Population structure and genetic diversity in males of the autochthonous Busha cattle in Croatia

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ABSTRACT

Busha is recognized as the most numerous autochthonous cattle breed in Croatia. In small populations, using the same sire repeatedly can increase inbreeding rates. This can lead to fewer high-quality, and high inbred breeding sires over time. Genealogical records of the Busha cattle breed registered from 1987 to 2022 were used to investigate the population structure and the genetic variability of the male individuals. The dataset included 10,411 animals, of which 3,962 were males. The average equivalent complete generation reached 2.92 in the total population and 3.05 in the reference population, when considering only living animals. The average inbreeding coefficient in the male population was 1.59%. Over the studied period, inbreeding rose to an average value of 2.07% in the last birth year cohort (2017-2022). The unequal contribution of the effective number of founders indicates the more frequent use of particular breeding sire lines. Nevertheless, despite the controlled inbreeding rate observed among breeding sires, and the absence of any evident population bottleneck, our study highlighted the need for breeding strategies to optimize the contribution of breeding animals in the next generations to ensure long-term conservation of the Busha cattle breed.

Key words: genetic diversity; cattle; Busha; sire; pedigree analysis

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Introduction

Autochthonous cattle breeds have a significant role in the global animal genetic resources. Their ability to adapt to different environments could be crucial in dealing with climate change and potential disease outbreaks, and preservation of the global food production chain (CURONE et al., 2018; SOINI et al., 2019). Significant advantages arise from both the preservation and conservation of local breeds, along with their genetic enhancement (BISCARINI et al., 2015). Busha cattle are one

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of the three Croatian autochthonous cattle breeds. They are bred in an environment with modest conditions and are well adapted to a harsh climate, roughage diet, and pasture conditions. Therefore, they have developed into a highly adaptable, resistant, long-lived breed of cattle with a small body frame, and low maintenance and production needs. The development of the Busha cattle began 6,500 years ago with the introduction of domesticated cattle from the Middle East into the existing wild local cattle populations of Southeastern Europe (HRISTOV et al., 2016). Apart from Croatia, this distinctive breed of cattle is also found in the Balkan region, specifically in Bulgaria, North Macedonia, Albania, Serbia, Bosnia and Herzegovina, Montenegro, and Kosovo, forming a single metapopulation (RAMLJAK et al., 2018). Different types have formed due to the various biogeographical regions and socio-cultural environments, and breeding goals, with the most noticeable differences being their morphological characteristics and the variety of coat colors (BUNEVSKI et al., 2016). The production traits of the native Busha cattle breed primarily emphasize their ability for milk production, with the secondary emphasis on meat production, although historically they were also used as working animals. Busha cattle dominated cattle production in Croatia until the middle of the 20th century. Then, their number rapidly declined to approximately 300 individuals (RAMLJAK et al., 2011). In 2003, a closed-type breed register was established to mitigate the decrease in population size, allowing for pure breeding. Subsequently, a conservation program started in 2004, supported by the government and scientific institutions. The primary breeding goal of the Busha cattle breed involves preserving genetic variability and maintaining the population with its distinctive standard morphological features (ČAČIĆ et al., 2012a).

Genetic diversity, defined as a measure of genetic differences between and within groups of animals, that is, breeds, is related to their adaptation and response to selection (GROENEVELD et al., 2010). Several causes influence genetic diversity, such as migration, mutation, selection, genetic drift, bottleneck, and inbreeding (AMOS and HARWOOD,

1998). Monitoring genetic diversity and trends in small populations is the basis for implementing any conservation program and selecting suitable breeding animals to reach sustainable breeding goals (GONZÁLEZ-CANO et al., 2022). The availability of non-related breeding females and males is crucial for assessing the risk of the considerable effect of inbreeding and the consequent reduction in genetic variability (HILL and MACKAY, 2004; HOWARD et al., 2017). In small, endangered populations, an increase in the rate of inbreeding can result from using the same sire repeatedly, leading to a reduced number of high-quality, and highly inbred breeding sires (WINDIG and KAAL, 2008). The loss of genetic diversity can cause a decrease in individual fitness, and negatively affect the populations lifespan in the long term (HUISMAN et al., 2016; DOEKES et al., 2019).

Pedigree analyses constitutes a classical approach to studying the evolution of genetic diversity, population structure, and breeding practices within a given breed (NYMAN et al., 2022). FRANKHAM et al. (1999) emphasize that analyses of pedigree data provide a better understanding of the history of the population, enabling the detection of past events that may have influenced them, such as identifying the founders and their contribution to the population, or genetic drift and bottlenecks, and help in understanding the potential implications for future conservation and breeding decisions.

The objectives of this study were to analyze the historical and present patterns in the population structure and genetic variability over a 36-year period within the population of male individuals of the autochthonous Busha cattle breed in Croatia.

