (Pandey *et al.* 2004), Aop116, Aop132, Aop943 (Segarra-Moragues *et al.* 2008), Am116, AM118, SM14, SM29, and SM60 (Kvesić *et al.* 2020), and AsT (Harmon *et al.* 2017). All loci were PCR-amplified using DFS-"Hot" Taq DNA Polymerase (GeneOn Bioscience, Ludwigshafen am Rhein, Germany) in 1X PCR buffer (20mM Tris-Cl, pH 8.3, 10mM (NH4)2SO4, 35mM KCl, 1.8 mM MgCl2, 0.1mg/ml BSA).

The PCR conditions were as follows: Multiplex 1 (MAP9, MAP46, Aop132, AsT) was amplified using a modified touchdown PCR program (according to (Chybicki *et al.* 2014) with initial denaturation at 95 °C for 2 min, five touchdown cycles (94 °C for 30 s, 61 °C (-1 °C/cycle) for 30 s and 72 °C for 1 min) and 28 regular cycles (94 °C for 30 s, 55 °C for 90 s and 72 °C for 1 min; Multiplex 2 (Aop116, Aop943, and MAP40) was amplified using conventional PCR profile comprising initial denaturation at 95 °C for 2 min and 29 cycles of 94 °C for 30 s, 56.5 °C for 90 s and 72 °C for 1 min; Multiplex 3 (Am116, AM118, SM14) was amplified using conventional PCR profile comprising initial denaturation at 94 °C for 30 s and 32 cycles of 59 °C for 30 s and 72 °C for 1 min. SM29 and SM60 were amplified individually (36 cycles comprising 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min for SM29, and 36 cycles comprising 94 °C for 30 s, 59.5 °C for 30 s and 72 °C for 1 min for SM60). The published reverse primers were modified by addition of 5' GTTTCTT tails to control Taq polymerase terminal transferase adenylation activity (Brownstein et al., 1996). PCR products were diluted 1:15 in deionized formamide and sized via a 3730XL DNA analyser using Genescan 400HDRox size standard (Applied Biosystems, UK). Exported data was analysed using Genotyper 2.0 software (Applied Biosystems, UK).

Plastid DNA sequencing

PCR reagents were performed in 20µl volumes by adding to the 10µl volumes PreCR Repair Mix 10µl aliquots of a mix containing 0.4U Phusion® High-Fidelity DNA Polymerase (New England Biolabs UK), a second aliquot of dNTPs (0.2mM final concentration), 1X manufacturer's supplied HF buffer, and 0.5 µM (final concentration) each forward and reverse primer (Table 1).

Table 1. Pre-amplification PCR primer pairs

locus	primer	sequence	Anneal T
ycf	ycf1_8F	GCGCATGGAGCCTTTGATTA	
ycf	ycf1_8R	GAGAAACCTCTCGCGACTCT	64°C
trn	trnH10_F	TTTGGTTATGGGCGAACGAC	
trn	psbA14_R	ATGTTAGGCGTAGCTGGTGT	64°C
rpl	rpL16_6F	CACGGCGTACATTTCGTGTC	
rpl	rpL16_2bR	AACCAACTCATTGCTTCGCA	62.6°C

Thermal profile was: initial denaturation 98°C , 30 seconds, 32 cycles; 98°C for 8 seconds anneal for 20 seconds; 72°C, 30 seconds; final extension: 72°C for 10 minutes. Half of each completed PCR product (10µl) was removed and added to 1.5µl agarose loading dye and resolved alongside a dilution series of λ DNA on a 1.8% agarose in 0.5X TBE buffer to determine each PCR product concentration. To the remaining 10µl of each PCR reaction, was added a mix containing 4.4U Exonuclease I, 1.1U Shrimp Alkaline Phosphatase (ThermoFisher Scientific UK), and MilliQ water to final volume of 10µl. Reactions were incubated for 90minutes at 37°C, followed by 15minutes at 85°C.

Each cycle sequencing reaction comprised 40 ng PCR product, 3.2 pmole single forward or reverse nested sequencing primer (table2), 0.5X BigDye Terminator Ready Reaction Mix (ABI BigDye Terminator v3.1 Cycle Sequencing Kit - ThermoFisher Scientific UK), and MilliQ water to final volume of 10µl. Thermal Cycling used 25 cycles of denaturation: 96°C for 10 seconds, annealing: 50°C for 5 seconds, axtension: 60°C for 4 minutes, repeat for a total of 25 cycles. To remove unincorporated dyes and other contaminants, 1X vol 3 M sodium acetate (pH 5.2) and 25 µL of 95% ethanol was added to each reaction. After 20 minutes at room temperature, reaction mixes were centrifuged at 14,000g for 15 minutes at 4°C. Supernatant was removed and pellets were washed twice with 100 µL 70% ethanol. After removal of the final supernatant the cleaned pellets were air-dried for 15minutes at room temperature and resuspended in 10µL Hi-Di Formamide. Samples were denatured at 95°C for 2 minutes, snap

cooled on ice and loaded into a 3730xl DNA Analyzer (ThermoFisher Scientific UK) fitted with a 50cm 48 lane capillary operating StdSeq36_POP-7 run module. Data was imported into Genious Prime 2021.1 to trim low quality sequence and combine forward and reverse reads.

All laboratory works were performed at Noahgene Ltd, Scotland.

Data analysis

Genotype subsampling

All samples analysed for microsatellites were arranged after the date of collection, and a lifespan of 70 years was assumed for each individual. In order to analyse variation in genetic descriptors over time, while still merging samples into statistical populations of reasonable size, the total data set was split into subsamples comprising periods of 30 years. In total, seven subsamples were extracted representing even time intervals of 1820–1849, 1850–1879, 1880–1909, 1910–1939, 1940–1969, 1970–2019 and 2020. No individuals were subsampled twice in any of the analyses. In the online supplementary information, the group assignments for all individuals are reported.

Population genetic diversity and null alleles

Overall genetic diversity and allelic summary statistics were computed in Cervus 3.0.7 (Kalinowski *et al.* 2007), estimated null allele frequencies in FreeNA (Chapuis & Estoup 2007), basic genetic statistics (expected heterozygosity (He), observed heterozygosity (Ho) and fixation index (F)) in GenAlEx (Peakall & Smouse 2006) and allelic richness and number of private alleles estimated by rarefaction in wHP-Rare (Kalinowski 2005). Student´s t-tests were used to test for differences between observed and expected heterozygosity, and to test for deviation from 0 of the fixation index (F). Variation in the nucleotide sequences of the microsatellite flanking regions may prevent the primer annealing to template DNA during amplification of the microsatellite locus by PCR, resulting in a null allele. To check for impact of such null alleles on the results, overall and pairwise Fst for each population and each locus was computed with and without correction as described in Chapuis & Estoup (2007) and the matrices were tested in a MANOVA for significant differences.

