

Brief Communication

Introgression of *ASIP* and *TYRP1* alleles explains coat color variation in Valais goats

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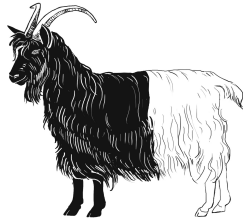
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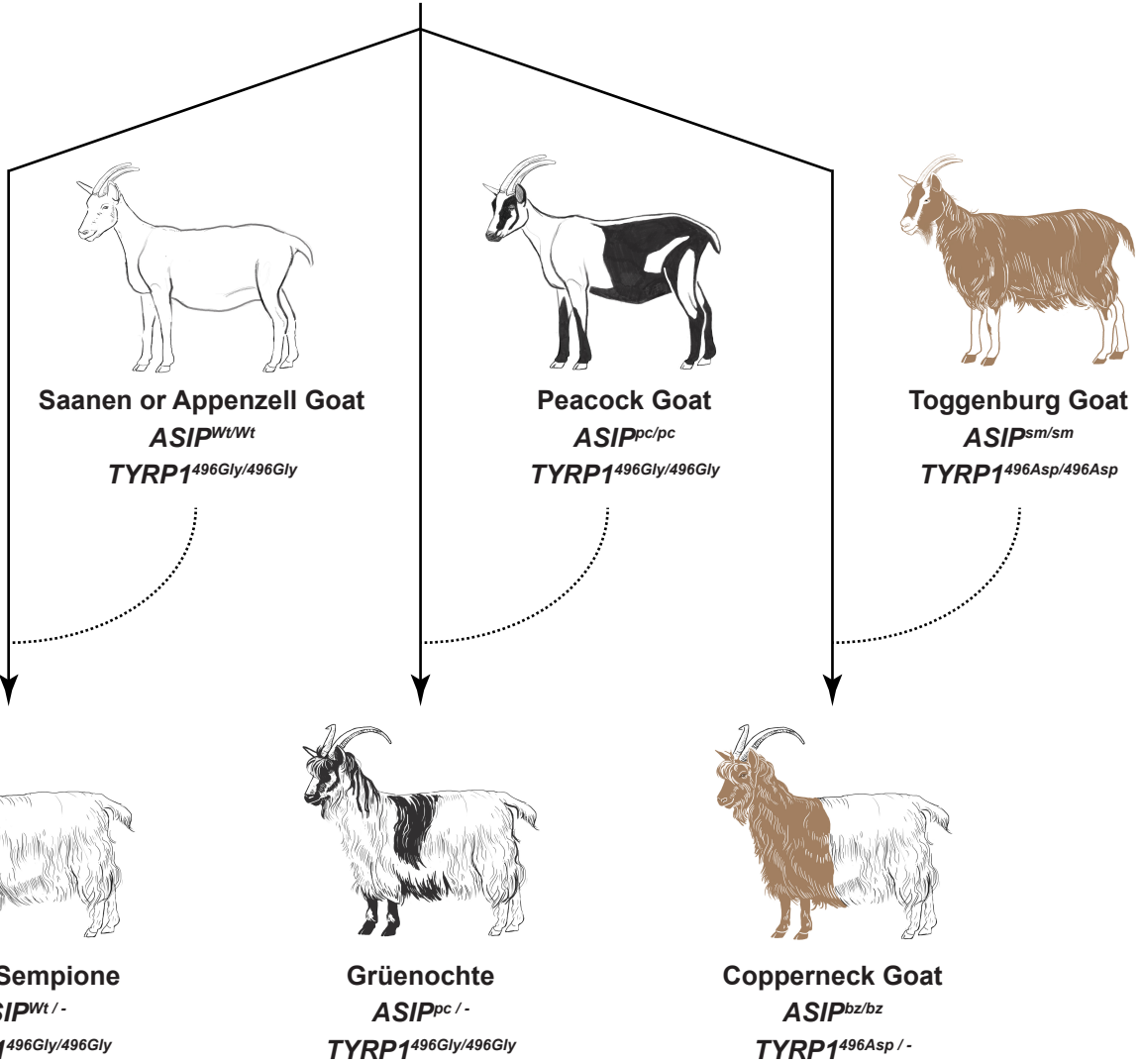
Running title: Coat color allele introgression in Valais goats



