Acta Biologica Plantarum Agriensis
ISSN 2061-6716 (Print), 2063-6725 (Online)
https://ojs.uni-eszterhazy.hu/index.php/ABPA
https://doi.org/10.21406/abpa.2023.11.1.396

11(1): 396–416 (2023)
Dedicated issue
Research article

MOLECULAR DIVERSITY AND GENETIC LINEAGE OF *PINUS SYLVESTRIS* POPULATION AT KUNPESZÉR, KISKUNSÁG NATIONAL PARK

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Abstract: We analysed genetic variation of the small Scots pine population from the forested area of Northern Kiskunság in comparison with other pine populations from the Carpathians and the Pannonian Basin that we studied earlier by microsatellite markers (SSR). Our results showed that genetic indices based on the five nuclear SSRs are similar to the values detected in other Hungarian pine populations. Based on STRUCTURE analysis, as well as PCoA the population from Northern Kiskunság fits into the Western Carpathians-Pannonian genetic lineage and we did not detect alien genoptypes as well as population specific genotypes. Although, the natural presence of Scots pine cannot be traced back to LGM/early Holocene, the habitat characteristics like the nutrient-poor sandy substrate and the extreme ecological conditions of the site may favor the natural presence of the species in the area. The local forest community including broadleaf species with the dominance of pedunculate oak mixed with Scots pine provide an aspect of a continental woodland community able to preserve valuable species and so an elevated biodiversity. Moreover, gene stock most probably adapted to the local environment might represent a high natural and economic value.

Keywords: Scots pine, heterozygosity, genetic lineage, landscape history, forest steppe

INTRODUCTION

Understanding patterns of genetic diversity and the current distribution of plant populations needs the study of past historical events and climate oscillations acting on the development of the vegetation on large and small geographic scales (Hewitt 2004; Birks-Willis 2008; Petit *et al.* 2008). Historical climate shifts forced species to migrate and to find suitable habitats, consequently their area have changed temporally as well as spatially (Cheddadi 2006; Gömöry *et al.* 2020; Feurdean *et al.* 2013). Phylogeographic investigations based on molecular methods were able to reconstruct historical genetic lineages of species from the Mid-Pleistocene to Early-Holocene and revealed the complex history and genetic structure of contemporary populations (Magri *et al.* 2006; Bagnoli *et al.* 2015; Lendvay *et al.* 2016; Tóth *et al.* 2019; Wachowiak *et al.* 2022).

Scots pine (*Pinus sylvestris* L.) is one of the most widely distributed tree species, with continuous presence along the Eurasian boreal region. The large temperate taiga forests of high economic importance however are considered to be of recent origin as they mostly developed in the last 14000–6000 years, during the climate warming of the Holocene when the glacial ice sheet has retreated and provided new habitats for boreal species in Northern Europe (Kullman 2008). Colonization of the northern regions by the boreal species was reported to originate from multiple directions, following several migration routes mostly from the southern regions (Bennett *et al.* 1991). For conifer species like Scots pine, the concept of surviving in small northern refuge territories was also accepted (Parducci *et al.* 2012). Based on molecular studies putative glacial refugia of the most widespread genetic lineages of Scots pine were detected from central continental Europe and authors report that colonization presumably originated from the (Sub) Mediterranean areas like the Balkan Peninsula, from around the eastern Alps and the surroundings of the Danube plain (western Pannonian Basin) (Cheddadi *et al.* 2006).

In the Early-Holocene when natural distribution of species changed dramatically, droughts along with rising temperatures, wildfire regimes strongly affected the survival of cold tolerant species in Europe. Increasing frequency of fires led to a shift from conifer towards deciduous tree dominance (Feurdean *et al.* 2012, 2020). Compelling evidences showed that particular characteristics of vegetation cover, especially percent of tree cover, were strongly related to biomass burning in the Holocene. This period had also a major impact on the population structure of Scots pine in Central-Europe. With its large ecological tolerance Scots pine was reported to withdraw into edaphically extreme habitats forming small, fragmented populations (Tóth *et al.* 2017). Started in the Bronze or Iron Age and reaching its maximum during the early modern period, land use intensification by humans caused further ecosystem hazards and deforestations. Forests were eradicated for the establishment of agricultural territories, urban centers, early industries and mining activities. East-Central Europe was strongly affected (Magyari *et al.* 2012). Moreover, in the need of timber, deforestation was followed by reforestation activities in Europe during the last more than 300 years, leading to seed exchange and seed trade (Koskela *et al.* 2014). These human activities strongly overcasted the natural distribution of the original genetic lineages. In consequence, the historical climatic change, along with the past and recent human activities led to ecosystem transformation or even to total decline at some localities, and the naturalness of the European forests changed. Most of the forests in EC Europe that persisted or have been reforested are managed forests. However, forests with various status of naturalness and with different species composition, including not only native species, have become into the focus as forests are considered the main contributors in mitigating the ongoing climate change.

