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SHORT COMMUNICATION

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# The African introgression of Murciano Granadina goats has a Moroccan origin and displays remarkable levels of inter-individual variability

**Abstract**

There is evidence that Murciano Granadina (MG), the most important caprine dairy breed in Spain, has been introgressed by African goats, but the precise geographic origin of such introgression has not been identified yet. Moreover, an accurate estimate of the magnitude of this African introgression is lacking, since current estimates are based on small numbers of sampled individuals. The aim of our work was to tackle these two issues by genotyping 500 MG goats with the Goat SNP50 BeadChip and comparing their genotypes with those of reference populations from Spain (*Bermeya*), France (*Saanen*), Morocco (*Barcha*, *Draa*, *Ghazalia*, *Noire de Atlas*, *Nord*, *Moroccan*), Egypt (*Barki*, *Oasis*, *Saidi*), Algeria (*Arabia*, *Makatia*, *M'Zabite, Kabyle*), Tunisia (*Tunisian native breeds*) and Sudan (*Desert*, *Nilotic*, *Taggar*). The population of 500 MG goats was subdivided into 10 datasets of 50 individuals to ensure that sample sizes of the target (MG) and reference populations are balanced. Performance of an unsupervised ADMIXTURE analysis demonstrated that MG goats have a North African ancestry, with an average proportion of 4.4 ± 2.3%. Next, we did a supervised ADMIXTURE analysis that revealed that the Moroccan genetic component reaches a proportion of 4.01 ± 3.9% in MG goats, while the Algerian (0.001 ± 0.001%), Egyptian (0.2 ± 0.1%), Sudanese (0.1 ± 0.1%) and Tunisian (0.3 ± 0.4%) components are present in extremely small proportions. The historical circumstances of this introgression event are currently unknown, but several plausible scenarios are outlined. Moreover, our results show considerable inter-individual heterogeneity regarding the magnitude of the Moroccan introgression of MG goats (0%– 12% depending on the MG data set under analysis). This result implies that reliable estimates about the introgression of autochthonous livestock by exotic breeds can only be obtained by extensively sampling target populations.

There is convincing evidence of the introgression of African goats into European breeds, being especially significant in Italy and Spain (Colli et al., 2018; Manunza et al., 2016; Martínez et al., 2016). One of the most important Spanish goat dairy breeds is Murciano Granadina (MG), which is mostly distributed in Andalusia. MG goats have black or mahogany coats, and generally they display eumetric proportions and lack horns, being well adapted to harsh climatic conditions with hot and dry summers and cold winters (https://www.mapa.gob.es/es/ganaderia/temas/zootecnia/razas-ganaderas/razas). During the 1960s–1970s, Granadina and Murciana goats were admixed to create the MG breed, the herdbook of which was approved in in 1975 (Fernández Rodríguez et al., 2009). Several studies have contributed evidence about the introgression of African goats into MG goats (Manunza et al., 2016), but the precise geographic origin of such introgression has not been defined yet. The goal of our work was to answer this question as well as to estimate the amount of African ancestry in MG goats.

To achieve this goal, we have extracted blood samples from 500 MG goats, mainly distributed in 15 farms located in the autonomous region of Andalusia (Southern Spain), and we have purified genomic DNA by using a previously published salting-out protocol (Luigi-Sierra et al., 2020). Subsequently, these 500 goats have been genotyped with the Goat SNP50 BeadChip (Illumina Inc., San Diego, CA, USA), which encompasses 53 347 single nucleotide polymorphism (SNP) markers, following the instructions of the manufacturer. In addition, we have retrieved Goat SNP50 BeadChip data from Moroccan (MOR; $N = 20$; Barcha, Draa, Ghazalia, Noire de Atlas, Nord, Moroccan), Egyptian (EGY; $N = 20$; Barki, Oasis, Saidi), Tunisian (TUN; $N = 21$; Tunisian native breeds), Spanish (BEY; $N = 20$; Bermeya) and French (SAA; $N = 40$; Saanen), and French ($N = 40$; Saanen [SAA]) breeds that were generated in the AdaptMap project (Stella et al., 2018). We have also gathered Goat SNP50 BeadChip data from Algerian (ALG; $N = 48$; Arabia, Makatia, M'Zabite,

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Kabyle) and Sudanese (SUD; $N=50$; Desert, Nilotic, Taggar) breeds that were available at the Dryad Digital repository (Ouchene-Khelifi et al., 2018; Rahmatalla et al., 2017). The genomic locations of the SNPs were obtained using the goat ARS1 genome (Bickhart et al., 2017) as reference, and the position and the name of each SNP was updated using the PLINK v 1.9 software (Chang et al., 2015).