Materials and methods

The data used in this research were compiled from the Directorate for Livestock and Food Quality of the Ministry of Agriculture of Croatia and the Veterinary Information System (VetIs) database, and include identification numbers for cows, bulls, and calves, and their dates of birth, sex, and area of origin. The data analysis covered the period from January 1987 to December 2022. After purging

the data by removing illogical or nonexistent values, the information for the total population (TP) of 10,411 animals was considered. Out of that number, 6,449 were females (61.95%), and 3,962 (38.05%) were males. From the total number of animals (TP), we distinguished the reference population (RP) including 6,064 animals, of which 5,390 were females (88.89%), and 674 (11.11%) were males. The reference population in the study consisted of currently living animals. A total of 3,160 individuals in the breeding population (BP) were recorded, consisting of 2,882 dams (91.20%) and 278 sires (8.80%), and the breeding population comprised breeding dams and sires that had at least one recorded offspring. Furthermore, from the total and the breeding population, we distinguished the male population (MP) and breeding sires for further analysis.

The population structure was characterized by analyzing various demographic parameters, including the total number of registered males, the number of living registered males, the overall number of sires used for breeding, birth cohorts as temporal periods in order to gauge the population's genetic structure over time, and the total number of offspring per sire line, categorized by sex. Birth cohorts were defined by the duration of the generation interval, approximately equal to six years.

Breeding areas were classified into regions on the basis of altitude, such as lowland Pannonian, mountainous Dinaric, and coastal Adriatic, which are Croatia's natural geographical regions (KLEMENČIĆ, 1993). The lowland Pannonian area is characterized by flat terrain and fertile soil below 200 meters above sea level. The coastal Adriatic area consists of hilly regions and plateaus with elevations ranging from 201 to 500 meters above sea level, and limited fertile soil. The mountainous Dinaric area includes mountains (501-1000 meters), foothills (1001-1500 meters), and high mountain areas (above 1500 meters). The parameters calculated within the geographical breeding area included the number of individuals born, the percentage of individuals born in the same area, and the percentage of individuals born in a different area. The use of sires across different areas is presented as a percentage of the sires with

the offspring originating from the same area, as well as the percentage of the offspring of sires relocated between these areas.

Pedigree completeness was determined by calculating the number of fully traced generations, the maximum number of generations traced, and the equivalent complete generation for each individual (CGE), defined as the sum of $(1/2)^n$, where 'n' denotes the number of generations between an individual and its known ancestor (BOICHARD et al., 1997). On the basis of a calculation by MACCLUER et al. (1983), pedigree integrity was assessed using the pedigree completeness index (PCI). The generation interval (GI) is defined as the average age of parents at the birth of their progeny kept for reproduction (JAMES, 1977). Additionally, we calculated the average age of parents at the birth of their offspring, regardless of whether the offspring were used for reproduction. The GI was calculated for the following pathways: sire to son and sire to daughter.

To explore inbreeding and the genetic influence of each individual in the population, we calculated two genetic parameters: the inbreeding coefficient (F) and the average relationship coefficient (AR) for the TP, RP and MP. F estimates an individual's likelihood of carrying two identical genes by descent, a computation derived from MEUWISSEN and LUO (1992). AR defines an individual's average genetic relationship with the rest of the population, thus providing insight into an animal's contribution within the entire pedigree, as annotated by GUTIÉRREZ and GOYACHE (2005). The individual increase in inbreeding (ΔF_i) was calculated. The effective population size (N_e) was estimated by calculating the regression coefficient of the individual inbreeding coefficient in the TP against: (i) the number of complete generations traced, (ii) the maximum number of generations traced, and (iii) the equivalent complete generations (CGE). Furthermore, N_e was computed for the TP via regression as follows: $N_e = 1/2*b$, where b is the regression coefficient of the individual F over CGE. Additionally, N_e was calculated by observing the individual increase in inbreeding across the population for the RP and MP. The rate of inbred animals (RIA) in the TP and

breeding sires was assessed by categorizing males with an F exceeding 0.05 as inbred, determined on the basis of the distribution pattern of individual inbreeding coefficients within the TP.

The effective number of ancestors (f_a) and the effective number of founders (f_e) were computed as the minimum count of founders and ancestors, respectively, responsible for the observed genetic diversity within the TP, RP and BP. The f_e/f_a ratio was calculated to evaluate potential population bottlenecks: a ratio close to one indicates stability in the population's effective contributing animals. Additionally, the number of ancestors accounting for 50% of genetic diversity was determined. The study assessed the genetic diversity among genealogical sire lines. There were 24 distinct lines; however, only 16 lines were represented by more than three offspring. The average pairwise co-ancestry coefficient (f_{ij}) was calculated within each genealogical sire line and between different lines. The founder genome equivalents (f_g) were computed for each genealogical line, representing the effective number of founders considering the nonrandom loss of founder alleles that contribute to the observed genetic variability.

The program ENDOG v.4.8 (GUTIÉRREZ and GOYACHE, 2005) was used to compute genetic diversity parameters. Statistical analysis was performed using the statistical programmes STATISTICA (2020) and R CORE TEAM (2024).

