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Genetic diversity of two native sheep breeds by genome-wide analysis of single nucleotide polymorphisms

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ABSTRACT

Wallachian and Sumava sheep are autochthonous breeds that have undergone a significant bottleneck effect and subsequent restoration efforts. The first objective of this study was to evaluate the degree of genetic variability of both breeds and, therefore, the current management of the breeding. The second was to determine whether these two breeds still retain their genetic uniqueness in relation to each other and other breeds, despite regenerative interventions. Our data consisted of 48 individuals of Sumava and 37 individuals of Wallachian sheep. The comparison data contained 25 other breeds (primarily European) from the HapMap dataset generated by the International Sheep Genomics Consortium. When comparing all 27 breeds, the Czech breeds clustered with 15 other breeds and formed a single branch with them according to Nei's distances. At the same time, however, the clusters of both breeds were integral and easily distinguishable from the others when displayed with principal component analysis (**PCA**). Population substructure analysis did not show any common genetic ancestry of the Czech national breeds and breeds used for regeneration or, eventually, breeds whose ancestral population was used for regeneration. The average values of F_{ST} were higher in Wallachian sheep (F_{ST} = 0.14) than in Sumava sheep (F_{ST} = 0.08). The linkage disequilibrium (**LD**) extension per autosome was higher in Wallachian than in Sumava sheep. Consequently, the N_e estimates five generations ago were 68 for Sumava versus 34 for Wallachian sheep. Both native Czech breeds exhibit a wide range of inbreeding based on the excess of homozygosity (F_{HOM}) among individuals, from −0.04 to 0.16 in Sumava and from −0.13 to 0.12 in Wallachian. Average inbreeding based on runs of homozygosity was 0.21 in Sumava and 0.27 in Wallachian. Most detected runs of homozygosity (**ROH**) were less than 5 Mb long for both breeds. ROH segments longer than 15 Mb were absent in Wallachian sheep. Concerning putative selection signatures, a total of 471 candidate genes in Wallachian sheep within 11 hotspots and 653 genes within 13 hotspots in Sumava sheep were identified. Czech breeds appear to be well differentiated from each other and other European breeds. Their genetic diversity is low, especially in the case of the Wallachian breed. Sumava is not so threatened by low diversity but has a larger share of the non-native gene pool.

Implications

The genetic diversity of small breeds can be threatened by inbreeding depression and inappropriately performed regeneration. The results of this study show that both breeds retain their genetic differences from the breeds directly used for regeneration or their closely related breeds after more than 50 years since the regenerative interventions. However, at the time, it is clear that this is not a permanent solution for populations of this type. The breeds are again threatened by inbreeding. This study demonstrates the importance of monitoring genetic variability in small breeds for early intervention in breeding management, which can prevent its further losses.

Introduction

In a worldwide climate-changing scenario, keeping animals adapted to harsh environmental conditions becomes increasingly important. In this sense, local sheep breeds constitute an important genetic resource due to their rusticity and adaptability to various agroecological environments. Despite this, more than 100 local

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breeds of sheep and goats have been lost in the last few decades, and a similar number of them face imminent extinction (FAO, 2022). This phenomenon is mainly caused by the spread of commercial breeds with specialised production, the overuse of a small number of rams (exacerbated by artificial insemination), and modern selection procedures (Kijas et al., 2012a).

In the Czech Republic, Sumava and Wallachian sheep are breeds characterised by their adaptability, resistance to harsh conditions and suitability to extensive management systems in submontane and montane regions (Ptáček et al., 2017). They are breeds with very good grazing ability and walkability. Both breeds have a tripartite efficiency (meat, milk, wool) and are included in the Czech genetic reserves, in the case of Sumava sheep since 1992 and Wallachian sheep since 1999. Sumava sheep has a genetic origin in Czech peasant sheep, but it did not obtain the status of the breed until 1986 (Horák, 2012). Since the second half of the last century, it has been regenerated by crossing with phylogenetically related breeds such as Texel, Cigaya, Lincoln, Kent, Leicester, or Improved Wallachian sheep (Milerski, 2019a). Ancestors Wallachian sheep came to the Czech lands around the 14th century from the Eastern Carpathians. As with the Sumava sheep, the improvement of Wallachian sheep started in the 1950s (Lincoln, East Frisian sheep), which resulted in the emergence of an independent Slovak Improved Wallachian sheep breed. However, in the Czech Republic, the original Wallachian sheep was renewed based on breeding a few individuals with the original phenotype (Milerski, 2019b).

The crucial assumption for preserving local breeds is maintaining their genetic diversity. However, breeding programmes find this challenging because these populations are usually very small, and inbreeding practices cannot be avoided (Liu et al., 2021). The knowledge of the genetic variability, historical development and kinship in the population is crucial for their conservation. For Walachian and Sumava sheep, previous studies have analysed within-breed genetic variability using pedigree and microsatellite information (Machová et al., 2021, 2020). Nowadays, the availability of medium- and high-density single nucleotide polymorphism (**SNP**) arrays provides an opportunity to accurately investigate genetic variability at the molecular level through the evaluation of genome-wide linkage disequilibrium (**LD**) extension (Chitneedi et al., 2017; García-Gámez et al., 2012; Getachew et al., 2020), effective population size (N_e) (Chitneedi et al., 2017; Pasandideh et al., 2020) and homozygosity analysis (Chitneedi et al., 2017; Dzomba et al., 2021; Getachew et al., 2020). In livestock genetics, runs of homozygosity (**ROH**) regions, consisting of continuous homozygous loci assumed to originate from the same ancestor, are commonly used for inbreeding detection (Addo et al., 2021; Rodríguez-Ramilo et al., 2019; Signer-Hasler et al., 2019). Long ROH segments indicate recent inbreeding (<5 generations), whereas shorter segments indicate a more historical effect (≤50 generations), because of interruption due to recombination (Mastrangelo et al., 2017; Meyermans et al., 2020b). The present study aims to characterise the genetic diversity and inbreeding levels in Sumava and Wallachian sheep using a medium-density (50 K) SNP array and to compare our results with previous estimates based on pedigree and microsatellite markers. Furthermore, these two breeds are compared with other breeds of sheep (Kijas et al., 2012a) to evaluate their relationship. Eventually, the information provided by this study will drive the breeding management of Sumava and Wallachian sheep.