A data set of 50729 SNPs was subsequently filtered with PLINK v 1.9 (Chang et al., 2015) to eliminate those meeting any of the following conditions: (1) SNPs with minor allele frequencies (MAF) lower than 0.05; (2) SNPs with missing call rates >0.05; (3) SNPs that did not deviate from Hardy–Weinberg expectations ($p$-value ≤0.001); and (4) unmapped SNPs. After filtering and quality control, we retained 39039 SNPs for further analyses. To investigate population structure, we performed principal component analysis (PCA) using the '--pca' command line in PLINK v1.9 (Chang et al., 2015). The PCA results were plotted with the '*ggplot2*' package of the RStudio program (RStudio Team, 2020). To avoid the overcrowding of the PCA with the 500 MG goats, we randomly extracted 50 MG goats with the Python '*randint()*' method (https://docs.python.org/3/library/random.html#functions-for-integers). Genotype data from the 50 MG goats selected at random were merged with those of Spanish, African, and SAA goats with PLINK v1.9 (Chang et al., 2015).

In the PCA shown in Figure 1, principal component 1, which accounts for 28.59% of the total variance, separated the SAA, Spanish (BEY and MG) and African (MOR, ALG, EGY, SUD, TUN) populations. Principal component 2, which accounts for 13.79% of the total variance, provided similar results. The degree of separation between the MG and BEY breeds from the African ones was similar to that between SAA and African breeds, suggesting that the introgression of African goats into MG goats was likely to be quite low. The ADMIXTURE v1.3.0 software (Alexander et al., 2009; Alexander & Lange, 2011) was used to determine the ancestry of MG goats. Since the MG population ($N=500$) is very large and unbalanced sample size can distort the results of ADMIXTURE analyses, we divided the data set of 500 MG goats into 10 datasets of 50 individuals. To do so, we followed the order of the total of 500 MG sample list i.e. dataset 1 = from the first MG in the list to the 50th, dataset 2 = from the 51st to the 100th, etc. The MOR, ALG, EGY, SUD, TUN, BEY, and SAA goat populations were set as reference populations, and each one of the 10 MG data sets was set as the target population. In other words, we carried out 10 independent ADMIXTURE analyses comprising one group of 50 MG goats plus the full set of reference populations. Two unsupervised and supervised ADMIXTURE analyses were performed. While the unsupervised analysis was useful for characterizing general population structure, the supervised analysis is expected to provide more precise estimates of ancestry (than the unsupervised one) because there is less uncertainty in the determination of allele frequencies (Alexander & Lange, 2011). Moreover, in the supervised analysis the display of results is simplified and run times are shorter because there are fewer parameters to estimate (Alexander & Lange, 2011). In both analyses, we tested the number of clusters (K) varying from two to 10 for the 10 datasets. Moreover, to infer the most likely K-value we estimated the cross-validation error for each $K$-value (Figure S1). The optimal K for all 10 datasets was $K=6$ (Figure S1). To carry out the supervised analysis, reference European and African populations of known ancestry were established and set to seven (MOR, ALG, EGY, SUD, TUN, BEY from Spain, and SAA from France) and the flag '*supervised*' was used in the command line.

