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# Population structure, genetic diversity, and reproductive efficiency in the autochthonous Busha cattle breed

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**Abstract:** Autochthonous cattle breeds represent important resources of genetic diversity. The Busha cattle breed is reared in the Balkan Peninsula and is characterised by high adaptability, resilience, longevity, small body size, and low maintenance and production requirements. During the 36-year observation period, the total population of the autochthonous Busha cattle breed in the Republic of Croatia amounted to 10 411 animals. Pedigree completeness for the total population, considering the first parental generation, was 97.1%. The average inbreeding coefficient was 2.44%, ranging from zero to 42.6%, while the average relatedness coefficient was 3.56% in the total population. The effective population size, calculated in the reference population based on the individual increase in inbreeding, was 63.3. The probabilities of gene origin in the reference population were $f_e$ – 38.0, $f_a$ – 33.0, and $f_g$ – 25.4, indicating a loss of genetic variability due to genetic drift ($f_g/f_e$ – 0.66) and a bottleneck ($f_e/f_a$ – 1.15). In the population of the autochthonous Busha cattle breed, the effective number of ancestors ($f_a$) was smaller than the effective number of founders ($f_e$). Increased relatedness among animals was observed, which could affect the long-term conservation of the population.

**Keywords:** conservation; bovine; fertility traits; genetic variation; inbreeding; pedigree analysis

Domestic cattle have long contributed to the development of human civilisation. Besides being a valuable source of meat, milk, leather, manure, and other products, they contribute to the preservation of habitats for many plant and animal species and thus they have a significant impact on the global environment and biodiversity (Leroy et al. 2018). Cattle have followed the human migrations, playing a key role in their adaptation to different biogeographical regions and sociocultural environments, resulting in numerous local populations, i.e. breeds (Felius et al. 2014). Local, autochthonous cattle breeds have unique genetic characteristics, representing valuable animal genetic resources (Papachristou et al. 2020). Busha is one of the three recognised Croatian autochthonous cattle breeds, with origins dating back around 6 500 years, when domesticated cattle from the Middle East were introduced to wild populations

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in Southeastern Europe (Hristov et al. 2018). Beyond Croatia, Busha is present throughout the Balkans, including Bulgaria, North Macedonia, Albania, Serbia, Bosnia and Herzegovina, Montenegro, and Kosovo, forming a single metapopulation (Ramljak et al. 2018). Adapted to harsh climates, roughage-based diets, and extensive farming systems, Busha has evolved into a small-framed, hardy, and long-lived breed with low maintenance and production requirements (Bunevski et al. 2016). Once dominant in Croatian cattle production, the breed nearly disappeared due to crossbreeding with more productive types, leaving only about 300 purebred animals. Despite closed breeding, introgression from other breeds remains evident (Ramljak et al. 2018). To prevent further decline, a closed-type herdbook was established in 2003, followed by a government-supported conservation program launched in 2004.

The importance of preserving genetic variability in autochthonous cattle breeds is reflected most of all in their adaptation, which is particularly important for facing challenges such as climate change or potential disease outbreaks (Curone et al. 2018; Hunter et al. 2018). Despite the effort, genetic erosion of the autochthonous cattle breeds has accelerated due to changes affecting the improvement of production systems and from the massive introgression of highly productive breeds, compromising their long-term viability (Shaw et al. 2025). Changes in population structure over a given period influence the occurrence of inbreeding, whose trend is the most commonly used factor for estimating the rate of genetic drift (Star and Spencer 2013). In small populations, it is inevitable that a small number of breeding animals, without an effective breeding strategy, will lead to an increase in inbreeding, which can ultimately result in potential inbreeding depression, and hence markedly reduce performance, particularly for fertility traits (Leroy et al. 2018). Moreover, inbreeding becomes a significant concern in small populations, especially when population growth occurs from a limited number of breeding animals that may already share some degree of relatedness (Howard et al. 2017).

The analysis of pedigree data is essential for understanding the historical kinship structure within the breed, while also serving as an indispensable reference point for achieving future breeding goals (Ablondi et al. 2022). This study aimed to assess the population structure, genetic diversity, and reproductive efficiency of the autochthonous Busha cattle breed in Croatia, based on 36-years of pedigree records, to evaluate the effectiveness of conservation efforts and to provide recommendations for the sustainable management of this endangered genetic resource.