Valais Blackneck Goat

ASIP^{bz/bz}

TYRP1^{496Gly/496Gly}



Abstract

The Valais Blackneck goat is a Swiss goat breed with a characteristic coat color phenotype. Before the revision of the breed standard in 1938, four different color varieties of Valais goats were known. Besides Blackneck animals resembling the modern breed standard, the brown and white Copperneck goat, the white Capra Sempione and the greyish Grüenochte comprised the historic Valais goats. The brown pigmentation of Copperneck goats had previously been traced back to an introgression of a mutant *TYRP1* allele from Toggenburg goats. In the present study, we identified additional introgression events of distinct *ASIP* alleles causing the remaining two rare coat color patterns within the Valais Blackneck goat breed. We identified the introgression of the A^{Wt} allele from Appenzell or Saanen goats in white Capra Sempione goats. Similarly, introgression of the A^{pc} allele from Peacock goats resulted in the greyish Grüenochte phenotype. These results demonstrate past hybridization events between breeds that are separated today. A perfect genotype-phenotype association in 393 Valais goats supported the causality of the genotyped variants for the different coat color phenotypes. Our study gives insights into the introgression of functionally relevant copy number variant (CNV) alleles controlling pigmentation between goat breeds with strikingly different coat color patterns.

Keywords: *Capra hircus*, gene flow, pattern, copy number variant, CNV, agouti

Introduction

Artificial selection of domesticated goats resulted in a variety of diverse goat breeds selected not only for milk, meat or fibers, but also for unique coat color phenotypes (FAO 2015; Burren *et al.* 2016). Due to their striking appearance, these goats were of special value to their owners and selected during breed formation. In many cases, coat color was the primary characteristic of new breeds, which subsequently developed into closed populations (Andersson 2013; Andersson 2016). Coat color variation was intensively studied in goats and several causative genetic variants for specific base colors or color patterns were reported (Adalsteinsson *et al.* 1994, Fontanesi *et al.* 2009; Becker *et al.* 2015; Dietrich *et al.* 2015; Menzi *et al.* 2016; Henkel *et al.* 2019).

Switzerland has ten local goat breeds showing diverse coat color phenotypes (Glowatzki-Mullis *et al.* 2008; Burren *et al.* 2016; Henkel *et al.* 2019). The Valais goat population originally comprised four color variants (Figure 1). The most common of these is the Valais Blackneck goat characterized by solid black pigmentation of the cranial half of the animal separated by a sharp vertical boundary of the depigmented white caudal half. We hypothesize that this depigmentation is due to a developmental defect resulting in a lack of melanocytes in the caudal half. The Blackneck phenotype became the only allowed phenotype of the modern breed standard in 1938. The other coat color variants of the Valais goats are the Copperneck goat, characterized by a brown and white pattern, the completely white Capra Sempione or Simplonziege and the greyish Grünochte. The three rare coat color variants were maintained by a few breeders and are now regaining increasing popularity. ProSpecieRara, a non-governmental organization dedicated to the conservation of rare local breeds established a separate herdbook comprising the three rare coat color variants of Valais goats in the year 2013 (www.prospecierara.ch).

We previously reported that the brown coat color of the Copperneck goat is due to the introgression of the dominant *TYRP1*^{496Asp} allele from Toggenburg goats (Becker *et al.* 2015; Dietrich *et al.* 2015). The goal of this study was the clarification of the genetic variation causing the coat color phenotypes in the white Capra Sempione and the greyish Grünochte.



Figure 1. Coat color varieties of Valais goats.

Materials and Methods

Ethics statement

All animal experiments were performed according the local regulations. All animals in this study were examined with the consent of their owners. Sample collection was approved by the “Cantonal Committee For Animal Experiments” (Canton of Bern; permit 71/19).

Breed definition and *ASIP* allele nomenclature

This study investigated four distinct coat color varieties of Valais goats, termed Blackneck, Copperneck, Capra Sempione and Grünenochte. Valais Blackneck goats form a major Swiss goat breed whose breed standard was revised in 1938 to include only the Blackneck coat color variety. In addition to the Valais Blackneck goat herdbook, another smaller herdbook of Valais goats

comprising the three rare coat color varieties was started in 2013. *ASIP* allele nomenclature was used as described previously (Adalsteinsson *et al.* 1994; Henkel *et al.* 2019).

Animals and sequencing

For this study, a total of 393 goat samples were used (Supplementary Table S1). Four representative, unrelated, female Valais goats of the different coat color patterns were selected for whole genome sequencing. Illumina TruSeq PCR-free DNA libraries with an insert size of 350 bp were prepared and sequenced on an Illumina HiSeq 2000 or NovaSeq 6000 instrument (Supplementary Table S2). On average, 200 million paired-end reads per goat were collected. The sequence data of the Valais Blackneck goat were already reported in an earlier publication of our group (Henkel *et al.* 2019).