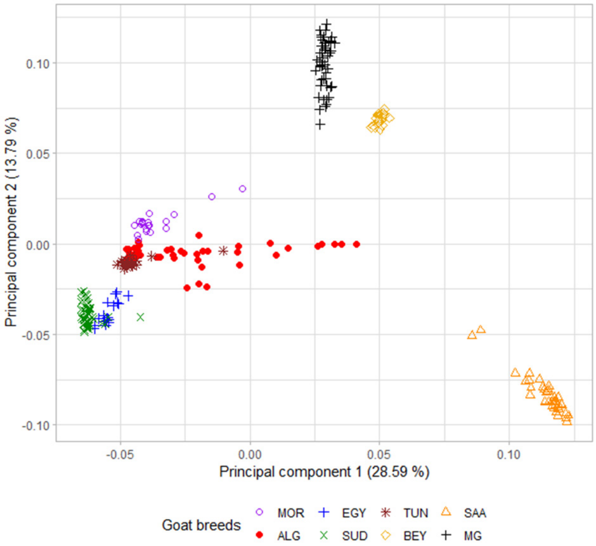

**FIGURE 1** Principal component analysis of 50 goats from the Murciano Granadina (MG) breed randomly extracted from a population of 500 MG individuals plus reference populations from Morocco (MOR), Algeria (ALG), Egypt (EGY), Sudan (SUD), Tunisia (TUN), France (SAA, Saanen), and northern Spain (BEY, Bermeya).

Maximum likelihood estimation of individual ancestries from Goat SNP50 BeadChip genotypes for each one of the datasets are shown in Figure S2 (unsupervised analysis) and Figure 2 (supervised analysis). In the unsupervised analysis ($K=6$), the genetic background that predominates in the MOR, ALG, and TUN goats (indicated in purple) was found in the 10 MG datasets with an average percentage of 4.4 ± 2.3% (Figure S2). In addition, the predominant background in the Spanish BEY breed (colored in green) was also present in the MG goats with an average percentage of 12.19 ± 6.2%. By contrast, in the supervised analysis the Spanish genetic background, represented by the BEY breed, was overwhelmingly predominant in MG goats (average proportion 95.1 ± 4%). Moreover, the MOR genetic component reached a proportion of 4.01 ± 3.9%, while the ALG (0.001 ± 0.001%),

