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Willdenowia
Annals of the Botanic Garden and Botanical Museum Berlin

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SSR markers distinguish critically endangered *Acer campestre* populations from cryptic invading gene pools

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Abstract: Garden escapes of *Acer campestre* spread as a cryptic invading gene pools and challenge the conservation of a unique ancient population in southern Scandinavia. The native gene pool consists of just 34 individuals and is listed as critically endangered. This population is more than 150 years old and represents a unique diorama into an almost extinct genetic diversity of the early 19th century. That the native individuals cannot be separated from the introduced by morphology makes it impossible to delimit populations worthy of conservation. Genetic structuring was based on six SSR markers and reveals that, although the native population is small, it does not suffer from inbreeding. This article reports a high group affinity (Q coefficient) of the known native gene pool and a new finding of a population not earlier identified as native. Because the population is old and the fruit set is strongly reduced, it is recommended to preserve the genetic material by *ex situ* grafting and introduction of carefully chosen individuals from related gene pools.

Key words: *Acer campestre*, admixture, cryptic invasion, microsatellites, SSR

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Introduction

Cryptic invasion (Saltonstall 2002) is when alien genotypes of an indigenous species are introduced to a local ecosystem. That happens typically as a result of good intentions when restoring or developing an ecosystem and indigenous species are chosen, but not of a local provenance. Morais & Reichard (2018) recognized three major impacts cryptic invasions may have on local ecosystems: (1) hybridization and evolutionary changes; (2) replacement; and (3) novel roles in biotic interactions. The authors concluded that the consequences of cryptic invasions are not well documented. However, they called attention to the possibility that hybridization between gene pools may cause homogenization of genetic diversity and loss of potentially unique endemic genotypes. An introduced gene pool may possess superior adaptation and finally replace the indigenous gene pool. Additionally, peripheral populations/genotypes adapted to its environment during millennia may hold specific traits (e.g. climatological) not replaceable by generic or core population genotypes (Safriel & al. 1994; Kawecki 2008).

During the last few centuries, *Acer campestre* L. (*Sapindaceae*) has become a popular tree for gardens and landscaping, and new genotypes have been introduced to Sweden from Germany and the Netherlands by the nursery trade. Since the 1980s, seedlings and young individuals, presumably originating from cultivated parents, have frequently been reported also from natural areas and the species is now rather common in the coastal regions of southernmost Sweden. In spite of this, the species is listed as Critically Endangered on the national Red List (Artdatabanken 2015, 2020), although with the notion that only the native population (Fig. 1A) may be consid-

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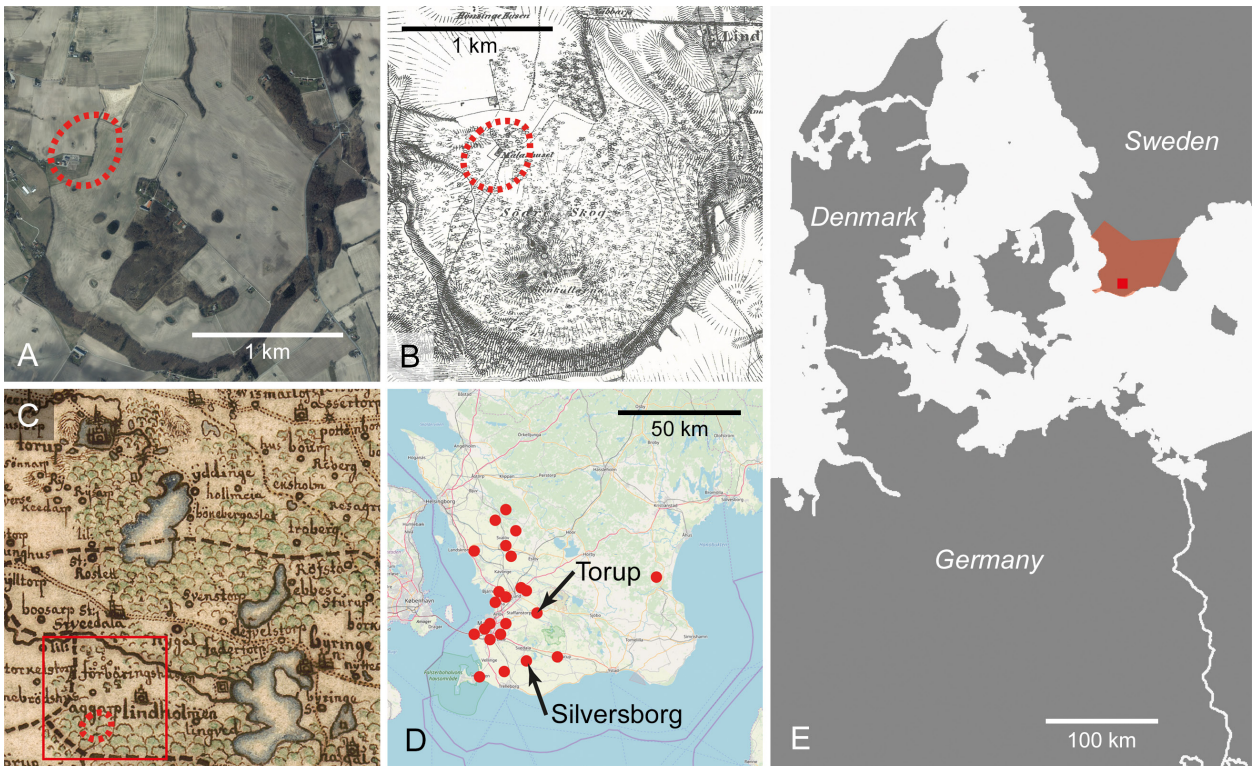

