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Genetic background of juniper (*Juniperus spp.*) consumption predicted by fecal near-infrared spectroscopy in divergently selected goats raised in harsh rangeland environments

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Abstract

Background Junipers (*Juniperus spp.*) are woody native, invasive plants that have caused encroachment problems in the U.S. western rangelands, decreasing forage productivity and biodiversity. A potential solution to this issue is using goats in targeted grazing programs. However, junipers, which grow in dry and harsh environmental conditions, use chemical defense mechanisms to deter herbivores. Therefore, genetically selecting goats for increased juniper consumption is of great interest for regenerative rangeland management. In this context, the primary objectives of this study were to: 1) estimate variance components and genetic parameters for predicted juniper consumption in divergently selected Angora (ANG) and composite Boer x Spanish (BS) goat populations grazing on Western U.S. rangelands; and 2) to identify genomic regions, candidate genes, and biological pathways associated with juniper consumption in these goat populations.

Results The average juniper consumption was 22.4% ($\pm 18.7\%$) and 7.01% ($\pm 12.1\%$) in the BS and ANG populations, respectively. The heritability estimates (realized heritability within parenthesis) for juniper consumption were 0.43 ± 0.02 (0.34 ± 0.06) and 0.19 ± 0.03 (0.13 ± 0.03) in BS and ANG, respectively, indicating that juniper consumption can be increased through genetic selection. The repeatability values of predicted juniper consumption were 0.45 for BS and 0.28 for ANG. A total of 571 significant SNP located within or close to 231 genes in BS, and 116 SNP related to 183 genes in ANG were identified based on the genome-wide association analyses. These genes are primarily associated with biological pathways and gene ontology terms related to olfactory receptors, intestinal absorption, and immunity response.

Conclusions These findings suggest that juniper consumption is a heritable trait of polygenic inheritance influenced by multiple genes of small effects. The genetic parameters calculated indicate that juniper consumption can be genetically improved in both goat populations.

Keywords Biological control, Genome wide association study, Monoterpene

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Background

For more than a century, junipers (*Juniperus spp.*) have encroached on the Western U.S. rangelands, changing the landscape and reducing forage resources [1, 2]. These plants exert a significant negative influence on the structure and productivity of the plant community [3] as they compete with valuable forage plants for water and essential nutrients. Thus, it becomes crucial to implement effective juniper management practices to ensure the productivity of rangelands [4]. An efficient approach for addressing the juniper invasion lies at the intersection of science, ecology, and economics [5]. Various strategies can be employed to reduce or eliminate juniper from pastures, including the use of chemical herbicides [6], mechanical interventions [7], or prescribed fire [8]. However, the efficacy of these methods may vary depending on the juniper species, and some approaches may entail considerable costs and environmental damage. In this context, using goats (*Capra hircus*) as biological agents to control these invasive plants can be an efficient strategy [9].

Goats have an extraordinary ability to control invasive plants because of their grazing behavior [9]. Preference for juniper by browsing animals is negatively correlated to the concentration of plant secondary metabolites [10, 11]. When consumed in sufficient quantities, they can negatively affect intake [12], rumen microbial function, and cause hepatic injury [13]. Despite these challenges, goats have demonstrated a remarkable ability to consume chemically defended plants. This makes them the most promising livestock species for targeted grazing programs, compared to sheep and cattle, as goats appear to be less affected by the odor of the plants compared to other species [14].

In extensive systems, accurately estimating the total consumption of specific plants poses a challenge due to uncertainties in controlling what animals ingest. Fortunately, recent technological advancements offer promising solutions to bridge this gap of uncertain information. One such approach is the application of fecal near-infrared reflectance spectroscopy (fNIRS) to predict diet composition. Compared to standard laboratory procedures, fNIRS brings significant advantages, such as being non-labor-intensive, enabling rapid execution, treating samples non-destructively, and reducing chemical waste [15]. fNIRS enables the measurement of essential attributes in the diet selected by grazing herbivores, including crude protein content, coefficient of digestibility, botanical composition, and, in some cases, voluntary intake [16]. This proprietary method presents a unique opportunity to accurately estimate the consumption of specific plants. By leveraging fNIRS technology, valuable insights into animal foraging behavior can be obtained, which will

improve the overall understanding of grazing ecology in extensive production systems.

Factors such as breed, sex, and animal age have been reported to influence juniper consumption by goats [12, 17]. Furthermore, there is evidence of individual variability, indicating that goats can be selected for increased juniper consumption [18]. However, there is limited research on the genetic background of this trait [19], including a lack of genomic-based variance components and identification of genomic regions and candidate genes associated with juniper consumption in goats. Therefore, the main objectives of this study were to: 1) calculate realized heritabilities and estimate variance components and genetic parameters, including heritability and repeatability, for predicted juniper consumption in divergently selected Angora (ANG) and composite Boer x Spanish (BS) goat populations grazing on Western U.S. rangelands; 2) investigate the relationship of juniper consumption prediction with a productive trait (i.e., weaning weight); 3) perform a genome-wide association study (GWAS) based on whole-genome sequence data (WGS); and 4) identify candidate genes associated with juniper consumption and the primary biological functions of these genes.

Methods

Animals and phenotypic information

The animals enrolled in this study were raised in herds managed by the Texas A&M AgriLife Research on typical juniper-infested rangeland in the Edwards Plateau of Texas, USA. All the procedures involving animals were approved by the Texas A&M University Institutional Agricultural Animal Care and Use Committee under protocols 2003–129 and 2018-021a. The predominant ecological site is a Low Stony Hill in an Oak/Mixed-brush Shortgrass seral state [20]. Woody plant canopy cover is about 35% and consists primarily of oak (*Quercus fusiformis*) and juniper (*Juniperus ashei* and *J. pinchotii*). Pricklypear (*Opuntia sp.*), algerita (*Mahonia trifoliata*), and other shrubby species are also common. Shortgrasses such as buffalograss (*Bouteloua dactyloides*), three-awns (*Aristida sp.*), and curlmesquite (*Hilaria belageri*), and the cool season Texas wintergrass (*Nassella leucotricha*) are also present in the pastures where the animals were raised. During the winter and long drought seasons, additional supplementation was provided to the goats based on an assessment of available forage quantity and quality. This supplementation encompassed either whole cottonseed or range cubes. Unlimited access to water and mineral mix was ensured.

Two goat populations were used for this study, including a Boer x Spanish (BS) composite population raised for meat production [21] and an Angora (ANG) population,

which is a sample from a breed known for its fiber-producing attributes [21]. To assess juniper consumption, fecal samples were gathered through rectal palpation, followed by drying at 55 °C in a forced-air oven for 48 h. Subsequently, the samples were grounded using a cyclone mill (Cyclotec 1093, Foss, Hilleroed, Denmark). These ground samples were packed into quarter-cup sample cells equipped with a near-infrared transparent quartz cover glass. The sample cells underwent 32 scans using a scanning reflectance monochromator (model 6500, NIR Systems Inc., Silver Springs, MD, USA). The reflected energy ($\log[1/R]$) was measured and averaged across 32 scans, recorded at 2-nm intervals spanning from 1,100 to 2,500 nm. The monochromator employing ISI NIR2 version 3 software (Infrasoft International, Port Matilda, PA, USA) was used for the analyses. This setup enabled the collection of spectra and prediction of the juniper percentage in the diets using a Partial Least Squares equation previously developed. The predictive equation had an r^2 value of 0.88, with a cross-validation standard error of 6.4% [17, 22]. Juniper consumption is usually higher in the dormant season, but the mean consumption over a 2-year period was highly correlated with spring samples [23]. In addition to seasonal variations in juniper consumption, there are also 7–9-day periodicities in juniper consumption [23]. Thus, the phenotypic estimate of the percentage of junipers in the diet was determined by sampling in the winter and spring seasons and collecting two samples three or four days apart in each season. When this study began, all goats were sampled each year. However, due to logistical constraints, sampling was reduced to four seasonal fecal collections (eight total collections) beginning with the first winter season after weaning. Both populations were raised on different ranches located in different counties, with varying plant populations and environmental conditions. Therefore, we avoided making direct statistical comparisons between them.