Temporal allele frequency clines and allele transfer

To investigate the fate over time of alleles present in the original gene pool, alleles present in the oldest timespan (1820–1849) were counted and compared to the later timespan of 1940–1969, before the great inflow of trees from the continent during the period of 1970–2020. To estimate potential gene flow from the historical populations to the contemporary populations, private alleles were counted for the historical samples and compared to the continental dataset as well as to the dataset of contemporary Swedish samples.

A principal components analysis (PCA) was conducted on the allele frequency covariance matrix with all detected alleles as variables and the seven time periods as samples. To explore how overall allele frequency variation changed over time, PC1 was plotted as a function of time.

Isolation by time (IBT) and effective population size

Mantel tests were conducted to investigate changes in population structure over time by comparing pair-wise data of genetic distance (Fst) and time distance, in addition, a linear regression was conducted between the first component in a PCA of the Fst matrix and time.

Effective population size (Ne) was estimated using the linkage disequilibrium-based approach implemented in LDNe software (Waples & Do 2008). The LD method is based on the stochastic association between alleles at different loci in a single population sample, caused by drift at a rate inversely proportional to Ne. The LD approach has become a genetic method of choice to estimate contemporary Ne in applied conservation biology, due to its sampling ease relative to temporal methods (especially for long-living species such as trees) and its estimation accuracy relative to other one-sample approaches (Santos-del-Blanco *et al.* 2022). This method is sensitive to low-frequency alleles, why the threshold was set to 0.1–0.2 as a minimum for allele frequencies. To obtain higher accuracy, individuals with high affinity to the introduced gene pool were omitted and samples from the period 1820–1879 were pooled to increase sample size. The effective population size was used to extrapolate a census size for each time period. The 0.1 ratio of Ne/census has been proposed by Hoban *et al.* (2021) for forest trees and was used for the extrapolation.

Genetic bottlenecks

The software Bottleneck 1.2.0.2 (Piry *et al.* 1999) was used to identify genetic bottleneck events for each period. The coalescent process was simulated under the two-phase model (TPM) with default settings. The significance of heterozygosity excess or deficiency was assessed by sign test and Wilcoxon test because these tests were found to be the most powerful and robust tests when used with less than 20 loci (Piry *et al.* 1999).

Spatio-temporal genetic structuring and gene pools

A Structure analysis (version 2.3.4) analysis (Pritchard *et al.* 2000) was conducted for the combined historical and contemporary dataset with admixture model and correlated allele frequencies. Burnin was set to 100 000 and the number of MCMC repetitions to 1 000 000. 10 simulations were run for each K ranging from 1–10. Optimal number of K was calculated by the delta K method (Evanno *et al.* 2005) as implemented in the web application Structure Selector (Li & Liu 2018).

Plastid sequences

Sequences from historical and present samples were aligned using the MAFFT algorithm (Katoh *et al.* 2019) for each of the two gene regions. Prior to further analysis, the aligned regions were concatenated, and haplotypes were identified using the software DnaSP (version 6.12.03) (Rozas *et al.* 2017) with default settings as invariable sites removed and sites with gaps not considered. Haplotype frequencies were computed for all periods stated above, and for the historical and present continental samples. Haplotype compositions were given as pie diagrams and plotted onto a map obtained in QGIS (version 3.16.2). To obtain comparable values of haplotype richness for different time periods, the number of haplotypes were estimated by rarefaction, computed in the EstimateS (version 9.1.0) software (Colwell 2013). As a complementary analysis, the haplotype dataset was also grouped into four different subsamples of even sizes, but uneven time spans.

Results

Herbarium samples

In total, 162 herbarium specimens collected from 1808 to 1998 were used for extraction of DNA. The sampling became somewhat skewed with a higher number of samples from the late 19th century, and fewer samples from the start and the end of the period (see online supplementary information).

Genotyping and plastid sequencing

The genotyping of the historical specimens generated complete genotypes for 148 samples. All loci were polymorphic except Map46 which was monomorphic and omitted in all further analyses. The total number of loci used in all downstream analyses was 12. The total number of alleles counted to 144 and the mean number of alleles per locus was accordingly 12. The locus MAP40 was the most variable one with 28 alleles and the EST-SSR derived locus AsT the least variable locus with only three alleles. Mean expected heterozygosity over loci exceeded observed heterozygosity except at loci 2, 9 and 12 which displayed deficits. The estimated null allele frequency ranged from zero to 0.12 (mean 0.06) with highest occurrence at loci 1, 5 and 11 (Table 2). The overall Fst adjusted for presence of null alleles was 0.016, as compared to the result obtained without adjustment which was 0.015. The MANOVA test for difference between the Fst matrices gave non-significant results ($p>0.05$) for all pairs. Since the presence of null alleles had limited effect on the results, no loci were discarded in any of the subsequent analysis, as the drawbacks of deleting the loci outbalanced the benefits of having more statistical power.

Table 2. Summary statistics for the twelve loci used in the current study. Number of alleles, observed and expected heterozygosity and null allele frequencies are reported.

Loc. No	Locus	Na	Hobs	Hexp	Null. Freq.
1	Aop116	13	0.63	0.81	0.11
2	Aop122	4	0.57	0.48	0.01
3	Aop132	18	0.71	0.83	0.09
4	Aop943R	14	0.73	0.85	0.09
5	AsT	3	0.36	0.51	0.11
6	MAP40	28	0.75	0.91	0.10
7	MAP9R	11	0.62	0.74	0.09
8	AM116	13	0.83	0.82	0.01
9	AM118	7	0.86	0.73	0.00
10	SM14	20	0.89	0.90	0.02
11	SM29	5	0.42	0.56	0.12
12	SM60	8	0.78	0.68	0.00
Mean		12	0.68	0.74	0.06

The plastid psbA-trnH region was successfully sequenced for 302 samples and covered 853 sites, of which 674 were conserved (80%) and 171 variable (20%). Complete sequences for the ycf1 region were obtained for 295 samples. The gene comprised 753 sites of which 627 were conserved (83%) and 121 variable (16%). When the two datasets were concatenated 288 compete sequences remained with 1618 sites. DnaSP found 150 unique haplotypes of which 102 represented just a single sequence and 25 just two sequences. The remaining haplotypes were found in between 3 and 23 samples each and these were retained for further analysis. A rooted maximum likelihood phylogeny of haplotypes was calculated in Mega X software (Kumar *et al.* 2016) with *Acer cappadocicum* as outgroup. The model selector favoured the Tamura 3-parameter with uniform rates and 1000 replicates were set for bootstrapping.