Material and methods

Our dataset contained 85 DNA samples of two Czech indigenous sheep breeds, Sumava (48) and Wallachian (37). Individuals were randomly selected from farms in Northern Moravia and Southwest Czechia, these breeds' most important breeding areas. One individual from each family was selected from each breeding (unrelated individuals). Sumava and Wallachian sheep come from 8 and 10 breedings, respectively. Nasal swaps were collected, which were subsequently processed by the Neogen laboratory. In addition to our original data, 25 different breeds (n = 1 028) from the Sheep HapMap dataset generated by the International Sheep Genomics Consortium (**ISGC**) were used to know the genetic relationships of our populations with other breeds of sheep, mainly European. The breeds taken from the ISGC dataset were as follows: African Dorper (21), African White Dorper (6), Australian Suffolk (109), Black Headed Mountain sheep (24), Castellana (23), Churra (90), East Friesian Brown (39), East Friesian White (9), Finnsheep (99), German Texel (46), Irish Suffolk (52), Karakas (18), Meat Lacaune (78), Merinolandshaf (24), Milk Lacune (103), New Zealand Romney (24), Norduz (20), Old Norwegian Spaelsau (15), Rasa Aragonesa (22), Sakiz (22), Scottish Texel (80), Spael-white (32), Swiss Black-Brown Mountain sheep (24), Swiss White Alpine sheep (24), and Valais Blacknose sheep (24).

Genotyping and quality control

DNA samples corresponding to the Sumava and Wallachian breeds were genotyped using GGP Ovine 50 K SNP bead chip (Neogen) and Illumina GenomeStudio Software v2.0.5, with a total number of 45 205 genotyped SNPs with known positions according to the sheep reference genome Oar_Texel_v4.0. Only SNPs with GC greater than 0.8 were used for further analysis. Quality control of these SNPs was performed with PLINK v1.9 software (Chang et al., 2015; Purcell and Chang, 2019). SNPs with minor allele frequency (MAF) < 0.1, genotyping rate < 0.9 and Hardy-Weinberg equilibrium (HWE) $P < 10^{-6}$ were excluded. In the second step, individuals with more than 10% of missing genotypes and sex chromosomes were also excluded from the data set.

To make our data mergeable with those obtained from ISGC genotyped on the 50 K Illumina ovine bead chip, it was necessary to use only common SNPs for both chips. We performed an additional quality control procedure to merge both datasets, applying the parameters described previously for Czech breeds, except for the Hardy-Weinberg equilibrium (**HWE**) threshold, which was increased to $P < 10^{-5}$. Additionally, LD pruning was performed for comparative analysis between breeds. Independent pairwise command parameters were set to the size of the SNP window of 50, the number of SNPs per step 5, and the r^2 threshold to 0.2. Based on these settings, 10 780 SNPs and 1 113 individuals were kept for further analysis.

Linkage disequilibrium

Using the SNPs remaining after the quality control step for Sumava and Wallachian sheep, we used Haploview v4.2 software (Barrett et al., 2005) to calculate LD for each pair of SNPs, using the commands ld-window-kb 1 000 and ld-window-r2 0. In the first step, a comparison among physical distances between pairs of markers was made (<20 kb, 20–40 kb, 40–60 kb, 60–100 kb, 100–200 kb, 200–500 kb, 0.5–1 Mb). In the second step, all pairwise LD combinations of SNPs with a distance smaller than 500 kb apart were computed separately for both breeds and all autosomes. The average LD and average LD of adjacent SNPs across each chromosome were also calculated.

Genetic diversity, inbreeding and effective population size

Genetic diversity within Sumava and Wallachian sheep populations was estimated by calculating the observed heterozygosity

(H_O) and expected heterozygosity (H_E) were computed with PLINK v1.9. The inbreeding coefficient (F_{HOM}) based on observed versus expected heterozygosity was also estimated using PLINK. To compare absolute levels to these relative levels of inbreeding, the inbreeding coefficient based on ROH (F_{ROH}) was also computed with SNP1101 v1.0 (Sargolzaei, 2014). Two different estimates for F_{ROH} were used. The first is based on a comparison of the total length of ROH with the full length of the genotype $F_{ROH/length}$, and the second is based on a proportion of SNPs included in the ROH to the total number of genotyped SNPs $F_{ROH/SNPs}$.

Effective population size (N_e) trends for these breeds were estimated from LD as implemented in SNeP v1.1 (Barbato et al., 2015). This approach of N_e estimation across generations is based on the relationship between LD decline and distance between adjacent markers in the presence of mutations (Corbin et al., 2012):

$$N_{T(t)} = (4f(c_t))^{-1}(E\left[r^2_{adj}|c_t\right]^{-1} - \propto),$$

where $N_{T(t)}$ is the effective population size t generations ago computed as $t = (2f(c_t))^{-1}$ (Hayes et al., 2003), and c_t is the specific recombination rate for a certain physical distance between the SNPs. For this study, Sved and Feldman's approximation was used (Sved and Feldman, 1973) for the recombination rate modification. r^2_{adj} is the LD adjusted for sampling bias, and α is a constant correcting the occurrence of mutations. For our analysis, values of minor allele frequency (**MAF**) and $\propto$ were set to 0.1 and 2.2, respectively, as investigated by (Corbin et al., 2012). To make our outputs more comparable to previous studies, the maximum distance between SNPs was set at 10 Mb to estimate N_e since the fifth generation (Addo et al., 2021; Deniskova et al., 2019; Liu et al., 2021; Mastrangelo et al., 2017). An adjustment of r^2 was also performed due to the limited sample size.

Population differentiation, principal component analysis, and model base structure

The pairwise F_{ST} matrix (Weir and Cockerham, 1984) among all 27 populations was calculated using HIERFSTAT (Goudet, 2005) in R (R Core Team, 2020). Genetic relatedness based on a variance-standardised relationship matrix was used to perform a principal component analysis (**PCA**).

The neighbour-joining trees were modelled based on Nei's distance matrix computed and visualised in R. SNPs without genomic information in at least one sample were removed (847 SNPs). The number of clusters was selected according to the lowest value of the Bayes information criterion.

Taking into account the expected historical admixture of the Czech autochthonous breeds (Sumava and Wallachian) with the East Friesian White and Brown, German Texel, and New Zealand Romney breeds, we selected these six populations to investigate their admixture and their genetic structure with ADMIXTURE v1.3.0 (Alexander et al., 2009). The most probable number of clusters (K) in the data set was obtained using the default cross-validation procedure based on the estimation of the prediction errors for each K (Alexander and Lange, 2011).

Runs of homozygosity analysis

The identification of ROH for Sumava and Wallachian sheep was carried out with the PLINK v1.9 program. The settings were as follows: minimum length of ROH – 1 Mb; maximum missing SNPs in ROH per window – 2; maximum heterozygous SNPs per window – 1; minimum scanning window hit rate – 0.05; the maximum gap between adjacent SNPs and minimum SNP density per ROH was set as in Abied et al. (2020) – 250 kb and 70 kb, respectively; scanning window and minimum number of SNPs in ROH (l) were 18 kb for both, as recommended by (Meyermans et al., 2020a). Our value 18 of the minimum number of SNPs (l) was calculated according to the formula (Purfield et al., 2017), originally proposed by (Lencz et al., 2007), to minimise false-positive ROH:

$$l = \log_e(\propto / n_s \times n_i)/(\log_e(1 - het))$$

where n_s is the number of genotyped SNPs per individual; n_i is the number of genotyped individuals of the breed; $\propto$ (set for our study at 0.05) means the percentage of false-positive ROH, and *het* is the mean heterozygosity computed for all SNPs. Values after the first pruning were substituted. The same value was obtained for both Wallachian and Sumava sheep.