A total of 2,048 BS (1,161 females and 887 males) and 1,480 ANG (1,026 females and 484 males) goats were included in the study. The BS goats had an average (standard deviation) of 3.15 (± 1.33) juniper consumption records measured at an average age of 1.53 (± 0.67) years. The ANG animals had an average of 2.77 (± 1.64) phenotypic records measured at an average age of 2.02 (± 0.83) years. A comprehensive breakdown of the record count per animal is presented in Table 1. The pedigree datasets included 2,427 (BS) and 1,594 (ANG) animals with individuals recorded up the 8th (average: 4.44 ± 1.32) and 6th (average: 2.35 ± 1.66) generation, respectively. The average pedigree inbreeding was 0.04 and 0.01 for BS and ANG, respectively. The data generated was edited to remove inconsistent or outlier records for further analyses. The

Table 1 Number of fecal samples per goat used for near-infrared spectroscopy determination of the percentage of juniper in the diets

Number of samples	Boer x Spanish composite	Angora
	Number of animals	Number of animals
1	111	372
2	752	461
3	188	193
4	885	303
5	15	50
6	41	89
7	19	21
8	15	18
9	8	2
10	4	1

final dataset included 6,457 and 4,076 records from BS and ANG animals, respectively.

Since 2003 and continued for over than 15 years, both goat populations were divergently selected to form HIGH and LOW selection lines, in which the HIGH line was selected to increase the percentage of juniper in the diet while the LOW line was selected to decrease the percentage of juniper in the diet. This was done to maximize the difference between divergent lines to improve our ability to identify physiologic and genetic differences between them.

Genomic datasets

Genomic data from 501 BS and 210 ANG goats were obtained using the NovaSeq 6000 platform at a coverage depth of ~1X. The initial dataset comprised information of 407,644 autosomal SNPs. The alignment to the reference genome ARS1 [24] was performed using SAMTools v1.19 [25] while sorting, realignment, and quality filtering were managed with PICARD v1.16 [26]. For imputation, Beagle V4.0 [27] was employed, applying a Genotype Probability (GP) threshold of ≥ 0.90 . After genotype imputation, 14,094,102 SNPs were obtained and used for the GWAS analyses.

A total of 74,199 SNPs were used for estimating variance components and genetic parameters. These SNPs were selected through a filtering process from the original dataset of 407,644 SNPs, excluding those with less than 20% of missing information and $MAF < 0.05$. Principal component analysis was conducted on the genotype information using PLINK v1.09 software [28]. As the PCA outcomes revealed two clearly distinct populations, all subsequent analyses were performed independently for both populations (BS and ANG).

Variance components and genetic parameters

Variance components and genetic parameters were estimated for each goat population using genomic data from 501 (BS) and 210 (ANG) goats and 74,199 SNPs (as described above). The quality control process also involved evaluating the call rate for individual and genotypes (<0.90) and identifying SNPs with an extreme departure from Hardy–Weinberg equilibrium (HWE p -value < 10^{-10}). As a result, we obtained 40,187 and 50,957 informative SNPs for the BS and ANG populations, respectively.

The variance components were estimated based on Bayesian methods, using the BLUPF90+family programs (GIBBS, POSTGIBBSF90) [29]. For the analyses, 1,000,000 cycles with a burn-in of 100,000 cycles and thinning of 100 samples were used. The “boa” R package [30] was used to evaluate convergence and posterior inference of the results. The single-step GBLUP approach (ssGBLUP) [31] was used to solve the mixed model equations. This method replaces the pedigree relationship matrix, combining pedigree and genomic relationship [32], as shown below:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where \mathbf{A} is the pedigree-based relationship matrix for all individuals in the population, \mathbf{A}_{22} is the pedigree-based relationship matrix of the genotyped animals, and \mathbf{G} is the genomic relationship matrix calculated as [33]:

$$\mathbf{G} = \mathbf{Z}\mathbf{Z}'$$

where \mathbf{Z} represents a matrix containing adjustments for allelic frequencies. These adjustment factors were incorporated to align the mean diagonal of matrix \mathbf{G} with \mathbf{A}_{22} , ensuring a close correspondence between the two matrices [34].

The following single-trait repeatability animal model was fitted:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{W}\mathbf{pe} + \mathbf{e}$$

where \mathbf{y} is the vector of phenotypic observations (pre-

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}\mathbf{a}_1 & 0 \\ 0 & \mathbf{Z}\mathbf{a}_2 \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{Z}_{m2} \end{bmatrix} \begin{bmatrix} 0 \\ \mathbf{m}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{W}_1 & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} \mathbf{pe}_1 \\ 0 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}$$

dicted juniper consumption); \mathbf{b} is the vector of systematic effects representing the contemporary group defined based on the date of fecal collection (month and year), sex (male and female), and age in years; \mathbf{a} is the random vector of additive genetic effects; \mathbf{pe} is the random vector of permanent environmental effects; \mathbf{e} is the random

vector of residual errors; \mathbf{X} , \mathbf{Z} , and \mathbf{W} are the incidence matrices associated with the systematic, additive genetic, and permanent environmental effects, respectively.

Contemporary groups with fewer than five individuals and records that deviated 3.5 standard deviations above or below the mean were excluded from further analyses. Residuals and permanent environment effects were considered to be independent and follow a normal distribution, characterized by a mean of zero and variances of $\mathbf{I}\sigma_e^2$ and $\mathbf{I}\sigma_{pe}^2$, respectively. The additive genetic effects were similarly assumed to adhere to a normal distribution, with a mean of 0 and a variance of $\mathbf{H}\sigma_a^2$.

From the variance components obtained, the narrow sense heritability (h^2) and repeatability (t ; for juniper consumption) estimates were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

$$t = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

Realized heritability estimates were also calculated based on the Thompson’s approach [35].

Genetic correlation with weaning weight

The analyses incorporated information on weaning weight from a total of 1,569 animals (805 females and 764 males) within the BS population, all born between 2006 and 2017. The animals were approximately 3.34 months old (with a standard deviation of ± 0.53 months) at the time of weaning weight measurement. Moreover, for the ANG population, the study included 598 animals (299 females and 299 males) born between 2009 and 2017. These animals were about 4.00 months old (with a standard deviation of ± 0.99 months) during the weaning weight assessment. Remarkably, 93.8% and 70.4% of the BS and ANG animals, respectively, had recorded information regarding juniper consumption. For further details on the descriptive statistics of weaning weight, please refer to Additional file 1: Table S1.

The genetic correlation with weaning weight was calculated based on bivariate analyses using the Bayesian methods implemented in the BLUPF90+family software [21]. The following model was applied:

where \mathbf{y}_1 and \mathbf{y}_2 are the vectors of phenotypic observations for juniper consumption and weaning weight respectively; \mathbf{b}_1 is the vector of systematic effects for juniper consumption representing the contemporary group defined based on the date of collection (month and year), sex (male and female), and age in years; \mathbf{b}_2 is the vector

of systematic effects for weaning weight representing the contemporary group defined based the year of birth and sex (male and female), the type of birth, and age in months as covariable; \mathbf{a}_1 and \mathbf{a}_2 are the vectors of direct additive genetic effects for juniper consumption and weaning weight respectively; \mathbf{m}_2 is the maternal genetic effects for weaning weight; \mathbf{pe}_1 is the vector of permanent environmental effects of juniper consumption; \mathbf{e}_1 and \mathbf{e}_2 is the vector of residual errors; \mathbf{X} , \mathbf{Z}_a , \mathbf{Z}_m , and \mathbf{W} are the incidence matrices associated with the systematic, additive genetic, maternal genetic, and permanent environmental effects, respectively. The residuals and permanent environment effects were assumed to be independent and follow a normal distribution, characterized by a mean of zero and variances of $\mathbf{I}\sigma_e^2$ and $\mathbf{I}\sigma_{pe}^2$, respectively. The additive genetic and maternal genetic effects were similarly assumed to adhere to a normal distribution, with a mean of 0 and a variance of $\mathbf{H}\sigma_a^2$ and $\mathbf{G}\sigma_m^2$, respectively.

Genome-wide association studies

A total of 14,094,102 SNPs were used for the genome-wide association studies (GWAS). Following the quality control described above, 5,309,518 and 8,052,245 SNPs were available for the BS and ANG populations, respectively. We utilized deregressed estimated breeding values (dEBVs) calculated based on the approach proposed by Garrick et al. [36] as pseudo-phenotypes. We used the GCTA software [37] based on the mixed linear model-based association analysis (MLMA) [38] following the model:

$$y_i = \mu + \mathbf{b}g_i + \mathbf{u}_i + \mathbf{e}_i$$

where \mathbf{y} is a vector of phenotypes; μ is the population mean, \mathbf{g}_i is the number of copies of the reference allele, \mathbf{b} is the allele substitution effect of the SNP, \mathbf{u}_i is the polygenic effect of the i^{th} individual considered to be normally distributed $\mathbf{N}(0, \mathbf{G}\sigma_g^2)$, where σ_g^2 is the polygenic genetic variance and \mathbf{G} is the genomic relationship

matrix as previously described; and \mathbf{e}_{ij} is a vector of residual effects with $\mathbf{e} \sim \mathbf{N}(0, \mathbf{I}\sigma_e^2)$. We applied the Bonferroni method to correct for multiple testing. The significance threshold ($p < 0.05$) considered the effective population size ($N_e = 80$, Brito et al. [39]), the average length of a chromosome and the number of chromosomes ($2n = 29$) at the chromosome wide level [40, 41]. This correction method accounts for multiple testing and adjusts the significance thresholds of the SNPs as demonstrated by Goddard et al. [41].

