**RUNNING HEAD:** *ASIP* copy number and goat pigmentation

# Estimating the copy number of the agouti signaling protein (*ASIP*) gene in goat breeds with different color patterns

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## Abstract

The agouti signaling protein (*ASIP*) gene has a crucial role in pigmentation by encoding a protein that binds the melanocortin 1 receptor and stimulates the synthesis of pheomelanin rather than eumelanin. Several studies have suggested that an increased copy number of the *ASIP* gene might explain the white pigmentation of certain goat breeds, as previously demonstrated in sheep. In the current work, we have identified the segregation of the *ASIP* CNV in Murciano-Granadina (black or brown coat), Malagueña (brown, blond or white coat) and Saanen (white coat) goats with available Illumina Goat SNP50 BeadChip (Illumina Inc., San Diego, CA) data by using the PennCNV v1.0.5 and QuantiSNP v2 tools. This result shows that the *ASIP* CNV segregates in non-white breeds. To gain new insights into this issue, we have estimated the copy number of the *ASIP* gene in 83 goats from 8 breeds with different coloration patterns using a real-time quantitative PCR approach. Our results showed an increased *ASIP* copy number not only in Saanen (3.50 ± 0.23 copies relative to the calibrator) and white Malagueña (3.51 ± 0.51 copies) goats, but also in the Murciano-Granadina breed (3.33 ± 0.58 copies) as well as in blond/brown individuals from the Malagueña (3.58 ± 0.73 copies) breed. The number of *ASIP* copies was not significantly different in these four caprine populations ($P > 0.05$). Moreover, we did not observe a trend towards increased *ASIP* copy number in breeds with predominantly white colors, such as Maltese (2.85 ± 0.28 copies), Jonica (2.82 ± 0.39 copies) and Blanca de Rasquera (2.37 ± 0.33 copies). Our results, combined with recent findings demonstrating the high structural complexity of the *ASIP* locus, indicate that additional functional and expression studies should be performed in order to fully understand the role of *ASIP* structural variation in goat pigmentation.

**Keywords**: *ASIP*, copy number variation, pigmentation, real-time quantitative PCR

## 1. Introduction

Copy number variation (CNV) plays a key role in the genetic determinism of several pigmentation phenotypes in domestic species (Freeman et al., 2006; Clop et al., 2012; Bickhart and Liu 2014; Zarrei et al., 2015). For instance, the loss of white pigmentation in the South African Boer breed is associated with a 1 Mb CNV mapping to the endothelin receptor type A (*EDNRA*) gene (Menzi et al., 2016). In sheep, the causal factor of the dominant white/tan ($A^{Wt}$) coat was mapped to a 190 kb tandem duplication encompassing the whole agouti signaling protein (*ASIP*) gene (Norris and Whan 2008). The transcription of the second copy of the ovine *ASIP* gene is controlled by the promoter of the itchy homolog E3 ubiquitin protein ligase (*ITCH*) gene and it shows a deregulated and ubiquitous pattern of expression associated with a white coloration (Norris and Whan 2008).

Classical genetic studies carried out in crossed goats revealed that the white color might be caused by the dominant $A^{Wt}$ (white/tan) allele of the *ASIP* locus (Adalsteinsson et al., 1994). Fontanesi et al. (2009) identified one CNV in the caprine *ASIP* gene encompassing at least 100 kb, and they suggested that in Saanen and Girgentana goats this CNV might be the causative factor explaining the white pigmentation of these two breeds. Noteworthy, Fontanesi et al. (2009) detected an *ASIP* copy gain in one individual from the Murciano-Granadina breed, which can be black or brown but never white, and they also demonstrated that not all the analyzed Saanen goats carried 2 additional *ASIP* copies. They proposed that there might be some degree of genetic heterogeneity in the *ASIP* locus and that the expression of *ASIP* alleles may be modulated by epistatic interactions (Fontanesi et al., 2009). In a subsequent study based on whole-genome resequencing data, Dong et al. (2015) showed that Yunnan Black goats and brown Australian Rangeland goats carry a single *ASIP* gene copy, while light colored Cashmere and Boer goats