Results

The numbers of cattle in the total and reference population over the studied period are given in Table 1. The total number of registered males over the 36 evaluated years was 3,962, ranging between 1 in 1995 and 649 in 2022 (complete year). From 1995, a gradual increase in registered males was recorded, with the highest peak in 2021 with 750 males, as shown in Fig. 1. The average number of females registered throughout the studied period in the TP and RP was higher than males (P=0.0001), being twice as abundant on average. In total, 278 Busha sires with at least one offspring were used for breeding over the studied period, and of that, 174 (62.59%) were still alive. From the point of view of reproductive activity, out of the total number of registered male individuals (N=3,962) during the entire observed period, 7.01% became breeding sires, with at least one recorded offspring. Of that number, 0.6% were the progenitors of genealogical sire lines.

Table 1. Descriptive data on the number of animals, males, females and animals with no progeny, for the Busha cattle in the total, and the reference and breeding populations

Parameters	TP	RP	BP
Number of animals	10,411	6,064	3,160
Number of males	3,962	674	278
Number of females	6,449	5,390	2,882
Numbers of animals with no progeny	7,251	3,458	-

TP - total population: total number of animals present in the database; RP- reference population: number of animals currently alive; BP – breeding population: breeding dams and sires that had at least one recorded offspring
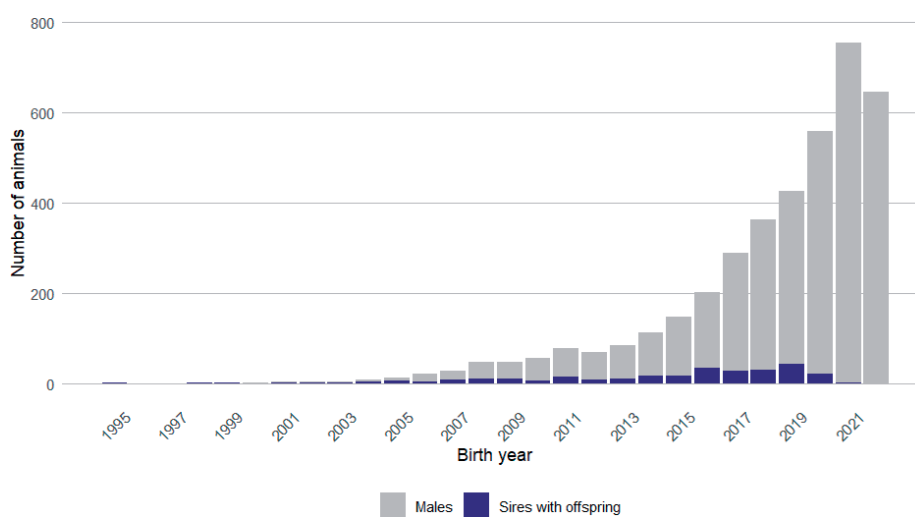
Fig 1. Number of registered males and sires with offspring of Busha cattle breed, per birth year over the studied period from 1995 to 2022 (complete year)

The male individuals in the breed registry are associated with 24 genealogical sire lines (SL) characterized in the Busha native breed breeding system (BARAĆ et al., 2012). Table 2 gives the total number of descendants of the 16 most influential SLs over the studied period. The most influential sire from SL1 had 300 offspring, half of all the registered sires had over 21 offspring, and 6.6% had only one offspring. Regardless of the GI, the breeding sire used longest belonged to SL12, was active for 16 years, and produced 108 descendants. The study notes the absence of descendants from SL20, SL21, SL22, SL23, and SL24 in the population when examining their presence compared to the 24 lines established. Moreover, a notable decrease in male offspring from SL13, SL15, SL16, SL18, and SL19 is observed. Geographic origin was known for 3,956 registered male Busha cattle individuals, and the proportion is shown in the Fig. 2. After examining the reasons for the decreased number of male individuals, it was found that 3,288 out of 3,962 male individuals were culled. The most frequent reasons for culling were found to be slaughter (N=2,496, 75.91%), stillbirths (N=239, 7.27%), and theft or disappearance (N=207, 6.30%).

The average GI computed from all pathways was 5.80±3.6 years. When considering the father-son pathway, the GI was 5.99±3.37 years in TP and 6.17±3.23 years in the RP. For the father-daughter pathway, GI was 6.28±3.44 years in the TP and 6.29±3.44 in the RP. The average age of the father when offspring are born was 6.40±3.50 years in the father-son pathway for TP and 6.29±3.36 in RP. In the father-daughter pathway, it was 6.31±3.50 in TP and 6.29±3.33 in RP.

Pedigree depth and completeness were assessed using the following parameters: there were, on average, a maximum of 4.62 generations traced in the TP. The CGE measuring pedigree information completeness equaled 2.92 in the TP and reached a mean value of 3.05 in the RP and 2.71 in the MP. The pedigree was most comprehensive in the latest generation (PCI>97%), and declined in earlier generations, with PCI approximately 80%, 64%, 31%, and 9% for the second, third, fourth, and fifth generations for the TP, respectively.