Mapping and Coverage plots

Mapping of the raw sequence data to the ARS1 reference genome assembly (Bickhart *et al.* 2017) was performed as described in Henkel *et al.* 2019. We visually inspected the short-read alignments in the Integrative Genome Viewer (IGV) to assess large structural variants (Thorvaldsdottir *et al.* 2013). We calculated the coverage of each base in a defined region of interest using samtools depth -b (Li H *et al.* 2009). Additionally coverage stats across the whole genome, including the average coverage were calculated using goleft covstats (<https://github.com/brentp/goleft>). We plotted the coverage using R plot type h version 3.4.1 (R Core Team 2018).

Genotyping

We designed primer pairs spanning the junction of the tandem copies of two CNVs in the *ASIP* gene to detect the presence of the mutant A^{Wt} and A^{pc} alleles involving amplifications of 155 kb and 115 kb, respectively (Supplementary Table S3; Henkel *et al.* 2019). PCR was performed using the primers specific for A^{Wt} or A^{pc} and ATG360 polymerase (ThermoFischer). The PCR products were analyzed using a 5200 Fragment Analyzer System (Agilent). These assays detected the

presence of the A^{Wt} or A^{pc} alleles, but could not discriminate between homozygous mutant or heterozygous animals. We genotyped the *TYRP1* variant NM_001285727.1:c.1487G>A, NP_001272656.1:p.Gly496Asp by direct Sanger sequencing of PCR amplicons as described (Becker *et al.* 2015).

Results

To elucidate the molecular basis and origin of rare coat color patterns in Valais goats, we analyzed whole genome sequence data of representative female goats with the four different coat colors (Figure 2). The black or brown base color phenotype was consistent with the genotypes at the previously reported *TYRP1*:p.Gly496Asp variant (Becker *et al.* 2015; Dietrich *et al.* 2015). The Blackneck and the Copperneck goat both carried the wildtype *ASIP* allele (A^{bz}) in a homozygous state.

Previously described mutant *ASIP* alleles were present in the white Capra Sempione and the greyish Grünenochte. The white Capra Sempione carried one copy of the A^{Wt} allele, which is commonly found in Saanen and Appenzell goats. A^{Wt} involves a triplication of ~155 kb comprising the coding sequence of the *ASIP*, *AHCY* and *ITCH* genes (Fontanesi *et al.* 2009; Henkel *et al.* 2019). Closer inspection of the short-read alignments revealed that the sequenced Capra Sempione had acquired additional smaller structural alterations compared to the original A^{Wt} allele, which comprised a duplication of 1'053 bp inserted into a 10,194 bp deletion of the original A^{Wt} CNV allele (Supplementary Figure S1).

The greyish Grünenochte goat carried one copy of the A^{pc} allele, which causes the characteristic black and white pattern of Swiss Peacock goats (Henkel *et al.* 2019). The A^{pc} allele consists of a central quadruplication of ~45 kb with triplicated adjacent flanking regions comprising ~28 kb and ~42 kb respectively.