Well-structured and species-rich forest communities are resistant (capable of self-maintenance), and their native populations are able to suppress invasive non-native species' propagation that would disintegrate community structure. Species that were native in some habitats but were removed, tend to recover when the land is not disturbed and may have competitive advantage against introduced species. Moreover, they are able to build up long-lasting ecosystems (Meli *et al.* 2017).

The concept of what constitutes a native species and as result, what is its present conservation value has become increasingly relevant due to recent challenges of biodiversity loss (McGeever *et al.* 2016). Forests of high age and with developed structure are considered valuable in terms of biodiversity conservation as they provide habitat for many species. While the naturalness of the forests can be characterized, by applying exact (objective and replicable) methods, the question whether or not a species is native to a region, might not be easily answered. Buried in the soil and conserved by reductive soil milieu, pollen records, plant remants and charcoal can provide important evidences for the presence or for the long-term persistence of some species at particular areas.

Unfortunately, at some locations where these conditions are not present, the clarification might be much more difficult. In addition to that, historical documents, military maps can be useful tools to tackle the changes in the vegetation during the past centuries (Molnár 2019; Molnár *et al.* 2022).

As in most parts of Europe, in the Pannonian Basin and the Carpathians, following the climate warming of the Holocene, Scots pine populations gradually declined and have been preserved in isolated, ecologically marginal habitats. These extreme habitat types are considered post-glacial refugia, where clear evidence has been obtained considering the continuous presence of the species. Historical decline of Scots pine populations was revealed mostly by palynological records (Willis 1994; Birks-Willis 2008), but the climate warming with a combination of other drivers like human activities including burning, deforestations or implementing plantations of non-native species or non-native genetic lineages of native species, led to a more complicated picture in the present distribution of the species. Although most existing populations of Scots pine today are considered to originate from direct forestations consisting of non-native genetic lineages, some surviving, native populations of this species (consisting of native genetic lineages) or once existed natural habitats of Scots pine are also presumed to persist from the beginning of the Holocene. In the western part of the Pannonian Basin, mostly at the premontaneous sites of the Eastern Alps ecological conditions and the edaphic conditions have been reported to be favorable for Scots pine. At these sites Scots pine forms mixed forests with broadleaf species like beech (*Fagus sylvatica*) and sessile oak (*Quercus petraea*) (Pócs 1967; Szmorad 2011). Moreover, the sandy substrate of the Great Hungarian Plain can be mentioned as a site characterized with edaphic conditions which might have favored the persistence of Scots pine throughout the Holocene. Indeed palynological-based vegetation reconstructions from the Kolon lake at Kiskunság report that *Pinus* pollen was continuous, circa one-third of the sum arboreal pollen consisting of *Pinus.* However, human impact in the last 8000 years was alarmingly intensive and the area near the Kolon lake was transformed gradually, making unsighted the distribution of the arboreal vegetation (Sümegi *et al.* 2022).

Arguments and debates have been preceeded the origin of Scots pine population at Fenyőfő sand area at the bottom of the Bakony

Hills (West-Hungary). Models characterizing the fundamental and the realized ecological niche of Scots pine, suggest that this Hungarian site is located within the optimal or close-to-optimal climatic conditions for Scots pine. Also the natural regeneration of Scots pine in Fenyőfő confirms that environmental conditions in this region are particularly favorable for this species (Misi *et al.* 2019). Although the presence of introduced (i.e., non-native) genetic lineages of Scots pine is indisputable at these locations, individuals of the old forest exhibit special growth and morphological characteristics and build up a forest steppe-type community structure (Majer 1988).

Molecular studies performed on the remnant, natural Scots pine population of the Carpathians and the Pannonian Basin were conducted with the aim of characterizing the genetic diversity of populations. Chloroplast and nuclear SSR markers revealed two genetic lineages. One lineage is represented by the Eastern Carpathian populations having strong links to the Balkan and extending towards the nordic regions. Another lineage comprises populations from the Western Carpathians, the Tatra and those of the Pannonian Basin including Fenyőfő. The later genetic lineage most probably originates from an East Alpine glacial refugia (Tóth *et al.* 2017, 2019).

