

Genomic diversity of two indigenous Croatian goat breeds

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Abstract

This study used genome-wide SNP data to characterise the Istrian and Croatian Coloured goats genetically. Population structure and conservation status were analysed using 11 Istrian and 33 Croatian Coloured goats and 506 additional animals belonging to 19 European goat breeds. Neighbour-net and admixture results show that the two breeds are unique and retain a great proportion of the ancestral genetic structure. Despite low N_e for Istrian goats, both breeds showed low levels of inbreeding ($F_{ROH} \sim 0.05$). Considering these results, the Istrian goat is at high risk of extinction and should be carefully monitored.

Introduction

The domestic goat (*Capra hircus*) is one of the first domesticated animals. A relatively small animal with modest requirements and good resistance to harsh environmental conditions, it migrated with humans and colonised Croatian territory around 7000 years BP (Amills et al., 2017). Since then, goat rearing has been a constant presence in Croatian livestock production, although there have been some difficult times. In 1954, the government banned goat keeping in open areas, which led to a drastic decline in goat populations in Croatia. The indigenous Croatian goats are raised in extensive systems with low production. The Croatian Coloured goat (GCH) has 2261 animals in selection, while the Istrian goat (GCI) has only 14 animals in selection (Croatian Agency for Agriculture and Food, 2021). Local breeds are part of the genetic and socio-cultural heritage of the local community.

For this reason, it is essential to preserve the genetic diversity of the remaining breeds, mostly captured in non-selected autochthonous breeds (Medugorac et al., 2009). To date, there is little knowledge about the genetic structure of indigenous Croatian goat breeds. This study used genome-wide genotype data to analyse the genetic diversity and conservation status of two Croatian indigenous goat breeds, the Istrian goat and the Croatian Coloured goat.

Materials & Methods

Sample collection and data genotyping. A total of 11 hair samples from GCI and 34 tissue samples from GCH were genotyped using the Illumina Goat 50K SNP Chip (Illumina INC., San Diego, CA). Quality control was performed using SVS ver 8.9.0 (SNP and Variation Suite, Golden Helix, USA). It included removal of all: i) loci on the mtDNA, sex chromosome and without position, ii) genotypes with a call rate <90%, iii) individuals with a call rate <95%. We used 506 additional samples downloaded from the Dryad repository (<https://doi.org/10.5061/dryad.v8g21pt>) representing 19 European goat breeds (VAL - Valdostana, ORO - Orobica, BIO - Bionda dell'Adamello, VSS - Valpassiria, ASP - Aspromontana, ARG - Argentata, GAR - Garganica, DIT - Di Teramo, CCG - Ciociara Grigia, SAR - Sarda, SIT - Saanen, NIC - Nicastrese, CRS - Corse, SFR - Saanen, FSS - Fosses, PTV - Poitevine, PYR - Pyrenean, PVC - Provencale, ARR - Arran, CRP - Carpatian).

Population structure. Population structure was determined using a model-based clustering algorithm computed in ADMIXTURE ver. 1.3.0 software (Alexander and Lange, 2011) for 2-25 populations. Prior to calculation, data was pruned in PLINK 1.9 software (Chang et al., 2015) using the indep-pairwise 50 5 0.03 command. Nei's distances (Nei, 1972) was calculated using the StAMPP package (Pembleton et al., 2013) in R software (R Core Team, 2020) and used to generate the Neighbour-net graph using SplitsTree ver 4.14.2 software (Huson and Bryant, 2006)

Conservation status. Individual genomic inbreeding (F_{ROH}) was assessed using ROH data and then averaged by breed. ROH was determined using SVS software with parameters previously defined in Ferenčaković et al. (2013). Effective population size (N_e) analysis was calculated based on LD using GONE (Santiago et al., 2020) and using the molecular co-ancestry method implemented in NeEstimator V2.1 (Do et al., 2014.) with default parameters.

Results and discussion

After quality control, 10 GCI and 33 GCH with 54216 genotypes remained. The merged Croatian and European dataset consisted of 548 goats and 44867 genotypes, and after pruning, 19530 SNP remained.