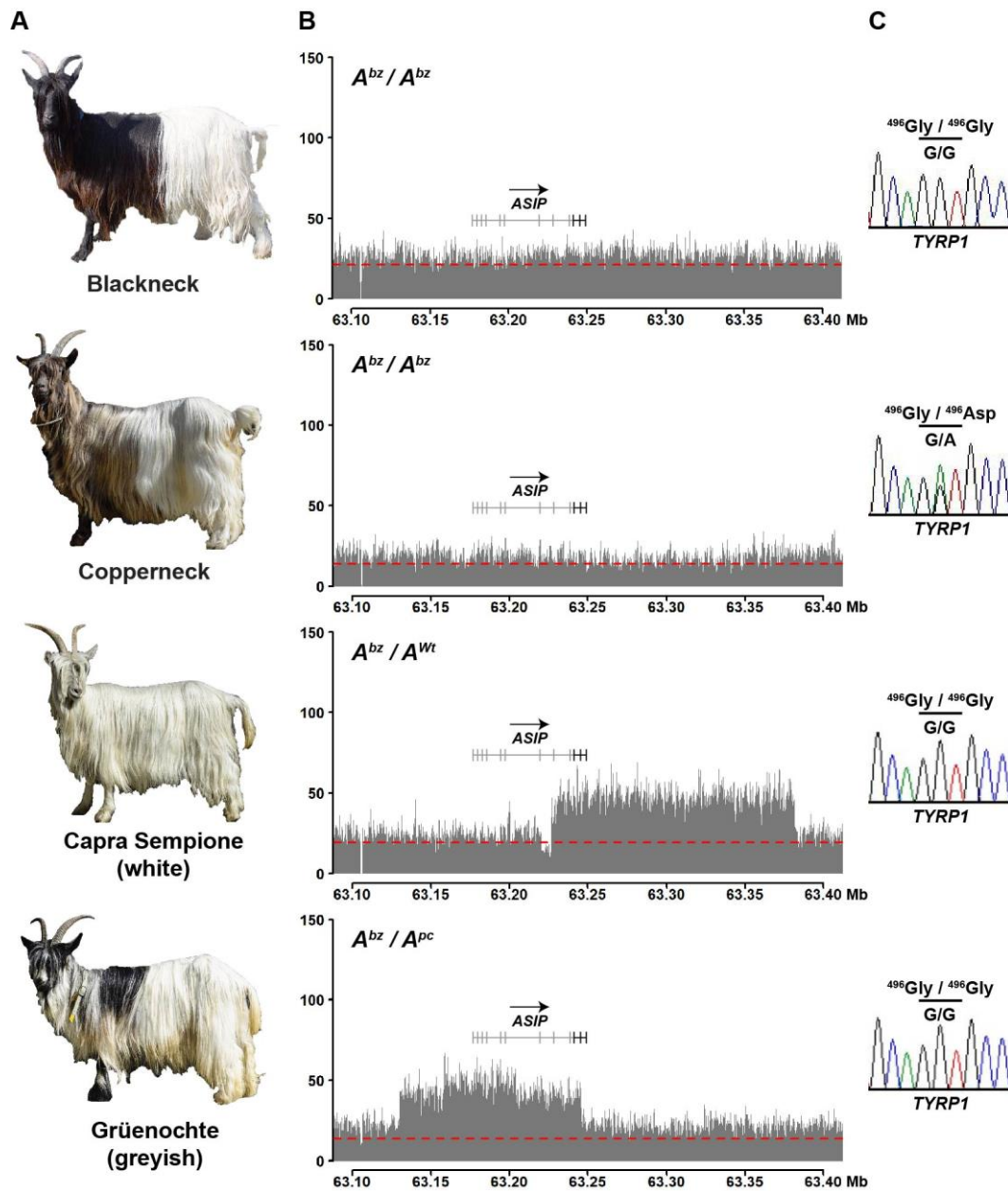


Figure 2. *ASIP* and *TYRP1* variants in Valais goats. **A** Coat color phenotypes of the four varieties of Valais goat. **B** Coverage plots of the *ASIP* locus in Valais goats revealed two distinct CNVs. The Blackneck and Copperneck coverage plot show uniform coverage characteristic for the wild type allele (A^{bz}). The coverage plot of the Sempione and Grünenochte goats revealed CNVs associated with mutant *ASIP* alleles (A^{Wt} and A^{pc}). The dashed red lines indicate the average coverage. **C** Sanger sequencing electropherograms illustrating the variation at *TYRP1*:c.1487G>A (p.Gly496Asp), which causes dominant brown coat color.

To confirm the suspected causal roles of the A^{Wt} and A^{pc} alleles for the white and greyish coat color in Capra Sempione and Grünenochte, respectively, we designed two diagnostic PCR genotyping assays and genotyped a total of 393 Valais goats for the presence of these two alleles (Table 1; Supplementary Table S1). This experiment revealed a perfect association between the presence of A^{Wt} and the white coat color in Capra Sempione as well as A^{pc} and the greyish coat color in Grünenochte. A^{Wt} and A^{pc} are dominant over the wildtype allele A^{bz} . Breeding records and pedigrees are in agreement with a dominant inheritance of the white and greyish coat color phenotypes over the Blackneck pattern. Our genotyping assay could not distinguish between heterozygous and homozygous mutant animals at the *ASIP* locus. However, the breeding records suggest that a substantial fraction of Capra Sempione and Grünenochte goats have one Blackneck parent and are therefore heterozygous. We did not observe any goat with a heterozygous A^{Wt}/A^{pc} genotype. Based on the known dominance hierarchy of caprine *ASIP* alleles, such a goat would be expected to display the white Capra Sempione phenotype.

Table 1. *ASIP* and *TYRP1* genotype association with specific coat color varieties in Valais goats.