Fig. 1. A: modern aerial photography of the locality of *Acer campestre* at Silversborg (red dotted line). Only remnants of earlier forests can be identified. – B: map from 1820 (Riksarkivet) showing a broad-leaved forest covering the area (red dotted circle delimits the approximate place of the present stand of *A. campestre*). – C: map from 1684 (Burman 1684) showing a widely forested landscape (red square shows approximate location of maps A and B). – D: distribution of collected samples for this study (Silversborg). – E: map overview of northern Europe with the sampling area in red.

ered for red-listing. However, to distinguish introduced escapes of planted, alien genotypes from indigenous plants solely by morphology is not trivial, or perhaps not even possible. The need to distinguish between native and escaped *A. campestre* has therefore become urgent.

Considering a changing climate with predicted warmer and drier summers, the meso- and somewhat xerophilic nature of *Acer campestre* may be favoured and further increase its rapid dispersal. To separate indigenous genotypes from introduced ones of the same species is of major concern in a conservation context. By ratification of the Convention of Biological Diversity (CBD), Sweden has taken responsibility to protect not only species, but also vulnerable genotypes.

Acer campestre is a medium-sized, deciduous tree occurring solitary in open fields or scattered in deciduous forests, preferably on base-rich soils (Zecchin & al. 2016). The species has its northern distribution limit in southernmost Scandinavia and is therefore considered indigenous to the Scandinavian flora. The species immigrated after the last glacial maximum, and in Sweden it has been found preserved in peat bogs (Gertz 1929). Overall, the species is found south to the Mediterranean area, Algeria included, west to France and the British Isles and east to Georgia (Zecchin & al. 2016). The Scandinavian gene pool has been identified as *A. campestre* subsp. *hebecarpum* (DC.) Pax based on hairiness of the samaras (Gertz 1929; artfakta.se). *Acer campestre* subsp. *leiocarpum* Pax is distinguished by glabrous samaras and has an origin in southeastern Europe (Krüssman 1984).

The landscape of southernmost Scandinavia is highly fragmented due to millennia of farming and agriculture. The few remaining deciduous forests in southernmost Sweden are closely associated with ancient manors and were protected from firewood lumbering and cattle-grazing to keep deer for pleasure hunting. Consequently, in Sweden just one small population of about 40 individuals (at Silversborg farm) is recognized as truly native (Fig. 1A, B). This stand was recorded as early as 1749 in a dissertation by Rosenblad (1749), at that time as a part of a larger woodland area (Fig. 1C, D). This stand was cut down in 1927 and the current trees are saplings from these stumps (Gertz 1929). It is likely that several saplings arose from the same stump and therefore neighbouring trees are clones and not discrete individuals.

Microsatellite markers (simple sequence repeat, SSR) are nowadays a standard tool for analysing population genetic structure among populations, and genetic diversity within populations of closely related taxa, in particular within species. For the genus *Acer* L. there are several previous SSR studies: Chybicki & al. (2014) on *A. campestre* in Poland; Guarino & al. (2008) on several *Acer* species in Italy; Khodwekar & al. (2015) on *A. saccharum* Marshall in the U.S.A.; Segarra-Moragues &

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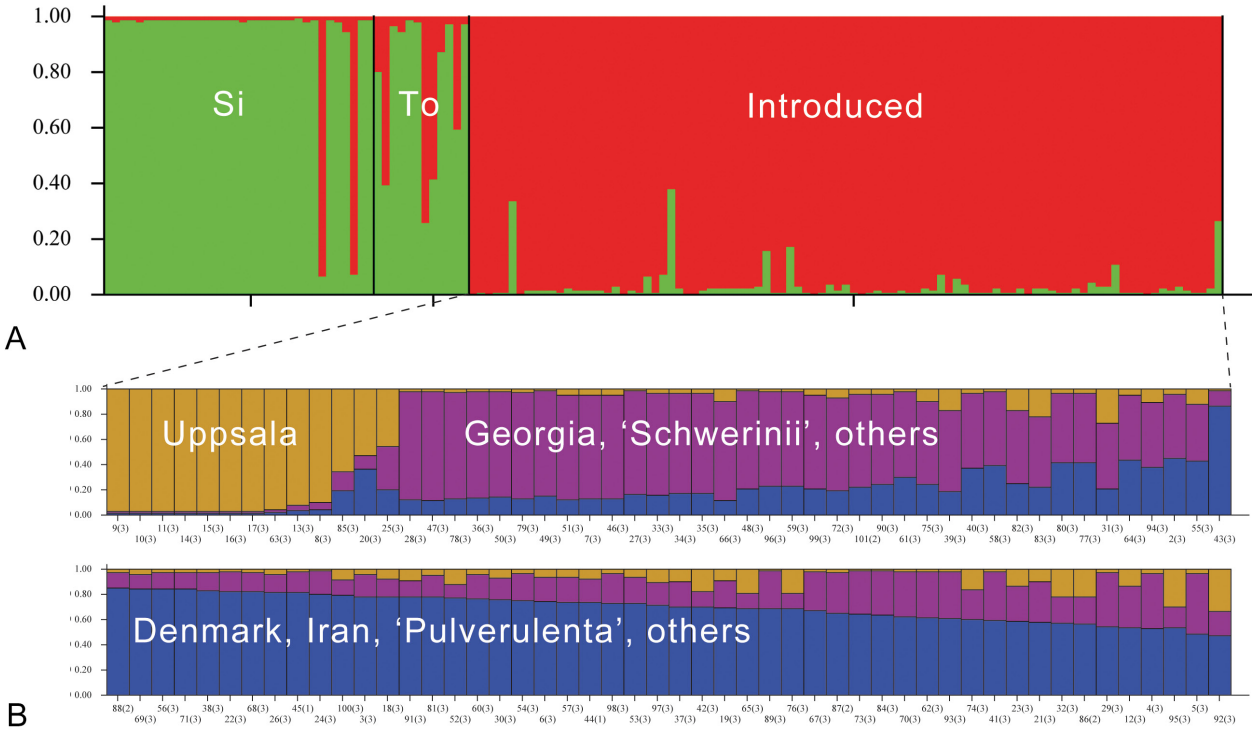