The main genetic parameters that characterize genetic variability for the TP, RP and MP of the Busha cattle are given in Table 3. The average F was equal to 2.44% (max 42.63%), the average AR was 3.56% (max 8.68%), and the average increase in

inbreeding (ΔF) 0.88% (max 25%), within the TP. The effective population size (N_e) in the TP resulted in 131.25, 74.96, and 74.24, when calculated using the increase in inbreeding by maximum generations, complete equivalent generations, and full generations traced, respectively. In the RP, the average F was equal to 2.45%, the average AR was 3.61%, and the ΔF_i was 0.84%. In contrast, for the MP, average inbreeding was 1.59%, and the average increase in inbreeding was 0.58%. The N_e calculated concerning the increase in inbreeding ΔF_i was 60.36 in the RP and 58.62 in the MP. The effective number of founders (f_e) was equal to 40, and the effective number of ancestors (f_a) was equal to 35 in the RP. When considering only the male population (MP), the effective number of founders (f_e) was equal to 36, and the effective number of ancestors (f_a) was equal to 32. The ratio between f_e and f_a was equal to 1.14 in RP and 1.12 in MP, respectively. The number of ancestors explaining 50% of the observed genetic diversity in the RP was equal to 13 animals. Conversely, when considering the male population, the number of ancestors explaining 50% of the observed genetic diversity was 11.
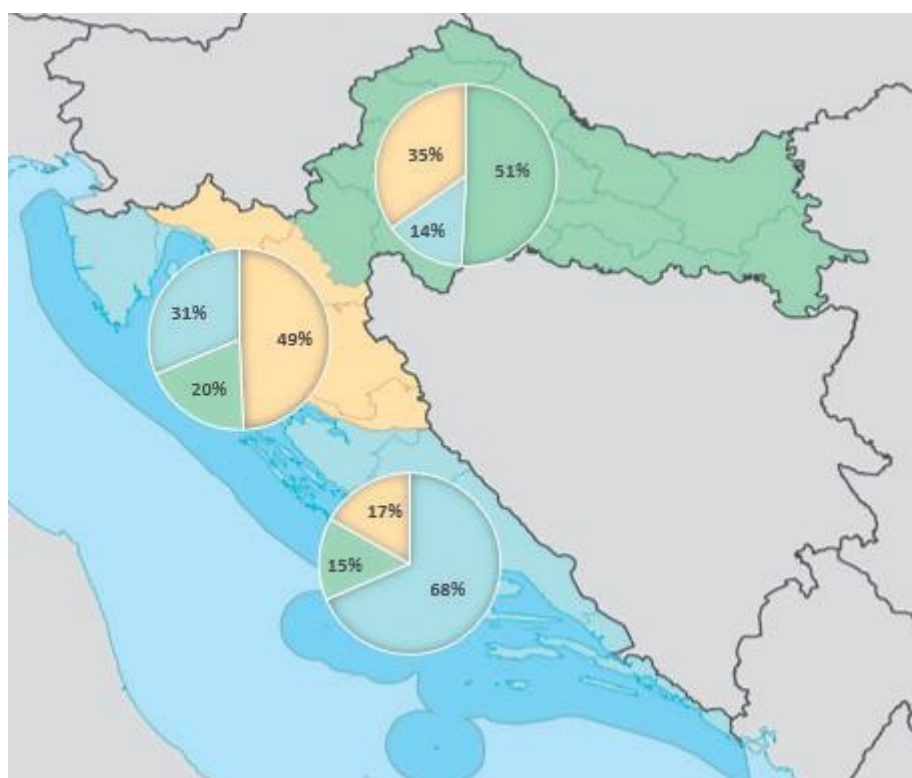

Fig 2. Demographic distribution and proportions of Busha cattle sires and their offspring in Croatia's lowland Pannonian, mountainous Dinaric, and coastal Adriatic areas are highlighted

The Busha males registered in different regions are denoted by green for lowland Pannonian (14.30%), yellow for mountainous Dinaric (47.40%), and blue for coastal Adriatic areas (38.30%). The pie chart depicts the proportions of offspring originating from the same area as the sire, and those whose sires came from other regions, showcasing breeding sire migration.