Data analysis

Population genetic diversity

The allelic richness (4.95-6.43, SD 0.49) and private allelic richness (0.15-0.58, SD 0.17) fluctuated over the centuries, but the expected heterozygosity (0.66-0.74, SD 0.03) tended to be rather stable (Fig. 1a-c). The latter can be explained by the expected heterozygosity is mostly dependent on the frequency of the most common alleles, which are normally changing slowly in frequency. Observed heterozygosity was somewhat lower than expected (over all mean difference 0.04) but when the mean between the two estimates was tested in a Student´s t-test no significant difference was detected for any time periods. The Fixation index (F) ranged from -0.003 to 0.12 (SD 0.04) but when deviation from 0 was tested in a t-test the result was non-significant for any of the time periods.

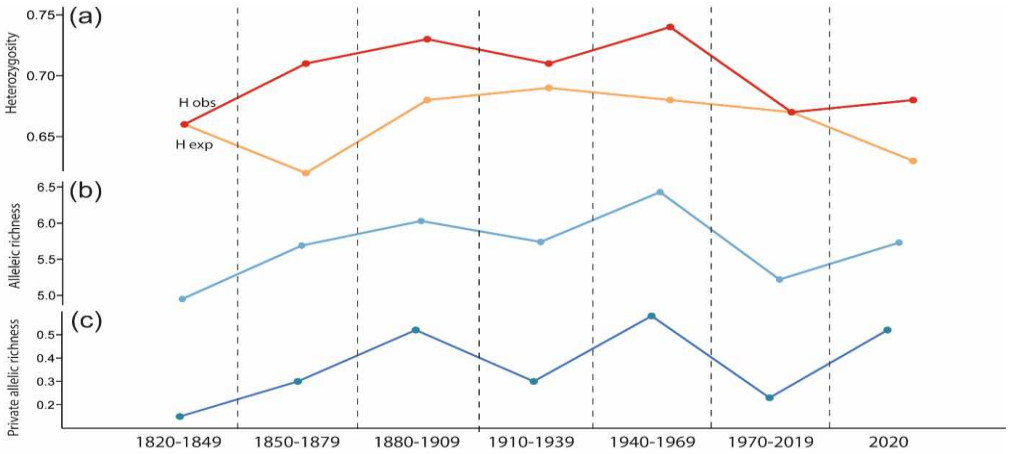


Fig. 1 Genetic diversity in *Acer campestre* over the 200-year sampling period; **a** observed and expected heterozygosity; **b** number of alleles after rarefaction; **c** number of private alleles after rarefaction.

Temporal allele frequency clines and allele transfer

When all alleles present in the earliest time period were compared to the frequencies of the same alleles in the gene pool of the contemporary sample (1980–2020), it was found that 66% of the alleles had decreased in frequency while 35% had increased (Fig. 2 and 3). Of the former, allele 3-141 had generally high frequency in the past but almost vanished in the contemporary sampling. This allele is rare on the Continent and only found in a few populations in France and Hungary. Alleles 4-140 and 12-236 are unique for the historical populations but absent from the contemporary population (Fig. 3) and is not present on the Continent; thus, any recent flux of these alleles are deemed as highly unlikely.

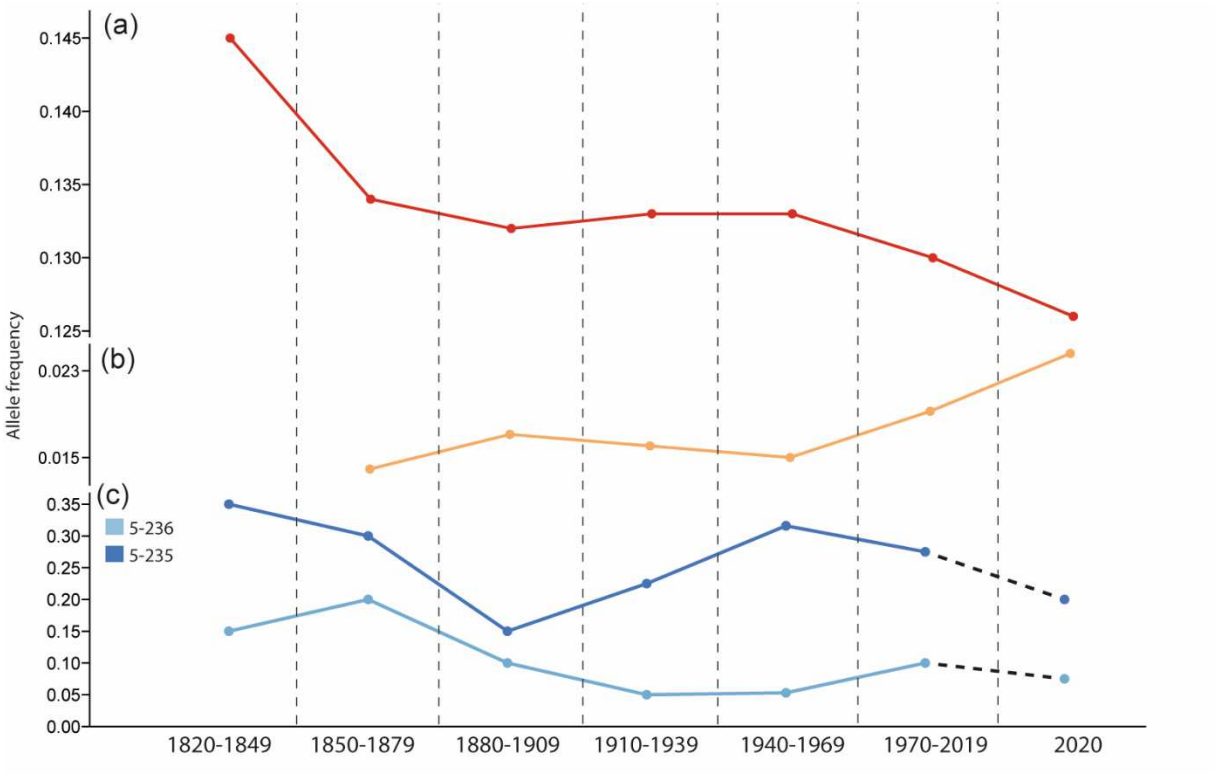


Fig. 2**a** Overall trend in frequency of alleles present in the most ancient period; **b** overall trend in frequency of alleles introduced after 1850; **c** two alleles unique to Sweden still extant in the modern population (dashed line indicate allele transfer to the modern population).