## MATERIAL AND METHODS

***Data collection.*** The study was conducted on the population of the autochthonous Busha cattle breed in the Republic of Croatia. For the analysis of population structure, reproductive efficiency, inbreeding and genetic variability, identification data were combined from the database of the Directorate for Livestock and Food Quality and the Veterinary Information System (VetIS) of the Directorate for Veterinary and Food Safety of the Ministry of Agriculture, Forestry and Fisheries of the Republic of Croatia. The quality control (QC) on the comprehensive database was performed on the identification numbers of individual animals, fathers and mothers, date of birth, date of culling, sex, and area of origin, and all incorrect and illogical data were removed. For the analysis, several populations were considered: (*i*) the total population (all registered animals) and (*ii*) the reference population [animals born within a one generational interval (approximately 6 years)]. The farms or breeding facilities where the animals were reared are located across different geographical areas of the Republic of Croatia, namely the lowland Pannonian [200 m above sea level (a.s.l.)], mountainous Dinaric (500–1 500 m a.s.l.), and coastal Adriatic regions (200–500 m a.s.l.). The animals were mainly bred in an extensive breeding system, while animals from certain farms spent the winter period in barns. During the breeding season, mating is primarily organised naturally, with bulls kept with the cows on pastures, and calves remaining with the cows after calving.

***Population structure.*** The population structure was assessed based on the total and reference populations. The number of females and males in each population, as well as the number of bulls and cows used for reproduction in the given populations was determined. The number of animals without offspring in the observed periods for the given populations was also calculated. The selection pressure of breeding bulls and cows, described as groups of animals born within approximately equal generation intervals, was calculated to assess reproductive efficiency. The age distribution of bulls and cows with respect to the number of offspring was estimated to gain insight into how long they remain reproductively active in breeding. In addition, the average number of offspring per cow and bull was also calculated.

***Genetic diversity.*** The quality of the pedigree data was assessed using the pedigree completeness index (PCI, %) (MacCluer et al. 1983) and equivalent complete generations (Maignel et al. 1996). Additionally, for each animal belonging to the given populations in this study, the number of traced complete generations and the number of traced maximum generations were calculated. The generation interval was calculated using a method based on the average age of parents at the birth of their offspring, which are later used in breeding, as defined by James (1977). The specified generation interval was calculated for the familiar relationships of father to son, father to daughter, mother to son, and mother to daughter.

For the given populations in this study, the number of founders ($f$) and ancestors was calculated. The effective number of founders ($f_e$) and the effective number of ancestors ($f_a$) were estimated according to Lacy (1989) and Boichard et al. (1997) as the minimum number of founders or ancestors required to explain the observed genetic diversity. The founder genome equivalent ($f_g$) was calculated according to Ballou and Lacy (1995). To investigate the occurrence and effects of bottlenecks in the populations, the ratio of $f_e$ to $f$ was computed. The ratio of the effective number of founders to the effective number of ancestors ($f_e/f_a$) was calculated to evaluate the founder/ancestor contribution. The ratio between $f_g$ and $f_e$ was calculated to assess the occurrence of genetic drift in the given population (Boichard et al. 1997). Additionally, the number of founders and ancestors explaining 50% of the genetic variability in the population was determined. For each individual, the genetic conservation index (GCI) was calculated using complete pedigree records back to the identified founders.

The inbreeding coefficient ($F$) is defined as the probability of an animal being homozygous for a locus by descent, and it was computed according to Meuwissen and Luo (1992). In this study, the percentage of animals with respect to their individual $F$ was calculated for the total and reference population: $F = 0.00$; $0.01 < F < 6.25$; $6.26 < F < 12.50$; $12.51 < F < 18.75$; $18.76 < F < 25.00$; and $F > 25.01$. The average relationship ($AR$), which is the mean relationship of each individual with the remaining population, was calculated. The effective population size ($N_e$) was estimated in the total population using the following methods: (*a*) by applying the regression coefficient of the individual inbreeding coefficient over: (*i*) the number of complete generations, and (*ii*) the number of equivalent complete generations; (*b*) as the individual increases in inbreeding. In the reference population, the effective population size was calculated using: (*i*) the individual increase in the inbreeding coefficient; (*ii*) the regression coefficient of the individual inbreeding coefficient over the number of equivalent complete generations; (*iii*) the logarithmic regression over the number of equivalent complete generations; (*iv*) the variance of family size. All the following analyses related to population structure and genetic variability were computed using ENDOG v4.8 (Gutierrez and Goyache 2005).