On the outskirts of the sand dune area of the Kiskunság National Park, in the forests communities Scots pine is present together with pedunculate oak trees forming forest-steppe type habitats. The Peszér forest and the other studied sites are located at the central part of the area between the Danube and the Tisza and are dominated by small patches of forests and mosaics of thickets with sand grasslands. Young eolian deposits of sand and loess form a deep substrate of approx. 100–120 meters making available the development of forests. Palynological records from the Selyemrét site (Dabas region), including C^{14} dating, reported significant amount of conifer pollen including Scots pine starting shortly after the LGM (Törőcsik *et al.* 2018). However, at the beginning of the Holocene pollen composition markedly changed in favor of broadleaf tree species like *Quercus, Corylus, Tilia, Fraxinus.* Forests community promoted locally the development of brown soils that persisted until the present.

For the period some 3400 years ago, a significant decrease of tree pollen was reported from the Selyemrét site. In this period most probably, the woody vegetation was already influenced by the increasing human activity in the area. Historical evidences starting from the Bronze Age are sporadic. However, it is presumed that forests were present in a matrix of grass community even during the last centuries (Sümegi *et al.* 2012). There are no evidences for continuous presence of Scots pine in the area but this highly tolerant pioneer species could hypothetically be sporadically present in some cryptic habitats starting from the Late Pleistocene. Pollen diagrams also have shown continuous presence of *Pinus* diploxylon in the region (Kustár *et al.* 2016). However, the poor representation of the pollen records on the young, mostly Pleistocene deposits of the Pannonian Basin as well as the wind dispersed pollen and seeds of the planted Scots pine stands have blurred clear resolution. Archive documents reported Scots pine plantations (Kerner 1877, Babos 1972).

The aim of this study was to evaluate genetic diversity and haplotype distribution of the Scots pine population in the protected forest steppe area of the Northern Kiskunság. Our hypothesis is that despite the millennia-long human disturbances, species' recovery and the long-term persistence of Scots pine populations was probable due to favourable habitat characteristics and the close-to optimal climatic and edaphic local conditions existing in the region.

We intended to test if the genetic composition of the stand integrates in one of the genetic lineages formerly described from the Carpathian Basin.

MATERIALS AND METHODS

Plant material and microsatellite genotyping

For the present study we sampled *Pinus sylvestris* individuals from the area of Kunpeszér, which consists of patches of mixed, pedunculate oak-pine forest (*Figure 1*). We collected needles from the oldest individuals altogether 25, by keeping a distance at least 100 meters. Samples were supplemented with the genetic data of 361 additional individuals (17 populations) from a previous study (Tóth *et al.* 2017, *Table 1*) for the data analysis. The additional populations came from the Central and Eastern European region, such as the Carpathians and the Pannonian Basin, one population from the northern region of the species distribution, Estonia, and a population from Bulgaria. Total genomic DNA was isolated from dried needles of 25 *P. sylvestris* individuals with the E.Z.N.A. SP Plant DNA Kit (Omega Biotek, Norcross, GA, USA) following the manufacturer's recommendations.

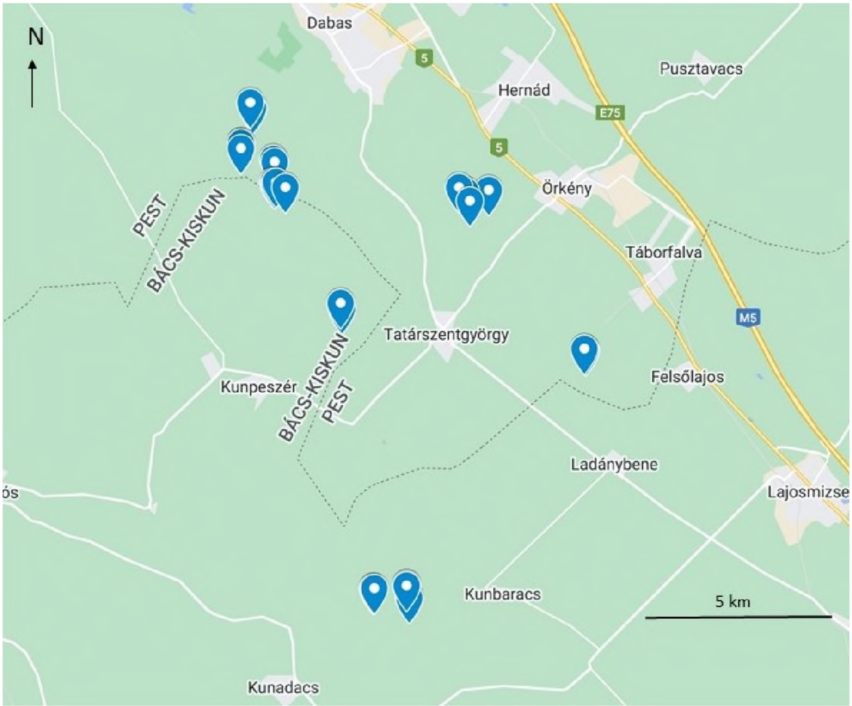

Figure 1. Sampled *Pinus sylvestris* individuals from the area of Kunpeszér, Kiskunság National Park. (Map: Google Maps).