Fig. 2. A: optimal K based on the Delta K model divides the dataset into two groups. Green is dominated by the native gene pool and red by introduced. Si = Silversborg; To = Torup. – B: introduced gene pools divided into three groups (Delta K = 3) reveals a cluster of the seed strain Uppsala (brown), a cluster of samples from Georgia, the cultivar 'Schwerinii' and other planted and naturalized individuals (lilac) and a cluster of samples from Denmark, Iran, the cultivar 'Pulverulenta' and other planted and naturalized individuals (blue).

al. (2008) on *A. opalus* Mill.; and Yang & al. (2015) on *A. yangbiense* Y. S. Chen & Q. E. Yang in China. Microsatellite markers have therefore proved to serve their purpose to map genetic diversity in the genus *Acer*. In the present study, microsatellite markers are therefore chosen to assess the genetic diversity of *A. campestre*.

The objective of this study is to explore the genetic diversity of *Acer campestre* occurring in the southernmost part of Sweden and identify ancient individuals or populations worthy of conservation. Furthermore, the results may guide in choosing new seed strains to be propagated and offered by Swedish nurseries to replace the present stock of plants with unknown genetic origin.

Material and methods

Plant material

Known stands and individuals of *Acer campestre* were chosen from the national Species Observations System (https://www.artportalen.se/). In particular, populations from forest areas, areas with multiple observations or old individuals were chosen for sampling. Typically, local populations comprise a single or few mature trees and countless juvenile seedlings. The total number of samples was 150 with the average of 4.7 samples per location. A detailed list of sampled trees is presented in Supplemental content online and a simplified list in Table 1.

The sampled trees can be divided into six groups. ***Cultivated*** individuals have been planted for garden or landscape purposes. Some such trees are known to have a continental origin, imported from preferably Germany or the Netherlands to Sweden by nurseries. However, one seed strain with unknown genetic origin (Uppsala) is commonly used for landscaping with stock plants growing in Sweden. This seed strain is propagated by seeds harvested from a stand of several old trees with chosen qualities as shape of tree crown and hardiness. The offspring is a result of open pollination. ***Champion trees*** are specific individuals with an estimated age of more than 150 years and DBH (diameter at breast height) exceeding 60 centimetres. A champion tree may once have been planted, but it is more likely that it is of local genetic origin because plant material for gardening was only rarely imported in those days. Old specimens are found in present-day parks, for example at Torup castle built in 1537. ***Self-seeded*** trees are spontaneously appearing in the landscape and near urban areas. ***Indigenous*** populations of *Acer campestre* in southern Sweden are difficult to prove and at present only the Silversborg stand is by tradition accepted. ***Cultivars*** are selected individuals with certain traits as leaf colour or habit shape propagated by cloning (e.g. grafting or cuttings). Some cultivars are widely used in gardens and landscapes in Sweden and, because they are fertile, they may disperse out into the landscape. The most common cultivars are 'Elsrijk',

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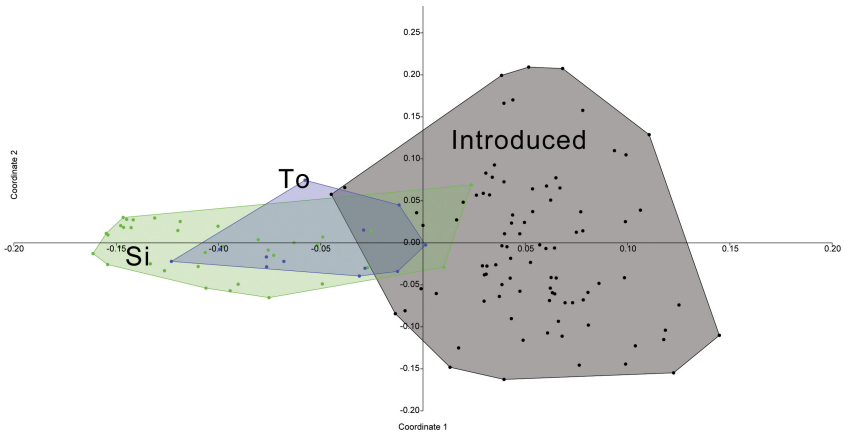

Fig. 3. Ritland kinship coefficients ordinated by PCoA show that the Silversborg and Torup samples group together versus samples from the introduced trees. PCo 1 explains 19% of the variation and PCo 2 explains 9% of the variation.