All identified ROH were subdivided into four categories by length (1–5 Mb, 5–10 Mb, 10–15 Mb, >15 Mb). Only the first three categories were needed for the Wallachian sheep. The average density of SNPs and the average number of ROH per individual were estimated for each category, as well as the count of ROH on each chromosome.

For the detection of ROH islands (or ROH hotspots, in other words), an approach based on the percentage of occurrence of SNPs in ROH was chosen. PLINK v1.9 generated output from ROH detection was used for the calculation – the number of certain SNP occurrences divided by the number of animals in each breed. The top 1% of most frequently observed SNPs in ROH were selected as potential genomic regions highly associated with ROH in each breed. SNPs with greater frequency than 1% and a distance $\leq$ 1 Mb between themselves were identified as ROH islands. The number of genes contained in each ROH island was determined in the National Center for Biotechnology Information database (NCBI, 2015) based on the range between the first and last SNPs of each ROH. Additionally, 0.5 Mb was subtracted/added to the chromosome coordinates of these two SNPs to avoid the exclusion of some genes based solely on the mismatch between the physical positions of the SNPs in the Oar_v4.0 genome assembly and our Neogen BeadChip. Only genes with a complete sequence in the given range were considered.

Results

Quality control

Of 45 205 SNPs genotyped in this study, 551 were duplicates, 134 SNPs were not mapped, and 241 were located on sex chromosomes. Thus, 44 654 SNPs mapped onto the 26 sheep autosomes were subjected to quality control. Of the 97 genotyped animals, all individuals had missed less than 10% of their genotype. The number of markers for Sumava removed during quality control was 8 405 SNPs: 1 134 SNPs were deleted due to low call rate (<0.90); 5 737 SNPs did not reach minimum MAF (<0.1); 53 markers were not in HWE ($P < 10^{-6}$). The number of markers for Wallachian removed during quality control was 12 610 SNPs: 1 267 SNPs were deleted due to low call rate (<0.90); 9 819 SNPs did not reach the minimal MAF (<0.1), and 43 markers were not in HWE ($P < 10^{-6}$). The total number of markers used in the Czech breed analysis was 36 249 SNPs in Sumava and 32 044 in Wallachian.

The total dataset for comparison with European breeds was created based on identical SNPs. These 19 634 SNPs were also subjected to quality control. Of the 1 113 genotyped animals, all individuals had less than 10% of their genotype missing and passed the quality control. The number of markers removed during the quality control was 8 148 SNPs: no SNPs were deleted due to the low call rate (<0.90); 100 SNPs did not reach the minimum MAF (<0.1); additional 8 048 markers were not in HWE ($P < 10^{-5}$).

Subsequently, LD pruning excluded 706 additional SNPs. Therefore, 10 780 SNP markers were kept for further analysis.

Linkage disequilibrium

The LD decay for both studied breeds is presented in Fig. S1. Autosomal SNP pairs were sorted into 20 kb bins according to their increasing mutual distance. Then, an average value of r^2 was calculated for each bin and plotted as a function of the genomic distance between markers (SNPs). The shortest distances (up to 120 kb, where ended the sharpest decrease) showed the highest r^2 according to expectations. r^2 reached a value of 0.59 in the first bin 0–20 kb in Sumava sheep; between 100 and 120 kb, it was 0.37 and only 0.27 in the last bin. Wallachian sheep had overall higher values of r^2, which started at 0.66 in the first bin, followed by a value of 0.45 between 100 and 120 kb and ended with 0.33 at 5 Mb. The decrease in r^2 and D' values depending on the physical distance of the marker pairs in both breeds is shown in Table S1.

Higher levels of average LD per autosome were observed in Wallachian than in Sumava sheep for all and adjacent SNPs. Overall average values of r^2 and D' in Wallachian sheep led in the intervals of 0.15–0.22 and 0.56–0.67, respectively. In Sumava sheep, these intervals were 0.08–0.17 for the coefficient r^2 and 0.37–0.53 for the coefficient D'. Furthermore, chromosome 6 reached a significantly higher level of LD in the Sumava breed compared to the other autosomes included in the analysis. However, the Wallachian breed did not show statistical differences in LD between autosomes. Table S2 compares the average values of LD on each chromosome for all SNP pairs and adjacent SNP pairs in both breeds.

Genetic diversity and effective population size for Sumava and Wallachian sheep

In the Sumava breed, 36 249 filtered loci with 96% polymorphic SNPs remained on average. This value was significantly lower in Wallachian sheep – only 74%. Furthermore, the observed heterozygosity (H_O) and the expected heterozygosity (H_E) were slightly higher in Sumava than in Wallachian sheep. Sumava showed $H_O = 0.42 \pm 0.11$ and $H_E = 0.43 \pm 0.08$. In the Wallachian sheep, $H_O = 0.41 \pm 0.12$ and $H_E = 0.40 \pm 0.09$. A total of 466 loci in the Sumava and 255 loci in the Wallachian breed deviated significantly from HWE ($P < 0.01$).

An estimation of the effective population size (N_e) of both breeds is depicted in Fig. 1. As expected, Sumava sheep had larger N_e for all displayed generations than Wallachian sheep but suffered approximately a steeper decline between the 60th and 600th generation. In the most recent time frame (five generations ago), the Sumava breed had $N_e = 68$, and the Wallachian breed had $N_e = 34$.

Runs of homozygosity in Sumava and Wallachian sheep

There were 1 886 ROH in total in the Sumava breed and 1 995 ROH in the Wallachian breed. The distribution of ROH across all chromosomes is shown in Fig. S2A and B for Sumava and Wallachian sheep, respectively. Most detected ROH were less than 5 Mb long for both breeds. On average, the ROH of the Sumava sheep were longer than that of the Wallachian and reached even larger absolute lengths. However, Wallachian sheep showed ROH longer on average in the categories 5–10 Mb and 10–15 Mb. Furthermore, the mean density of SNPs in ROH was comparable in both breeds. All these characteristics are explained in detail in Table 1.

There were no shared ROH islands shared between the Wallachian and Sumava breeds. Regarding the number of genes contained in each ROH island ±0.5 Mb, a total of 471 candidate genes were identified in Wallachian sheep distributed to 11 hotspots and 653 genes within 13 hotspots in Sumava sheep, as shown in Table S3. The SNPs within the ROH hotspots above the 1% threshold for Sumava and Wallachian sheep are shown in Fig. 2.