The molecular diversity of the 386 Scots pine tree was analysed using five nuclear microsatellite (nSSR) markers (psyl16, psyl17, psyl19, psyl36, psyl42, Sebastiani *et al.* 2012), that proved to be variable in the former study (Tóth *et al.* 2017). Pine samples from the former study were re-ran together with the new samples to have the cross-check of the sizes.

Table 1. List of the 18 populations *of Pinus sylvestris* included in the microsatellite study.

Code	Country	Region	Residential area	Latitude (°N)	Longitude (°E)
HKU	Hungary	PB	Kunpeszér	47.08	19.31
HFE	Hungary	PB	Fenyőfő	47.35	17.77
HVE	Hungary	PB	Pethőhenye	46.87	16.92
HZA	Hungary	PB	Szalafő	46.87	16.30
HOR	Hungary	PB	Csörötnek	46.93	16.35
HKO	Hungary	PB	Kőszeg	47.34	16.45
RFE	Romania	EC	Fantana, Brazilor	46.50	25.26
RPO	Romania	EC	Poiana, Stampei	47.30	25.12
RMO	Romania	EC	Baile Tusnad	46.13	25.91
RCO	Romania	EC	Lacu rosu, Bicaz	46.80	25.79
RPA	Romania	SC	Voineasa	45.38	23.91
RBI	Romania	CIM	Rosia	46.84	22.37
SKV	Slovakia	WC	Kvacany	49.18	19.54
SME	Slovakia	WC	Zuberec	49.27	19.63
STU	Slovakia	WC	Svarin	49.02	19.91
SLI	Slovakia	WC	Liptovszky Hrádok	49.04	19.74
BYU	Bulgaria	RL	Yundola	42.07	23.83
ESE	Estonia	CE	Selgise	58.58	27.00

RL: Rila Mountains, CE: Central Estonian Plain, PB: Pannonian Basin, WC: Western Carpathians, EC: Eastern Carpathians, SC: Southern Carpathians, and CIM: Central-Island Mountains (Apuseni).

Amplification of microsatellite loci was performed in a 22.75 µl PCR mix with 1 µl genomic DNA, 15.4 µl Mili-Q ultrapure water (Merckmillipore, Billerica, MA, USA), 2.5 µl (10x) Dream Taq Green PCR buffer (ThermoFisher, Waltham, MA, USA), 0.5 µl (10 mM) dNTP mix (ThermoFisher, Waltham, MA, USA), 1 µl (2.5 mM) MgCl2, 0.25 µl (1%) bovine serum albumin (Termo Fisher Scientific, Waltham, MA, USA), 1-1 µl (10–10 mM) from each primer and 0.1 µl Dream Taq GREEN DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA). The amplification profile consisted of a first step at 94°C for 4 min, and 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 40 s, followed by a final 72°C for 8 min. Amplification were performed on an AerisTM Thermal Cycler (Esco Micro Pte., Singapore). Microsatellite fragments were separated in an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and genotype scores were obtained with the Peak Scanner Software 1.0 (Applied Biosystems) and manual inspection. We cross-checked the nSSR size variants by running together the samples from the earlier and recent study.

Data analysis

We calculated standard diversity indexes (Na: number of alleles, Ne: number of effective alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F: fixation index) for each stand and the analysis of molecular variance (AMOVA) was performed using GenAlEx v6.5 (Peakall and Smouse 2012). Genetic differentiation was calculated following Weir and Cockerham (1984) with the R package *hierftat* (Goudet 2015). Population divergence represented by the pairwise F_{ST} were used for hierarchical cluster analyses and represented by dendrogram with the '*stats*' package (R Team 2013).

We also calculated Nei's genetic distance between populations using GeneAlEx v6.5 for principal coordinates analysis (PCoA) to get a summary of the population genetic structure.

The BARRIER 2.2 program (Manni *et al.* 2004) was used to tackle the geographic regions with pronounced genetic discontinuity between the populations, i.e. the barriers. The geographical coordinates and genetic distances from the SSRs of the 386 samples were connected by Delauney triangulation in the way that each connection had an associated distance. Using Monmonier's maximum distance algorithm, barriers were then identified.