harbor multiple *ASIP* copies. However, the scope of this experiment was limited by the low number of analyzed individuals. Zhang et al. (2018) also compared *ASIP* copy number in two white (Saanen and Liaoning) vs. two black (Leizhou and Dera Din Panah) goat breeds and reported a lower *ASIP* copy number in the latter, suggesting that this CNV has causal effects on pigmentation. Indeed, a selection scan performed in white vs. colored (black and red) goats made it possible to detect a selective sweep co-localizing with the *ASIP* gene (Bertolini et al., 2018). More recently, Henkel et al. (2019) identified four different CNVs, close to or encompassing the *ASIP* locus, that showed correspondence with the white or tan ($A^{Wt}$, Appenzell and Saanen), Swiss markings ($A^{sm}$, Grisons Striped and Toggenburg goats), badgerface ($A^{b}$, Chamois Colored and the St. Gallen Booted goat), and peacock ($A^{pc}$, Peacock goat) alleles of the *ASIP* locus. The CNV segregating in Saanen and Appenzell goats encompasses 154.6 kb and covers the entire coding regions of the *ASIP*, *AHCY* and *ITCH* genes. In Grisons Striped and Toggenburg goats, eight tandem copies of a 13.4 kb sequence from the 5'-flanking region of *ASIP* ($A^{sm}$ amplification) locus were identified (Henkel et al., 2019). In the case of Chamois Colored and St. Gallen Booted goats, there was a five-fold amplification of 45.6 kb ($A^{b}$ amplification) positioned ~61 kb downstream of the $A^{sm}$ amplification (Henkel et al., 2019). In contrast, in Peacock goats a quadruplication of the same ~45 kb region having five copies in the $A^{b}$ allele was detected (Henkel et al., 2019). Moreover, Henkel et al. (2019) observed that in Grisons Striped ($A^{sm}$), Chamois Colored ($A^{b}$) and Peacock goats ($A^{pc}$), the eumelanistic skin displayed low levels of *ASIP* mRNA expression, while pheomelanistic skin areas showed at least 10-fold higher *ASIP* expression than their eumelanistic counterparts. Noteworthy, the white pigmented Saanen goat ($A^{Wt}$) showed the highest levels of *ASIP* mRNA expression. However, there was no significant correlation between *ASIP* mRNA levels and the intensity of the pheomelanistic coloration.

In a previous study (Guan et al., 2020), we performed a CNV scan in a population of 1,036 Murciano-Granadina goats and found evidence of a CNV mapping to the *ASIP* locus. The goal of the current work is to characterize the segregation of the *ASIP* CNV in several goat breeds with different coat colors (Saanen, Jonica, Carpathian, Maltese, Blanca de Rasquera, Derivata di Siria, Malagueña and Murciano-Granadina) in order to find out whether dark-colored breeds have lower *ASIP* copy numbers than the ones with white coats.

## 2. Materials and Methods

### 2.1. Animal material

We performed a first experiment exclusively focused on three breeds with available color records, i.e., Murciano-Granadina (N = 559, 400 black and 159 brown), Malagueña (N = 53, 11 dark blond, 16 light blond, 16 white and 10 brown) and Saanen (N = 42, all white). These individuals were subjected to a CNV scan based on Goat SNP50 BeadChip (Illumina) data in order to identify the presence (or absence) of a CNV in the *ASIP* locus.

A second experiment aiming to estimate *ASIP* copy number by quantitative real-time PCR (qPCR) in a sample of 83 goats from eight breeds with different color patterns (please see below) was carried out. More specifically, goats under analysis belonged to the following breeds (**Figure S1**): Saanen (white, N=10), Jonica (white or rosy, sometimes with tawny spots in the head and neck, N=9), Carpathian (polychromatic, N=9), Maltese (white, with a raven-black area on the top and sides of the head, N=6), Blanca de Rasquera (white or white with black spots, N=9), Derivata di Siria (brown/blond, sometimes white pied, N=10), Malagueña (white, N=10;

blond/brown, N=10) and Murciano-Granadina (black/brown, N=10). Genomic DNAs from these goats had been obtained in previous studies (Badaoui et al., 2014; Manunza et al., 2016).