Gene annotation and functional analyses

To annotate the SNP associated with juniper consumption, we utilized the GALLO package [42] with a genomic window of 100 Kb upstream and downstream of the significant SNPs. The identified genes from the gene annotation were then used for functional analyses using the DAVID tool [43]. These analyses enabled the identification of the biological processes, molecular functions, cellular components, and pathways in which these genes are involved.

Results

Descriptive statistics

The descriptive statistics for juniper consumption, categorized by breed and sex, are presented in Table 2. In the ANG population, sex had a significant influence on juniper consumption, with males consuming more than females.

The average consumption of juniper per sex, and line for BS and ANG is presented in Table 3. On average, juniper consumption increased by 105.8% for females from 2005 to 2017 and 127.5% for males from 2006 to 2017 in BS animals selected for high consumption of juniper (HIGH; Table 3). Also, for the lines selected for low consumption of juniper (LOW), there was a decrease in the consumption of 61.6% in females from 2005 to 2017 and 23.9% in males from 2006 to 2017 (Table 3). However, line was not recorded for all ANG animals, especially

Table 2 Descriptive statistics for percentage of juniper consumption by breed and sex

Breed	Sex	N	Min	Max	Mean	SD
Boer x Spanish	F	4,027	-19.30	75.70	22.24	17.14
Boer x Spanish	M	2,424	-19.80	74.40	22.56	20.86
Angora	F	2,949	-23.60	36.00	4.65	10.18
Angora	M	1,127	-15.50	36.30	10.82	11.57

N Number of observations

Min Minimum percentage of juniper consumption observed

Max Maximum percentage of juniper consumption observed

Mean Average of juniper consumption

SD Standard deviation

Table 3 The average consumption of juniper per goat, sex, and line

Breed	Sex	Year	Line	N	Min	Max	Mean
Boer x Spanish	F	High	1951	-15.00	75.70	28.73	16.95
	F	Low	2076	-19.30	67.90	17.56	14.33
	M	High	1185	-16.40	74.40	30.32	19.95
	M	Low	1239	-19.80	66.90	15.24	19.23
Angora	F	High	401	-21.50	58.50	10.62	7.87
	F	Low	304	-31.30	44.10	5.55	7.85
	M	High	93	2.90	50.00	23.17	8.66
	M	Low	24	2.50	44.00	19.89	6.64

N Number of observations

Min Minimum percentage of juniper consumption observed

Max Maximum percentage of juniper consumption observed

Mean Average of juniper consumption

SD Standard deviation

from 2010 to 2017. This gap hinders a comprehensive analysis of juniper consumption trends in ANG. The Additional file 1: Table S1 present the average consumption of juniper per year, sex, and line.

Variance components and genetic parameters

The variance components and genetic parameters for juniper consumption are provided in Table 4. The heritability of juniper consumption was 0.43 ± 0.02 and 0.19 ± 0.03 for BS and ANG populations, respectively.

The repeatability for juniper consumption was 0.45 ± 0.02 and 0.28 ± 0.02 in the BS and ANG populations, respectively. With respect to the genetic correlation with weaning weight, a negative and weak genetic correlation (-0.04 ± 0.11) was observed in the BS population, while the correlation was moderate and negative (-0.31 ± 0.20) in the ANG population. When calculating variance components for each line separately for the BS population (line information was not available for all ANG animals), the heritability estimates decreased from 0.43 to 0.22 for each line (Table 4). The realized heritability estimates are 0.34 ± 0.06 and 0.13 ± 0.03 for BS and ANG, respectively. The variance components and genetic parameters for weaning weight are presented in the Additional file 2: Table S2.

Genome-wide association studies results

Figure 1 displays the Manhattan plot illustrating the GWAS analysis of juniper consumption in the BS population. A total of 571 significant SNPs were distributed across all autosomal chromosomes that exhibited a significant effect on juniper consumption. The two most significant peaks are located on the *Capra hircus* chromosome 13 (CHI13: 75,272,313–79,825,590) and CHI17

(327,164–378,403). These SNPs are associated with 231 positional genes, including 168 protein-coding genes, 34 long intergenic non-coding RNAs, 11 small nuclear RNAs, five immunoglobulin V genes, seven small nucleolar RNAs, three microRNAs, one miscellaneous RNA, and one processed pseudogene. A detailed description of the genes and their classifications is presented in Additional File 3: Table S3.

For the main genomic peaks identified, there are six genes located on CHI13 (*ENSCHIG00000002030*, *ENSCHIG00000002361*, *ENSCHIG00000002371*, *ENSC HIG00000005214*, *EYA2*, *ZMYND8*), and 26 genes on CHI17 (*ENSCHIG00000003453*, *ENSCHIG00000003521*, *LOC102174962*, *ENSCHIG00000008572*, *ENSCHIG0000 0009334*, *ENSCHIG00000011400*, *ENSCHIG000000174 61*, *ENSCHIG00000017632*, *ENSCHIG00000020716*, *ENS CHIG00000024061*, *ENSCHIG00000025831*, *ENSCHIG0 0000023467*, *ENSCHIG00000011805*, *ENSCHIG0000002 0629*, *ENSCHIG00000010666*, *ENSCHIG00000011513*, *ENSCHIG00000015496*, *LOC108637841*, *KSR2*, *FICD*, *SART3*, *ENSCHIG00000003166*, *ENSCHIG00000005872*, *ENSCHIG00000006007*, *SH3D19*, *FHIP1A*). Notably, five genes located on CHI3 and CHI10 (*LOC102185841*, *LOC102190566*, *LOC102186401*, *LOC108636907*, *LOC1 02186483*) are associated with the olfactory receptor system.

Table 5 provides an overview of the functional analyses for the positional genes linked to juniper consumption within the BS population. The genes identified play a role in seven biological processes, eight molecular functions, three cellular components, and three pathways that are specifically associated with juniper consumption, including intestinal absorption (GO:0050892).

Figure 2 presents the Manhattan plot for juniper consumption in the ANG population. A total of 116 SNPs

Table 4 Variance components and genetic parameters estimated for juniper consumption in Boer x Spanish composite and Angora goat populations

	Boer x Spanish		Angora	
	Mean	SD	Mean	SD
h²	0.43	0.02	0.19	0.03
High	0.22	0.05	-	-
Low	0.22	0.04	-	-
Realized h²	0.34	0.06 ^a	0.13	0.03 ^a
High	0.13	0.02 ^a	-	-
Low	0.19	0.03 ^a	-	-
pe	0.03	0.01	0.09	0.02
High	0.14	0.04	-	-
Low	0.15	0.04	-	-
σ²_g	35.80	2.37	9.40	1.39
High	16.26	3.59	-	-
Low	15.63	3.33	-	-
σ²_{pe}	2.12	1.08	4.41	1.16
High	9.56	2.24	-	-
Low	9.95	2.29	-	-
σ²_e	45.80	0.98	35.00	0.93
High	45.43	1.43	-	-
Low	40.72	1.22	-	-
t	0.45	0.02	0.28	0.02
High	0.36	0.02	-	-
Low	0.38	0.02	-	-
r_g	-0.04	0.11	-0.31	0.20

h² Heritability
HIGH Selected line for high consumption of juniper
LOW Selected line for low consume of juniper
pe ratio of permanent environment
σ²_g genetic variance
σ²_{pe} permanent environment variance
σ²_e residual variance
t repeatability
r_g genetic correlation between juniper consumption and weaning weight
^a standard error of the realized heritability

were identified to have a significant impact ($p < 0.05$) on juniper consumption. These SNPs are associated with 183 genes, consisting of 138 protein-coding genes, 22 long intergenic non-coding RNAs, seven small nuclear RNAs, four immunoglobulin germline genes, three microRNAs, three processed pseudogenes, two pseudogenes, two ribosomal RNAs, and two small nucleolar RNAs. Similar genomic regions were identified for both populations. The CHI13 region contains 14 genes (*ENSCHIG00000001274*, *ENSCHIG00000002347*, *ENSC HIG00000002357*, *ENSCHIG00000003660*, *ENSCHIG00 000005214*, *ENSCHIG00000009331*, *ENSCHIG0000001 0780*, *ENSCHIP00000012347*, *GINSL*, *ANKEF1*, *ITGA8*,

PCMTD2, *ZMYND8*, *FZD8*), and the one on CHI17 contains 14 genes associated with juniper consumption (*ENSCHIG00000003453*, *ENSCHIG00000003521*, *ENSC HIG00000004635*, *ENSCHIG00000008572*, *ENSCHIG000 00008793*, *ENSCHIG00000009334*, *ENSCHIG00000011 400*, *ENSCHIG00000011805*, *ENSCHIG00000017461*, *EN SCHIG00000017632*, *ENSCHIG00000020716*, *ENSCHIG 00000023467*, *ENSCHIG00000024061*, *ENSCHIG000000 25831*). A detailed description of all the position genes along with their respective classifications is presented in Additional File 4: Table S4.