The genetic relationship between the analysed breeds is represented with the Neighbour-net (Figure 1). Consistent with PCA (result not shown), several clear clusters are revealed in which the breeds form sub-branches. The GCI was clustered with the SIT and SFR, while the GCH was clustered with the CRP goat and is very close to the BIO and VSS. The longest branch formed Ireland ARR goat due to island isolation, similar to the results of Colli et al. (2018).

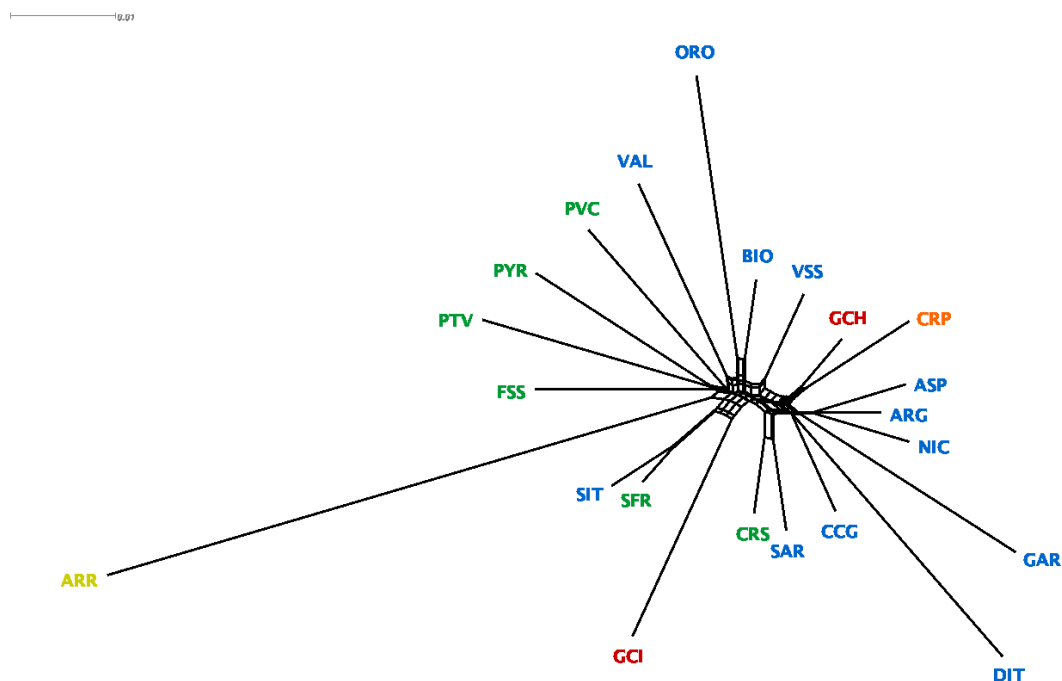


Figure 1. Neighbour-Net graph of Nei's distances between breeds. Each color represents one country: Croatia, Italy, France, Ireland, Romania.

The ADMIXTURE plots showed the results for K=3, K=8 and K=14 (Figure 2). First breed split occurred at K=3, and continued as the K value increased. After calculating CV errors, K=14 was identified as the best-fitting representation. K=14 confirmed clusters that diverged at lower K values and exhibited varying breed admixture degrees. The breeds ARR, DIT, FSS, GAR, ORO, PTV, PVC, PYR, SFR, SIT, SAR, VAL were assigned to separate clusters. Other breeds studied showed a high degree of admixture, as previously described in Nicoloso et al. (2015) and Colli et al. (2018). GCI showed a common genetic background of mixed origin similar to BIO and VSS with minor components of ORO, PVC, and Saanen breeds. The admixture pattern of GCH was similar to ARG, ASP, CRP, and NIC with a minor component of SAR. The Croatian goat breeds retained a large part of the historical genetic structure.