### 2.2. CNV calling based on Goat SNP50 BeadChip genotyping data

Goat SNP50 BeadChip (Illumina) data from Murciano-Granadina goats were generated in a previous study (Guan et al., 2020), while in the case of the Saanen goats chip genotypes were kindly provided by Dr. Gwenola Tosser-Klopp from INRA (Castanet-Tolosan). Genomic DNAs of Malagueña goats were genotyped by using the Illumina Goat SNP50 BeadChip (Tosser-Klopp et al., 2014) in accordance with the instructions of the manufacturer (Illumina Inc., San Diego, CA). Following Attiyeh et al. (2009), B allele frequencies (BAF) and signal intensity ratios (log R Ratio or LRR) were obtained with the GenomeStudio software 2.0.4 (Illumina, https://emea.illumina.com). After converting SNP coordinates to the latest version of the goat reference genome (ARS1, Bickhart et al., 2017), we filtered out unmapped and non-autosomal SNPs and those with a call rate lower than 98%. By doing so, CNV mapping was independently carried out with sets of 49,877 SNPs (Saanen, N=42) and 50,701 SNPs (53 Malagueña goats). Specifically, we employed the EnsembleCNV pipeline (Zhang et al., 2019) to assemble initial calling data from PennCNV v1.0.5 (Wang et al., 2007; Diskin et al., 2008) and QuantiSNP v2 (Colella et al., 2007) into CNV regions (CNVRs) with a heuristic algorithm (threshold of minimum overlap = 30%). The CNVR boundaries were subsequently refined by considering the local correlation structure of the LRR values of the SNPs mapping to CNVRs (Zhang et al., 2019). Then, we reassigned CNV calls initially obtained with both PennCNV and QuantiSNP to

each refined CNVR, so the final set of CNVRs only comprised those simultaneously detected by both callers.

### 2.3. Estimating *ASIP* copy number by quantitative real time PCR

We performed a second experiment based on real-time qPCR to estimate *ASIP* copy number in the three breeds mentioned before (Murciano-Granadina, Malagueña and Saanen) plus five additional populations (Jonica, Carpathian, Maltese, Blanca de Rasquera and Derivata di Siria) for which DNA was available. Primers were designed with the Primer Express Software (Applied Biosystems) to amplify specific regions of the caprine *ASIP* gene and two reference genes (**Table S1**): melanocortin 1 receptor (*MC1R,* Fontanesi et al., 2009; Liu et al., 2018) and glucagon (*GCG*, Ballester et al., 2004; Ramayo-Caldas et al., 2010). Specifically, polymerase chain reactions (PCR) were carried out in a final 15 µL volume containing 7.5 ng genomic DNA, 7.5 µL 2 × SybrSelect Master Mix (Applied Biosystems), 300 nM of each forward and reverse primer and ultrapure water. Each sample was analyzed in triplicate. Assays were loaded in 384-well plates and run in a QuantStudio 12K Flex Real-Time PCR System instrument (Applied Biosystems). The thermal cycling was 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 seconds and 60°C for 1 min. The specificity of the PCR reactions was assessed with a melting curve analysis procedure based on the following thermal profile: 95°C for 15 seconds, 60°C for 15 seconds and a gradual increase in temperature with a ramp rate of 1% up to 95°C. By performing ten-fold serial dilutions of a goat DNA pool template, the generated standard curves showed a comparable amplification with efficiencies ranging from 107.2% to 108.8%. The relative copy number of the *ASIP* gene was inferred with the qbase+ software (Biogazelle, Ghent, Belgium) by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The average of the four

samples with the lowest *ASIP* copy numbers was employed as calibrator for relative quantification.