Inbreeding in Sumava and Wallachian sheep

The average inbreeding values for both Czech breeds are shown in Table 2. Both breeds exhibit a wide range of inbreeding (F_{HOM}) among individuals, from – 0.04 to 0.16 in Sumava and from – 0.13 to 0.12 in Wallachian (data not shown). Values obtained from F_{ROH} were much higher on average, reaching 21% in Sumava and 27% in Wallachian sheep (data not shown). Based on the proportion of SNPs in ROH ($F_{ROH/SNPs}$), the inbreeding estimate reached, on average, slightly higher values than the one that considered their length ($F_{ROH/length}$). Inbreeding based on the excess of homozygosity mainly had very low to negative values, despite the presence of animals with relatively high F_{HOM} in the dataset.

PCA results are shown in Fig. 3. The principal components 1, 2 and 3 explained 24.4, 19.11 and 18.6%, respectively, of the total

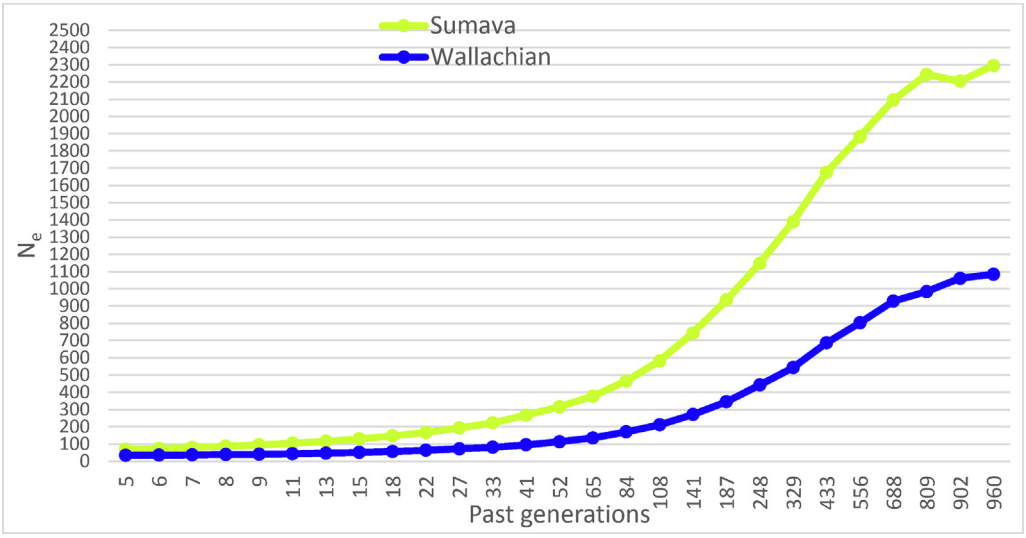

Fig. 1. Estimates of the effective population size (N_e) for Sumava and Wallachian sheep for 5–960 generations ago.

Table 1
Descriptive statistics for ROH in Sumava and Wallachian sheep.

	N ROH	Mean density	ROH/individual	Mean ROH length (b)
Sumava				
1–5 Mb	1 626	58.839	21.021	2 913 387
5–10 Mb	234	61.066	3.271	2 951 468
10–15 Mb	23	59.575	0.313	3 036 947
15–20 Mb	3	48.874	0.042	6 369 466
Total	1 886	59.109	24.646	2 925 116
Wallachian				
1–5 Mb	1 873	60.264	18.541	2 448 172
5–10 Mb	104	61.178	0.919	2 288 401
10–15 Mb	18	57.635	0.270	2 305 839
Total	1 995	60.288	18.541	2 438 559

Abbreviations: ROH = runs of homozygosity.

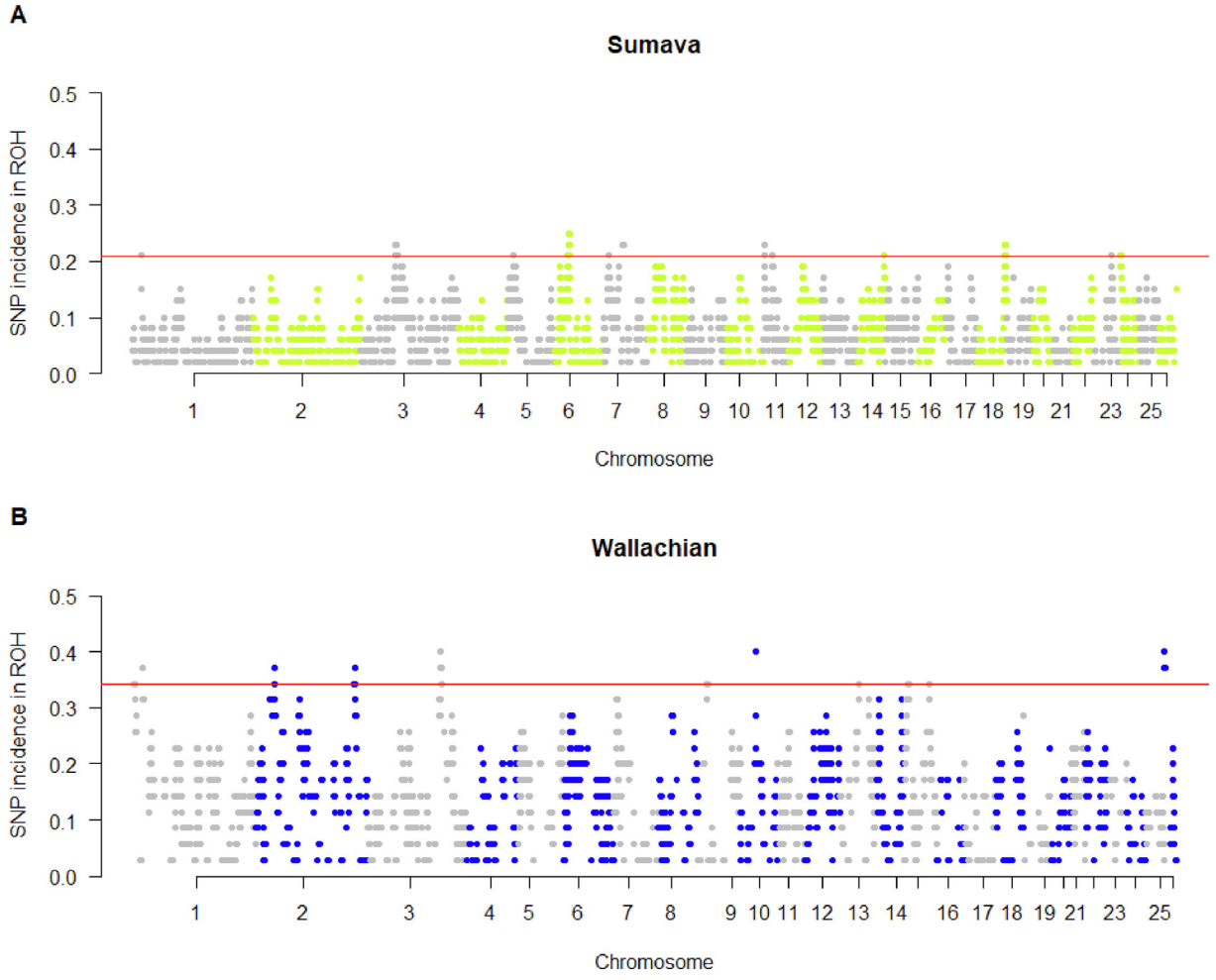

Fig. 2. Runs of homozygosity (ROH) hotspots lie above the redline representing a 1% threshold of SNPs incidence in the total number of ROH: (A) 0.208 for Sumava sheep and (B) 0.343 for Wallachian sheep.