The gene *OR2Z1*, which is related to the olfactory receptor, has been annotated within the genomic regions where the SNPs exhibit a significant impact on juniper consumption. Table 6 presents the gene ontology terms associated with the genes identified in the ANG population regarding juniper consumption. The genes identified are significantly involved in four biological processes, six molecular functions, two cellular components, and nine metabolic pathways associated with juniper consumption.

Discussion

The objective of this study was to investigate the genetic background of predicted juniper consumption by BS and ANG goats in a divergently-selected population. In terms of measuring juniper consumption, it is important to interpret the results in the context of an interval scale. In the field of measurement, an interval scale implies that values can be ordered systematically, and the differences between them hold meaning and remain consistent across the entire range of measurements, even without an absolute zero point [17]. When interpreting the results, the values represent the percentage of juniper in their diets. On average, the composition of juniper consumed in the diets was 22.4% and 7.01% in BS and ANG diets, respectively.

Juniper consumption differences were observed in the ANG population regarding sex. In this population, males consumed more juniper than females (223%). Previous research on the effect of sex on fNIR predictions of juniper consumption reported that, when on the same diet, males had a 50% higher predicted percentage of juniper in the diet compared to females, which may account for some of the difference between populations [17]. Although not statistically comparable as the animals were not kept in the same pastures, numerically the BS population consumed more juniper than ANG, and previous studies reported similar findings [15, 44] that the composite animals [45, 46] might be more effective biological agents for juniper control. During droughts, pastoralists are reluctant to reduce their livestock numbers

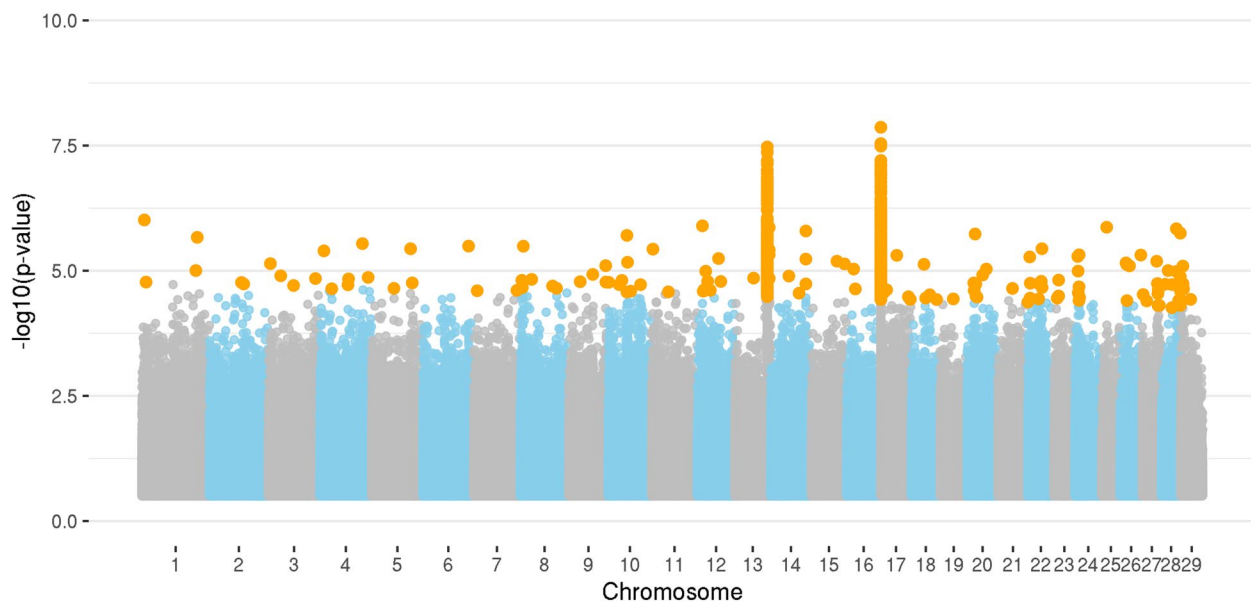


Fig. 1 Manhattan plot for juniper consumption in a crossbred Boer x Spanish goat population based on whole-genome sequence data. Legend: Orange dots indicate the significant markers for juniper consumption

partly because they believe their animals are uniquely suited to the environment and management system they were raised in and believe this adaptiveness is genetic and environmental [47, 48]. These results provide a potential validation for this belief.

As observed in Table 3 and Additional File 1: Table S1, there has been a consistent improvement in juniper consumption by the lines selected for high consumption over the years. This improvement can be attributed to the selection that was applied in the populations for this trait since 2003 [49] and the positive results of the selection process. Over the course of nearly 15 years, animals were carefully chosen based on their estimated breeding values (EBVs) to accentuate the desired phenotypes while effectively managing the inbreeding coefficient to stay below 5%. This finding is consistent with previous studies and reinforces the idea that juniper consumption has varying levels of genetic control [18, 50]. This implies that the trait can be actively selected and exhibit significant improvements across generations. In this study, animals from both lines within population were first analyzed together. This was done because there was an exchange of breeding animals across lines and the lines were selected during a limited number of generations. Furthermore, the animals included in this study originated from a single farm and they might not represent the genetic background of the studied trait in the breeds. However, the results obtained do indicate that the trait is under genetic control and one can expect the same pattern in other goat populations. To confirm this hypothesis, future

studies in other goat populations should be conducted to evaluate the genetic background of juniper consumption in more representative populations. We also calculated variance components per line for the BS population and observed heritability estimates of 0.22 for both HIGH and LOW lines. The realized heritability estimates also indicate that juniper consumption is heritable in both goat populations.

When considering weaning weight, the consumption of juniper showed a weak to moderate negative genetic correlation for both BS and ANG populations. In the case of BS, this implies a slight, almost negligible trend for genetic variants influencing juniper consumption to be linked with weaning weight. Conversely, for ANG, a moderate and negative association was observed. This suggests that does that consumed more juniper weaned lighter kids resulting in a lower EBV for weaning weight. Nonetheless, existing evidence suggests that animals that begin consuming juniper immediately after weaning tend to exhibit increased juniper consumption over time [51].

The identification of numerous SNPs and genes associated with juniper consumption indicates that the consumption of juniper is influenced by many genes with small effects. While the exact mechanism enabling goats to tolerate juniper consumption has not been fully established [14, 52], and the impact on the animals' physiological systems can be quite variable. Goats are usually not significantly affected by the odor of juniper plants [14, 53]. In this study, five genes associated with olfactory receptor activity were identified for the BS population

Table 5 Functional analyses conducted on the genes linked to juniper consumption in the Boer x Spanish goat population