## 3. Results and discussion

By performing CNV mapping, we confirmed the existence of a CNVR co-localizing with the *ASIP* gene in white Saanen, black/brown Murciano-Granadina and white/blond/brown Malagueña goats (**Figure S2**). This is a clear indication that, as pointed out by Fontanesi et al. (2009) there is increased copy number of the *ASIP* locus not only in goats from white breeds, such as Saanen, but also in Murciano-Granadina individuals that are black or brown. To gain new insights into this issue, we decided to carry out a real-time qPCR quantification of *ASIP* copy number in a panel of 83 goats from 8 breeds with diverse coat colors. The average estimates of *ASIP* relative copy number per breed and individual relative to the calibrator are shown in **Figures 1A** and **1B**, respectively. It is important to emphasize that our data should not be interpreted in terms of absolute copy numbers. As previously said, all copy number estimates are calibrated with regard to the four individuals with lowest copy numbers. As expected, we observed high *ASIP* copy numbers in the Saanen breed (3.50 ± 0.23 copies, relative to the calibrator), a result that is consistent with data reported by Fontanesi et al. (2009). Likewise, a similar high copy number was observed in pure white individuals from the Malagueña breed (3.51 ± 0.51 copies). However, we also detected high *ASIP* copy numbers in brown/blond Malagueña goats (3.58 ± 0.73 copies) and in black/brown Murciano-Granadina goats (3.33 ± 0.58 copies). Interestingly, the highest copy number was found in a light blond Malagueña goat (5.00 ± 0.18 copies). Performance of an ANOVA test with the “aov” function implemented in R

(https://www.r-project.org/) revealed that *ASIP* copy numbers are not different amongst the four populations cited above ($P > 0.05$, **Table 1**). Moreover, several breeds, which exhibit a white or predominantly white coat (e.g. Jonica, Maltese and Blanca de Rasquera), showed lower *ASIP* copy numbers than brown/blond Malagueña and black/brown Murciano-Granadina goats (**Figure 1**). The correlation between copy number estimates derived from Goat SNP50 BeadChip data and qPCR analysis in 30 individuals (**Table S2**) analyzed with both techniques was very low and non-significant ($r = 0.07$, $P = 0.71$). In the case of SNP arrays the relationship between signal and copy number is not linear and subject to saturation effects (Attiyeh et al., 2009). Furthermore, inferring gains and losses on the basis of signal intensity alone involves the establishment of arbitrary intensity cutoff values (Attiyeh et al., 2009). Although SNP arrays are useful to detect CNV at a genome-wide scale, real-time qPCR is probably better suited to accurately genotype individuals. Moreover, the genomic region containing the *ASIP* gene is highly complex at the structural level (Henkel et al., 2019). Our qPCR test is designed to amplify a small region spanning part of intron 2 and part of exon 3 of the *ASIP* gene. Any CNV outside this region, *e.g.* $A^{sm}$ allele of the *ASIP* gene which involves 8 tandem copies of a 13 kb sequence from the 5'-flanking region of the *ASIP* (Henkel et al. 2019), would not be detectable by qPCR, while it could be identified through the analysis of SNP array data. So, the lack of consistence between qPCR and SNP array copy number estimates could be due to multiple technical factors. Despite this lack of consistence at the individual level, both SNP array and qPCR data indicate that high *ASIP* copy numbers can be detected in goats from non-white breeds.