However, decrease in individual alleles are compensated for by increase of other alleles over time (Fig. 2b). For instance, two of the alleles at loci 4 and 10 are found at a rather low frequency in the past but are increasing considerably in the contemporary sampling, possible due o the introduction of material from the continent (Fig. 3), where these alleles are rather common. Allele 4-146 is frequent in contemporary populations in France, Germany, England and Hungary and allele 10-94 is frequent in France and England.

A few rare alleles are confined to the old samples in the dataset. Allele 6-168 is found in one sample from 1856 with the frequency 0.05 (Fig. 3), allele 4-159 in one sample from 1860 and allele 4-160 in one sample from 1866, both of the latter with frequency 0.025. Overall, comparing the frequecies of the 81 alleles that were present in the earliest time period with their corresponding frequencies in the most recent historical period (1940-1980), it was found that 11 had disappeared (or were not found in the sampled material) (13%), 31 had decreased in frequency (38%), 12 had kept their frequencies (15%) and 27 had increased in frequency (33%).

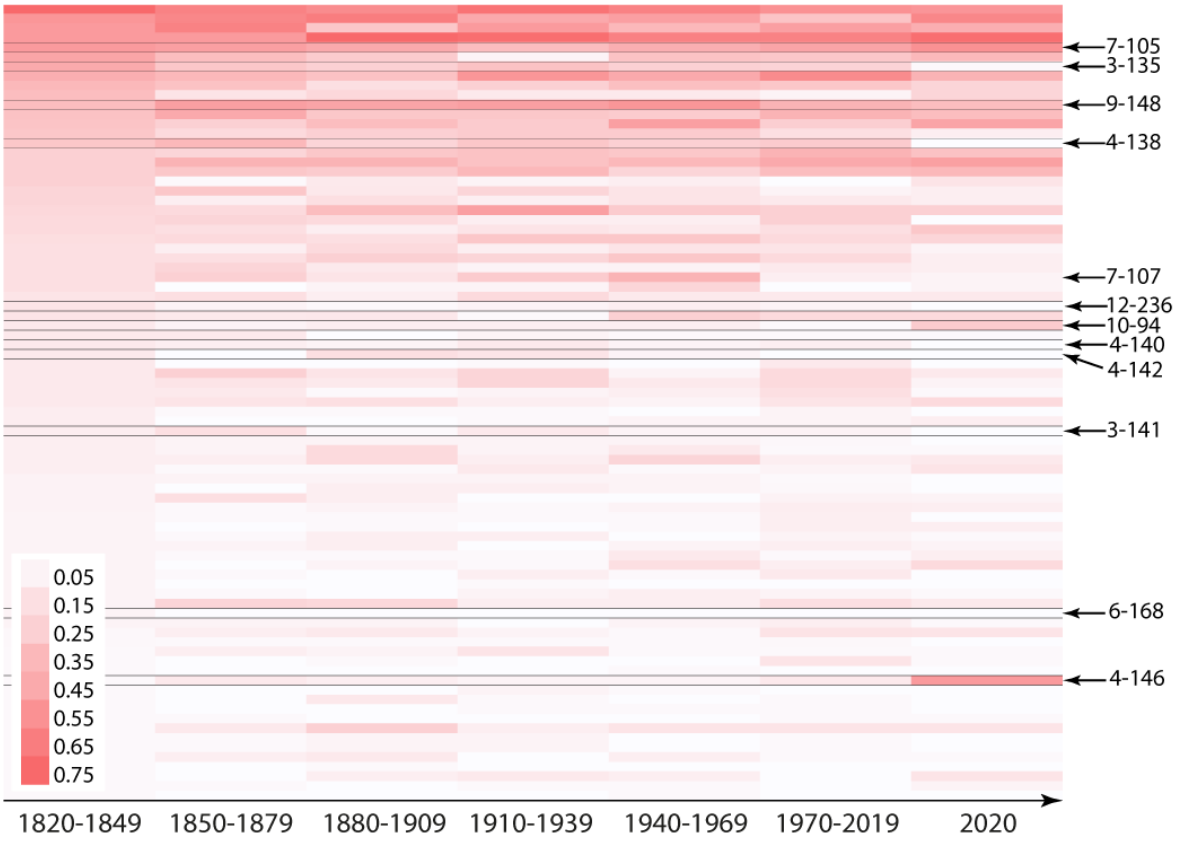


Fig. 3. An allele fate matrix illustrating the frequency of 81 alleles occurring in the most ancient period and how they change over time. Red indicates high frequency, pinkish low frequency and white absence of allele, frequencies are coded by colour ramp in lower left. Alleles discussed in the text are marked with arrows.

Gene transfer from historical samples to contemporary ones was evident in allele 5-235 (Fig. 2), a once common and well spread allele transferred to several samples of the contemporary population. Also, allele 5-236 was a rather common allele private to Scania and that was found in two individuals of the contemporary population (Fig. 2C).

The PCA clearly separated the most ancient period (1820), the most recent (1970 & 2020) and the mid periods (1850–1929) as separate groups (Fig. 4a). The first principal component explained 39% of the variance and arranged the samples on a timeline and when PC1 was plotted as a function of time a clear overall allele frequency cline was evident (Fig. 4b). Note that PC1 represents an overall change of allele frequency, i.e., the change of one dominating allele group to another, and not a general decline (Fig. 4 b). Component loadings indicated that the most recent time periods (1970 and 2020) are characterised by relatively high frequencies of allele 4-146, that the earliest period is characterised by high frequencies of the alleles 6-148 and 7-105, and that the mid periods are characterised by high frequencies of alleles 7-107, 9-148, 4-138 and 3-135. The age of the temporal subpopulations correlated with their positions along the first principal component, and it could be concluded that the overall allele composition changes gradually over time.