## RESULTS

***Population structure.*** Based on records from the consolidated database, the population structure was assessed for the total and reference populations, as shown in Table 1. The first recorded ancestor dates back to 1987, while the youngest offspring included in the study was born in December 2022. Over the 36-year period, the total population of the autochthonous Busha cattle breed in the Republic of Croatia was recorded at 10 411 animals: 3 962 (38.0%) were males, and 6 449 (62.0%) were females. The reference population consisted of animals born within one generational interval, totalling 8 146 animals, of which 3 236 (38.7%) were males and 4 909 (61.3%) were females.

Furthermore, as shown in Table 1, the number of animals without offspring in the total and reference populations surpassed the number with offspring. The average number of offspring per sire in the total population was 36.0, and in the reference population it was 19.5.

The proportion of dams relative to the total number of registered female animals ($n$ = 6 449) was 44.7%, with only 2.41% being lineage founders. The first registered female animals were designated as the founders of pedigree lines. The average number of offspring per dam in the total population was

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Table 1. Demographic structure of the Busha cattle breed in the Republic of Croatia for the total and reference populations

| Parameters | Population | |
|---|---|---|
| | total | reference |
| Number of animals | 10 411 | 8 146 |
| Number of males | 3 962 | 3 236 |
| Number of females | 6 449 | 4 909 |
| Number of sires | 278 | 157 |
| Number of dams | 2 882 | 1 568 |
| Number of animals with offspring | 3 160 | 1 725 |
| Number of animals without offspring | 7 251 | 6 408 |

3.53, ranging from a minimum of one to a maximum of 15.0, whereas in the reference population the average decreased to 2.32. Furthermore, 56.6% of dams had more than three offspring, while 18.1% had only one offspring. It was also noted that some breeding cows remained in reproduction for extended periods, with up to 15 calvings during their reproductive lifespan. In the case of breeding bulls, the maximum number of offspring recorded for a single sire was 300.

The selection pressure of sires and dams in the autochthonous Busha cattle breed, calculated in five-year intervals, is presented in Table 2. In this study, selection pressure was calculated as the proportion of male and female offspring used for breeding, assuming a sex ratio of 1 : 1. Analysing the first three periods, the selection pressure was lower in dams compared to the sires, with an average of 74.0% of dams later producing offspring. This result indicates a moderate selection pressure in dams, meaning that nearly 80.0% of all calved and registered animals in each period were selected for breeding. The average age of sires in the total population at the time of offspring birth was 5.80 years, while that of dams was 5.98 years.

***Genetic variability.*** The informativeness and completeness of the pedigree are depicted in Figure 1. Considering the first parental generation in the total population, the pedigree completeness index (PCI) was 97.1%. Higher PCI was observed in the reference population, with recorded values of 99.8% in the first parental generation, 97.4% in the second, and 76.0% in the third parental generation.

Table 2. Reproductive indicators and selection pressure in sires and dams of the autochthonous Busha cattle breed in the total population, based on the number of registered animals from 1987 to 2022

| Birth year | Total sires | Sires with offspring | Average offspring per sire$^a$ | Selection pressure$^b$ | Total dams | Dams with offspring | Average offspring per dam$^c$ | Selection pressure$^d$ | Total number of offspring |
|---|---|---|---|---|---|---|---|---|---|
| A | B | C | D | E | F | G | H | I | J |
| 1987–1992 | – | – | – | – | – | – | – | – | – |
| 1993–1998 | – | – | – | – | – | – | – | – | – |
| 1999–2004 | 5.00 | 3.00 | 2.00 | 0.6 | 8.00 | 4.00 | 1.25 | 0.80 | 10 |
| 2005–2010 | 27.0 | 27.0 | 18.2 | 0.11 | 216 | 176 | 2.27 | 0.71 | 491 |
| 2011–2016 | 87.0 | 83.0 | 22.4 | 0.08 | 861 | 684 | 2.26 | 0.70 | 1 951 |
| 2017–2022 | 229 | 122 | 33.1 | 0.03 | 2 654 | 1007 | 2.85 | 0.26 | 7 577 |

$^a$Estimated – J/B; $^b$Estimated assuming a 1 : 1 sex ratio – C/(J/2); $^c$Estimated – J/F; $^d$Estimated assuming a 1 : 1 sex ratio – G/(J/2)