Table 2
Comparison of two types of inbreeding in Sumava and Wallachian sheep.

Breed	F_{HOM} ± SD	$F_{ROH/length}$ ± SD	$F_{ROH/SNPs}$ ± SD
Sumava	0.01 ± 0.039	0.06 ± 0.037	0.07 ± 0.036
Wallachian	−0.02 ± 0.059	0.14 ± 0.045	0.14 ± 0.048

Abbreviations: ROH = runs of homozygosity; F_{HOM} = coefficient of inbreeding based on the excess of homozygosity; $F_{ROH/length}$ = coefficient of inbreeding based on ROH length; $F_{ROH/SNPs}$ = coefficient of inbreeding based on the proportion of SNPs in ROH.

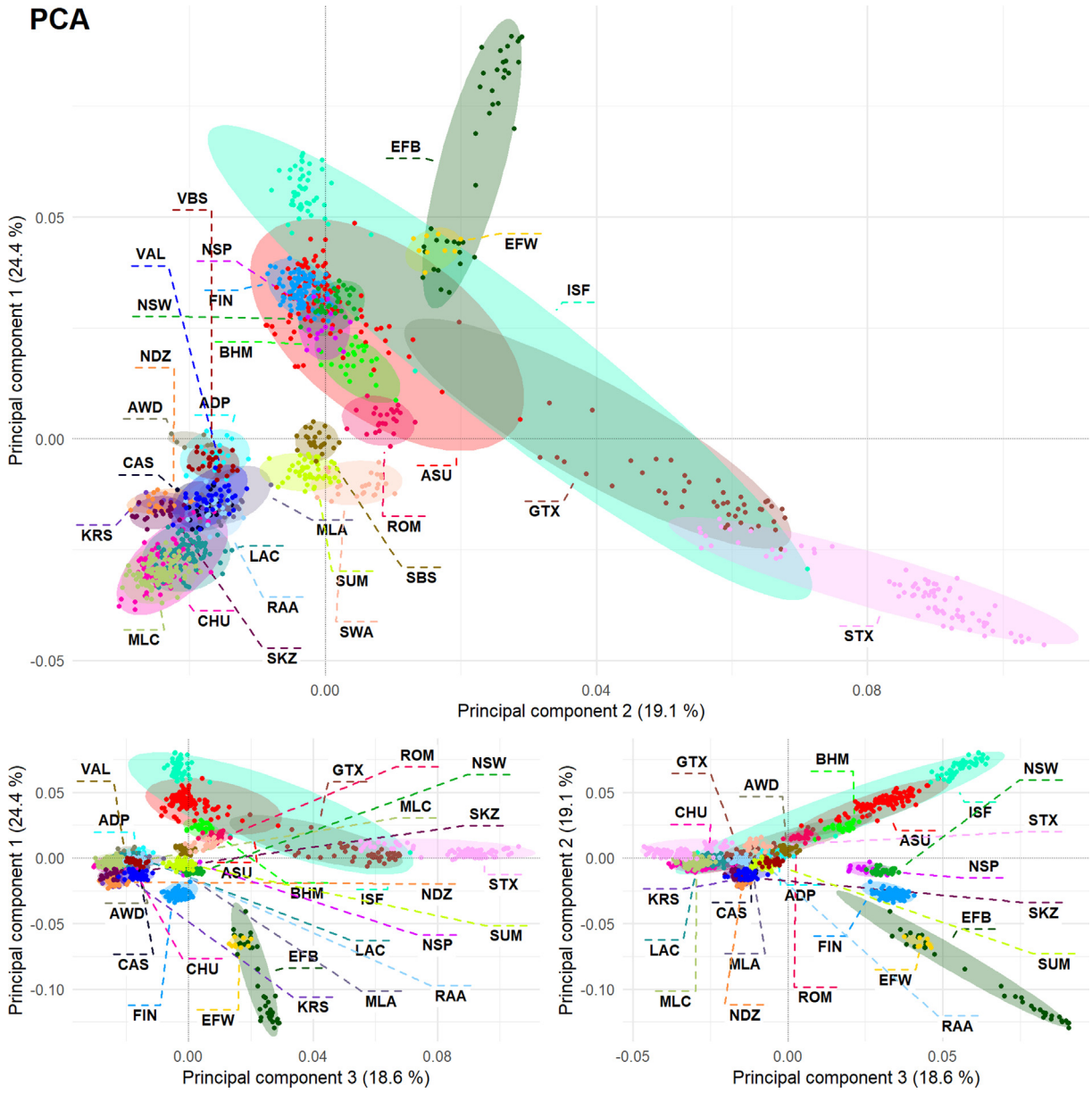

Fig. 3. Results of the principal component analysis based on a variance-standardised relationship matrix performed on the 27 sheep breeds included in this study. Abbreviations: ADP = African Dorper; AWD = African White Dorper; ASU = Australian Suffolk; BHM = Black Headed Mountain sheep; CAS = Castellana; CHU = Churra; EFB = East Friesian Brown; EFW = East Friesian White; FIN = Finnsheep; GTS = German Texel; ISF = Irish Suffolk; KRS = Karakas; MLC = Meat Lacaune; MLA = Merinolandshaf; LAC = Milk Lacune; ROM = New Zealand Romney; NDZ = Norduz; NSP = Old Norwegian Spaelsau; RAA = Rasa Aragonesa; SKZ = Sakiz; STX = Scottish Texel; NSW = Spaelwhite; SUM = Sumava sheep; SBS = Swiss Black-Brown Mountain sheep; SWA = Swiss White Alpine sheep; VAL = Wallachian sheep; VBS = Valais Blacknose sheep.

variation associated with breed distinctiveness according to their variance-standardised relationship. Sumava and Wallachian sheep tend to group with Sakiz, African Dorper, African White Dorper, Karakaz, Norduz, Valais Blacknose sheep, German Texel, New Zealand Romney, Swiss Black-Brown Mountain sheep, Swiss White Alpine sheep, Merinolandshaf, Meat Lacaune and Castellana.