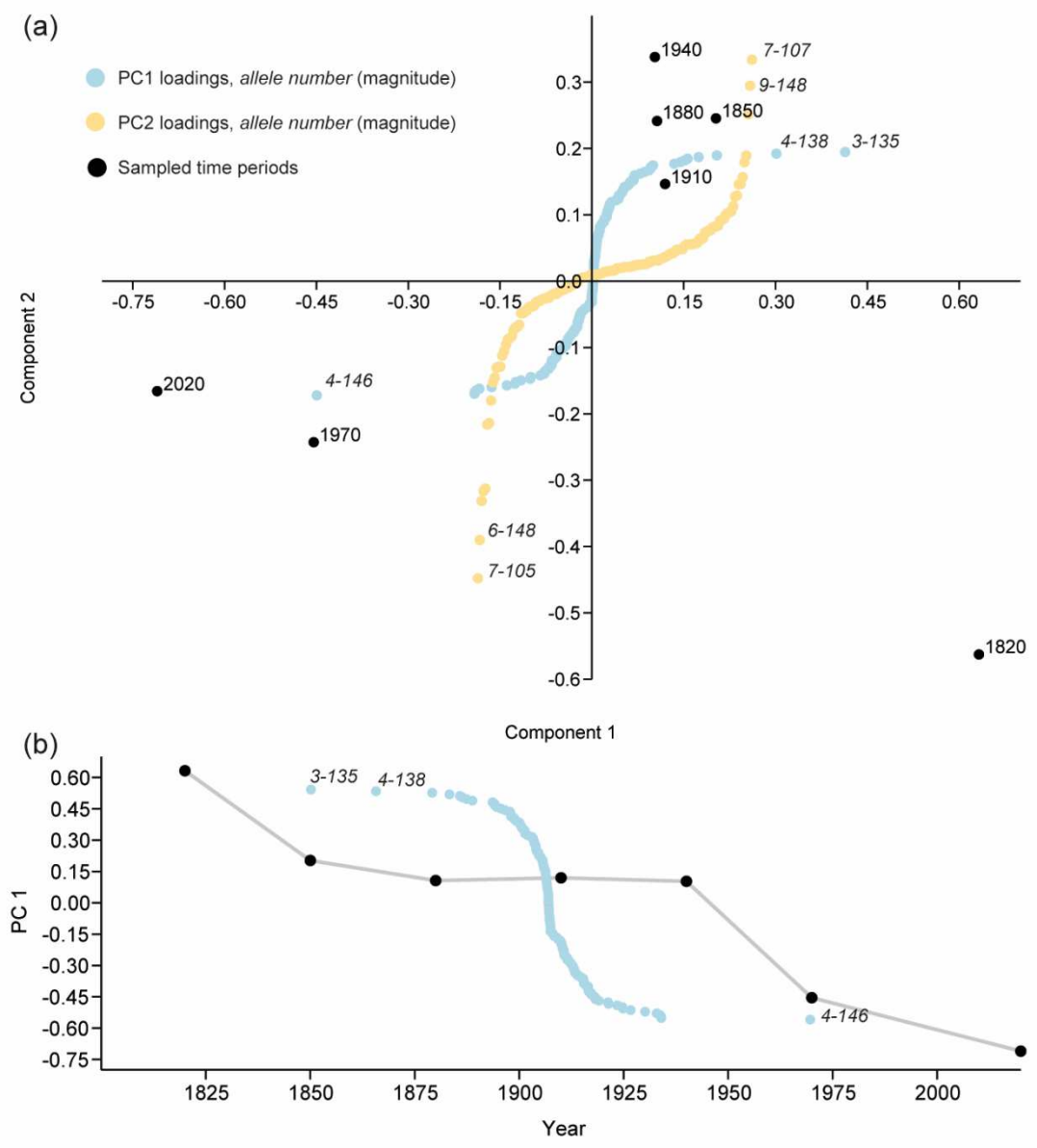


Fig. 4. Principal components analyse of overall allele frequency; **a** PCA scatter plot with sampled periods and the impact of alleles on the first to components (PC 1 explains 39% of the variance and PC 22%), loading plots corresponds to the respective axis scale; **b** the first principal component as function of time and the loadings plot indicating alleles with high impact on certain periods.

Isolation by time (IBT) and effective population size

The Mantel tests revealed a positive correlation between genetic difference and time (r2=0.57, p=0.0001) and when the first principal components of Fst values was plotted as function of time the correlation was positive (r2=0.86, p=0.0011), indicating that the genetic structure of the field maple population has changed over the centuries.

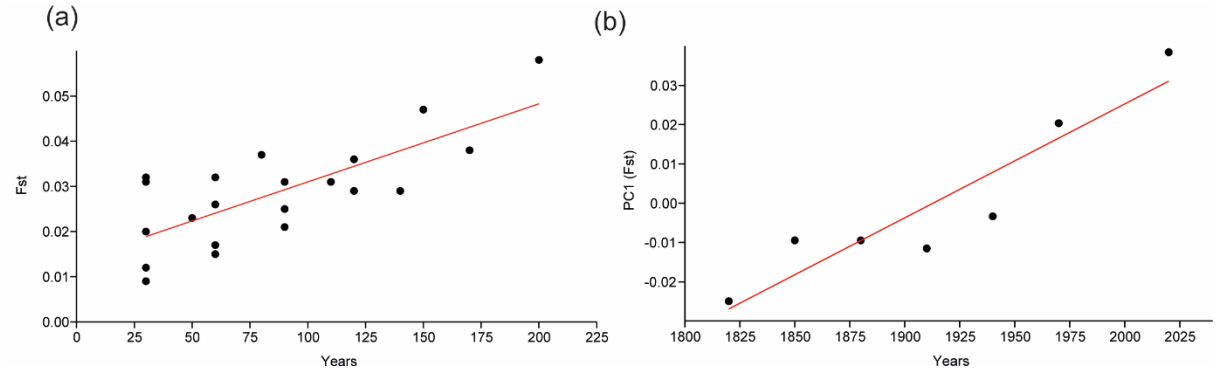


Fig. 5. Genetic differentiation as correlation of time reveals a positive correlation with increasing genetic differentiation over the sampling period; **a** Mantel test scatter plot between pairwise Fst and sampling period; **b** first principal component of a Fst genetic differentiation matrix as a function of time.

Effective population size for the pooled dataset of samples from 1820–1880 was estimated to 72 with 95% confidence interval (56–100). When the effective population size was extrapolated for calculation of census size, the total number of historical field maple trees in Scania turned out be low, about 700 trees with a range from 500 to1000.

Genetic bottlenecks

The studied population does not harbour any signatures of genetic bottlenecks for any time periods according to the Sign test and the Wilcoxon test. For the Sign test, p was calculated as in the range of 0.39–0.59 and for Wilcoxon 0.09–0.34.

