**Application:** By studying the genetics of indigenous populations, we offer valuable data to guide breeding strategies that ensure sustainable, profitable sheep farming.

**Introduction:** Autochthonous sheep breeds are well-adapted to local environments and agro-ecological conditions compared to cosmopolitan breeds, making them preferred in Mediterranean countries like Greece. Preserving rare alleles of indigenous breeds is essential for their resilience and adaptability in harsh environments, poor-quality pastures, and extreme temperatures. In this context, we analysed the most numerous Greek sheep breeds to unveil their genetic variation patterns, through population structure, ancestry, evolutionary history, and inbreeding levels. Three cosmopolitan breeds frequently reared in Greece in large flocks were included, to identify possible admixture patterns and ancestral connections with Greece’s national flock. We also examined whether geographical barriers, transhumance or human management have shaped the existing genetic material of sheep flocks in Greece over the years.

**Materials and Methods:** A total of 384 sheep samples originating from nine Greek sheep breeds (Boutsko, Chios, Kalarritiko, Karagkouniko, Katsika, Lesvou, Pelagonias, Serres, Thrakis) and three cosmopolitan breeds (Assaf, Lacaune, Charollais) were analysed using different versions of Illumina’s OvineSNP50K bead arrays (v1, v2, and v3). We retained only the common SNPs among versions (N=53,137 SNPs). Quality filtering was performed in PLINK v1.90 (Chang et al., 2015), setting the thresholds as follows: SNP call rate at 98%, minor allele frequency at 1%, and deviations from Hardy-Weinberg equilibrium at a p-value of 1x10-6. Additionally, samples with SNP missingness >10% were removed. The genetic background of sheep populations was tested both within and between breeds. Within each breed we analysed the i) observed (Ho) and expected (He) heterozygosities to calculate genetic diversity within each breed using the ARLEQUIN software v3.5.2.2 (Excoffier and Lischer, 2010), ii) Wright’s inbreeding coefficient FIS (Individual within Subpopulation) to measure excess of homozygotes or heterozygotes using PLINK v1.90, iii) effective population size (Ne) over 1,000 generations to assess relevant diversity histories using SNeP (Barbado et al., 2015) and iv) Runs of Homozygosity (ROHs) to identify inbreeding levels using PLINK v1.90, for a total length of 1Mb ROH, 15 variants in the scanning window, allowing 1 heterozygous SNP and 1 missing call to estimate homozygosity. Population structure was assessed by principal component analysis (PCA), while admixture analysis was performed to test for the genetic structure of the populations using the ADMIXTURE v1.3 software (Alexander and Lange, 2011) and the cross-validation (CV) error method, assuming a number of subpopulations (K) ranging from 2 to 12. The genetic differentiation of breeds was assessed using Wright’s pairwise FST values. Historical geneflow and ancestral connections were investigated between breeds using the Treemix software v1.12 (Pickrell and Pritchard, 2012) for different migration events, starting from 0 to 20 migration events, by grouping SNPs in windows of 500.

**Results:** Overall, the national flock of Greece’s sheep is characterized by moderate levels of genetic heterozygosity with mean values for the nine populations being Ho=0.356 and He=0.362. Chios breed, the only sheep breed that has been subjected to genetic improvement breeding schemes over the last decades in Greece, presented the lowest number of Ho (0.326) and He (0.344) values. On the contrary, the highest values were acquired for Karagkouniko breed (Ho=0.378, He=0.372). Within breed heterozygosity results were also verified by mean FIS values with the highest value obtained for Chios breed (FIS=0.155) and the lowest for Karagkouniko breed (FIS=0.022). Patterns of homozygosity and inbreeding levels revealed that Thrakis breed had on average the greatest number of ROH length (8.25Mb), followed by Lesvou (7.49Mb) and Serres (7.32Mb) breeds, suggesting more recent inbreeding events. Population structure analysis revealed the genetic proximity of Boutsko and Kalarritiko breeds, as anticipated based on historical data and the region of origin of these breeds (mountainous areas in Western Greece). Admixture analysis revealed a partitioning of the Serres, Kalarritiko, and Pelagonias breeds, corresponding to the farms from which the individual animals originated. Ancestral Ne displayed a gradual decreasing trend over the last 1,000 generations for all the studied populations. In the distant past, for Kalarritiko breed Ne was estimated to be 3,411, gradually reaching Ne=153 13 generations ago. All Greek breeds presented a wide genetic pool of considerable sizes ranging from 66 (Boutsko and Katsika) to 153 (Kalarritiko), assuring for long-term population viability. Genetic differentiation of breeds and geneflow analysis revealed three distinct clusters of sheep breeds, confirming also population structure results. In particular, sheep that originate from the mountainous areas of Western Greece, were found closely related (Kalarritiko, Boutsko, Katsika, Pelagonias), whereas, breeds from Eastern Greece and the Aegean Sea were found in close proximity. The greatest genetic distance was acquired in all pairwise comparisons of Greek breeds with the Charollais breed. Among Greek breeds, the greatest genetic differentiation was observed between Chios and Boutsko breeds, which is expected based on their phenotypic, historical, and pedigree data. Geneflow analysis indicated that Chios and Lesvou partially shaped the Karagkouniko population through distant gene flow events.

**Conclusions:** To address the genetic landscape of Greek sheep breeds we analysed ~50K SNPs to unveil their population structure and ancestral connections. To date, our work comprises the most comprehensive genetic characterization of sheep diversity in Greece. Our results expand our knowledge on the molecular variability of sheep breeds and present data on geographical partitioning and gene flow events. Although Greece’s national flock is characterized by large introgression from cosmopolitan breeds, we conclude that long-term preservation of sheep diversity is feasible by designing the appropriate breeding schemes.

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