'Green Column' and 'Schwerinii', and the seed strain Uppsala (the use of simple quotation marks and capital letters follow ICNCP 9th edition (Brickell & al. 2016) to distinguish cultivars and trademarks from naturally appearing taxa). ***Continental Eurasia*** constitutes several references of populations or individuals to compare the core area with. These specimens are sampled from living collections in arboreta.

From each tree, leaves and, if present, samaras were collected in August and September. Generally young or heavily pruned individuals were just represented by leaves. The samples were dried at room temperature, labelled, vacuum sealed and stored in a regular domestic freezer until DNA extraction.

DNA extraction and SSR genotyping

Dried leaf tissue (60 mg) was disrupted by shaking at 600 rpm in the presence of a 5 mm steel bearing. Genomic DNA was extracted using a Qiagen DNeasy 96 Plant Kit (Qiagen, Manchester, U.K.) following the manufacturer's protocol. The six microsatellite loci MAP9, MAP40, MAP46 (Pandey & al. 2004), Aop116, Aop132 and Aop943 (Segarra-Moragues & al. 2008) were PCR-amplified in two multiplexes using DFS-"Hot" Taq DNA Polymerase (GeneOn Bioscience, Ludwigshafen am Rhein, Germany) in 1X PCR buffer (20 mM Tris-Cl, pH 8.3, 10 mM $(NH_4)2SO_4$, 35 mM KCl, 1.8 mM $MgCl_2$, 0.1 mg/ml BSA). Multiplex 1 (MAP9, MAP46, Aop132) was amplified using a modified touchdown PCR programme (after Chybicki & 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 a 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. PCR products were diluted 1:15 in de-ionized formamide and sized via a 3730XL DNA analyser using Genescan 400HDRox size standard (Applied Biosystems, U.K.). Exported data was analysed using Genotyper 2.0 software (Applied Biosystems, U.K.). All laboratory work was carried out at Noahgene ltd., Scotland, U.K.

Data analysis

For data analysis, the following steps were conducted: (1) clone identification; (2) genetic structuring; (3) investigation of genetic variation and diversity; (4) test morphology relative to SSR data.

To distinguish clones from individuals will help future conservation efforts and eliminate the risk that the results of this study will be swamped by unintended genotyped clones. Identification of clones was conducted in Cervus 3.0.7 software (Kalinowski & al. 2007). A single allele difference can either be caused by a genotyping error or by a somatic mutation. Therefore, in cases where just one allele in one locus was different between two individuals, they were treated as identical. The individual probability of identity is the probability given the genotype of one individual that a second individual will have the same genotype. It is calculated in two forms, one assuming that the two individuals are unrelated and a second, more conservative form assuming the two individuals are full siblings. Cervus calculates these non-exclusion probabilities for each locus and the combined non-exclusion probabilities across all loci: these represent the average probability of not excluding a single randomly chosen unrelated individual from parentage at one or more loci. All identified clones were omitted prior to further analysis.

The samples were structured by a Bayesian model-based method as implemented in Structure software (Pritchard & al. 2000). Genotypes were clustered assuming correlated allele frequencies and the admixture model. The posterior distribution was approximated with 500 000 iterations after 50 000 samples were disregarded for burn-in. The analysis was performed assuming the number of clusters K = 2–19, with 5 repetitions for each K. The optimal K was determined based on the Delta K approach (Evanno & al. 2005) using the Structure Harvester web application (Earl & von Holdt 2012). Succeeding analyses of individual Delta K clusters were conducted by K = 2–9 and optimal K was again determined by the same approach.

The statistical significance of difference between the clusters obtained from the Structure analysis was tested by a population differentiation test using the exact G test and Fisher's exact probability test (Markov chain param-