Genetic structuring and gene pools

The Structure and Delta K results indicate that the optimal numbers of clusters equal to three (Fig. 6). One gene pool (blue in Figure 6) corresponds to samples collected during the earliest period and in particular to plants collected at locality B (Brännemölla), but is represented by successively fewer samples from more recent periods, from 56% in the earlies period to just 14% in the contemporary sample. The second gene pool (purple) is strongly restricted to the population at locality S (Silversborg, the only population today considered native) but decreases from 37% maximum to 12% of the contemporary sampling. The third gene pool (orange) is represented by introductions from the continent and has a low proportion of 20% in the early 19th century but increase significantly (p=0.001, r2=0.83) to 74% in the contemporary sample. Historical individuals assigned to this gene pool are mainly collected in cities and represent probably the very first introductions of *Acer campestre* as a garden plant.

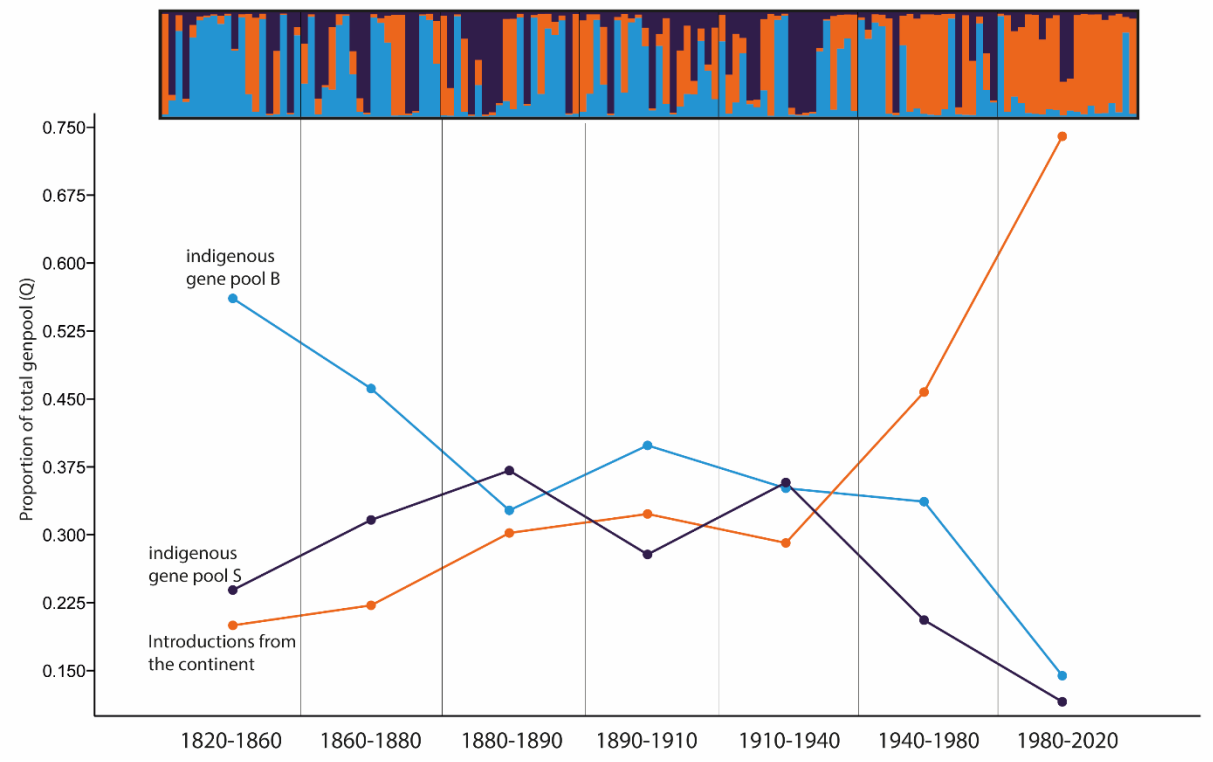
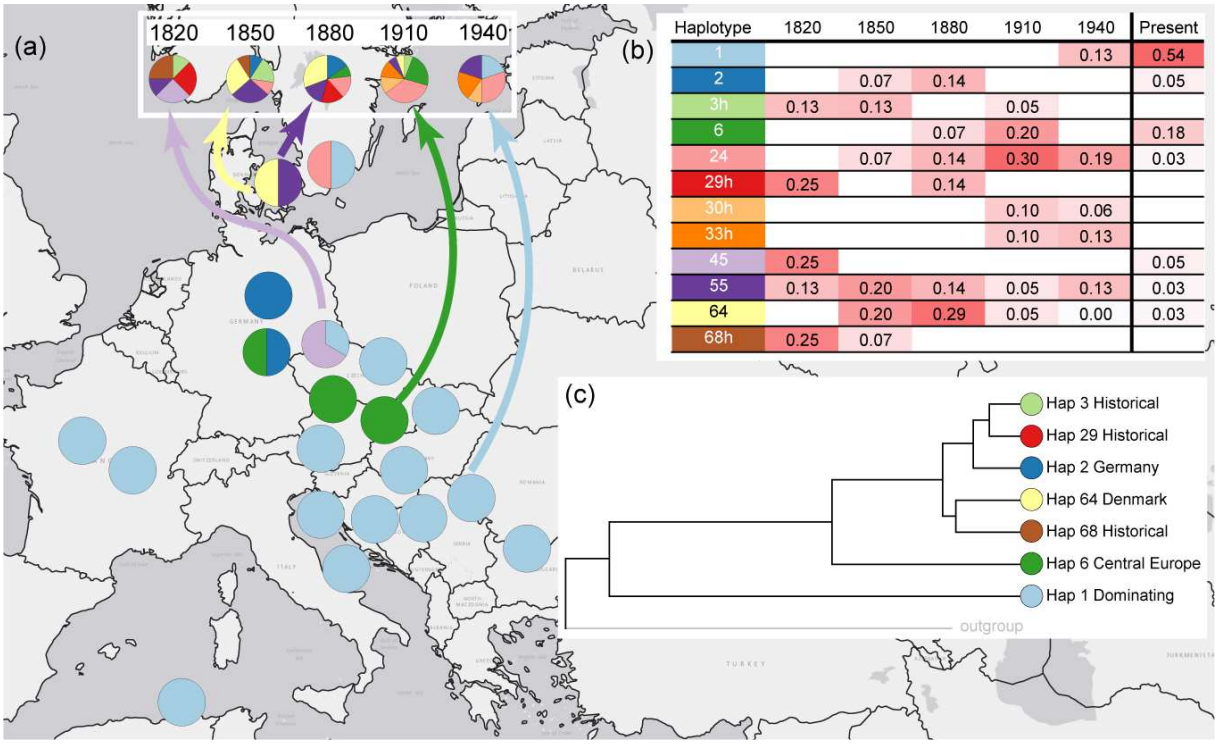


Fig. 6. Structure bar plot where each sample is represented by a vertical line which has been partitioned into K = 3 gene pools. Individuals were ordered according to period of sampling shown on the x axis. The graph below illustrates the change of dominating gene pool.