There are previous studies indicating the absence of a linear relationship between *ASIP* copy number and goat pigmentation. For instance, Zhang et al. (2018) reported that white Liaoning Cashmere goats have higher average *ASIP* copy numbers than black Leizhou goats, but

several black Leizhou goats harbored higher *ASIP* copy numbers than their white Liaoning Cashmere counterparts. Similarly, Fontanesi et al. (2009) indicated that not all Saanen goats investigated in their experiment carried 2 additional copies of the *ASIP* gene and, even more, they also identified a Murciano-Granadina individual (MGb7) with an increased *ASIP* copy number similar to that estimated in Saanen goats. So, our data and results presented by other authors (Fontanesi et al., 2009; Zhang et al., 2018) cast a doubt about the existence of a direct and tight relationship between *ASIP* copy number and white coat color in goats. As pointed out by Fontanesi et al. (2009), this could be due to a complex inheritance pattern involving epistasis and other genetic factors modulating pigmentation. Recently, Henkel et al. (2019) detected at least four CNVs located near or encompassing part of the caprine *ASIP* gene, which are associated with different color patterns. These authors showed that in Grisons Striped goats ($A^{sm}$), Chamois Colored goats ($A^{b}$) and Peacock goats ($A^{pc}$), the eumelanistic skin displayed a weak *ASIP* mRNA expression, while the pheomelanistic skin regions in these three goats had at least 10-fold higher *ASIP* expression than the corresponding eumelanistic samples. Moreover, the uniformly white Saanen goat ($A^{Wt}$) had the highest *ASIP* mRNA expression. In summary, these authors were able to correlate the eumelanistic/pheomelanistic pigmentation of skin with *ASIP* mRNA expression, but evidence correlating *ASIP* copy number estimates with *ASIP* mRNA expression in the skin were not reported. In the absence of evidence linking copy number with mRNA expression levels, it is difficult to assume that the CNV has a causal role on pigmentation because it is unknown whether increased copy number translates into an increased function. Indeed, the duplication of a gene does not necessarily involve an increase of its expression because compensatory mechanisms might be at play or regulatory elements might be lost during the duplication process (Clop et al., 2012).

## 4. Conclusion

As a whole, our results and those published by other authors (Fontanesi et al., 2009; Dong et al., 2015; Zhang et al., 2018; Henkel et al., 2019) evidence that the potential role of structural variation in the *ASIP* locus on goat pigmentation has not been fully elucidated yet. In our study, we have not detected a consistent pattern by which light colored goat breeds display higher *ASIP* copy numbers than those observed in dark-colored breeds such as Murciano-Granadina. This could be due to the existence of genetic factors masking the effects of increased *ASIP* copy number or, alternatively, to the fact that increased *ASIP* copy number does not imply an increase in *ASIP* expression or function. For the four CNVs mapping close or overlapping the *ASIP* gene, it would be crucial to investigate if copy number correlates with *ASIP* mRNA expression in the skin.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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## Availability of data

Genotypes of the 53 Malagueña goats used for CNV calling can be accessed in the Figshare database (https://doi.org/10.6084/m9.figshare.13072223.v1).

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**Table 1.** Estimates of *ASIP* relative[1] copy number in eight goat breeds

| Coloration pattern | Population[2] | Code | Country | Number of individuals | Copy number range[2] | Mean ± SD[3] |
|---|---|---|---|---|---|---|
| Pure white | Saanen | SAA | Switzerland | 10 | 3.04-3.82 | $3.5 \pm 0.23^{a}$ |
| | White Malagueña | W_MLG | Spain | 10 | 2.57-4.42 | $3.51 \pm 0.51^{a}$ |
| Predominantly white | Blanca de Rasquera | RAS | Spain | 9 | 1.89-3.04 | $2.37 \pm 0.33^{c}$ |
| | Jonica | ION | Italy | 9 | 2.08-3.33 | $2.82 \pm 0.39^{bc}$ |
| | Maltese | MAL | Italy | 6 | 2.56-3.36 | $2.85 \pm 0.28^{abc}$ |
| Solid dark-colored | Murciano-Granadina | MUG | Spain | 10 | 2.68-4.7 | $3.33 \pm 0.58^{ab}$ |
| | Non-white Malagueña | NW_MLG | Spain | 10 | 2.84-5 | $3.58 \pm 0.73^{a}$ |
| | Derivata di Siria | DER | Italy | 10 | 2.03-3.27 | $2.42 \pm 0.40^{c}$ |
| Polychromatic | Carpathian | CAR | Romania | 9 | 2.01-3.16 | $2.63 \pm 0.33^{c}$ |