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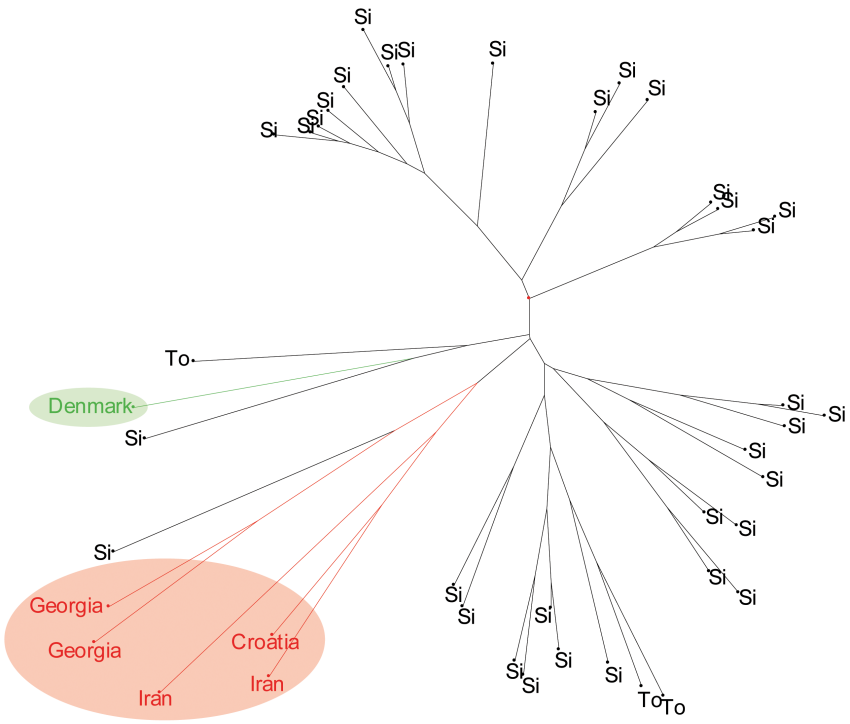

Fig. 4. A neighbour joining tree clusters the Eurasian samples together versus Denmark, Silversborg and Torup.

eters: 1000 dememorizations; 100 batches, 1000 iterations per batch, conducted in Genepop 4.2).

A threshold for native individuals was set by using the ancient population of Silversborg as a reference. This relies on the assumption that the Silversborg population was isolated from introduced gene pools by time (it was present in the middle of the 18th century) and by distance (the rural location had a great distance to gardens with introduced individuals). The threshold for the native population was therefore set to a membership coefficient (Q) of > 0.9. A similar approach was used by Schnitzler & al. (2014), whereas Sloop & al. (2011) and Reudink & al. (2007) interpreted hybrid zones from the Structure bar plot without threshold value.

The allele data was also explored by Ritland kinship coefficients (Ritland 1996) between individuals computed in SPAGeDi software (Hardy & Vekemans 2002) and visualized by PCoA (principal coordinate analysis) scatter plot in PAST 4.02 (Hammer & al. 2001). The software DARwin 6 was used to draw a neighbour joining tree based on the genetic distance simple matching (Perrier & al. 2003) for the indigenous gene pool and the Eurasian samples to explore how the indigenous individuals are related to other gene pools with known origin.

Genetic variation was investigated based on the Structure results using the standard genetic indices expected heterozygosity (H_e), observed heterozygosity (H_o), fixation index (F), inbreeding coefficient (F_{is}) and allelic richness (AR) as implemented in GenAlEx (Peakall & Smouse 2006), Genepop (Raymond & Rousset 1995) and Fstat (Goudet 1995).

Whether it is possible to distinguish the native gene pool considering the hairiness (presence of setae) of the samaras has been debated. Nevertheless, the native gene pool has been identified as *Acer campestre* subsp. *hebecarpum* based on this character. Therefore, the presence of setae on the samaras was recorded as present/absent for all samples represented by samaras (in total 71 samples, see Supplemental content online). For the same set, the Ritland kinship coefficients were calculated for the SSR data and the difference between the two groups (with and without setae) were tested by PERMANOVA (permutational multivariate analysis of variance, 9999 permutations and Euclidian similarity index) in PAST 4.02, an approach similar to the statistical comparison of the genetic diversity of populations conducted by Ritchie & al. (2014). Also, a population differentiation test was conducted using the exact G test for genotypic differentiation in Genepop 4.2 and the amount of pairwise differentiation was calculated by Fst. Because the variance tests and the G test are powerful, the methods may give a false positive result (for further discussion, see Balloux & Lugon-Moulin 2002). To assess the results further, a new Structure analysis was conducted with the same 71 samples assuming K = 2 to represent clusters with setae and another without.

Results

Characteristics of microsatellite loci

The total number of alleles per locus ranged from one (Map46) to six, indicating one monomorphic locus and five polymorphic loci (Table 2). Mean expected heterozygosity was higher than observed heterozygosity (H_e 0.817, H_o 0.640) for all loci and two loci showed Hardy-Weinberg proportions. Null allele frequencies ranged from −0.095 to 0.147. All allele sizes are presented in Supplemental content online.

Clone identification

The clone identification conducted in Cervus 3.0.7 revealed 17 samples from Silversborg identical to ten unique individuals when checked by all loci but one as

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matching. The probability of identity among individuals (p) was 4.90×10^{-5} and among siblings 0.015. After the clone samples were removed, 34 discrete individuals were left in the Silversborg population.

Genetic structuring

Optimal K based on the Delta K model revealed that two clusters (K = 2) best explained the structure of the data set (Fig. 2A), one group (1) dominated by individuals from the indigenous population (Silversborg) with individuals from Torup and the other group (2) consisted of all other samples of planted and naturalized individuals.