	Term	N genes	p-value	Genes
Biological Process				
GO:0010634	Positive regulation of epithelial cell migration	3	0.010	ENSCHIG00000011473, ENSCHIG00000026002, ENSCHIG00000022660
GO:0007179	Transforming growth factor beta receptor signaling pathway	4	0.010	ENSCHIG00000007488, ENSCHIG00000011473, ENSCHIG00000022660, ENSCHIG00000017970
GO:0009411	Response to UV	3	0.016	ENSCHIG00000011503, ENSCHIG00000016647, ENSCHIG00000025781
GO:0006909	Phagocytosis	3	0.024	ENSCHIG00000019616, ENSCHIG00000013665, ENSCHIG00000014924
GO:0050892	Intestinal absorption	2	0.058	ENSCHIG00000009946, ENSCHIG00000019616
GO:0043066	Negative regulation of apoptotic process	5	0.079	ENSCHIG00000004678, ENSCHIG00000014974, ENSCHIG00000025831, ENSCHIG00000026002
GO:0010468	Regulation of gene expression	3	0.090	ENSCHIG00000007488, ENSCHIG00000021385, ENSCHIG00000011473
Molecular Function				
GO:0051959	Dynein light intermediate chain binding	3	0.005	ENSCHIG00000017543, ENSCHIG00000021643, ENSCHIG00000022612
GO:0045505	Dynein intermediate chain binding	3	0.006	ENSCHIG00000017543, ENSCHIG00000021643, ENSCHIG00000022612
GO:0008569	ATP-dependent microtubule motor activity, minus-end-directed	3	0.008	ENSCHIG00000017543, ENSCHIG00000021643, ENSCHIG00000022612
GO:0005267	Potassium channel activity	3	0.011	ENSCHIG00000003466
GO:0005524	ATP binding	19	0.013	ENSCHIG00000014297, ENSCHIG00000017543, ENSCHIG00000023716, ENSCHIG00000008083, ENSCHIG00000016664, ENSCHIG00000021643, ENSCHIG00000016963, ENSCHIG00000015887, ENSCHIG00000022612, ENSCHIG00000014954, ENSCHIG00000020551, ENSCHIG00000024773, ENSCHIG00000019938, ENSCHIG00000025781, ENSCHIG00000005472, ENSCHIG00000012319, ENSCHIG00000004524, ENSCHIG00000012697, ENSCHIG00000011473
GO:0046872	Metal ion binding	19	0.021	ENSCHIG00000014650, ENSCHIG00000017941, ENSCHIG00000014974, ENSCHIG00000022976, ENSCHIG00000024542, ENSCHIG00000024773, ENSCHIG00000021135, ENSCHIG00000025203, ENSCHIG00000020183, ENSCHIG00000025440, ENSCHIG00000025781, ENSCHIG00000012697, ENSCHIG00000017295, ENSCHIG00000019075, ENSCHIG00000004901, ENSCHIG00000010780, ENSCHIG00000011473, ENSCHIG00000019650, ENSCHIG00000010382
GO:0004197	Cysteine-type endopeptidase activity	3	0.029	ENSCHIG00000018930, ENSCHIG00000011503, ENSCHIG00000026002
GO:0005539	Glycosaminoglycan binding	2	0.058	ENSCHIG00000011473, ENSCHIG00000019067
Cellular Component				
GO:0005886	Plasma membrane	24	0.059	ENSCHIG00000017543, ENSCHIG00000019327, ENSCHIG00000014078, ENSCHIG00000022504, ENSCHIG00000020318, ENSCHIG00000024417, ENSCHIG00000021187, ENSCHIG00000015813, ENSCHIG00000014802, ENSCHIG00000020690, ENSCHIG00000003793, ENSCHIG00000026073, ENSCHIG00000004678, ENSCHIG00000011139, ENSCHIG00000004524, ENSCHIG00000000113, ENSCHIG00000012697, ENSCHIG00000013522, ENSCHIG00000017295, ENSCHIG00000011473, ENSCHIG00000017970, ENSCHIG00000013154, ENSCHIG00000016363, ENSCHIG00000000529
GO:0035869	Ciliary transition zone	2	0.084	ENSCHIG00000000494, ENSCHIG00000006328

Table 5 (continued)

	Term	N genes	p-value	Genes
GO:0016021	Integral component of membrane	45	0.088	ENSCHIG00000023329, ENSCHIG00000019327, ENSCHIG00000014078, ENSCHIG00000019622, ENSCHIG00000009140, ENSCHIG00000014974, ENSCHIG000000021187, ENSCHIG00000024773, ENSCHIG00000014717, ENSCHIG00000005144, ENSCHIG000000025440, ENSCHIG00000020690, ENSCHIG00000003793, ENSCHIG00000013959, ENSCHIG00000000464, ENSCHIG00000002685, ENSCHIG00000011354, ENSCHIG00000019075, ENSCHIG00000017791, ENSCHIG00000011473, ENSCHIG00000013154, ENSCHIG00000018122, ENSCHIG00000016363, ENSCHIG00000022504, ENSCHIG00000008083, ENSCHIG00000016664, ENSCHIG00000018308, ENSCHIG00000025118, ENSCHIG00000015813, ENSCHIG00000024762, ENSCHIG00000014802, ENSCHIG00000021652, ENSCHIG00000024122, ENSCHIG00000007488, ENSCHIG00000026073, ENSCHIG00000000255, ENSCHIG00000003466, ENSCHIG00000012753, ENSCHIG00000013522, ENSCHIG00000012697, ENSCHIG00000015461
Pathways				
chx04724	Glutamatergic synapse	4	0.042	ENSCHIG00000024417, ENSCHIG00000015813, ENSCHIG00000009680, ENSCHIG00000020690
chx04024	cAMP signaling pathway	5	0.083	ENSCHIG00000008083, ENSCHIG00000016664, ENSCHIG00000021423, ENSCHIG00000019792, ENSCHIG00000017295
chx04014	Ras signaling pathway	5	0.084	ENSCHIG00000023716, ENSCHIG00000012319, ENSCHIG00000019842, ENSCHIG00000021423, ENSCHIG00000024417

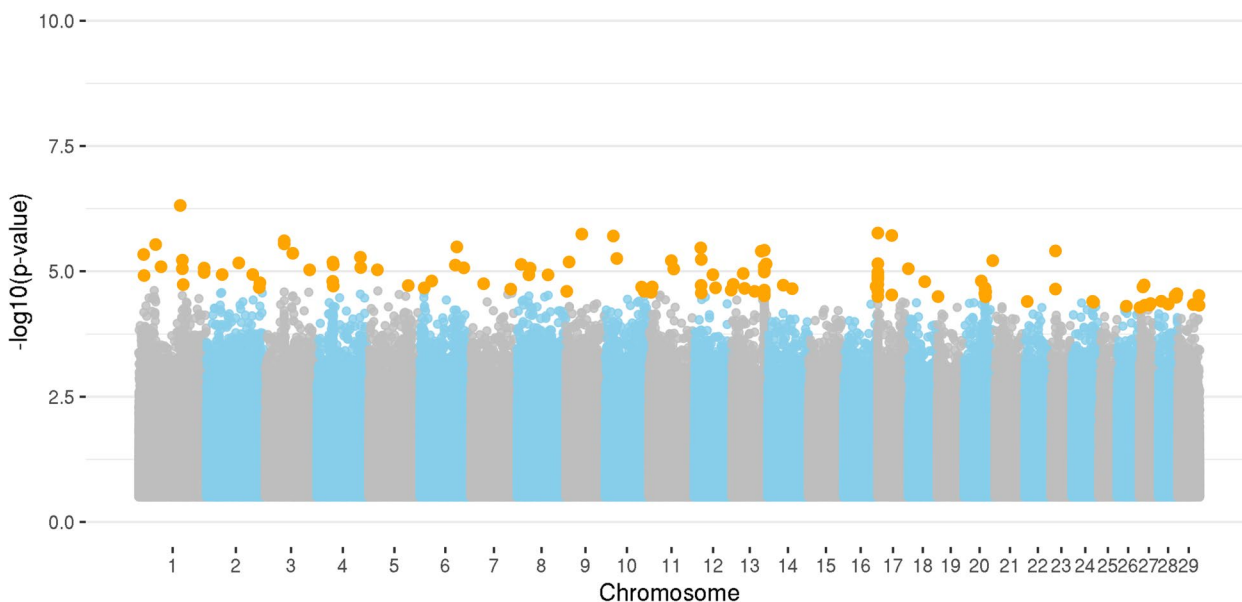


Fig. 2 Manhattan plot for juniper consumption in an Angora goat population based on whole-genome sequence data. Legend: Orange dots indicate the significant markers for juniper consumption

Table 6 Functional analysis conducted based on the genes linked to juniper consumption in the Angora goat population