[1]For each individual, *ASIP* copy number was measured in triplicate and the average of the four samples with the lowest *ASIP* copy numbers was used as calibrator. [2]The specific colors of each breed can be found in **Figure S1**. Blanca de Rasquera goats are white or white with black spots; Jonica goats are white or rosy, sometimes with tawny spots in the head and neck; Maltese goats are white, with a raven-black area on the top and sides of the head; Murciano-Granadina goats are black or brown; Non-white Malagueña goats are blond or brown; Derivata di Siria goats have a light red coat, possibly white pied; Carpathian goats can be white, gray, reddish, black, or spotted. [3]*ASIP* copy number averages with different letters are significantly different ($P < 0.05$) according to an ANOVA test. SD: standard deviation.

**LEGENDS TO FIGURES**

**Figure 1A** Boxplot depicting the relative copy number of the *ASIP* gene in eight goat breeds. The *y* axis represents the median and the distribution of *ASIP* copy number in eight goat breeds (*x*-axis). The average of the four samples with the lowest *ASIP* copy numbers was used as calibrator. **1B**. The relative copy number of the *ASIP* gene in 83 individuals from eight breeds. The *x* and *y* axes represent sample ID and relative quantification of *ASIP* copy number (mean ± standard error, with each sample analyzed in triplicate). As a calibrator, we have used the four samples with the lowest copy numbers estimated by qPCR. The following abbreviations have been used: SAA, Saanen (white, N=10); ION, Jonica (white or rosy, sometimes with tawny spots in the head and neck, N=9); CAR, Carpathian (polychromatic, N=9); RAS, Blanca de Rasquera (white or white with black spots, N=9); MAL, Maltese (white, with a raven-black area on the top and sides of the head, N=6); DER, Derivata di Siria (brown or blond, sometimes white pied, N=10); MUG, Murciano-Granadina (brown/black, N=10); white Malagueña (W_MLG, N=10); blond or brown Malagueña (NW_MLG, N=10). The pigmentation patterns of these populations are reported in **Figure S1**.