Table 2. Proportion of registered male and female descendants and the total number of descendants of the 16 most influential sires of Busha cattle population from 1995 to 2022 (complete year)

Sire line (SL)	Number of sire	Number of descendants	Male	Female
	N^1	N^2	%	%
SL4	40	1487	40.29	59.71
SL9	28	1164	46.22	53.78
SL5	32	1104	46.38	53.62
SL3	40	1094	38.40	61.60
SL8	23	915	35.96	64.04
SL1	12	883	35.33	64.67
SL11	23	673	45.47	54.53
SL12	22	607	43.00	57.00
SL6	16	526	36.12	63.88
SL17	9	509	44.20	55.80
SL14	17	484	38.64	61.36
SL2	2	220	33.64	66.36
SL7	6	220	23.64	76.36
SL10	3	135	34.81	65.19
SL13	1	15	40.00	60.00

N^1 – number of sire per sire line; N^2 – total number of descendants per sire line

Table 3. Genetic parameters for the Busha cattle breed in the total, and the reference and male populations

Genealogical parameter	TP	RP	MP
Population size	10,411	6,064	3,962
Number of founders (f)	396	222	268
Number of ancestors	200	214	259
Equivalent complete generations (CGE)	2.92	3.05	2.71
Average inbreeding (F) %	2.44	2.45	1.59
Average relatedness (AR) %	3.56	3.61	3.86
Effective number of founders (f_e)	38	40	36
Effective number of ancestors (f_a)	33	35	32
Founder genome equivalents (f_g)	28.11	26.94	20.90
f_e/f	0.09	0.18	0.13
f_e/f_a	1.15	1.14	1.12
f_g/f_e	0.74	0.67	0.58
Individual increase in inbreeding (ΔF_i) %	0.88	0.84	0.58
Effective population size (N_e)	74.96[a]	60.36[b]	58.62[b]

TP - total population; RP - reference population; MP - male population; N_e[a] - calculated over equivalent complete generations (CGE); N_e[b]- calculated by observing the individual increase in inbreeding

When considering sires as breeding animals within the TP in the studied period, 26 sires out of 278 were inbred (9.35%). The coefficient of inbreeding (F) and the rate of inbred animals (RIA), calculated per birth year cohort, steadily increased within the breeding sires of the total population, reaching an F value of 2.07% and an RIA equal to 12.62% in the last birth cohort (Table 4).

Table 4. Average inbreeding coefficient, rate of inbred sires and highest inbreeding coefficient calculated for each generation for the total population and the breeding sires within the total population

Birth year cohort	Total population (TP)			Breeding sire within TP		
	Inbreeding[a] %	RIA[b] %	Highest F[c]	Inbreeding[a] %	RIA[b] %	Highest F[c]
2005-2010	1.26	5.80	25.00	0.00	0.00	0.00
2011-2016	2.81	17.95	37.50	1.01	0.31	25.00
2017-2022	2.51	13.10	42.36	2.07	12.62	25.00

TP: total population; Inbreeding[a]: mean inbreeding coefficient calculated per generation. RIA[b]: rate of inbred sires within a generation as a percentage; if a sire showed an inbreeding coefficient higher than 0.05, it was considered as inbred. Highest F[c]: the highest individual inbreeding coefficient found within a generation.
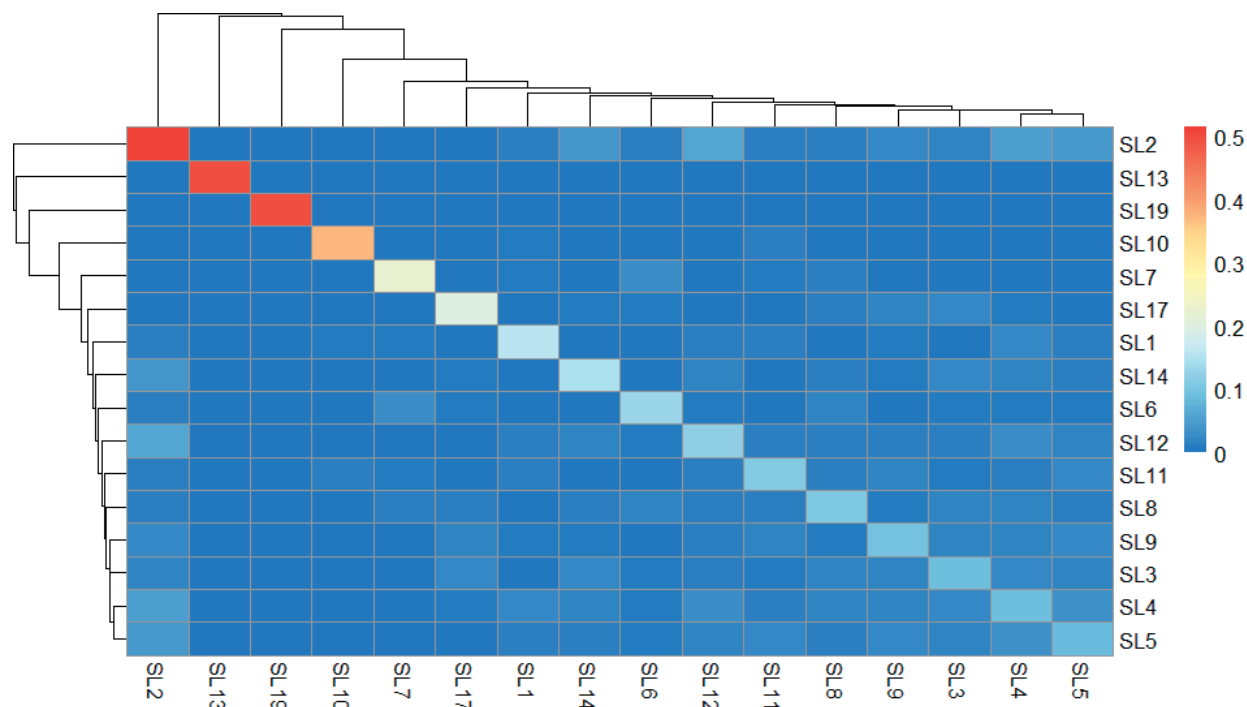