When cluster 1 (native gene pool) was analysed separately by Structure and optimal K was determined by the Delta K method, no strong sub-clusters were revealed. However, cluster 2 (introduced) was divided into 3 sub-clusters by the Delta K method (Fig. 2B). Sub-cluster 2.1 (brown in Fig. 2B) was totally dominated by individuals of the seed strain Uppsala, sub-cluster 2.2 (lilac) was represented by samples from Georgia, the cultivar 'Schwerinii', an individual tentatively identified as 'Schwerinii', a champion tree from Torup and several planted and naturalized individuals. Sub-cluster 2.3 (blue) was represented by samples from Denmark, Iran, the cultivar 'Pulverulenta' and several planted and naturalized individuals.

The significant difference between Silversborg and introduced was confirmed by the population differentiation test using the exact G test and Fisher's exact probability test ($p < 0.001$ for both).

The PCoA of Ritland kinship coefficients for the whole dataset showed a similar pattern as the Structure analysis (Fig. 3). Torup and Silversborg clustered together on the negative score of coordinate 1 (explaining 19% of the variation) and introduced on the positive score with some individuals overlapping (Fig. 4). Therefore, coordinate 1 may be interpreted to represent the variation between introduced and native gene pools. Coordinate 2 explained 9% of the variation and represents the variation among the introduced samples.

A neighbour joining tree drawn in DARwin 6 based on simple matching (Perrier & al. 2003) for Silversborg, Torup and Eurasian samples showed a strong clustering for the Eurasian individuals versus clusters for Silversborg, Torup and the Danish sample (Fig. 4).

Genetic variation and diversity

Based on the results from the Structure analysis, genetic variation was calculated for all samples from Silversborg regarded as a population and all others as introduced. For Silversborg, the average number of alleles calculated to 4.83, observed heterozygosity 0.62, expected heterozygosity 0.59 and fixation index –0.04 (Table 3). The global test for Hardy-Weinberg equilibrium (Markov chain parameters: 1000 dememorizations; 100 batches, 1000 iterations per batch) was neither significant due to heterozygote deficit nor excess. The introduced group showed a higher average number of alleles and higher allelic richness, but also a somewhat higher expected than observed heterozygosity (Table 3).

Morphology

The PERMANOVA with 999 permutations for Ritland kinship coefficients gave $p < 0.001$, total sum of squares 22.55, within-group of squares 21.66 and F 2.844. The exact G test for genotypic differentiation gave $p < 0.001$ (Table 4) and Fst among the groups gave 0.016. However, the result of the Structure analysis confirmed the low Fst in no clear clustering inferring presence of setae. The best run of three gave a cluster ($Q > 0.5$) with 60% of the samples with setae and the other ($Q < 0.5$) with 16% with setae.

Discussion

The objective of this study was to explore the genetic diversity of *Acer campestre* in the southernmost part of Sweden and identify ancient individuals or populations worthy of conservation. The most important findings of genetic structuring, variation, morphology and conservation will be discussed here.

The native gene pool showed a similar allelic richness to *Acer campestre* in Poland using the same SSR markers (4.80 versus 4.56) (Chybicki & al. 2014) but a higher observed heterozygosity (0.62 versus 0.38 to 0.65, mean 0.47) indicating a reasonably high genetic variation. Results from Italy covering six *Acer* species show a contrary low genetic variation with just 1.14 average number of alleles and low observed heterozygosity (0.29) (Guarino & al. 2008), results confirmed by Ducci & al. (2010) reporting average number of alleles to 1.86 and observed heterozygosity to 0.19.

Low measure of F_{is} in the Silversborg population suggested random mating but also indicated a small excess of heterozygotes, which is to be expected in a small population of an outcrossing species. However, this may be interpreted as a stochastic effect because the population is small. That the observed heterozygosity exceeds the expected heterozygosity is in accordance with the just partial self-compatibility system and promotes avoidance of inbreeding and the maintenance of intra-population genetic variability. Similar patterns are described in e.g. *Sorbus torminalis* (L.) Crantz (Jankowska-Wroblewska & al. 2016).

Although the population at Silversborg is small (34 unique individuals) the excess of heterozygotes indicates that the population does not suffer from inbreeding. This may be explained by the present stand being a small, relict fragment of a once large population with high genetic variation and today a very low regeneration.

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Table 1. Accessions of *Acer campestre* used in this study. Cult. = cultivated in city, garden or park; Cvs. = cultivars selected for gardening; Cham. = champion tree, estimated age > 150 years, DBH > 60 cm; Self. = self-seeded juvenile plant; Indi. = indigenous; Eurasia = reference samples from continental Eurasia. Total number of samples: 150.