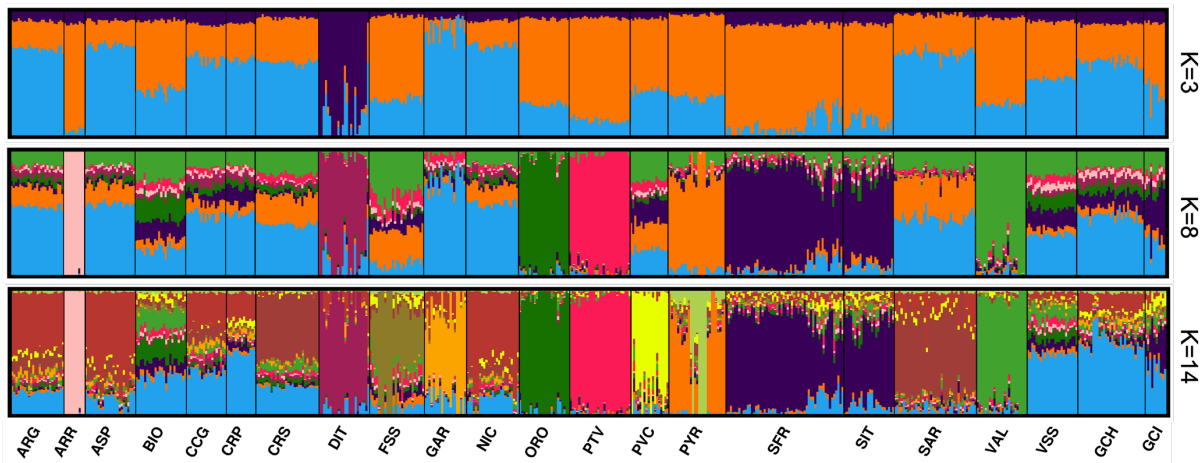


Figure 2. Population structure of 21 selected goat breeds using ADMIXTURE.

The conservation status of Croatian goats was estimated using genomic inbreeding (F_{ROH}) and effective population size (N_e). The distribution of F_{ROH} and estimates of N_e are presented in Table 1. Effective population size was shown for generation 1 (NeEstimator, GONE) and generation 5 (GONE) to be comparable with other authors. GCI had a lower N_e value than the GCH, which was expected due to the smaller population size of the GCI. The results for the GCI should be viewed with caution because the sample size is small. We observed an initial pattern of decreasing N_e in both breeds, but then a small increase in N_e was observed in GCH, probably due to a bottleneck. In general, highly productive breeds (Angora, Boer, Sannen, Alpine) show lower effective population size than local breeds with lower production (Colli et al., 2018). N_e should be at least 100 to ensure that an animal population is viable in the long term in terms of genetic diversity (Meuwissen, 2009), which means that the GCI is at high risk of extinction.

Table 1. Effective population size and inbreeding coefficients for Croatian goat breeds.

Population	N_e			F_{ROH}		
	NeEstimator	GONE		>2Mb	>8Mb	>16Mb
		Generation 1	Generation 5			
CGI	5.40	27.21	∞	0.06	0.05	0.04
CGH	119.20	477.10	439.27	0.04	0.03	0.02

Considering the small N_e it is surprising that the GCI, together with CGH, has a very low genomic inbreeding of 0.06 and 0.04, respectively. The low inbreeding could be explained by

high genetic admixture. Similar F_{ROH} values were obtained for native Italian ARG (Bertolini et al., 2018; Cortellari et al., 2021; Mastrangelo et al. 2021) SAR, BIO, VSS, NIC (Bertolini et al., 2018, Cortellari et al., 2021) and French CRS (Bertolini et al., 2018) breeds. F_{ROH} values decreased with increasing minimum ROH length, suggesting lack of recent anthropogenic influence. In summary, Croatian goat breeds have great genetic diversity, with a large part of historical admixture resulting from an extensive production system. Decrease in recent inbreeding ($F_{ROH} > 16\text{Mb}$) suggest no recent human selection. Caution should be taken when implementing breeding programmes, especially for the GCI, as the small N_e could seriously affect the overall biological fitness of the breed. This study was supported by ANAGRAMS: IP-2018-01-8708, project No.QK1810253 and Sir je IN: KK.01.1.1.04.0058.

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