Fig. 3. Heat map of average pairwise co-ancestry (f_{ij}) within and between genealogical sire lines (SL)
The distribution of coefficients of co-ancestry is shown by the color histogram, and the stronger red color indicates that individuals from the genealogical sire lines are more related to each other
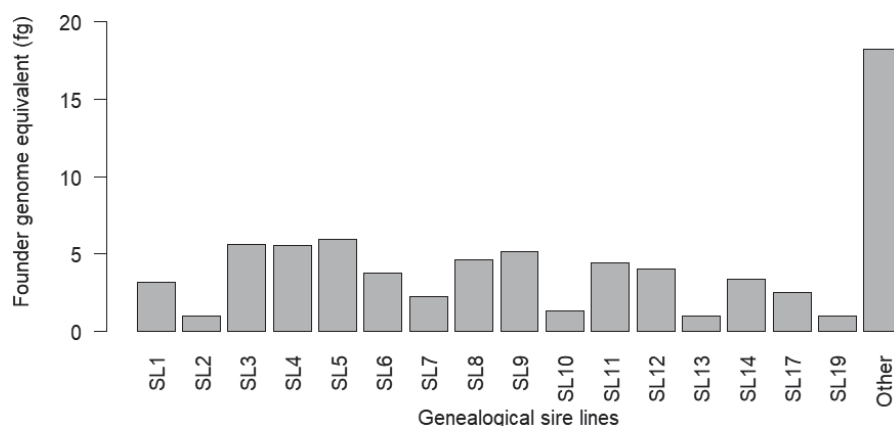
Fig 4. Founder genome equivalents (f_g) by sire genealogical lines

Genetic variability was analyzed within and between each subpopulation of the described 24 genealogical sire lines, defined as subpopulations. The highest number of sires was in SL3 and SL4 (N=40), and a sire from SL2 had the highest F_i (37.5%). The average pairwise co-ancestry coefficients (f_{ij}) between genealogical lines are presented in Fig. 3. The average pairwise co-ancestry coefficient was calculated for the 16 most influential sire lines, including all N_i x N_j pairs of a given breeding population. The highest pair co-ancestry was between SL2 and SL12 (f_{ij}=0.059%) and SL2 and SL4 (f_{ij}=0.047%). Thus, the lowest pair co-ancestry was computed between SL14 and SL7 (f_{ij}= 0.001%). We also computed founder genome equivalents (f_g) for the breeding sires (Fig. 4). The SL with less than three offspring were included in the 'Other lines' joint group. The highest f_g value was found in SL5, represented by 32 sires, reaching a value of 5.92 when excluding the 'Other' category.

Discussion

Our study revealed the population structure and genetic variability of male individuals and breeding sires within the Busha cattle breed in Croatia. The trend of an increase in the number of registered animals in the Busha cattle population (Fig. 1) indicates the heightened efforts in preservation and the growing awareness of the breed's significance. This progress can be attributed to national policy strategies, conservation programs, and herdbook establishment. However, selection intensity varied between male and female Busha cattle individuals: over 36 years, 27.68% females, but only 2.67% of males were used for breeding. This practice resulted in a ratio of sires to cows of approximately 0.096 sires for every female cow used for breeding in the population of Busha cattle. One of the probable reasons for this is the selective breeding practice that favors a small number of males for reproduction. Similar findings regarding the number of breeding males confirm the widespread use of a small number of natural service sires, with artificial insemination not being applied in most local breed populations (CAÑAS-ÁLVAREZ et al., 2014; FABBRI et al., 2019).

The distribution of registered sires in the Busha cattle population varied across geographical areas. The highest percentage of animals were bred in the mountainous Dinaric area, and breeding sires were predominantly used for reproduction within their respective areas. The results indicate a certain degree of migration between the areas, as found in

our study (Fig. 2), and these patterns suggest that certain regions may have a more significant role in breeding Busha male individuals, potentially influenced by environmental factors, historical practices, or economic considerations (SENCZUK et al., 2020).

The findings from our study indicate the absence or decline of specific SLs. The oldest sire is from SL3, and was born and registered in 2007, while the youngest breeding sire belongs to SL14 (born in 2021). The decreasing number of breeding sires has an influence on breeding efficacy and is a potential challenge in maintaining genetic diversity within the population, as described by DOUBLET et al. (2020). The complete disappearance or small number of descendants from a specific SL represents a reduced reservoir or gene pool, and this loss could reduce the total genetic variability, which cannot be revitalized (JACQUES et al., 2023). Thus, further research should align findings on the breeds' sire and dam lines with the current status of the animals in the national breeding program.