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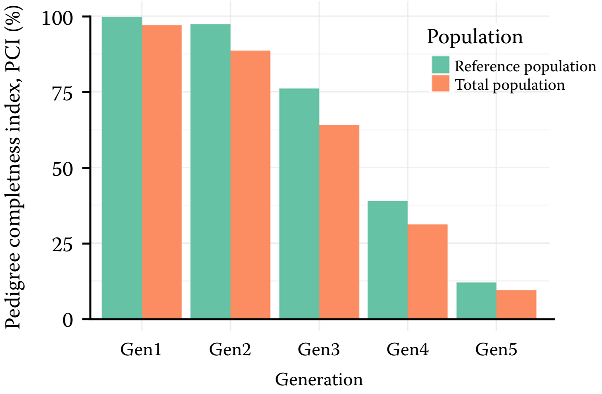

Figure 1. Pedigree completeness index (PCI), expressed as the percentage of known ancestors across five generations (Gen1–Gen5) in two populations

Table 4. Distribution and proportion of individuals according to the range of individual inbreeding coefficients in the total and reference populations

| Parameter | Population | |
|---|---|---|
| | total | reference |
| $F = 0.00$ | 6 142 (59.0%) | 4 227 (51.9%) |
| $0.01 < F < 6.25$ | 3 267 (31.4%) | 3 142 (38.6%) |
| $6.26 < F < 12.50$ | 483 (4.64%) | 370 (4.54%) |
| $12.51 < F < 18.75$ | 144 (1.38%) | 135 (1.65%) |
| $18.76 < F < 25.00$ | 245 (2.35%) | 152 (1.87%) |
| $F > 25.01$ | 130 (1.25%) | 120 (1.47%) |
| Number of inbred animals | 4 269 | 3 919 |
| Inbred animals (%) | 41.0% | 48.1% |

Main genealogical parameters for the Busha cattle breed in the total and reference populations are presented in Table 3. In the total population, the mean value of equivalent complete generations was 2.92, while in the reference population it was 3.27. Considering the total population, the actual number of founders was 396 and the $f_e$ value was equal to 40.0. The number of $f_e$ (38.0) and $f_a$ (33.1) was similar for the reference population compared to the total population, as shown in Table 3. The $F$ in the total population was 2.44%, while the $AR$ was 3.56%. Higher values were recorded in the reference population, with $F$ at 2.54% and $AR$ at 3.74%. Table 4 presents the distribution of individuals with respect to the given populations in this study and according to the defined ranges of individual inbreeding coefficients. The majority of the population (59.0%) had an individual inbreeding coefficient equal to zero, and a significantly smaller proportion of animals (4.98%) had an inbreeding coefficient greater than 12.5%.

Table 3. Main genealogical parameters for the Busha cattle breed in the total and reference populations

| Genealogical parameter | Population | |
|---|---|---|
| | total | reference |
| Maximum generations traced | 4.62 | 5.19 |
| Equivalent complete generations | 2.92 | 3.27 |
| Complete generations traced | 2.07 | 2.32 |
| Number of generations known | 9 | 9 |
| Average inbreeding coefficient ($F$), % | 2.44 | 2.54 |
| Individual increase in inbreeding ($\Delta F_i$), % | 0.83 | 0.83 |
| Average relatedness coefficient ($AR$), % | 3.56 | 3.74 |
| Number of founders ($f$) | 396 | 222 |
| Effective number of founders ($f_e$) | 40.0 | 38.0 |
| Effective number of ancestors ($f_a$) | 33.4 | 33.1 |
| Number of ancestors | 200 | 217 |
| Founder genome equivalent ($f_g$) | 28.1 | 25.4 |
| $f_e/f$ ratio | 0.09 | 0.17 |
| $f_e/f_a$ ratio | 1.19 | 1.15 |
| $f_g/f_e$ ratio | 0.74 | 0.66 |
| Genetic conservation index (GCI) | 5.65 | 6.38 |
| Number of ancestors explaining 50% of variability | 12 | 12 |
| $N_e$ (via individual increase in inbreeding) | 131 | 63.3 |

$N_e$ = effective population size

In this study, the genetic conservation index (GCI) for the total population was 5.65, and in the reference population the GCI value was 6.38 (Table 3).