Temporal and geographical haplotype distribution

The historical data set comprised a total of 59 different haplotypes of which 18 haplotypes were considered common (more than four samples/haplotype) (Fig 7). The most frequent haplotype (1) is widely distributed in central Europe but was not introduced to Sweden until the 1960s. The second most common haplotype (6) is today found in Czech Republic and Germany and appears in Sweden at low frequency (just one sample) in the 1880s and more commonly in the 1910s. When overall means for the historical data set were computed for the frequency of most common haplotypes for each time period, the trend was clearly declining, meaning that all original haplotypes diminished and replaces by introduced haplotypes. The maximum likelihood phylogeny of the most common haplotypes indicated that northern European haplotypes (Denmark (64), Germany (2) and historical (3, 29 & 68)) diverged separately from the central European (6) and the dominating haplotype (1), Fig 7c.



Haplotype	1820	1850	1880	1910	1940	Present
1					0.13	0.54
2		0.07	0.14			0.05
3h	0.13	0.13		0.05		
6			0.07	0.20		0.18
24		0.07	0.14	0.30	0.19	0.03
29h	0.25		0.14			
30h				0.10	0.06	
33h				0.10	0.13	
45	0.25					0.05
55	0.13	0.20	0.14	0.05	0.13	0.03
64		0.20	0.29	0.05	0.00	0.03
68h	0.25	0.07				

Fig 7**a** Present distribution of the 12 most common haplotypes in Acer campestre Europe. Arrows indicate putative origins of certain central European haplotypes to the historical samples in southern Sweden; **b** table of haplotype frequencies of the 12 historical haplotypes, colours in the left column corresponds to those of the map, haplotypes indicated with 'h' are only present in the set of historical samples; **c** maximum likelihood phylogeny of the seven most common haplotypes indicates a late divergence of the northern European haplotypes.

Out of the total of 59 haplotypes found in the historical data set, eight were present already during the earliest period 1820-1840. Two of these still occur at low frequency within the present distribution of the species in Denmark and Czech Republic. From the 1850-1859 period 13 haplotypes were detected, of which two still occur within the present distribution in Germany and Denmark.

The haplotype composition in the historical data set indicated an inflow of haplotypes during the 1860-1890 and a loss of haplotypes during the later period of 1930-1940 (Fig 8 **a**). The haplotype count dataset corrected for uneven sampling size was despite rarefaction heavily biased by the unbalanced sampling, but although, indicated a gene inflow during the mid period (Fig 8 **b** red line).

The Mantel tests revealed a positive correlation between haplotype difference and time (p 0.0038, r^2 0.49), Fig 8 **b**, and a PCA of the haplotype frequency indicated that haplotypes 24, 33 and 59 were associated with the later sampling periods, 3 and 68 with the oldest and 64 with the mid period.

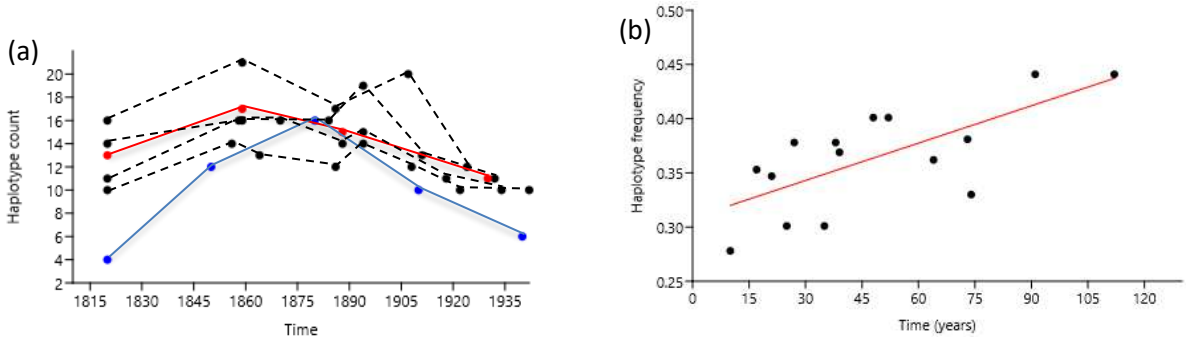


Fig 8**a** Absolute numbers of haplotypes found in four different historical sampling pools (dashed line) over the last two centuries indicating an influx of haplotypes in the period of 1860-1890. Red indicates mean number of haplotype count and blue haplotype richness after rarefaction; **b** Correlation between haplotype frequency and time in the historical dataset. A mantel test indicates a positive correlation, interpreted as a change of haplotype composition over the last 120 years.

Discussion

The genetic diversity of the Swedish *Acer campestre* population from the period 1820–2020 showed high expected heterozygosity (mean 0.67, Fig. 1) compared to the continental population (mean 0.57 reported in Wahlsteen *et al.* (2023)). The allelic richness remained relatively stable and varied between 5.0 and 6.5, and each period contributed a few private alleles (Fig. 1). When individual alleles in the historical sampling were compared to the contemporary sample, 66% decreased and 35% increased (Fig. 2). The same pattern became evident in the plastid haplotypes, where all historical haplotypes declined in frequency with time (Fig 8a). Three old and rare alleles were detected (Fig. 4) and when the most ancient period was compared to the latest historical (1910–1940) 31 decreased, 11 disappeared, 12 were stable and just 27 had increased (Fig. 3). Fifteen alleles were private to the historical period, and of these, only two had been perpetuated to the contemporary landscape (Fig. 2c), while the others had disappeared. Similarly, seven large plastid haplotypes (Fig. 7) were private to the historical samples and not present in the contemporary European distribution. The effective population size has apparently always been low, estimated to be 72 for the most ancient period, and when extrapolated to individuals the census size was estimated to be about 700 trees. However, the estimation of Ne should be interpreted with caution; for instance, work by Santos-del-Blanco *et al.* (2022) implies that the LD method generates unstable results, strongly influenced the marker system employed. Despite the low effective population size, no recent genetic bottleneck was detected.

The most dramatic change was found in the turnover of the gene pools. The oldest sampling was dominated by the native gene pool B with a small contribution of another native gene pool (S) and trees of an assumed continental origin. By time, the continental gene pool became dominant, while

the native was marginalized (Fig. 5). This exchange of the gene pool is strongly supported by the isolation by time regression, where the Mantel tests showed a significant correlation between genetic differentiation and time (Fig. 4 and 8b).