Culling data indicated that most male individuals are sent for slaughter, usually within a specific age range (from 2 to 8 months old). Comprehending the reasons for culling is vital for improving breeding purposes and management approaches (OUÉDRAOGO et al., 2021), and our study highlights the predominant practices in utilizing male cattle for meat production and their possible economic importance.

The average values of the GI within the sire pathways indicate varied patterns, with some sires being rapidly replaced, while others were used for reproduction over an extended period. As is well known, longevity is a distinctive characteristic of autochthonous breeds; most cattle breeds can continue reproducing until they reach older ages (CAROLINO et al., 2020). The overall GI within the Busha cattle population was observed to be 5.80±3.62 years. Notably, sires were found to be used for a shorter duration in reproduction compared to females, with father-son relationships averaging at 5.99±3.37 years and father-daughter relationships 6.28±3.44 years. The use of breeding sires for a longer period, whilst avoiding inbreeding, can result in the improvement of conservation strategies

and breeding programs. The longer GI observed in cows compared to sires exceeds the findings reported by CAÑAS-ÁLVAREZ et al. (2014) for seven Spanish local beef cattle breeds, where their average GI ranged from 3.75 in Sayaguesa to 7.83 in Morucha. According to IVANKOVIĆ et al. (2022), the average GI recorded for Istrian cattle was 5.93±3.65 years, varying from 4.77±3.07 (sire-son) to 6.69±3.84 (dam-son). These GI values were higher than for commercial breeds such as Angus and Nellore (FALLEIRO et al., 2014), as well as the majority of studies conducted on cattle populations in different geographic locations, including the Maremana breed (FIORETTI et al., 2020) or Limousin and Charolais cattle in Italy (DE REZENDE et al., 2020).

The accuracy of estimating relatedness within populations depends on the completeness of pedigrees; the significant absence of parents in the pedigree leads to substantial underestimation of inbreeding. As expected, in recent years animals have deeper and higher-quality pedigree data. In the Busha cattle population, pedigree completeness reached 96% in the latest generation, with a CGE value equal to 2.92, similar to that observed in the Istrian cattle population (CGE=2.99) (IVANKOVIĆ et al., 2022). This indicates a more comprehensive and higher-quality pedigree in recent years, likely due to breeding systematization, considering the previously determined values with CGE of 1.12 as described in ČAČIĆ et al. (2012b).

Controlling the inbreeding rate is one of the primary goals of conservation programs (CERVANTES et al., 2016). The average inbreeding for the sires born in the 2011-2016 birth cohort was 1.01%. As the number of animals increased in the following years, the average inbreeding coefficient amounted to 2.07%. In the study by JARNECKA et al. (2021) for Polish red cattle, the average inbreeding values were higher (F=4%), and the average inbreeding coefficient was higher for males (6%) and lower for females (2%). IVANKOVIĆ et al. (2022) reported that the F in the population of Istrian cattle, was 3.14%, ranging from a minimum of 1.41% to a maximum of 3.19% over 16 years. According to these authors' findings, Istrian cattle's AR was 4.00%, ranging from 3.31%

to 4.27%. Nevertheless, the inbreeding coefficient in our study was higher than those in commercial beef breeds, such as Charolais in Italy (F=0.96%) (FABBRI et al., 2019) and Swedish, Irish, French, and Danish Charolais (BOUQUET et al., 2011). Autochthonous cattle breeds usually have higher inbreeding coefficients because they have a smaller population size and a more limited gene pool than commercial breeds.

According to the FAO (2015), the inbreeding rate per generation should be below 1.00 % to avoid substantial loss of genetic material over time. This can be prevented by selection, especially when the population size is small. In our study, for the total population of the Busha cattle breed, a ∆F greater than 1 % was found, and the AR ranged between 0.01 and 8.68%, indicating that some individuals share a certain percentage of alleles in the population. Using the same sires in reproduction in the long term can contribute to a higher average relationship and rates of inbreeding between breeding animals, potentially exacerbating a reduction in genetic diversity over time (FORNERIS et al., 2021). Furthermore, the individual increase in inbreeding (∆Fi) was smaller between the sires (0.58%) than in the TP and RP. The AR values observed in the TP, RP, and MP in our study were lower than in the Italian local beef breeds population, which ranged from 3.46 to 7.25% (FABBRI et al., 2019).

Another measure commonly used to assess the genetic variability within the population of a breed is the effective population size (N_e) (LEROY et al., 2013). The Busha cattle breed in our study had higher values than the proposed threshold (N_e=50) (MEUWISSEN, 1999), especially when observing the 'realized' N_e based on CGE. The population size, considered large enough, with an N_e of 74.24, indicates the notable presence of genetic variability within the TP. In several regional populations of diverse strains within the Busha cattle breed, it has been noted that these populations are classified as endangered to highly endangered (RAMLJAK et al., 2018). In a more recent study, MASTRANGELO et al. (2018) analyzed genomic data for some Italian local and cosmopolitan breeds, reporting N_e estimates ranging from 8.7 for Mucca Pisana to 62.2 for Sarda and 1021.3 for Sardo

Bruna, extending to 67.8 for Charolais and 468.9 for Limousine. Despite the fact that the values of N_e in our study seem to highlight variability in the inbreeding coefficient, estimating the effective population size using genomic tools would offer a more comprehensive understanding of the level of genetic variability.