The effective population size ($N_e$) in the total population was calculated from individual $F$ values, based on the number of all known generations, and it was 131. Based on the number of complete generations, it was 74.7, while based on the number of equivalent complete generations, it was 75.0. The effective population size in the reference population, calculated using the individual increase in inbreeding ($F_x$), was 63.3. The effective population size in the total population, calculated using the regression coefficient of the individual inbreeding coefficient on the number of equivalent complete generations, was 48.4. In contrast, the value computed using logarithmic regression, considering the number of equivalent complete generations (“realised $N_e$”), was 44.4. Additionally, the effective population size, calculated from family-size variance, was 517.

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## DISCUSSION

Autochthonous cattle breeds are at risk of losing their genetic integrity due to admixture with high-producing breeds. Comprehensive knowledge of breed population pedigrees enables sustainable management of the breed genetic variability and preservation of their genetic uniqueness (Wellmann and Bennewitz 2019).

Thirty-six years of pedigree data have been studied to investigate demographic and genetic parameters (population size, generation interval, inbreeding coefficient, effective population size) to have a long-term overview of the effect of planned mating schemes established for the conservation of genetic variability in the population of Busha cattle breed. By creating a pedigree from combined databases, we assessed the changes that have occurred, with a particular focus on inbreeding.

The results of the pedigree completeness index were observed over nine generations, which also represented the maximum number of known ancestors. A high proportion of pedigree completeness was recorded in the last parental generation of the total population, while the completeness of the pedigree in the reference population was comprehensive. As expected, the proportion of known ancestors gradually decreased in both the total and reference populations with increasing pedigree depth, and it was higher in more recent generations than in early periods of the breed registry. This can be explained by the increase in the population size due to reproductive activity, as well as by the systematic recording of animal data in the pedigree. According to Boichard et al. (1997), pedigree completeness decreases with increasing depth. If 10% of the ancestor data in the pedigree is unknown, the estimated inbreeding value does not accurately reflect the actual value of this indicator in the population.

Given the low average number of equivalent complete generations (Table 3), the informativeness of the Busha breed pedigree remains incomplete and insufficient for the accurate assessment of inbreeding indicators, individual relatedness, and effective population size. The average number of equivalent complete generations in the total population of Busha cattle (2.92) was similar to that of Istrian cattle (2.99) (Ivankovic et al. 2022). Although slightly higher values of the average number of equivalent complete generations were recorded in the reference population (3.27), they were still lower than in Spanish autochthonous breeds such as Lidia (4.20) (Cortes et al. 2014) and Galician breeds such as Cachena, Caldela, Frieiresa, Limia and Vianesa (4.50–5.00) (Garcia-Atance et al. 2023), but higher than in Bruna del Pirineus (1.00) (Canas-Alvarez et al. 2014). The lower values for the number of equivalent complete generations recorded in this study point to the small population size of the Busha breed and to the lack of data on ancestors at the beginning of the establishment of the registry and of animal registration. The higher the average number of equivalent complete generations, the more detailed and higher-quality the pedigrees are in providing data on ancestors in past generations, which is crucial for understanding the genetic structure of the population.

In this study, a smaller number of founders was observed in the reference population (222) compared to the total population (396). This suggests a reduction in genetic variability in the reference population relative to the total population. The value of the indicator of the $f_e/f$ ratio was recorded, showing the significant difference between the total (0.09) and reference population (0.17). The observed value in the total population and in the reference population suggests that only 9.0% and 17.0% of the initial genetic variability of the founders has been retained over generations, presumably as a result of the frequent use of specific breeding animals in the population and because a significant number of founders did not have any offspring in subsequent generations despite the systematisation of breeding. Based on the results and the indication of an imbalance in founder contributions to the current population, it can be assumed that the Busha cattle breed has undergone a loss of genetic variability as a consequence of a bottleneck.