Term	N genes	p-value	Genes
Biological Process			
GO:0002376 Immune system process	3	0.005	ENSCHIG00000001274, ENSCHIG000000014141
GO:0007160 Cell–matrix adhesion	3	0.029	ENSCHIG000000020502, ENSCHIG000000017512, ENSCHIG000000019633
GO:0008206 Bile acid metabolic process	2	0.057	ENSCHIG000000020709, ENSCHIG000000000506
GO:0045596 Negative regulation of cell differentiation	2	0.096	ENSCHIG000000025831
Molecular Function			
GO:0004222 Metalloendopeptidase activity	6	0.000	ENSCHIG000000020502, ENSCHIG000000025021, ENSCHIG000000015652, ENSCHIG000000019633, ENSCHIG000000015701, ENSCHIG000000001217
GO:0005524 ATP binding	14	0.052	ENSCHIG000000014981, ENSCHIG000000016126, ENSCHIG000000023289, ENSCHIG000000014206, ENSCHIG000000015009, ENSCHIG000000014879, ENSCHIG000000007275, ENSCHIG000000008522, ENSCHIG000000003216, ENSCHIG000000013314, ENSCHIG000000011751, ENSCHIG000000016690, ENSCHIG000000012374, ENSCHIG000000013396
GO:0042626 ATPase activity, coupled to transmembrane movement of substances	2	0.068	ENSCHIG000000003216, ENSCHIG000000025227
GO:0046914 Transition metal ion binding	2	0.078	ENSCHIG000000021789, ENSCHIG000000013424
GO:0008017 Microtubule binding	4	0.083	ENSCHIG000000008522, ENSCHIG000000010675, ENSCHIG000000011751, ENSCHIG000000005959
GO:0004707 MAP kinase activity	2	0.084	ENSCHIG000000012374, ENSCHIG000000013396
Cellular Components			
GO:0045177 Apical part of cell	3	0.040	ENSCHIG000000025789, ENSCHIG000000000147, ENSCHIG000000018661
GO:0005764 Lysosome	4	0.073	ENSCHIG000000018060, ENSCHIG000000009558, ENSCHIG000000013798, ENSCHIG000000005959
Metabolic Pathways			
chx01523 Antifolate resistance	5	0.000	ENSCHIG000000014981, ENSCHIG000000016126, ENSCHIG000000013314, ENSCHIG000000014206, ENSCHIG000000016690
chx04976 Bile secretion	6	0.000	ENSCHIG000000002450, ENSCHIG000000014981, ENSCHIG000000016126, ENSCHIG000000013314, ENSCHIG000000016690, ENSCHIG000000022594
chx04657 IL-17 signaling pathway	5	0.002	ENSCHIG000000021789, ENSCHIG000000013424, ENSCHIG000000014206, ENSCHIG000000012374, ENSCHIG000000013396
chx02010 ABC transporters	4	0.006	ENSCHIG000000014981, ENSCHIG000000016126, ENSCHIG000000013314, ENSCHIG000000016690
chx04071 Sphingolipid signaling pathway	4	0.031	ENSCHIG000000015908, ENSCHIG000000020626, ENSCHIG000000009558, ENSCHIG000000012374
chx04024 cAMP signaling pathway	5	0.046	ENSCHIG000000014981, ENSCHIG000000016126, ENSCHIG000000013314, ENSCHIG000000016690, ENSCHIG000000012374
chx05418 Fluid shear stress and atherosclerosis	4	0.049	ENSCHIG000000000147, ENSCHIG000000014206, ENSCHIG000000025722, ENSCHIG000000012374
chx01240 Biosynthesis of cofactors	4	0.065	ENSCHIG000000002450, ENSCHIG000000025722, ENSCHIG000000022594, ENSCHIG000000007275
chx00830 Retinol metabolism	3	0.083	ENSCHIG000000002450, ENSCHIG000000023075, ENSCHIG000000022594

(*LOC102185841*, *LOC102190566*, *LOC102186401*, *LOC108636907*, *LOC102186483*), and one for *ANG* (*OR2Z1*). The identification of these genes could provide insights into the molecular mechanisms underlying the high juniper consuming goats' ability to perceive and tolerate the consumption of juniper. Goats have a complex nasal cavity and presumably a high sensitivity to odors [54], which may allow them to detect the amount of plant secondary metabolites such as condensed tannins, mono- and sesquiterpenes in juniper and select

plants that have lower levels of these defensive chemicals. Goats prefer *J. ashei* over *J. pinchotii* [44] and the former has 30 – 50% less volatile oils than the latter [44, 55], and browsed *J. ashei* has 60% less volatile oils than unbrowsed plants [11].

Another effect of juniper consumption is associated with the negative consequences that arise from exposure to high levels of monoterpenes [4]. Upon ingestion of juniper and subsequent passage through the rumen, plant secondary metabolites, particularly monoterpenoids,

can be rapidly and easily absorbed through the intestinal wall without interacting with digestive enzymes due to their physicochemical properties [56]. In the BS population, the biological process of intestinal absorption (GO:0050892) was found to be influenced by leptin (*ENSCHIG00000019616—LEP*) and glucosaminyl (N-acetyl) transferase 3 (*ENSCHIG00000009946—GCNT3*). When camphor was intra-uminally dosed, total serum camphor was five times lower in HIGH goats compared to LOW goats [57] and when fed a diet with a constant amount of monoterpenes, HIGH animals had a higher concentration of monoterpenes in their feces than LOW [58]. The leptin gene is known to affect feed intake and energy homeostasis, playing a crucial role in nutrient absorption [59]. Interestingly, when it comes to plant-derived molecular components, the presence of terpenoids enhances leptin sensitivity [60], leading to a decrease in animal consumption. Regarding glucosaminyl (N-acetyl) transferase 3, it is a gene that belongs to the N-acetylglucosaminyltransferase family. It plays a role in reducing the expression of catabolic genes involved in glucosamine metabolism [61]. Moreover, it is associated with exerting an anti-inflammatory effect on intestinal epithelial cells [62], and in liver damage [63]. These findings suggest that the genes possess mechanisms that enhance the tolerance of juniper components, thereby mitigating the negative effects of its consumption.

For the peak observed on CHI13 (75,272,313–79,825,590), most of the genes are long intergenic non-coding RNAs and small nucleolar RNAs. These genes have the potential to regulate neighboring genes, suggesting their involvement in enhancer-like activity [64, 65]. In the BS population, the identified genomic regions in this peak harbor the *EYA2* and *ZMYND8* genes, which play crucial roles in cell maintenance and regulation, contributing to growth and development processes [66, 67]. In the ANG population, although the significant genomic region was not as pronounced as in the BS population, several other genes including *GINS1*, *ANKEF1*, *ITGA8*, *PCMTD2*, *ZMYND8*, and *FZD8* were found within the region. These genes are involved in cellular maintenance and activities such as replication [68] and cell recovery [66, 67].

For BS, the genomic region located on CHI17 (327,164–378,403) harbors a small nucleolar RNA and two small nuclear RNAs, which play important roles in gene regulation [65]. Additionally, five immunoglobulin V genes overlap with this region, contributing to the differential capacity of generating an immune response to restore normal activities after intoxication [69]. There was also one processed pseudogene identified, which may function as a promoter for neighboring genes

[70]. Some of the genes within this genomic region are uncharacterized proteins specific to the goat species. The remaining genes are protein-coding, with some yet to be fully recognized. Notably, the *KSR2* gene appears to be linked to consumption. *KSR2* is involved in multiple signaling pathways and plays a role in energy homeostasis and insulin resistance [71]. There are studies reporting that the consumption of terpenoids can be beneficial for health, promoting a therapeutic potential for insulin resistance and hyperglycemia [72]. In the ANG population, the region associated with this peak was primarily characterized by four immunoglobulin V genes and one processed pseudogene. Furthermore, we identified nine protein-coding genes, all of which have not been annotated yet. More extensive investigation is required to uncover the specific functions of these genes and their implications in the response to juniper consumption.

Notably, our findings underscore a relationship between juniper consumption and the gene ontology related to bile acid metabolic processes, as well as bile secretion metabolic pathways. A function of bile is to facilitate the elimination of toxic substances from the organism [73]. This observation implies that these metabolic processes could potentially contribute to the preference for juniper consumption in these particular genetic lines of goats.

Although not evaluated in this study, it is important to consider other factors that contribute to goats' ability to consume juniper. One such factor is the rumen microbiome, which plays a crucial role in degrading, deactivating, and detoxifying plant metabolites [74]. Microbial activity can aid in the consumption of plants like juniper. Additionally, a significant proportion of goats is raised in harsh environmental conditions, where plants accumulate secondary metabolites as a defense mechanism against herbivores, heat, and water stress [75]. This suggests that the interaction between the microbiome, the animal, and the environment may contribute to the adaptability of goats to tolerate the negative effects of juniper consumption and being able to act as a controlling agent.