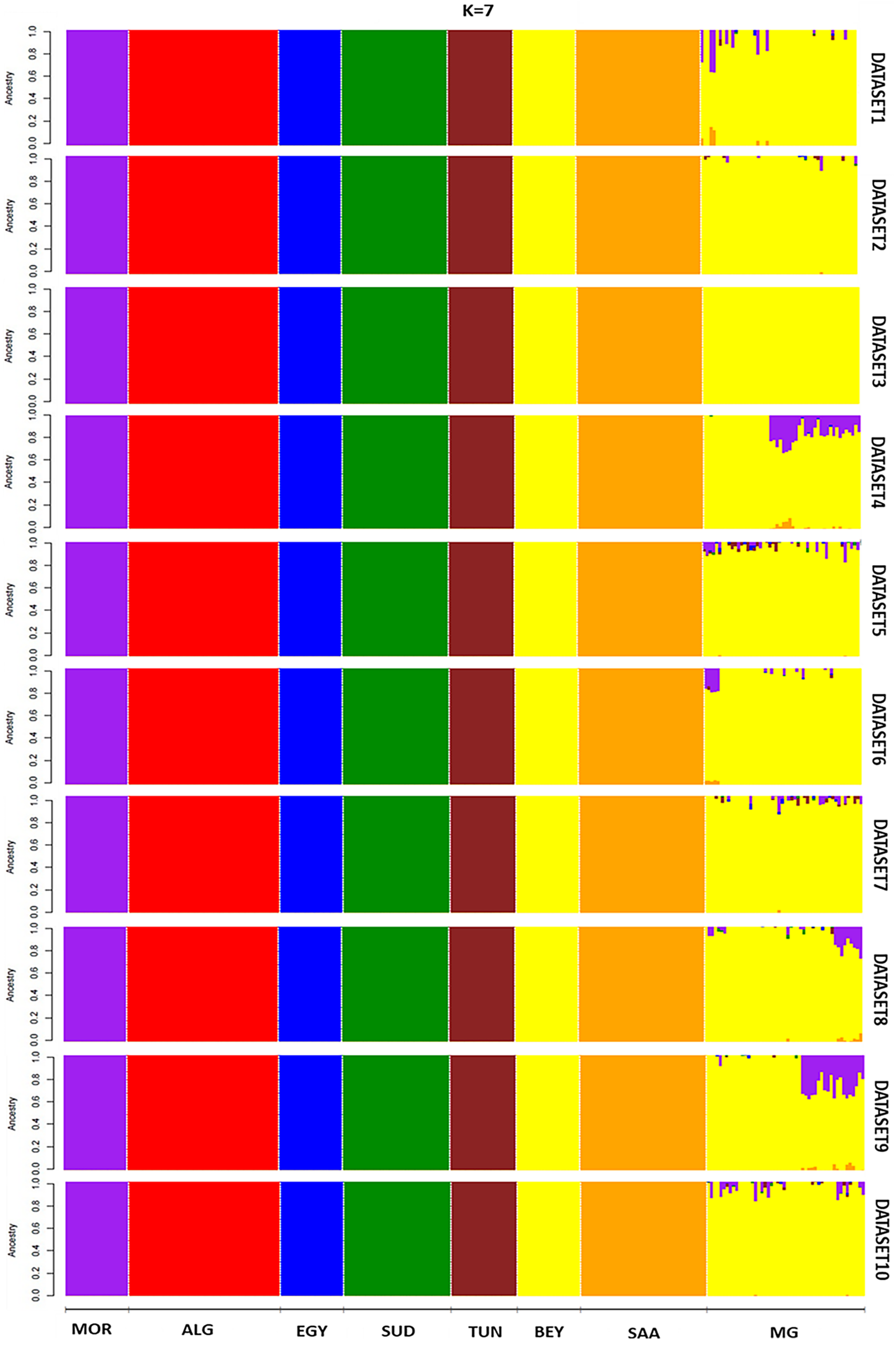

FIGURE 2 Supervised ADMIXTURE analysis of 10 groups of Murciano Granadina goats (MG) with 50 individuals each (datasets 1–10) plus reference populations from Morocco (MOR), Algeria (ALG), Egypt (EGY), Sudan (SUD), Tunisia (TUN), Central Europe (SAA, Saanen), and northern Spain (BEY, Bermeya). The levels of African (essentially Moroccan) introgression in MG goats are highly variable across datasets 1–10.

EGY (0.2±0.1%), SUD (0.1±0.1%), and TUN (0.3±0.4%) components were present in extremely small proportions. In a previous study, Manunza et al. (2016) reported that MG goats were substantially introgressed by TUN goats but in their analysis North Africa was exclusively represented by TUN goats and the number of investigated MG goats was much smaller than ours. This latter observation is important because we have shown that admixture levels can vary considerably from one individual to another.

The percentage of MOR ancestry in MG goats was highly variable and dependent on the selected MG data set e.g., data set 3 did not show any sign of African admixture, while in data sets 4 and 9 the MOR ancestry component reached values of 10% and 11.7%, respectively. Signatures of African introgression into European breeds have been detected in cattle (Decker et al., 2014), sheep (Ben et al., 2019), and goats (Colli et al., 2018; Manunza et al., 2016; Martínez et al., 2016). Manunza et al. (2016) investigated the diversity and demographic history of seven Spanish goat breeds and detected moderate levels of African introgression (~25%) into the Andalusian Malagueña and MG breeds. Our results confirm the African ancestry of MG goats although of a much lower magnitude (~4%). More importantly, we also demonstrate the existence of a significant inter-individual variability regarding the magnitude of such African admixture. This means that large sample sizes are needed to accurately estimate the degree of exotic introgression into livestock breeds. The African component identified in the genomes of MG goats had fundamentally a MOR origin, while breeds from Tunisia, Algeria, Sudan, or Egypt did not make any significant contribution to it. When we calculated f4 statistics with the qpdstat program, which is included in the ADMIXTOOLS software package (Patterson et al., 2012), we obtained positive and highly significant values, i.e. f4 (MOR, ALG; MG, SAA)=0.0043862444 (Z-score=27.777064), f4 (MOR, TUN; MG, SAA)=0.0011471014 (Z-score=7.233554), f4 (MOR, SUD; MG, SAA)=0.0009029751 (Z-score=4.955694) and f4 (MOR, EGY; MG, SAA)=0.0021544189 (Z-score=11.135624). The f4 statistic reflects the covariance of allele frequency differences between two pairs of populations and here a positive value should be interpreted as evidence of gene flow between MOR and MG goats or, far less likely, between Swiss SAA and any of the North African tested populations. The close geographical proximity between southern Spain and Morocco (Strait of Gibraltar=13 km), the existence of a bidirectional flow between Africa and Spain goat populations (Martínez et al., 2016), and the Admixture results obtained in the current work point to MOR goats as the most probable source of African introgression into MG goats. Moreover, investigating the potential effect of selection for dairy traits on the patterns of African introgression observed in MG goats would be relevant. For example, the analysis of the genomes of Large White pigs has demonstrated that regions of Asian origin often contain genes related to lipid metabolism, thus suggesting that artificial selection on fat traits shaped the genomic landscape of Asian introgression into this swine breed (Bosse et al., 2015).