Values of the fixation index were computed for each pair of breeds. The resulting matrix of relative genetic distinctiveness is displayed in Table S4. The most different breeds, according to the average of the F_{ST} values, seemed to be both African Dorpers and both breeds of East Frisian sheep, followed by Sakiz, Wallachian sheep, and Valais Blacknose sheep. In contrast, Sumava sheep showed low differentiation from Rasa Aragonesa, Castellana, Australian Suffolk and both Lacaune populations. The highest F_{ST} values were observed when comparing African Dorper with East Friesian White and East Friesian Brown sheep – $F_{ST} = 0.215$ and $F_{ST} = 0.213$, respectively. The smallest difference ($F_{ST} = 0.022$) was found between Milk and Meat Lacaune.

Fig. 4 shows the clustering of populations by neighbour-joining trees based on Nei's genetic distances among individuals when comparing 15 (Fig. 4A) and 6 (Fig. 4B) breeds, respectively. The 15 breeds are those that cluster together in PCA analysis. The six populations are those that, based on historical information, have an expected relationship to Czech autochthonous breeds. The graphical representation of the entire data set (27 populations) includes six clusters and is depicted in Fig. S3. In the last graph, German Texel and Scottish Texel clustered together, Norduz with Finnsheep and the two Spaels. Individual branches were observed for both Suffolks and the two East Friesian populations. The rest of the included breeds remained undistinguished. For the 15 selected

breeds, only three populations made their own branches: German Texel, Valachian Blacknose, and Wallachian sheep. Sakiz, African Dorpers, Karacas and Norduz created another cluster and the remaining 7, the last one. The smallest dendrogram focused on six breeds and consisted of only two multibreed branches. Sumava and New Zealand Romney shared one of these clusters, and the second was composed of both breeds of East Friesian sheep.

Model-based structure analysis

For population structure analysis (Fig. 5), the same six breeds were chosen as for Fig. 4B. The most likely number of clusters was $K = 7$. The common pattern of genetic ancestry showed only East Friesian Brown and White; the rest of the breeds clustered separately. A minor admixture of New Zealand Romney could be found in Sumava and German Texel.

Discussion

The conservation of livestock genetic resources constitutes a challenging mission, even more so for breeds with limited population size in a country where sheep farming constitutes only a minority of livestock production. Regular evaluation of the results of these efforts is therefore desirable. This study builds on two previous studies dealing with indigenous breeds of Czech sheep (Machová et al., 2021, 2020). It provides the first overview of their population structure and diversity based on SNP analysis. To obtain a global context for our data, the 25 breeds of the ISGC database were used to assess genetic similarity and the possible relation between two Czech autochthonous breeds and other European breeds. The number of samples represents 0.5–1% of the studied populations. Due to the small population size of both breeds and the selection of unrelated individuals, it is a sufficient amount for the given type of genetic diversity type, and it is in agreement with other published studies in other indigenous breeds (Ben Jemaa et al., 2019; Eydivandi et al., 2020; Kumar et al., 2018; Mukhina et al., 2022).

Sumava sheep had overall lower levels of inbreeding than Wallachian sheep, which agrees with our previous results (Machová et al., 2021, 2020). This could be due to its larger population or/and regeneration efforts, which were more intensive than in Wallachian sheep. High levels of F_{HOM} are caused by animals with heterozygosity higher than randomly expected at the genome-wide level. No correlation between F_{HOM} and F_{ROH} observed by other authors (Ghoreishifar et al., 2020; Zhang et al., 2015) was found in our data. F_{ROH}, as the absolute rate of inbreeding, reflected comparable levels of inbreeding in Sumava with those found in Ovino delle Langhe (0.052), Valle del Belice (0.067), and Sopravissana (0.052) studied by Persichilli et al. (Persichilli et al., 2021). Similar results were found in the Swiss indigenous breeds Bundner Oberlander (0.615), Swiss Black- Brown Mountain (0.641), and Swiss Mirror sheep (0.762) (Signer-Hasler et al., 2019). While the inbreeding rate in Sumava can be classified as a better average among autochthonous breeds, Wallachian sheep are among those with higher values. When we compare our results with the Polish Olkuska breed, F_{ROH} values 0.096, 0.124, and 0.082 found for three sub-populations of this breed are higher than in Sumava but lower than in Wallachian (Sobieraj-Kmiecik et al., 2020). In other Polish breeds, the F_{ROH} values were similar to those obtained from Wallachian: Świniarka (0.17), Wrzosówka (0.10) and Polish Merino of Colored Variety (0.15) (Gurgul et al., 2021).

The identification of specific ROH showed, in some respects contrasting results with the mean values of inbreeding. Even though the Wallachian showed higher values of inbreeding, including the one based on ROH, the average number of ROH per individual was higher in the Sumava sheep. In addition, the average length in Sumava reached 2.9 Mb, while Wallachian was only about 2.4 Mb. Animals from the Sumava breed also had more ROH than those from the Wallachian breed. Thus, these results suggest that while Wallachian ROH estimates are mainly products of the distant past, Sumava length ROH indicate a relatively recent inbreeding, which could have happened approximately-three generations ago, according to cattle estimates (Ferenčaković et al., 2013). The ROH islands can be used to identify specific selection signatures throughout the breed genome, elucidating the genetic nature of unique breed-specific traits (Liu et al., 2021). Domestication, artificial selection, and environmental influences have shaped these breeds' characteristics for centuries. Both Czech breeds do not share any ROH hotspots, although they came from the same, relatively small state. Nothing like this has been observed in national breeds, even from significantly larger countries such as South Africa (Dzomba et al., 2021), Russia (Yurchenko et al., 2019), China (Liu et al., 2021), and India (Saravanan et al., 2021), where it would be easier to believe a historically separated development. The most probable explanation for the results obtained in Czech breeds will be the insufficient size of the sample, a too strict selection threshold for ROH islands, or the random selection of genetically very distant individuals.

Sheep generally have lower LD values than other domestic animals, such as cattle, pigs, and dogs (Al-Mamun et al., 2015). In autochthonous Czech breeds, a rapid decline of LD was observed after 25 kb. On average, higher levels of LD were observed in Sumava than in Wallachian sheep. In the context of other breeds, LD levels studied on SNPs distant < 50 kb and on 50 K SNP panel, Sumava belongs to the average, while Wallachian showed high levels of LD. For example, close values to the Sumava's mean r^2 value ($r^2 = 0.1$) were found in Xinjiang type of Chinese Merino ($r^2 = 0.13$) (Liu et al., 2017), Frizarta ($r^2 = 0.09$) (Kominakis et al., 2017), Australian Merino ($r^2 = 0.12$) (Al-Mamun et al., 2015) and Santa Inês ($r^2 = 0.05$) (Alvarenga et al., 2018). Higher levels of LD, like those from Wallachian sheep, are common for local breeds such as Barbaresca ($r^2 = 0.18$) (Nel et al., 2022), Churra ($r^2 = 0.17$), (García-Gámez et al., 2012) or Zandi ($r^2 = 0.18$) (Ghoreishifar et al., 2019). The average r^2 found in Sumava between adjacent SNPs lies somewhere between the values recorded by Zhao et al. (Zhao et al., 2014) in Sunite ($r^2 = 0.12$), German Mutton Merino ($r^2 = 0.20$) and Dorper ($r^2 = 0.22$). However, the values of Sumava are somewhat underestimated from this comparison because the distance between syntenic SNPs in the study of Zhao et al. (Zhao et al., 2014) was shorter (~57 kb) than in ours (~70–80 kb – data not shown). The values among adjacent SNPs in Wallachian were expectedly higher ($r^2 = 0$ 0.26), close to the breeds evaluated on the High-Density SNP chip, like Lamb Supreme ($r^2 = 0.27$), Primera ($r^2 = 0.26$), or Texel ($r^2 = 0.26$) from New Zealand (Brito et al., 2017).