Another physiological aspect that may contribute to goats' ability to handle the negative effects of juniper consumption is their high capacity to absorb less and excrete metabolites more efficiently compared to other species [76]. When components of juniper that were not utilized by rumen microorganisms reach the intestine, they continue the digestion process. These components are then absorbed, and any toxic compounds are processed by the liver, where they are metabolized, detoxified, and excreted [77]. Numerous mechanisms involving enzymes and proteins, some of which are related to the genes

associated with juniper prediction, are involved in this process. Although some of these mechanisms are yet to be fully characterized, they appear to be strongly linked to juniper consumption. While this study offers significant insights into the genetic factors influencing juniper consumption, it is important to address certain limitations and challenges that were encountered during the study's execution. Despite the utilization of a highly accurate predictive technique to analyze animal consumption patterns, its predictions might not be 100% precise. This potential variability could introduce bias, particularly in cases where the dataset size is limited. Another notable consideration is the gap in our knowledge concerning the animals' weights during the experiment. Regrettably, our dataset lacked information regarding the animals' weights when collecting fecal samples. This aspect represents an intriguing avenue for further investigation, as it could potentially shed light on the relationship between consumption patterns and animal development. The inclusion of weight data during the fecal sample collection phase could enhance the comprehensiveness of our findings. To compensate for the absence of weight data, we relied on information related to weaning weight, a trait that exhibits a strong correlation with weight in different ages [78]. While this alternative offered valuable insights, it is important to recognize that direct weight measurements during the experiment would have provided a more comprehensive perspective on how juniper consumption impacts animal development. Addressing this limitation in future studies could contribute to a more holistic understanding of the relationships involved.

Conclusions

There were numerical differences in juniper consumption between BS and ANG goats, with BS exhibiting higher juniper consumption. Juniper consumption is heritable and can be increased through selective breeding. Various SNPs and candidate genes associated with juniper consumption were identified in both populations. The analyses revealed a potential connection between juniper consumption and genes associated with olfactory receptors. Furthermore, genes implicated in crucial processes such as intestinal absorption, including *Leptin* and *glucosaminyl (N-acetyl) transferase*, as well as those involved in bile secretion and bile metabolic processes, were also identified. Multiple uncharacterized genes were found to be related to juniper consumption, which opens opportunities for further genome annotation studies. Overall, these findings highlight the potential for genetic selection to change dietary preferences in goats, resulting in animals that are better adapted to their environment for more sustainable production and rangeland restoration.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-024-10009-7>.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

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Authors' contributions

HAM and LFB (Brito) conceived, designed, and conducted the data analyses. JWW, DFW, and DGW contributed to the data generation and interpretation of the results. HAM wrote the first version of the manuscript. HAM, DGQ, LFB, LFB, and FEC contributed to the interpretation of the results and edited the manuscript. All authors reviewed and approved the final version of the paper.

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Availability of data and materials

All the data needed for the interpretation of the results are presented in the paper and in its supplementary materials. The raw datasets can be made available for research based upon reasonable requests (Dr. Luiz Brito; E-mail: britol@purdue.edu).

Declarations

Ethics approval and consent to participate

This study is reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>). All procedures involving animals were approved by the Texas A & M University Institutional Agricultural Animal Care and Use Committee under protocol 2003–129.

Consent for publication

This is not applicable.

Competing interests

The authors declare no competing interests.

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References

- Engle DM, Coppedge BR, Fuhlendorf SD. From the dust bowl to the green glacier: human activity and environmental change in great plains grasslands. 2008. p. 253–71.
- Ansley RJ, Pinchak WE, Ueckert D. Changes in redberry juniper distribution in northwest Texas (1948 to 1982). *Rangelands*. 1995;17:49–53.
- Coultrap DE, Fulgham KO, Lancaster DL, Gustafson J, Lile DF, George MR. Relationships between western juniper (*Juniperus occidentalis*) and understory vegetation. *Invasive Plant Sci Manag*. 2008;1:3–11.

4. Campbell ES, Taylor CA, Walker JW, Lupton CJ, Waldron DF, Landau SY. Effects of supplementation on juniper intake by goats. *Rangel Ecol Manag.* 2007;60:588–95.
5. Leis SA, Blocksome CE, Tidwell D, Fuhlendorf SD, Briggs JM, Sanders LD. Juniper invasions in grasslands: Research needs and intervention strategies. *Rangelands.* 2017;39:64–72.
6. Ueckert DN, Whisenand SG. Individual plant treatments for controlling redberry juniper seedlings. *J Range Manag.* 1982;35:419.
7. Flippo D, Gunturu S, Baldwin C, Badgujar C. Tree trunk detection of eastern red cedar in Rangeland environment with deep learning technique. *Croatian J For Eng.* 2023;44:357–68.
8. Bates JD, Davies JW. Seasonal burning of juniper woodlands and spatial recovery of herbaceous vegetation. *For Ecol Manag.* 2016;361:117–30.
9. Hart SP. Recent perspectives in using goats for vegetation management in the USA. *J Dairy Sci.* 2001;84(E170):176.
10. Estell RE, Fredrickson EL, Tellez MR, Havstad KM, Shupe WL, Anderson DM, et al. Effects of volatile compounds on consumption of alfalfa pellets by sheep. *J Anim Sci.* 1998;76:228.
11. Adams RP, Muir JP, Taylor CA, Whitney TR. Differences in chemical composition between browsed and non-browsed *Juniperus ashei* Buch. Trees. *Biochem Syst Ecol.* 2013;46:73–8.
12. Pritz RK, Launchbaugh KL, Taylor CA. Effects of breed and dietary experience on juniper consumption by goats. *J Range Manag.* 1997;50:600.
13. Straka E, Scott CB, Taylor CA Jr, Bailey EM Jr. Biological control of the toxic shrub juniper. Poisonous plants and related toxins. UK: CABI Publishing; 2004. p. 436–42.
14. Narjisse H, Maleček JC, Olsen JD. Influence of odor and taste of monoterpenoids on food selection by anomic and intact sheep and goats. *Small Rumin Res.* 1997;23:109–15.
15. Ikoyi AY, Younge BA. Faecal near-infrared reflectance spectroscopy profiling for the prediction of dietary nutritional characteristics for equines. *Anim Feed Sci Technol.* 2022;290:115363.
16. Dixon R, Coates D. Review: near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. *J Near Infrared Spectrosc.* 2009;17:1–31.
17. Walker JW, Campbell ES, Lupton CJ, Taylor CA, Waldron DF, Landau SY. Effects of breed, sex, and age on the variation and ability of fecal near-infrared reflectance spectra to predict the composition of goat diets 1,2. *J Anim Sci.* 2007;85:518–26.
18. Waldron DF, Taylor CA, Walker JW, Campbell ES, Lupton CJ, Willingham TD, et al. Heritability of juniper consumption in goats 1. *J Anim Sci.* 2009;87:491–5.
19. Rook AJ, Dumont B, Isselstein J, Osoro K, WallisDeVries MF, Parente G, et al. Matching type of livestock to desired biodiversity outcomes in pastures – a review. *Biol Conserv.* 2004;119:137–50.
20. USDA. USDA-Natural Resources Conservation Service. Low Stony Hill 19–23 PZ. 2006.
21. Lashmar S, Visser C, Van Marle-Köster E. Validation of the 50k Illumina goat SNP chip in the South African angora goat. *S Afr J Anim Sci.* 2015;45:56.
22. Walker JW, McCoy SD, Launchbaugh KL, Fraker MJ, Powell J. Calibrating fecal NIRS equations for predicting botanical composition of diets. *J Range Manag.* 2002;55:374.
23. Walker JW, Waldron DF, Campbell ES, Taylor CA, Lupton CJ, Landau S. Intermediate periodicities in juniper consumption and sampling strategies to estimate the diet of free-grazing goats. *Rangel Ecol Manag.* 2013;66:209–15.
24. Dong Y, Xie M, Jiang Y, Xiao N, Du X, Zhang W, et al. Sequencing and automated whole-genome optical mapping of the genome of a domestic goat (*Capra hircus*). *Nat Biotechnol.* 2013;31:135–41.
25. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. *Gigascience.* 2021;10:10.
26. Picard toolkit [Internet]. Broad Institute. 2019. Available from: <https://broadinstitute.github.io/picard/>.
27. Browning BL, Browning SR. Improving the accuracy and efficiency of identity-by-descent detection in population data. *Genetics.* 2013;194:459–71.
28. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559–75.
29. Misztal I, Lourenco D, Lourenco D, Masuda Y. Manual for BLUPF90 family of programs. Athens: University of Georgia; 2014. p. 1–149.
30. Smith BJ. boa: an R package for MCMC output convergence assessment and posterior inference. *J Stat Softw.* 2007;21:1–37.
31. Misztal I, Legarra A, Aguilar I. Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. *J Dairy Sci.* 2009;92:4648–55.
32. Lourenco D, Legarra A, Tsuruta S, Masuda Y, Aguilar I, Misztal I. Single-step genomic evaluations from theory to practice: using SNP chips and sequence data in BLUPF90. *Genes (Basel).* 2020;11:790.
33. VanRaden PM. Efficient methods to compute genomic predictions. *J Dairy Sci.* 2008;91:4414–23.
34. Vitezica Zg, Aguilar I, Misztal I, Legarra A. Bias in genomic predictions for populations under selection. *Genet Res (Camb).* 2011;93:357–66.
35. Thompson R. Estimation of realized heritability in a selected population using mixed model methods. *Genet Sel Evol.* 1986;18:475.
36. Garrick DJ, Taylor JF, Fernando RL. Deregressing estimated breeding values and weighting information for genomic regression analyses. *Genet Sel Evol.* 2009;41:55.
37. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: A tool for genome-wide complex trait analysis. *Am J Hum Genet.* 2011;88:76–82.
38. Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL. Advantages and pitfalls in the application of mixed-model association methods. *Nat Genet.* 2014;46:100–6.
39. Brito LF, Jafarikia M, Grossi DA, Kijas JW, Porto-Neto LR, Ventura RV, et al. Characterization of linkage disequilibrium, consistency of gametic phase and admixture in Australian and Canadian goats. *BMC Genet.* 2015;16:67.
40. Hayes BJ, Bowman PJ, Chamberlain AC, Verbyla K, Goddard ME. Accuracy of genomic breeding values in multi-breed dairy cattle populations. *Genet Sel Evol.* 2009;41:51.
41. Goddard ME, Hayes BJ, Meuwissen THE. Using the genomic relationship matrix to predict the accuracy of genomic selection. *J Anim Breed Genet.* 2011;128:409–21.
42. Fonseca PAS, Suárez-Vega A, Marras G, Cánovas Á. GALLO: an R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci. *Gigascience.* 2020;9:1–9.
43. Huang D, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, et al. The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol.* 2007;8:R183.
44. Riddle RR, Taylor CA, Kothmann MM, Huston JE. Volatile oil contents of ashe and redberry juniper and its relationship to preference by Angora and Spanish Goats. *J Range Manag.* 1996;49:35.
45. Mellado M. Dietary selection by goats and the implications for range management in the Chihuahuan desert: a review. *Rangel J.* 2016;38:331.
46. McIntosh MM, Spiegel SA, McIntosh SZ, Castaño Sanchez J, Estell RE, Steele CM, et al. Matching beef cattle breeds to the environment for desired outcomes in a changing climate: a systematic review. *J Arid Environ.* 2023;211:104905.
47. Elizondro J, Varvaro G, Gras G. Regenerative ranching maximum sustainable profit by ranching in nature's image. 2019.
48. Wilmer H, Fernández-Giménez ME, Ghajar S, Taylor PL, Souza C, Derner JD. Managing for the middle: rancher care ethics under uncertainty on Western Great Plains rangelands. *Agric Hum Values.* 2020;37:699–718.
49. Seidel DS, Walker JW, Musser JM, Whitney TR, Callaway TR. Impact of camphor on the in vitro mixed ruminal microorganism fermentation from goats selected for consumption of low and high levels of *Juniperus* spp. 1. *Transl Anim Sci.* 2022;6(3):txac098.
50. Ellis C, Jones R, Scott C, Taylor C, Walker J, Waldron D. Sire influence on juniper consumption by goats. *Rangel Ecol Manag.* 2005;58:324–8.
51. Tidwell KW, Scott CB. Sire influence on redberry juniper consumption by kid goats. *Rangel Ecol Manag.* 2021;74:96–9.
52. Wojtunik-Kulesza KA. Toxicity of selected monoterpenes and essential oils Rich in these compounds. *Molecules.* 2022;27:1716.
53. De Rosa G, Moio L, Napolitano F, Grasso F, Gubitosi L, Bordi A. Influence of flavor on goat feeding preferences. *J Chem Ecol.* 2002;28:269–81.
54. Octura JER, Maeda K, Wakabayashi Y. Structure and zonal expression of olfactory receptors in the olfactory epithelium of the goat (*Capra hircus*). *J Vet Med Sci.* 2018;80:913–20.
55. Stewart WC, Whitney TR, Scholljegerdes EJ, Naumann HD, Cherry NM, Muir JP, et al. Effects of *Juniperus* species and stage of maturity on nutritional, in vitro digestibility, and plant secondary compound characteristics. *J Anim Sci.* 2015;93:4034–47.