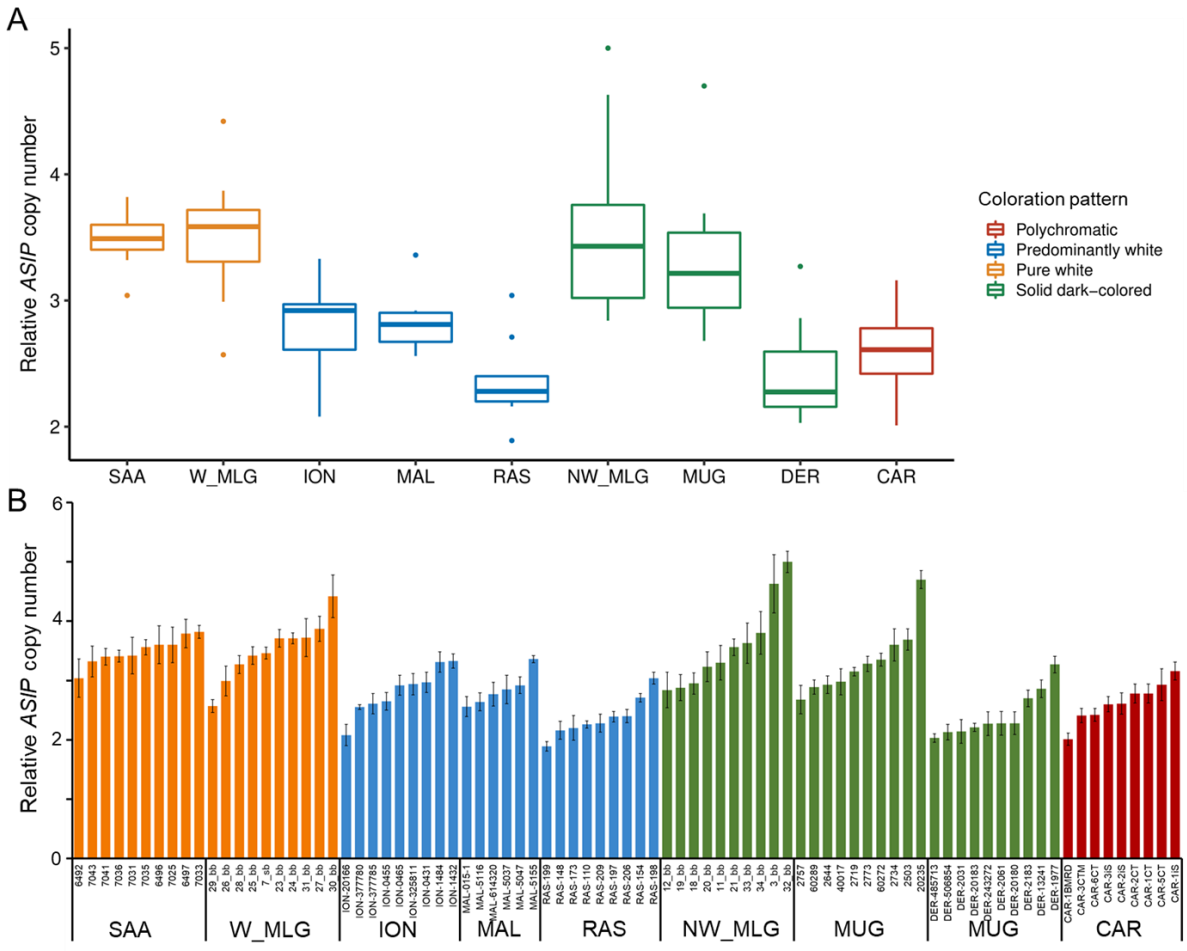

A
Relative ASIP copy number
5
4
3
2
SAA
W_MLG
ION
MAL
RAS
NW_MLG
MUG
DER
CAR
Coloration pattern
Polychromatic
Predominantly white
Pure white
Solid dark−colored
B
Relative ASIP copy number
6
4
2
0
SAA
W_MLG
ION
MAL
RAS
NW_MLG
MUG
MUG
CAR

**Table S1.** List of primers used for the relative quantification of *ASIP* copy number by real-time qPCR

| Gene | Forward | Reverse |
|---|---|---|
| *ASIP* | 5’-CTCGGCGTTTCCCCACA-3’ | 5’-GGGTCCGTGCCACGTTCT-3’ |
| *GCG* | 5’-AACATTGCCAAACGTCATGATG-3’ | 5’-GCCTTCCTCGGCCTTTCA-3’ |
| *MC1R* | 5’-CATCTCCATCTTCTACGCCCTG-3’ | 5’-CTGCAATGATCCTCCACGC-3’ |

**Table S2.** Comparison of the copy number estimates obtained with the Goat SNP50 BeadChip (together with the BAF and LRR parameters) vs real-time quantitative PCR in Malagueña (MLG) and Murciano-Granadina (MUG) goats.