Measures based on identity by descent and the probability of gene origin can provide meaningful results about a population's past and future genetic events. The relationships between N_e, f_e, and f_a provide information about the occurrence of bottlenecks in a population. A f_e/f_a ratio close to 1 indicates a high balance between the founders' contributions and, consequently, an absence of the bottleneck effect (BOICHARD et al., 1997), and a high f_e/f_a ratio indicates the disproportionate use of breeding animals (STEPHENS and SPLAN, 2013). Here, the f_e/f_a ratios for the TP, RP, and MP were equal to 1.15, 1.14, and 1.12, indicating no obvious bottleneck in the current population. Similar results were found in the study by IVANKOVIĆ et al. (2022) for Istrian cattle, who reported the f_e/f_a ratio to be 1.03. Hence, it may be assumed that this is a consequence of the relative increase in the number of animals over the years and the current population size. The f_g/f_e ratio indicates genetic drift resulting from the random selection of alleles in a limited population. In the Busha cattle population, the f_g/f_e ratio for the TP, RP, and MP was lower than f_e/f_a, demonstrating the potential influence of genetic drift on the loss of genetic variability. In instances where genetic drift has been stabilized, it is expected that f_e would be near $N_e/2$, a pattern supported by the findings in our study (CABALLERO and TORO, 2000; GIONTELLA et al., 2018). Among the 16 most influential genealogical SLs (Fig. 3), a lower paired average co-ancestry (f_{ij}) was observed between certain lines. In contrast, higher f_{ij} values were found within the sires from the same genealogical lines. This pattern suggests deliberate mating strategies and the selection of sires on the basis of their relationships with individuals from other SLs, and their relatedness with the breeding cows. Additionally, it indicates the existence of genetic diversity among the breeding sires in the population. A slightly uneven founder contribution

(f_g), as observed in our study (Fig. 4), results from certain founders being represented in only a small number. Amore equitable representation of founders would increase genetic variation. This observation could be due to variations in reproductive success, possibly influenced by differing management practices.

Conclusions

Our study revealed the past and current trends in the population structure and genetic variability of the male individuals in the Busha cattle population. The quality of the pedigree improved with an increase in population size. Inbreeding observed among breeding sires was somewhat higher than the recommended level, but no apparent population bottleneck was found. Disproportionate use of breeding sires and their contribution to the gene pool was recorded, emphasizing the importance of looking at the dynamics of offspring from sire lines for future breeding. To prevent a further reduction in genetic variability and the number of individuals contributing, using breeding sires for planned mating from the wider, metapopulation of the Busha cattle breed would aid in preserving the population size and reinforce the existing conservation strategies.

Declaration of competing interest

All authors declare that they have no conflicts of interest.

Acknowledgements

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SAŽETAK

Buša je prepoznata kao najbrojnija autohtona pasmina goveda u Hrvatskoj. U malim ugroženim populacijama učestalo korištenje istih bikova u rasplodu može utjecati na povećanje uzgoja u srodstvu, što s vremenom može rezultirati manjim brojem visokokvalitetnih rasplodnih bikova koji nisu u srodstvu. Za istraživanje strukture populacije i genetske varijabilnosti muških jedinki korišteni su genealoški zapisi pasmine goveda buša registriranih od 1987. do 2022. godine. Skup podataka uključivao je 10 411 životinja, od kojih su 3962 bila mužjaci. Prosječan ekvivalent potpunih generacija iznosio je 2,92 u ukupnoj populaciji i 3,05 u populaciji trenutačno živućih jedinki. Prosječan koeficijent uzgoja u srodstvu iznosio je 1,59 % u populaciji rasplodnih bikova. Tijekom promatranog razdoblja koeficijent uzgoja u srodstvu porastao je na prosječnu vrijednost od 2,07 % u kohorti posljednje godine rođenja (2017. - 2022.). Nejednak doprinos efektivnog broja osnivača upućuje na učestalije korištenje pojedinih uzgojnih linija bikova. Unatoč kontroliranoj stopi uzgoja u srodstvu uočenoj među rasplodnim bikovima i nepostojanju evidentnog uskoga grla u populaciji, provedeno istraživanje istaknulo je potrebu za uzgojnim strategijama koje bi optimizirale doprinos rasplodnih životinja u sljedećim generacijama kako bi se osiguralo dugoročno očuvanje pasmine goveda buša u Hrvatskoj.

Ključne riječi: genetska raznolikost; govedo; buša; bikovi; analiza rodovnika