Genotype	Blackneck (n = 266)	Copperneck (n = 98)	Capra Sempione (white) (n = 13)	Grünenochte (greyish) (n = 16)
A^{bz} / A^{bz}	266	98	–	–
$A^{Wt} / -$	–	–	13	–
$A^{pc} / -$	–	–	–	16
<i>TYRP1</i>^{Gly496/Gly496}	266	–	10	16
<i>TYRP1</i>^{Gly496/Asp496}	–	87	3	–
<i>TYRP1</i>^{Asp496/Asp496}	–	11	–	–

Discussion

The present study clarified the molecular basis for the four different coat color varieties in Valais goats. Previous research had established that the difference in black or brown base coat color is due to the *TYRP1*:p.Gly496Asp variant. The dominant *TYRP1*^{Asp496} allele resulting in the synthesis of brown eumelanin was most likely introgressed from Toggenburger goats (Becker *et al.* 2015, Dietrich *et al.* 2015). The additional variation resulting in the white or greyish coat color is due to altered pigment type switching caused by mutant *ASIP* alleles. While Blackneck and Copperneck goats exclusively express eumelanin in their pigmented cranial body half, the white Capra Sempione may be assumed to synthesize a very light phaeomelanin in the cranial half of the body, similar to what is seen in white Saanen or Appenzell goats. The “greyish” coat color of the Grünenochte closely resembles the pattern of Peacock goats in the cranial body half, while the caudal body half is devoid of melanocytes as in the other varieties of Valais goats. The similarity of the pattern in Grünenochte and Peacock goats had been difficult to recognize due to the extremely long hair of Valais goats and the fact that the pigmentation of Valais goats show a highly variable degree of bleaching with age and/or sunlight exposure.

The molecular cause for the depigmentation of the caudal half of all Valais goats is still unknown. Further research is required to elucidate the molecular genetics of this striking pattern.

Our study identified introgression events of two distinct *ASIP* alleles into Valais goats. We observed a variant *A*^{Wt} allele in the sequenced Capra Sempione. We speculate that this variant *A*^{Wt} allele is carried by all Capra Sempione but it might also represent a private variation of the sequenced animal. The observed genotype distribution and available pedigree data indicated an autosomal dominant mode of inheritance. These findings support what is already known about the copy number variants at the agouti locus of goat and sheep (Adalsteinsson *et al.* 1994; Norris *et al.* 2008; Fontanesi *et al.* 2009; Henkel *et al.* 2019).

The origin of the *A*^{Wt} allele is most likely in the Swiss Appenzell or Saanen goats, as both are nearly fixed for this allele, which is responsible for their characteristic white coat color (Fontanesi *et al.* 2009; Henkel *et al.* 2019). The *A*^{pc} allele was presumably introduced from Peacock goats as it gives

them their characteristic black and white pattern (Henkel *et al.* 2019). The Swiss goat herding practices represent a potential mediator for the observed introgression. During summer, goats from different farms and breeds are jointly kept on pastures in the mountains, the so-called Alps. This offers a contact zone for the normally closed Swiss goat breeds.

Introgression events of domestic alleles into wild animals are known in various mammalian species and were for example observed in *Capra ibex* (Grossen *et al.* 2014), mouflons (Feulner *et al.* 2013) or in North American gray wolves (Anderson *et al.* 2009). The introgression of the two mutant *ASIP* alleles into Valais goats probably happened before the revision of the breed standard in 1938, as anecdotal reports of the Valais goat population suggested a phenotypically quite diverse population during the first half of the 20th century. Offspring of such hybridizations between Appenzell/Saanen and Blackneck or Peacock and Blackneck may have been of particular interest to their owners and remained in the Valais goat population until 1938, when the breed standard was refined to exclusively allow animals with the Blackneck phenotype.

The presented results show a previously unsuspected introgression of different *ASIP* alleles into Valais goats and provide a molecular characterization of historic breed developments in Switzerland.

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computing infrastructure. Furthermore, we thank Christian Gazzarin for photos and Sarah Stangl for drawings of the different goats.

Data Availability

We deposited the primary data underlying these analyses as follows:

NCBI SRA: SRX1560776, SRX5250095, SRX7715598 and SRX9449177 (see Supplementary Table S2 for more details)

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Supplementary Material

Supplementary Figure 1. Details of the A^{Wt} allele in the sequenced *Capra Sempione*.

Supplementary Table 1. Individual goat samples and their genotypes.

Supplementary Table 2. Sequenced goat genomes.

Supplementary Table 3. Primer sequences.