56. Kohl KD, Pitman E, Robb BC, Connelly JW, Dearing MD, Forbey JS. Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. *J Comp Physiol B*. 2015;185:425–34.
57. Campbell EJ, Frost RA, Mosley TK, Mosley JC, Lupton CJ, Taylor CA, et al. Pharmacokinetic differences in exposure to camphor after intraruminal dosing in selectively bred lines of goats1. *J Anim Sci*. 2010;88:2620–6.
58. Walker JW, Shoemaker BM, Quadros DG, Thorne JW, Cahill NC. Selecting goats for juniper consumption did not improve their liver phase I detoxification. *J Anim Sci*. 2023;101:skad180.
59. El-Zein O, Kreydiyyeh SI. Leptin inhibits glucose intestinal absorption via PKC, p38MAPK, PI3K and MEK/ERK. Yang C-M, editor. *PLoS One*. 2013;8:e83360.
60. Aragonés G, Ardid-Ruiz A, Ibars M, Suárez M, Bladé C. Modulation of leptin resistance by food compounds. *Mol Nutr Food Res*. 2016;60:1789–803.
61. Suryanarayana Rao A. An appraisal for the need to start independent research institutes and study programs in metabolic engineering. *Adv Biochem*. 2021;9:60.
62. Nagaoka I. Glucosamine suppresses interleukin-8 production and ICAM-1 expression by TNF- α -stimulated human colonic epithelial HT-29 cells. *Int J Mol Med*. 1998;22:205–11.
63. Clegg DO, Jackson CG. Glucosamine. *Encyclopedia of Dietary Supplements*. CRC Press; 2018;279–86.
64. Ransohoff JD, Wei Y, Khavari PA. The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol*. 2018;19:143–57.
65. Bratkovič T, Božič J, Rogelj B. Functional diversity of small nucleolar RNAs. *Nucleic Acids Res*. 2020;48:1627–51.
66. Luo M, Bao L, Chen Y, Xue Y, Wang Y, Zhang B, et al. ZMYND8 is a master regulator of 27-hydroxycholesterol that promotes tumorigenicity of breast cancer stem cells. *Sci Adv*. 2022;8:5295.
67. De Munter S, Köhn M, Bollen M. Challenges and opportunities in the development of protein phosphatase-directed therapeutics. *ACS Chem Biol*. 2013;8:36–45.
68. Bu F, Zhu X, Zhu J, Liu Z, Wu T, Luo C, et al. Bioinformatics analysis identifies a Novel role of *GINS1* gene in colorectal cancer. *Cancer Manag Res*. 2020;12:11677–87.
69. Mikocziova I, Greiff V, Sollid LM. Immunoglobulin germline gene variation and its impact on human disease. *Genes Immun*. 2021;22:205–17.
70. Kazazian HH. Processed pseudogene insertions in somatic cells. *Mob DNA*. 2014;5:20.
71. Pearce LR, Atanassova N, Banton MC, Bottomley B, van der Klaauw AA, Revelli J-P, et al. KSR2 mutations are associated with obesity, insulin resistance, and impaired cellular fuel oxidation. *Cell*. 2013;155:765–77.
72. Fang Z-J, Shen S-N, Wang J-M, Wu Y-J, Zhou C-X, Mo J-X, et al. Triterpenoids from *Cyclocarya paliurus* that enhance glucose uptake in 3T3-L1 adipocytes. *Molecules*. 2019;24:187.
73. Ong ES, Espat NJ. Bile secretion. *Surgery of the liver, biliary tract and pancreas*. Philadelphia: Saunders Elsevier; 2007. p. 72–8.
74. Gallo A, Giuberti G, Frisvad J, Bertuzzi T, Nielsen K. Review on mycotoxin issues in ruminants: occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. *Toxins (Basel)*. 2015;7:3057–111.
75. Giger-Reverdin S, Domange C, Broudiscou LP, Sauviant D, Berthelot V. Rumen function in goats, an example of adaptive capacity. *J Dairy Res*. 2020;87:45–51.
76. Elsheikh HA, Ali BH, Homeida AM, Hassan T, Hapke HJ. Pharmacokinetics of antipyrine and sulphadimidine (sulfamethazine) in camels, sheep and goats. *J Vet Pharmacol Ther*. 1991;14:269–75.
77. Gu X, Manautou JE. Molecular mechanisms underlying chemical liver injury. *Expert Rev Mol Med*. 2012;14:4.
78. Singh H, Pannu U, Narula HK, Chopra A, Naharwara V, Bhakar SK. Estimates of (co)variance components and genetic parameters of growth traits in Marwari sheep. *J Appl Anim Res*. 2016;44:27–35.

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