Locality	Origin	No. of samples	Coordinates (WGS84)
Sweden			
Burlöv, Stjärnelundsvägen	Cult., Self.	3	55°37'10.8"N, 13°05'26.3"E
Kristianstad, Maglehem	Self.	1	55°45'04.78"N, 14°09'09.60"E
Kävlinge, Lilla Harrie	Cult., Self	6	55°47'21.8"N, 13°12'11.9"E
Kävlinge, Södervidinge	Self.	3	55°49'29.4"N, 13°05'38.1"E
Landskrona, Saxtorp	Self.	1	55°50'02.0"N, 12°57'27.9"E
Lomma	Cult.	4	55°40'43.5"N, 13°04'16.9"E
Lomma, Alnarp	Cult.	1	55°39'27.2"N, 13°04'49.1"E
Lomma, Habo ljung	Cult., Self.	6	55°41'34.8"N, 13°03'24.1"E
Lund, S:t Hans, Klosterängshöjden	Cult., Self.	4	55°43'31.3"N, 13°13'29.0"E
Lund, Stora Råby	Cult, Self.	6	55°41'17.6"N, 13°13'23.4"E
Malmö, Abbekåsgatan	Cult	1	55°35'32.80"N, 13°01'16.27"E
Malmö, Almåsa	Cult., Self.	3	55°33'53.6"N, 13°08'19.1"E
Malmö, Bunkeflo, Brofästet	Cult., Self.	3	55°33'46.1"N, 12°54'38.0"E
Malmö, Kungsparken	Cham.	2	55°36'13.4"N, 12°59'31.4"E
Malmö, Norregatan	Cult.	3	55°36'22.60"N, 13°00'35.41"E
Skurup, Stjärneholm	Cham.	6	55°29'17.0"N, 13°27'19.6"E
Svalöv, Källs-Nöbbelöv	Cult.	3	55°53'32.9"N, 13°04'49.5"E
Svalöv, Norrvidinge	Cult., Self.	5	55°50'45.2"N, 13°06'55.3"E
Svalöv, Trolleholm	Self.	2	55°54'51.12"N, 13°16'17.42"E
Svedala, S Lindholmen, Silversborg	Cham., Indi.	45	55°29'03.1"N, 13°15'09.4"E
Svedala, Värby, Bara, Torup	Self. Cham.	12	55°34'01.4"N, 13°12'46.0"E
Vellinge, Höllviken, Räng, Ljunghusen	Cult., Self.	5	55°23'16.7"N, 12°54'53.1"E
Vellinge, Östra Grevie	Self.	3	55°27'59.6"N, 13°08'22.6"E
Other countries			
Croatia, Papuk	Eurasia	1	45°30'32.5"N, 17°39'40.4"E
Denmark, Fakse Bugt, Roneklint	Eurasia	1	55°07'34.1"N, 12°07'24.3"E
Georgia, Tiblisi	Eurasia	2	41°51'05.4"N, 44°54'27.5"E
Iran, Elbrus Mts., Kandavan Pass, Siah Bisheh	Eurasia	2	36°12'43.4"N, 51°18'30.8"E
Cultivars			
'Elsrijk'	Cvs.	1	N/A
'Green Column'	Cvs.	4	N/A
'Pulverulenta'	Cvs.	1	N/A
'Schwerinii'	Cvs.	1	N/A
Uppsala (seed strain)	Cvs.	11	N/A

The result of the genetic structuring revealed that most trees from Silversborg clustered together, confirming a genetic affinity among the trees (Fig. 2A). Although K = 2 indicated two individuals with low Q to the Silversborg group, a separate run for just Silversborg gave no clear structuring. That several individuals from the castle of Torup strongly grouped with Silversborg may indicate a past genetic bridge when the distribution of the species was widespread. Torup castle has a history from the early 16th century and the forests have been managed for hunting and therefore saved against firewood lumbering. The grandest individual of *Acer campestre* in southern Sweden, by the moat of Torup, showed a strong affinity to the Silversborg group with a high Q coefficient.

When the relation between the indigenous individuals of Silversborg and Torup was tested with the Eurasian samples, the only Danish sample clustered with the indigenous (Fig. 4). The Danish sample had a moderate affinity (Q 0.38) to the Silversborg population when K = 2, indicating a past linkage. The relation to Danish and other continental populations would be of great interest to investigate in the future to better understand the dispersal of the species.

When the native gene pool was compared to the remaining samples of planted and naturalized trees, it appears that the average number of alleles is much higher (11.50 versus 4.83, Table 3) and the expected heterozygosity is higher than for the indigenous. This is typical when comparing small, indigenous populations to several introduced gene pools that originate from various cultivated and natural gene pools, e.g. Kang & al. (2007), Rosenmeier & al. (2013) for *Cytisus scoparius* (L.) Link, Valtuena & al. (2011) for *Meconopsis cambrica* (L.) Vig.

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Table 2. Characteristics of microsatellite markers for the native gene pool. Range in base pairs, number of observed alleles (A_{obs}), observed heterozygosity (H_o), expected heterozygosity (H_e), Hardy-Weinberg equilibrium test (HW, dash indicates that test was not conducted), null allele frequencies. Computed in Cervus.

	Range	A_{obs}	H_o	H_e	HW	Null allele frequency
Aop132	124–155	6	0.658	0.775	–	0.0763
Map46	153	1	0	0	–	–
Map9	99–108	4	0.500	0.681	NS	0.1467
Aop116	112–116	3	0.789	0.671	NS	–0.0950
Aop943	135–151	4	0.658	0.711	–	0.0330
Map40	114–174	6	0.892	0.771	–	–0.0806
Mean	–	4	0.640	0.817	–	–

Table 3. Genetic structure of studied populations. Average number of alleles for each population, observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F), inbreeding coefficient (F_{is}), allelic richness (AR). Computed in: [1]GenAlEx 6.5, [2]Genepop 4.2, [3]Fstat 2.9.4.