| Breed | ID | qPCR | BeadChip | LRR | BAF |
|---|---|---|---|---|---|
| MLG | 30_bb | 4.42 | 3 | -0.511 | 0.5956 |
| MLG | 31_bb | 3.72 | 3 | -0.5149 | 0.5793 |
| MLG | 29_bb | 2.57 | 3 | 0.139 | 1 |
| MLG | 12_bb | 2.84 | 3 | -0.0092 | 0.84 |
| MLG | 19_bb | 2.88 | 3 | -0.1056 | 0.7832 |
| MLG | 25_bb | 3.42 | 3 | -0.0554 | 0.7878 |
| MLG | 27_bb | 3.87 | 3 | -0.4121 | 0.4432 |
| MLG | 28_bb | 3.27 | 3 | -0.0489 | 0.8293 |
| MLG | 32_bb | 5 | 3 | 0.0346 | 0.809 |
| MLG | 7_sb | 3.46 | 3 | 0.0039 | 0.938 |
| MLG | 18_bb | 2.95 | 2 | 0.0012 | 0.8212 |
| MLG | 20_bb | 3.23 | 2 | -0.15 | 0.7031 |
| MLG | 11_bb | 3.3 | 2 | -0.3394 | 0.5709 |
| MLG | 21_bb | 3.56 | 2 | -0.1678 | 0.5622 |
| MLG | 33_bb | 3.63 | 2 | -0.1891 | 0.9477 |
| MLG | 34_bb | 3.8 | 2 | -0.413 | 0.4885 |
| MLG | 3_bb | 4.63 | 2 | -0.078 | 0.7602 |
| MLG | 26_bb | 2.99 | 2 | -0.0341 | 0.9738 |
| MLG | 23_bb | 3.71 | 2 | -0.4107 | 0.5714 |
| MLG | 24_bb | 3.71 | 2 | 0.0885 | 0.9731 |
| MUG | 2644 | 2.93 | 3 | -0.2266 | 0.4959 |
| MUG | 2773 | 3.28 | 3 | -0.1165 | 0.5693 |
| MUG | 20235 | 4.7 | 3 | -0.0966 | 0.5466 |
| MUG | 60289 | 2.89 | 3 | 0.2606 | 1 |
| MUG | 2757 | 2.68 | 2 | -0.3066 | 0.7359 |
| MUG | 40017 | 2.98 | 2 | -0.2594 | 0.6692 |
| MUG | 2719 | 3.15 | 2 | -0.4582 | 0.003 |
| MUG | 60272 | 3.35 | 2 | 0.0511 | 0.8401 |
| MUG | 2734 | 3.6 | 2 | -0.1255 | 0.7789 |
| MUG | 2503 | 3.69 | 2 | -0.3205 | 0.5826 |

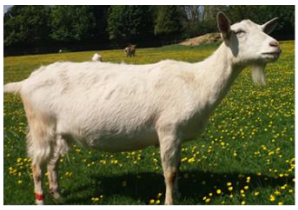
**Figure S1** Pictures of several goat breeds used in the qPCR experiment. The picture of the Saanen breed was retrieved from: https://commons.wikimedia.org/, and the remaining pictures were provided by Dr Jordi Jordana, Dr Juan Manuel Serradilla, Dr Baltasar Urrutia and Dr Juan Carrizosa. Moreover, the pigmentation patterns of additional breeds can be found at the following links:

Carpathian: https://www.iga-goatworld.com/uploads/6/1/6/2/6162024/03_grosu_h_romania_09.04.2014.pdf.

Derivata di Siria: http:// www.agraria.org/caprini/derivatadisiria.htm.

Jonica: http://www. agraria.org/caprini/jonica.htm.

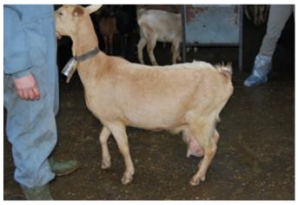
Maltese: http://eng.agraria.org/goat/maltese.htm.



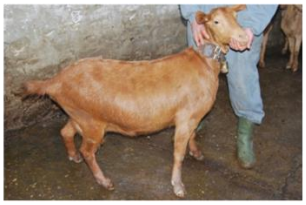
Saanen

Blanca de Rasquera

White Malagueña



Light blond Malagueña



Dark blond Malagueña

Brown Malagueña

Brown Murciano-Granadina

Black Murciano-Granadina

**Figure S2** Predicted copy number in the caprine *ASIP* locus. Each bar in the *x*-axis represents one individual and the *y*-axis indicates the estimated copy number based on the analysis of Illumina Goat SNP50 BeadChip data (Illumina Inc., San Diego, CA). SAA: Saanen; MLG: Malagueña; MUG: Murciano-Granadina.