At individual level, inter-population structure among the population of Kunpeszér and other 17 population from the previous work (Tóth *et al.* 2017) was investigated using Bayesian clustering with STRUCTURE v.2.3.4. (Pritchard *et al.* 2000) by testing 10 independent runs for a given number of inferred clusters K, from K = 2 to 18. STRUCTURE runs consisted of 500000 MCMC generations, after a burn-in period of 100000 iterations with LOCPRIOR model described by Hubisz *et al.* (2009). Admixture model and uncorrelated allele frequencies model were used. STRUCTURE HARVESTER (Earl and von Holdt 2012) was used to apply the Evanno method (Evanno *et al.* 2005) to detect the value of K that best fit the data in case of STRUCTURE runs. The 10 runs of the best K were averaged and visualized with the web application Pophelper (Francis 2017).

RESULTS

Genetic variation, as estimated by expected heterozygosity (H_E), differed moderately among populations (*Table 2*), ranging from 0.38 to 0.57 with a slightly lower value below the mean (H_E=0.50) in the Kunpeszér population (HKU) (H_E=0.48). Slightly higher observed heterozygosity values were estimated ranging from 0.43 to 0.66 (mean H_O=0.51) with a higher value than the mean at HKU (H_O=0.56). Including the study population, some populations (HKU, HZA, STU, SLI, SKV, RCO, RBI, RMO and RFE) showed higher H_O values than H_E, indicating heterozygous excess. F values were low, (F=–0.18) to moderate (F=0.10) with a negative F (–0.12) at HKU. Mean number of alleles (Na) was 4.54 (ranging from 3 to 6) with a slightly higher value at HKU (Na=5.40), while number of effective alleles was slightly below the mean (Ne=2.74, ranging from 1.99 to 3.4) at HKU (Ne=2.57).

AMOVA analysis including all populations showed that 22% of the genetic variance resides among populations and 77% within populations (*Table 3*).

Genetic differentiation among populations is shown in *Figure 2*. Higher differentiation was observed at some East- and Southern Carpathian populations (RFE, RPO, RMO, RPA), but among these the weakest differentiation ($0.02 < F_{ST} < 0.03$) was detected at RCO. Also, higher population differentiation was revealed in the Western Carpathian populations (SLI, SKV, SME and STU). The differentiation of HKU from the other Hungarian populations was slightly stronger ($0.03 < F_{ST} < 0.07$, HZA, HOR, HFE) and the strongest with HKO (F_{ST}=0.09). The differentiation among the other Hungarian population was weaker ($-0.01 < F_{ST} < 0.04$).

Table 2. Genetic diversity indices in the 18 *Pinus sylvestris* populations. (Abbreviations: N: number of samples, Na: Number of alleles, Ne: number of effective alleles, H_E: Expected heterozygosity, H_O: Observed heterozygosity, F: fixation index. Minimum, maximum, and mean values across the populations are also shown).

Population	N	Na	Ne	H_O	H_E	F;
HKU	25	5.4	2.57	0.56	0.48	-0.12
HFE	22	4.6	2.78	0.47	0.52	0.03
HVE	19	5	2.86	0.5	0.55	0.1
HZA	18	4.6	2.61	0.5	0.47	-0.06
HOR	22	5	2.95	0.45	0.5	0.04
HKO	17	6	3.4	0.53	0.54	-0.01
RFE	18	3.4	2.05	0.52	0.46	-0.11
RPO	14	3.8	2.16	0.44	0.44	0.04
RMO	19	3	1.99	0.47	0.38	-0.18
RPA	14	4.4	2.98	0.43	0.51	0.09
RBI	7	3.8	2.61	0.51	0.49	-0.04
RCO	13	4.2	2.77	0.64	0.54	-0.15
SKV	18	4.6	3.08	0.53	0.5	-0.05
SME	20	4.4	2.86	0.52	0.52	0.03
STU	19	5.2	3.37	0.58	0.56	-0.06
SLI	10	4.6	2.94	0.66	0.57	-0.15
BYU	25	4.8	2.64	0.51	0.51	-0.03
EST	27	5	2.63	0.44	0.47	0.03
MIN	7	3	1.99	0.43	0.38	-0.18
MAX	27	6	3.4	0.66	0.57	0.1
MEAN	18	4.49	2.75	0.51	0.50	-0.03

Table 3. Summary of the AMOVA results for the 18 *Pinus sylvestris* populations.

Source	df	SS	MS	Est. Var.	%
Among Pops	17	4339062.398	255238.965	5146.160	22%
Among Indiv	368	13042864.794	35442.567	17640.973	77%
Within Indiv	386	62000.000	160.622	160.622	1%
Total	771	17443927.192	-	22947.755	100%

In the previous study by Tóth *et al.* (2017), based on the F_{ST} values two main groups were detected. One group consisted of the Hungarian populations (HFE, HVE, HOR, HZA, HKO, including HKU), populations from the Western Carpathians (SKV, SLI, SME, STU), one population from the rocky surface of the Eastern Carpathians (RCO) and RBI from the Western Island Mountains. Within this cluster, the populations from different regions were mixed into two groups. The other main group consisted of three populations from the Eastern Carpathians (RFE, RMO, RPO), the Bulgarian population (BYU) and the Estonian population (EST).