Population	Average no of alleles[1]	Ho[1]	He[1]	F[1]	Fis[2]	AR[3]
Native gene pool	4.833	0.62	0.59	–0.05	–0.04	4.83
Introduced	11.50	0.50	0.68	0.27	0.27	10.15

Table 4. Result of the exact G test for genotypic differentiation between groups of samaras with or without setae.

p-value						Chi[2] (all)	*p* (all)
Aop132	Map46	Map9	Aop116	Aop943	Map40	42.7	0.0000257
0.09841	0.6485	0.00182	0.27776	0.00237	0.0071		

The K = 2 model derived from Structure (Fig. 2A) show a very low admixture between the introduced group and the Silversborg population, although two individuals had a Q for the group lower than 0.07. The very low Q value for these individuals indicates an affinity to other gene pools. One probable reason for the low Q may be that those individuals have been planted later. However, such events have not been recorded. Both of the two individuals group with the lilac cluster (Fig. 2B) in the Structure K = 3 analysis, but it is not possible to make any strong conclusions on origin because both the lilac and blue groups contain individuals with Eurasian origins. Further, the Q-values should be interpreted carefully because the number of SSR markers are low with just low to moderate polymorphism. However, The Torup population has a high admixture with the introduced group, probably because of recurrent planting during the last decades. Several individuals have a close affinity to Silversborg and may be remnants of a large, ancient population. Admixture between the indigenous population and the introduced are especially evident at Torup. One champion tree planted east of the castle shows a high affinity to the lilac group with samples from Georgia and the cultivar 'Schwerinii'; evidently this tree has an introduced history to the park.

The introduced gene pools are compound by several groups, and most samples do not have a strong affinity according to the Structure analysis. However, the seed strain Uppsala commonly used in cities and gardens groups clearly with high Q value (brown, Fig. 2B). This is natural for a seed strain where propagation is made from a small population of stock trees.

For the introduced gene pools there is generally a negligible admixture from the indigenous group, but low Q-values may be found in some samples. The Danish sample (Q 0.38 to native) probably indicates remnants of a past, extinct population bridging the two countries. The traces of the native gene pool in the cultivar 'Pulverulenta' (Q 0.34 to native) which can be traced to the Muskau Arboretum in Poland and Germany as early as 1864 is harder to explain. Remaining samples with admixture from the native gene pool are from a plantation in an urban area (Q 0.18) and an overgrown old nursery (Q 0.11 to native), both difficult to explain the traces of the indigenous gene pool. The generally negligible admixture of the indigenous gene pool to the introduced is most easily explained by the large distance and isolation of the indigenous population. The Silversborg population is surrounded by farmlands several kilometres to the next stand of *Acer campestre*, a crucial constraint for an insect-pollinated species dispersed by wind. Therefore, the admixture at Torup is more logical with a history of gardening and conservation of forests for hunting.

The division of the species based on hairs on the samara has a history from the late 19th century (Schwerin 1893). *Acer campestre* subsp. *leiocarpum* is distinguished by glabrous samaras and has an origin from southeastern Europe (Krüssman 1984). *Acer campestre* subsp. *hebe-*

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carpum has hairy fruits and is considered indigenous to Scandinavia (artfakta.se). Individuals with hairy fruits are found in half of all locations investigated, southern origins included (Croatia and Georgia) and the Silversborg population. To identify native trees solely based on this character is therefore not reliable. The investigated samples differed significantly in the variance test and the exact G test, but the result could not be confirmed when the morphological data was inferred to the clusters generated by Structure suggesting a false positive result. The G-test can detect very fine differences of allele frequencies among subpopulations. Accordingly, it is not surprising to find significant genetic differences among a set of subpopulations, even if these differences may not necessarily be biologically meaningful. Fst among the two groups was calculated to 0.016, indicating a very low differentiation between the groups with and without setae. Consequently, the design used here to test differentiation between individuals with and without setae did not contribute to solve the question of taxonomic level of the two subspecies.

The conservation issues for the species in southern Sweden may be delimited by the results of this study. The problem of distinguishing native and introduced gene pools in the field may not be a big problem because the results show that only two localities keep the native gene pool (Silversborg and Torup). The sampling in this study covers probably the most essential parts of southern Sweden where native gene pools may be expected to appear. So long as Silversborg and Torup are not lumbered, the gene pool would be saved for several decades. However, all trees in the proven native gene pool are old, most more than 100 years, and just a few produce seeds regularly. When visiting Silversborg in 2019, just nine individuals were fruiting and most of the pairs of the samaras were reduced to just a single samara with a viable seed. The reason for this is probably not the genetic structure, because a slight excess of homozygotes does not indicate inbreeding depression. The most likely reason for limited fruiting is probably the age of the trees.

A possible back up for the unique genetic diversity of the native gene pool could be an *ex situ* plantation of grafted individuals. This will not save the gene pool from an approaching climate change or evolve the gene pool over time, but would back up the genetic material. Such a gene bank might be combined with carefully chosen individuals from other Scandinavian populations to facilitate an artificial gene flow. The gene bank might later serve as a seed bank for propagation of a lineage adapted for Scandinavian gardens and parks.

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Supplementary File S1. Localities, coordinates and allele sizes, Microsoft Excel spreadsheet (XLSX format).

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