The empirical data do not unambiguously confirm the initial hypothesis that the genetic diversity would decrease due to genetic drift, low recruitment and diminishing population size. Contrary, the historical samplings turned out to be relatively stable measured as expected heterozygosity (Fig. 1). This is most likely explained by a comparatively high recruitment of trees with origin from the continent, a phenomenon later increasing because of an industrialized nursery business. However, similar findings were reported by Cozzolino *et al.* 2007 for a terrestrial orchid: five haplotypes were reported for the contemporary sampling and just three for the historical (1832–1948). The authors speculated that recent mutations, immigrants or sampling bias could have caused the discrepancy. In our study, the pooling of historical samples into artificial populations proved challenging and significantly impacted the haplotype counts and frequencies (Fig 8b). However, regardless sampling strategy, the haplotype count seems to decline in the most recent sampling period after an inflow during the mid periods.

In a comprehensive review, Pautasso (2009) pointed out that habitat fragmentation ultimately affects genetic diversity due to alteration in landscape features, in turn leading to reduced gene dispersal. Loss of genetic variation through random genetic drift and increased selfing can then cause local extinction of small populations. Such a genetic loss is not evident in the light of the empirical data of the current study. This pattern may be seen in the light of the long life span of our study species – after all, 200 years is not a very long time for population genetic processes to act on a population of long-lived trees. However, our results clearly show that the allele composition has changed with time (Fig. 4 and 8) to fewer but more frequent alleles (Fig. 3 & 2): 66% of all alleles decreased compared to the contemporary sampling and of the alleles in the most ancient population, 38% decreased, and 13% disappeared compared to the most recent historical period (1940–1980). The reason for this decline in allele frequency and composition is not further investigated, but sampling error (“false” recruitments) and genetic drift are plausible explanations. For populations with effective size in the range of 40–60, drift has an obvious impact on individual alleles (Allendorf *et al.* 2013). Thus, in our data alleles tend to decline and vanish over time (Fig. 2); and the presence of old and rare alleles and haplotypes may reflect once common and frequent alleles in the 1700s, just caught by the current sampling. Such alleles may be represented by 6-160, 4-159 and 4-160 and haplotypes 29 and 45, all present at low frequency in the 1850^{ies} and 1860^{ies}.

The change in allelic composition (Fig. 3 & 4) and turnover of gene pools explain the positive correlation observed between genetic differentiation and time (Fig. 5 and 8b). Here, the term isolation by time (IBT) is used to express the change in genetic differentiation in the same population over centuries (similar to Duforet-Frebourg & Slatkin 2016), contrasting the definition used by Hendry & Day (2005) which refer to genetic differentiation by breeding time (IBBT) within the same year. However, the stochastic mutations due to a small effective population size (i.e. genetic drift) has probably a strong impact, as the unstratified sampling where new individuals not belonging to the original population act as "false" recruitments. When the effective population size was extrapolated to estimate the total census size, the resulting number was unexpectedly low. For the most ancient period, the total population should equal to ca 700 trees, and even if the highest confidence interval is considered, also 1000 trees is a low number for a tree on a landscape level. Budd *et al.* (2015) discuss their findings for another long-lived forest tree distributed in the northern hemisphere and conclude that a long generation time has helped to sustain genetic diversity, but the benefits of longevity may be at least partially offset in the longer term by a greater need for high genetic diversity in long-lived sessile organisms such as trees, because for these species, environmental change is likely to occur over time scales that are shorter than their generation length. Thus, efficient historical dispersal in combination with longevity have likely contributed to the relatively high levels of genetic diversity in the peripheral, fragmented populations, the authors conclude.

Empirical data did not confirm that the contemporary sampling represents an own gene pool deriving from the continent. Instead, the contemporary gene pool appears to trace back to the most ancient period, revealing the very first introductions of *Acer campestre* as a garden plant (Fig. 6). This hypothesis is strengthened by the observation that all the early sampling of individuals with high affinity to this gene pool were collected in cities. The process when the original gene pool of a native species is replaced by an alien gene pool has been termed a cryptic invasion (Saltonstall 2002) and later authors have recognized major impacts of cryptic invasions on local ecosystems such as hybridization and evolutionary changes, replacement and novel roles in biotic interactions (Morais & Reichard 2018).

Implications for conservation

Using historical herbarium materials in population genetics makes it possible to directly estimate the genetic diversity of historical populations and allows for a better estimation of historical effective population sizes, diversity, and levels of gene flow (Nakahama 2021). As the estimations of these population parameters may inform about conditions before the onset of major human perturbations of the landscape caused by the industrialization and the agricultural modernization, they are

essential for making realistic conservation plans (Leonard 2008; Wandeler *et al.* 2007). The implications for future conservation of the endangered *Acer campestre* population can be concluded in two main paragraphs:

- Today the remains of *Acer campestre* in Sweden consists of a small stand of 36 individuals (Wahlsteen 2021). Such a small population will be challenging to sustain in the long term, but a small population size seems to be the normal state of *Acer campestre* in Sweden. Efficient historical dispersal in combination with longevity have likely contributed to the relatively high levels of genetic diversity despite the peripheral, fragmented locality. An important outcome of this study is that a small population of about 500-1000 trees can be enough to sustain the species at the national level in at least a short term.
- The drastic change of dominating gene pool (Fig. 4 & 6) is likely the most urgent threat, if not to the species as such, at least to the conservation-relevant unit constituted by the original Swedish population. This represents a profound, landscape genetic shift we cannot predict the consequences of. The most severe impact the future landscape faces is a replacement effect where non-native *Acer campestre* outcompete other local woodland tree species at the same time as the native population of the same species is becoming genetically swamped with loss of conservation values such as local adaptations.

Supplementary information

The online version contains a spread sheet with allele lengths, localities, year of collection, gene pool affinity and subsampling as supplementary material available at…

Acknowledgments

Our sincere thanks to the staff of the herbaria, which contributed with samples to this study: Stefan Ekman (UPS), Claes Persson (GB) and Åke Rühling (OHN).

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Statements and Declarations

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

The authors have no relevant financial or non-financial interests to disclose.

Material preparation, data collection and analysis were performed by Eric Wahlsteen. The first draft of the manuscript was written by Eric Wahlsteen and all authors commented on previous versions of the manuscript. All laboratory work was conducted at Noahgene Ltd and supervised by Halina Sobolewska.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary Files

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