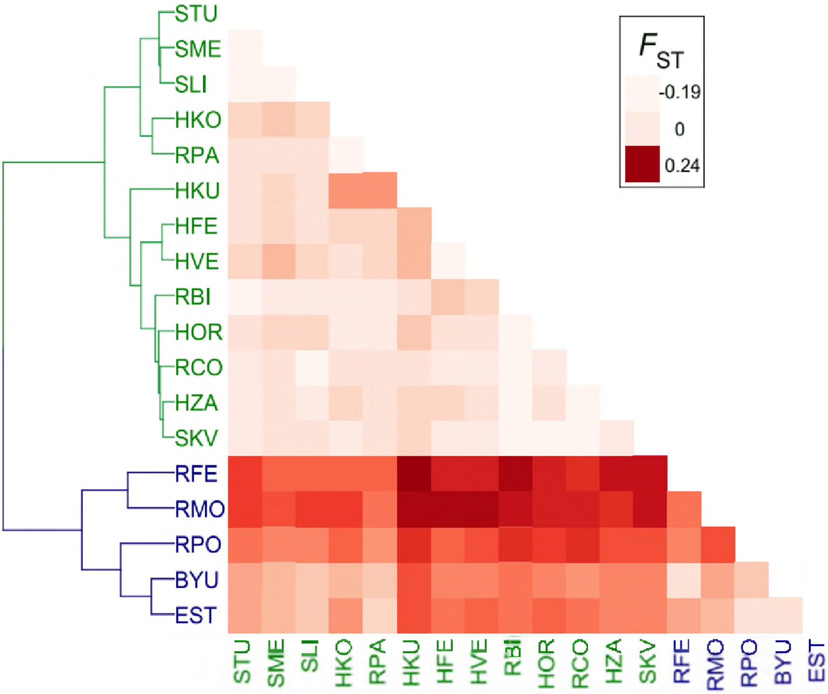

Figure 2. Pairwise F_{ST} heatmap and dendrogram based on F_{ST} values among the 18 *Pinus sylvestris* populations. The heatmap color code represents the F_{ST} matrix considering different discrete F_{ST} bins, from low to high genetic differentiation ($-0.19 \leq F_{ST} \leq 0.24$). F_{ST} values were calculated following Weir and Cockerham (1984).

The differentiation of the two groups is shown by PCoA analysis (*Figure 3*). HKU genetically is very close to the Hungarian populations and fits into the region of the Pannonian Basin.

According to the PCoA, HKU showed to be the closest to Zalaság population (HZA), from SW Hungary.

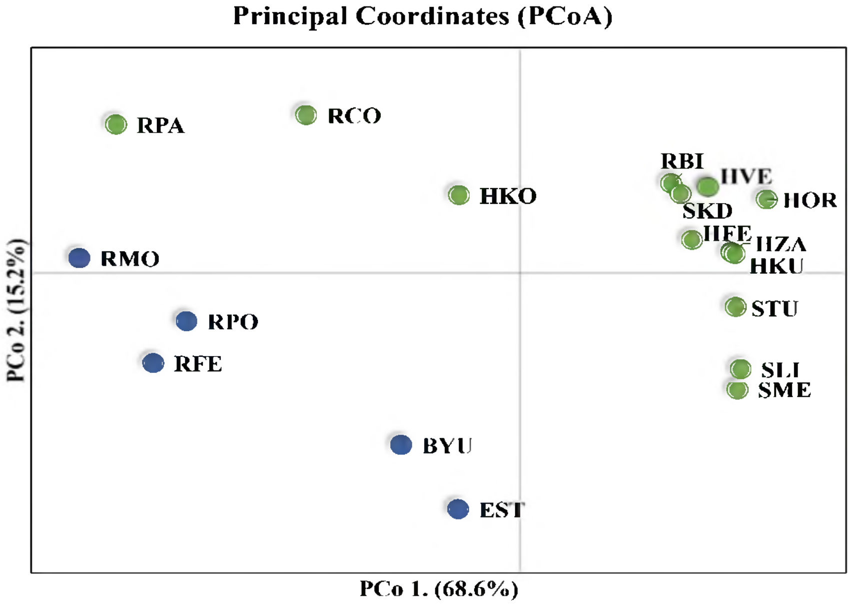

Figure 3. Population genetic structure among 18 *Pinus sylvestris* populations revealed by the principal coordinates analysis (PCoA) based on Nei's genetic distance. Colors refers to the clusters identified by the hierarchical cluster analyses (*Figure 1*).