The estimation of historical effective population size (N_e) by the rate of LD decay is a widely spread method of modelling the evolution of genetic diversity of populations (Chitneedi et al., 2017; Liu et al., 2017; Nel et al., 2022; Prieur et al., 2017). The declining trend N_e in both studied breeds is a predictable phenomenon, as both Czech breeds are affected by reducing the effective size of the population - a small number of breeding males, the bottleneck effect, and the absence of migration (Charlesworth, 2009). Although an increase in N_e has already been identified in livestock populations (Brito et al., 2017; Prieur et al., 2017), most authors have observed similar decline trends as in our study (Ghoreishifar et al., 2019; Kominakis et al., 2017; Liu et al., 2021; Moosanezhad Khabisi et al., 2021; Pasandideh et al., 2020; Purfield et al., 2017), because the only long-acting mechanism that reduces LD (and thus increases N_e computed from LD) is the long-term effects of random mating and recombination (Qanbari, 2020;

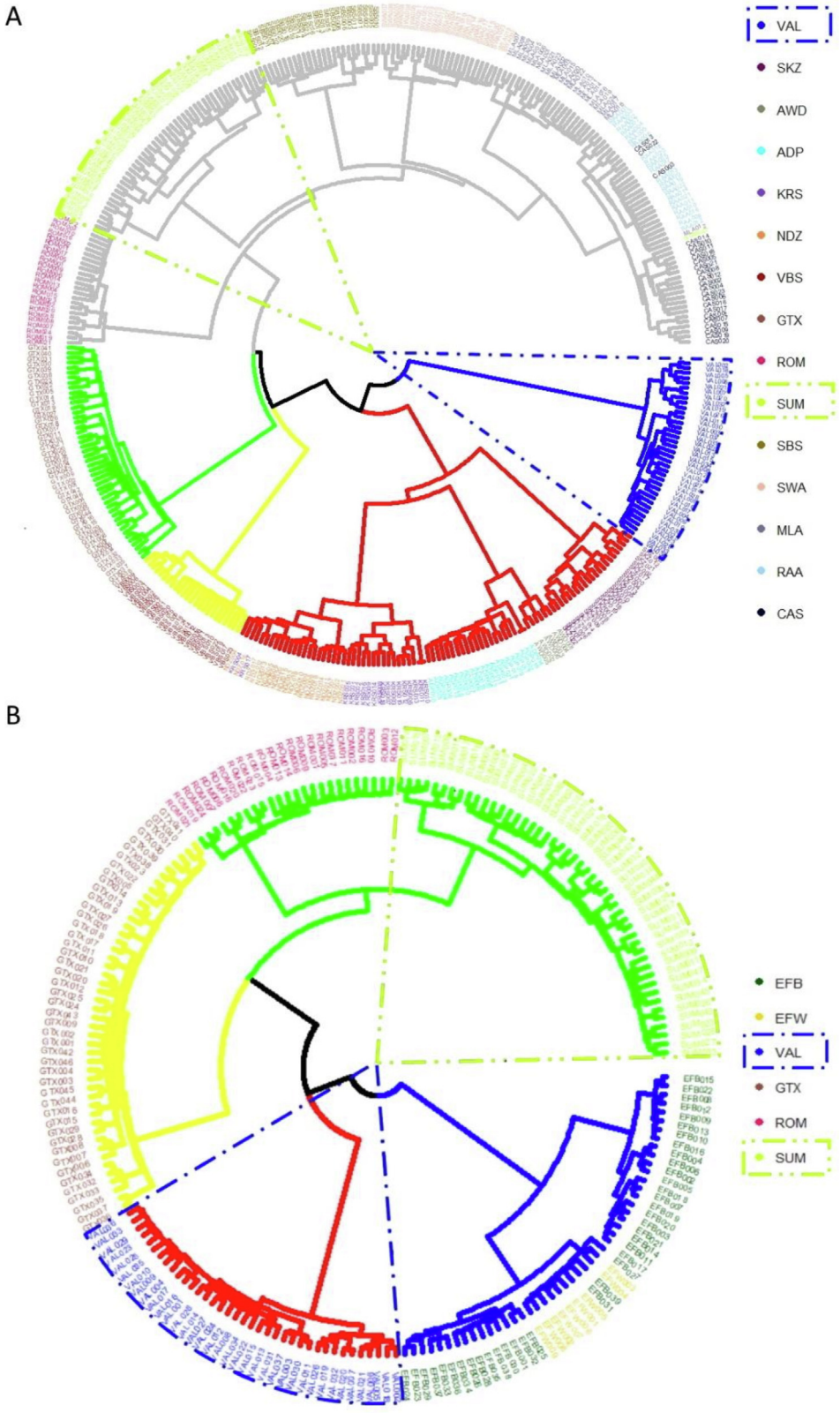

Fig. 4. Dendrogram based on Nei's genetic distances between animals of 15 sheep breeds in 5 branches: blue branch = Wallachian sheep; red branch = Sakiz, African Dorper, African White Dorper, Karakas, Norduz; yellow branch = Valais Blacknose sheep; green branch = German Texel; grey branch = New Zealand Romney, Sumava sheep, Swiss Black-Brown Mountain sheep, Swiss White Alpine sheep, Merinolandshaf, Meat Lacaune, Castellana.Dendrogram based on Nei's genetic distances between animals of 6 breeds in the four branches: blue branch = East Friesian Brown, East Friesian White; red branch = Wallachian sheep; yellow branch = German Texel; green branch = New Zealand Romney, Sumava sheep. Abbreviations: ADP = African Dorper; AWD = African White Dorper; CAS = Castellana; EFB = East Friesian Brown; EFW = East Friesian White; GTS = German Texel; KRS = Karakas; MLC = Meat Lacaune; MLA = Merinolandshaf; ROM = New Zealand Romney; NDZ = Norduz; SKZ = Sakiz; SUM = Sumava sheep; SBS = Swiss Black-Brown Mountain sheep; SWA = Swiss White Alpine sheep; VAL = Wallachian sheep; VBS = Valais Blacknose sheep.