Regarding the historical circumstances that led to the introgression of MG goats with MOR blood, several non-mutually exclusive scenarios are possible. The genetic analysis of Iberian Bronze Age cattle from the Atapuerca archaeological site provides evidence for the existence of prehistoric contacts over the Strait of Gibraltar that involved the exchange of domestic animals (Anderung et al., 2005). The Roman occupation of the Iberian Peninsula, which started in 218 BCE and ended in the 5th century, might have also favored the transportation of livestock from North Africa to Hispania. Indeed, the Roman army was supplied with animals for riding, cargo and/or transport by military-logistics centers distributed all across the Roman empire (Colominas et al., 2022). Interestingly, Colominas and Edwards (2017) reported the identification of the African mitochondrial T1 haplotype in archaeological cattle remains from the Roman trading post of Empúries (Catalonia). A third plausible historical scenario is the Muslim invasion of Spain, in the 8th century, which resulted in the establishment of 40 000–50 000 Arabs, from northeast Africa and Middle East, and hundreds of thousands of Amazigh settlers in Spain (Franco Moreno, 2005). Although we do not know for sure whether African small ruminants were transported to Spain, studies based on archaeofaunal assemblages coming from Andalusian contexts consistently point out the predominant presence of sheep and goat remains, providing evidence that these two species were the most consumed (Carvajal López, 2016; Moreno, 2013). Granadina goats, together with Merino sheep, Spanish Pure Breed Horse, and the Fighting Bull, already existed as breeds in the 15th century (Rodero et al., 1992). In 1439, the King Juan II of Castile authorized the yearly exportation of 7000 sheep and goats and 1000 cattle to the Emirate of Granada, and this permission was renewed in 1442 (Fernández Arriba, 1986). However, such commerce suffered important restrictions whenever hostilities between the Kingdoms of Castile and the Emirate of Granada intensified (Fernández Arriba, 1986). Moreover, we do not know if such livestock exchanges resulted in the introgression of Granadina goats by African goats, since very little is known about livestock husbandry during the Nasrid dynasty that ruled the Emirate of Granada from 1232 to 1492. Although our data support the transportation of African livestock into Spain, the genetic analysis of dated archaeological remains will be needed to confirm it.

**KEYWORDS**
admixture, goat, high-density SNP arrays, introgression, Murciano Granadina

## AUTHOR CONTRIBUTIONS

**E. Petretto:** Formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **M. G. Luigi-Sierra:** Data curation; formal analysis. **G. M. Vacca:** Conceptualization; writing – review and editing. **A. Martínez:** Conceptualization; resources; writing – review and editing. **J. V. Delgado:** Resources; supervision; writing – review and editing. **A. Castelló:** Data curation; formal analysis; investigation. **M. Pazzola:** Conceptualization; writing – review and editing. **J. Jordana:** Conceptualization; supervision; writing – review and editing. **M. L. Dettori:** Conceptualization; writing – review and editing. **M. Amills:** Conceptualization; project administration; resources; supervision; validation; writing – original draft; writing – review and editing. **J. Fernández Álvarez**: Resources.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

Murciano Granadina goat SNP50 BeadChip genotypes are accessible at https://doi.org/10.6084/m9.figshare.18095825. The AdaptMap genotypic datasets can be accessed at: https://datadryad.org/stash/dataset/doi:10.5061/dryad.v8g21pt. ALG goat genotype data are available: https://doi.org/10.5061/dryad.5pt8nt8. Raw genotyping data of SUD goat breeds are available in: (Rahmatalla et al., 2017 – Additional file 10).

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## SUPPORTING INFORMATION

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