BARRIER analysis revealed four main barriers among the studied populations. These four barriers of high statistical support delimited, (1) the Balkan population (BYU), then two barriers (2, 3) separated the Eastern and Southern Carpathian populations including Estonian population (EST), but surprisingly excluding RBI from the Western Island Mountains. The Kunpeszér (HKU) area with the other populations from the Pannonian Basin were delimited from all other studied populations by a strong barrier (4), (data not shown).

Evaluation of the nuclear SSR variation with STRUCTURE revealed the highest ΔK for K=2 (*Figure 4*). The two main groups developed similarly to the results by Tóth *et al.* (2017); first group consisting the Eastern Carpathian (RFE, RPO and RMO) populations, the Bulgarian population (BYU) and the Estonian population (EST), while the second group consists of the western

Hungarian populations (HFE, HZA, HVE, HOR and HKO), Western Carpathian populations (SLI, SKV, SME and STU) and Southern Carpathian populations (RPA), the Eastern Carpathian RCO plus RBI. The population of Kunpeszér (HKU) from Central Hungary fits into the second group grouping within other Hungarian populations.

At K=3, two Eastern Carpathian populations (RMO, RPO) differentiated from the other Eastern Carpathian RFE. Also, BYU and EST were admixed with three clusters. The group with the Hungarian populations remained uniform at K=3, showing the same structure as in K=2. HKU is embedded among other Pannonian populations.

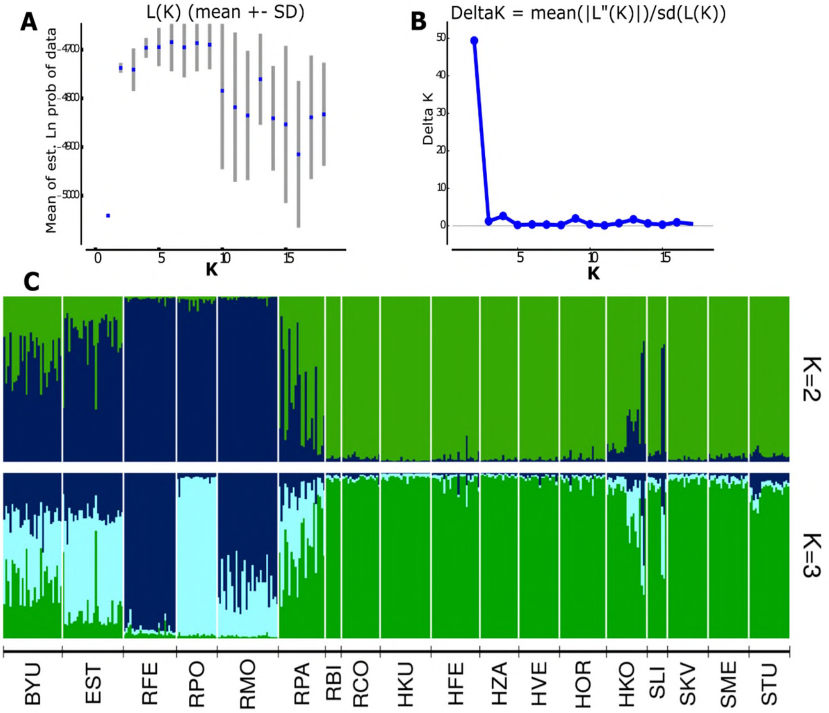

Figure 4. Population genetic structure among the 18 *Pinus sylvestris* populations using Bayesian clustering with STRUCTURE (Pritchard *et al.* 2000). (A) mean values of L(K) plus/minus standard deviation and (B) mean values of L(K) Evanno *et al*'s ΔK statistics obtained from 10 runs. (C) Results of assignment tests for numbers of clusters K = 2 and K = 3. Individual specimens are represented by vertical bars. Each genetic cluster is represented by a different colour.