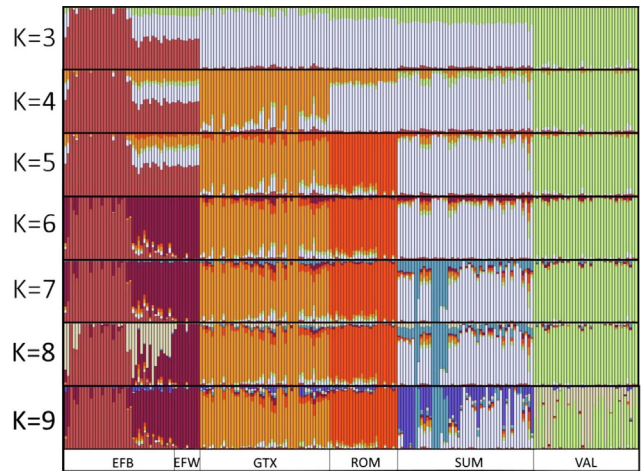

Fig. 5. Model-based clustering of 6 sheep breeds: East Friesian Brown, East Friesian White, German Texel, New Zealand Romne, Sumava sheep, Wallachian sheep, for modelled ancestral population $K = 3 - 9$, while the most likely number of clusters was $K = 7$. Abbreviations: EFB = East Friesian Brown; EFW = East Friesian White; GTS = German Texel; ROM = New Zealand Romney; SUM = Sumava sheep; VAL = Wallachian sheep.

Slatkin, 2008). Considering the last 40 generations, our findings are very similar to those obtained from the German White-headed Mutton population with the same methodology (Addo et al., 2021). The German White-headed Mutton had N_e slightly over 50 animals in the past five generations, which remained from the original 200 after a 35-generation-long decline (Addo et al., 2021). The same trend of N_e, with significantly lower values compared to other Sicilian breeds, was found in Barbaresca sheep by Mastrangelo et al. (2017b). On the contrary, Kyrgyz breeds showed notably higher values of N_e, between 176 and 660 animals in the 5th generation (Deniskova et al., 2019). Nosrati et al. (2021) compared breeds among different locations and continents, and breeds from Central Europe showed the lowest N_e drop curves; even so, the two breeds we monitored would belong to the lowest among them.

In the present study, the relationship between the two indigenous Czech breeds and other European sheep breeds was investigated with various approaches (F_{ST}, PCA, Admixture, neighbour-joining trees), and all of them pointed to their apparent uniqueness. Both Czech breeds made homogeneous clusters without significantly deviating individuals in the PCA graphical output based on the variance-standardised relationship matrix. However, the proximity of these breeds and among about 13 other European breeds was evident. The neighbour-joining trees revealed only one individual of Sumava sheep, who shared a common branch with the Castellana and Rasa Aragonesa breeds. On a smaller scale, the entire population of Sumava showed greater proximity to the New Zealand Romney than the other breeds used for its regeneration - East Friesian and Texel. However, not all of them were present in our dataset, and a significant genetic influence of other regenerative breeds thus cannot be excluded.

On the contrary, Admixture analysis revealed distinct ancestries in Sumava, which remained stable in several K-values. Some even affected almost the entire monitored genotype of the individual. Nevertheless, this admixture should not be interpreted as the influence of one additional breed because the value of K estimates the smallest number of populations involved in creating variability of our selected six breeds, which is probably smaller than the number of different events (bottlenecks, populations splits and mergings, gene flow between a new population and its ancestral population, ...) that significantly affected the sample. In future, it would be appropriate to check whether this is a remnant of historical outcrossing or modern, created by including crossed animals in the herdbook.

Calculated F_{ST} values can be divided into three categories based on the degree of isolation of populations (from the least to the most isolated): 0–0.05, 0.05–0.15, and 0.15–0.25 (Grasso et al., 2014). Breeds from the same region traditionally have lower fixation indexes between pairs of them due to great gene flow among populations, as was confirmed for the following sheep breeds: Berber and D'Man from Morocco; Hamra, Beni-Guil and Ouled-Djellal from Algeria; Ouled-Djellal from Morocco; and Sardi and Timahdite from Northwest Africa, whose pairwise F_{ST} does not exceed 0.67 value (Belabdi et al., 2019). For goats kept in the same region, the F_{ST} estimates were lower (<0.05) (Ouchene-Khelifi et al., 2018). Although the same pattern was observed in various sheep breeds and locations in the world, for example, in Tibet breeds, $F_{ST} = 0.03 - 0.09$ (Xiong et al., 2020), in Sicilian breeds, $F_{ST} = 0.03 - 0.05$ (Mastrangelo et al., 2014), and in Kazakh breeds, $F_{ST} = 0.02 - 0.04$ (Pozharskiy et al., 2020). This is not the case for the two Czech breeds analysed here, whose $F_{ST} = 0.10$ indicates only moderate gene flow between them in a range typical for well-differentiated breeds originating from different regions of Europe (Kijas et al., 2012b). There is a strong correlation and consistency that allows direct comparison between F_{ST} values obtained from microsatellites and SNPs (Laoun et al., 2020; Zimmerman et al., 2020). Compared to our previous study, the F_{ST} values obtained from the SNPs array were almost double those obtained from the microsatellites between Wallachian and Sumava and between them and other breeds as well (Machová et al., 2020). The number of individuals used in the previous study was significantly greater (Wallachian = 340, Sumava = 474), but the overall variability detectable by the markers was significantly lower, even though polyallelic microsatellites were used (Machová et al., 2020).

Conclusions

In this study, we evaluated the genetic variability of two indigenous Czech sheep breeds based on a 50 K SNP-Chip panel and compared it with 25 different breeds. Both breeds appeared to be well differentiated between each other and among other European breeds. Due to inbreeding and the effective population size, their genetic diversity is low, especially in the case of the Wallachian breed. Sumava, on the other hand, is facing a significant foreign gene pool. This study identified several regions with a high degree of homozygosity; further studies should focus on these regions to determine breed-specific genes under the influence of selection. The degree of genetic diversity within Sumava and Wallachian breeds is roughly consistent with the results obtained from pedigrees and microsatellite markers, contrary to the degree of inter-population genetic diversity (F_{ST}), which was significantly different.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2022.100690.

Ethics approval

Not applicable.

Data and model availability statement

None of the data was deposited in an official repository. The datasets used and analysed during the current study are available from the corresponding author upon reasonable request. Data obtained from Sheep HapMap are available from the International Sheep Genomics Consortium (ISGC). Still, restrictions apply to the availability of these data, which were used under license for the current study and therefore are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of the International Sheep Genomics Consortium (ISGC).

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Declaration of interest

None.

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