DISCUSSION

The results of our genetic analysis performed by microsatellites markers supported our hypothesis that Scots pine population of the northern Kiskunság (HKU) fits well into the genetic cluster that includes all Hungarian populations. Expected heterozygosity value of the population was close to the overall mean and the allele composition was slightly lower compared to populations from Western Hungary including Fenyőfő (*Table 1*). Overall, fixation indexes were low and there was a heterozygosity excess, supporting open pollination and lack of inbreeding effects. As expected, heat map based on F_{ST} values and PCoA based on Nei's genetic distance indicated that the Kunpeszér population is related to Hungarian populations and diverge significantly from the peatbog populations of the Eastern Carpathians, from Bulgarian and Estonian samples all described as originating from a Balkan glacial refugia. Hungarian Plain and the Eastern Alps is known as another detectable glacial refugia for conifers like Scots pine (Tóth *et al.* 2017). Our former results based on microsatellite variation confirmed that populations from Hungary are most probably descendants of the East-Alpine refugia including the Hungarian Plain, mentioned by Cheddadi *et al.* (2006). Although, it is not possible to prove local, natural origin of the Scots pine trees from Kunpeszér and the neighbouring area, the fact that they cluster with all other Hungarian populations (*Figure 4*), and do not exhibit any alien, population specific genoptypes makes them valuable forest biodiversity components in the Kiskunság National Park. Scots pine trees within the studied territory are growing among pedunculate oak (*Quercus robur* L.) individuals as part of a matrix of forest and steppe-like grasslands. The trees are of old age exhibiting special habit characteristic for individuals that grow in arid conditions and in low community density. Although the region (Great Hungarian Plain) was strongly affected by human activities in the past, the landscape and the neighbouring territories have preserved the forests where tree populations could survive locally during the whole Holocene. In turn, according to landscape historical descriptions by Kerner (1877) and Babos (1972), who have mentioned earlier introduced plant material, we cannot exclude either that the gene-stock of the HKU originates from Western Hungary, from Írottkő or Őrség area.

According to the results, it is not possible to decide whether the investigated specimens are descendants of a population having survived the Holocene locally or they originate from other native populations from Hungary.

It has been accepted that the elevated population allele diversity represents an important precondition for an effective and fast genetic adaptation of the population to the local environment. The studied HKU population exhibited similar genetic diversity to other studied populations from the Pannonian region including population from the sand area in Fenyőfő. However, further diversity studies are required involving not just neutral DNA markers but also adaptive genotypic and phenotypic traits to understand and evaluate the resilience and adaptive potential of the population (Mátyás 2006).

In case of largely distributed species with disjunct area like Scots pine geographically isolated and ecologically marginal populations can exhibit divergent gene stock by holding specific alleles (Prus-Glowacky *et al.* 2012). Moreover, these marginal populations sustaining in extreme environment may have higher ecological plasticity, and their genes can play an important role in the adaptation during the climate change. Accordingly, peripheral genotypes represent a high conservation value. The peripheral forest tree populations are "natural laboratories" where complex interactions can be monitored such as demographic processes and natural selection that result in local adaptation (Fady *et al.* 2016).

Extreme conditions in the time of the climate change may appear not just at the periphery of the species' distribution area but also in non-peripheral locations ending up in the decline of trees. In some cases, assisted migration is the only way to prevent population extinctions (Tollefsrud *et al.* 2021). In Spain climate change models forecasted important shifts in the environmental variables and the studies anticipated the higher plasticity of the local gene stock (Notivol *et al.* 2020). The use of genetic resources from local provenances was highly recommended. In forest tree species with high level of intra-population and low level of inter-population variation, such as *Pinus sylvestris*, the traditional population based (local and predictive) seed sourcing methods provide better performance in dendromass production. The admixture and composite or even climate adjusted methods, which are based on the combination of different seed sources and recommended in face of climate change, are not suitable for peripheral populations of *Pinus sylvestris* due to the absence of populations that follow the predicted climate gradient. "This information should be taken into consideration in forest policies by adapting the transfer guidelines of FRM in order to improve the recommendations for future climatic "scenarios" (Notivol *et al.* 2020).

The Great Hungarian Plain is strongly affected by the climate change, therefore the gene stock adapted to the local environment may represent a high natural and economic value. Although the natural presence of Scots pine in the area is not trackable the habitat characteristics may favor long-term survival of the species. The local forest community including broadleaf species with the dominance of pedunculate oak mixed with Scots pine provide an aspect of a continental woodland community able to preserve valuable species and so an elevated biodiversity. Any introduced alien genetic material by pine plantations can suppress the gene-stock already adapted to the local conditions. As well local fires acting during the long-lasting drought periods or human induced activities (like military exercises), may also cause decrease in the population genetic diversity and results in bottleneck effect. Therefore, the protection of the larger forest stand in the area where natural seedling regeneration can take place is strongly suggested.

Acknowledgments – We would like to express our thanks to professor Tamás Pócs who always has encouraged us in the Scots pine research, participating in collection trips on numerous occasions. He was always interested to hear about the results with our Scots pine research. The basis of our work was his coenological and floristical study that he carried out in Western Hungarian habitats of Scots pine.

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(submitted: 20.02.2023, accepted: 01.05.2023)