The $f_e/f_a$ ratios ranged from 1.19 in the total population to 1.15 in the reference population, indicating an unbalanced contribution of the founders to the genetic structure of the population and evidence of a pronounced loss of genetic variability. The values recorded when observing the ratio of the effective number of founders to effective ancestors in the Busha breed show similarities with other populations of native breeds, such as Avilena Negra Iberica (1.15) and Morucha (1.24) (Canas-Alvarez et al. 2014), and the Mertolenga breed (1.48) (Carolino et al. 2020). Lower values were observed in the Istrian cattle breed (1.03), indicating no apparent population bottleneck (Ivankovic et al. 2022). In contrast, Nyman et al. (2022) found a higher value for the $f_e/f_a$ ratio (ranging from 1.16 to 3.35) in the Norwegian Red cattle breed. A bottleneck occurs when the effective number of ancestors ($f_a$) is smaller than the effective number of founders ($f_e$), resulting in the $f_e/f_a$ ratio greater than 1.00. The number of ancestors explaining 50.0% of the variability in the total population was 12.0, meaning that nearly 0.12% of all registered animals were considered for half of the population genetic diversity (Table 3). The above indicates that the ancestors themselves are likely to be used frequently in breeding, increasing the risk of inbreeding and reducing genetic diversity. Furthermore, the $f_g/f_e$ ratio in the given populations suggests the occurrence of genetic drift (Table 3). High values of the founder genome equivalent indicate the preservation of genetic diversity and population stability, whereas low values may signal a risk of losing genetic variability and inbreeding due to an imbalance in the genome representation or the loss of individual founders over time (Bosse and van Loon 2022). In this study, $f_g$ in the reference population was 25.4. This value suggests that, among 38.0 founders in the reference population, if all had equal contributions and there were no allele losses, 25.4% would contribute to the current genetic variability in the population. Additionally, this study found the $f_g/f_e$ ratio of 0.74 in the total population and 0.66 in the reference population. These results indicate the occurrence of genetic drift in the Busha breed during the studied period. Lower values of the $f_g/f_e$ ratio (from 0.15 to 0.54) and the occurrence of genetic drift were recorded by Cortes et al. (2014) in the Spanish Lidia cattle breed. According to Star and Spencer (2013), genetic drift in populations with a small number of animals leads to faster changes in allele frequency, resulting in a significant reduction in genetic variability.

The average generation interval was 5.80 years in the total population, whereas it was 5.55 years in the reference population. The age at which individuals in the reference population became parents was 3.71 years. Considering the duration of the generation interval between the maternal relationship and the offspring, it was longer than that between the father and the offspring. Similar results were recorded in the study of Italian autochthonous breeds Calvana, Mucca Pisana, Pontremolese, and Sarda, as well as in the Charolais and Limousin breeds (Fabbri et al. 2019) and in six Spanish autochthonous cattle breeds, as described by Garcia-Atance et al. (2023). The longer generation interval in cows observed in most autochthonous cattle populations could be a result of breeding practices, including breeding dams at later ages, indicating late-maturity traits, as well as longer herd lifespans and retention in breeding for more than 10.0 years. Longer generation intervals could also be a result of breeder bias in favour of the reproductive use of certain animals. A shorter generation interval in the father-son relationship than in the mother-daughter relationship may indicate the earlier replacement of sires with their male offspring in reproduction, compared to the female population of animals. According to Carolino et al. (2020), longevity is considered a desirable characteristic in autochthonous cattle breeds, but it can have a negative impact on genetic progress due to the longer retention of breeding animals in the herd.

In this study, we found an $F$ value of 2.54% in the reference population. The $F$ values were higher in cattle breeds such as Mucca Pisana (7.25%), Calvana (5.10%), and Pontremolese (3.64%) (Fabbri et al. 2019), and Maremmana (4.90%) (Biscarini et al. 2020). Smaller values were recorded for the Sardo Bruna (1.23%), Sardo Modicana (1.60%), and Sarda (1.90%) cattle breeds (Fabbri et al. 2019), compared to the Busha breed. The average $F$ in the reference population of the Istrian cattle breed was 3.14%, ranging from a minimum of 1.41% in 2002 to a maximum of 3.19% in 2018 (Ivankovic et al. 2022). A large proportion of animals (59.0%) exhibited $F$ equal to zero, whereas 4.98% showed $F$ greater than 12.5% (Table 4). In contrast, Carolino et al. (2020) reported that 85.0% of animals in the Mertolenga breed had $F$ greater than zero. Although zero inbreeding coefficients are considered a desirable outcome in the study, their interpretation depends on how well the ancestors are known in the pedigree. If ancestral pedigree data are missing, this results in a low number of equivalent complete generations, and the low value of this indicator limits the ability to precisely estimate the actual inbreeding coefficient (Villanueva et al. 2021). Several studies have proposed strategies for controlling and minimising the effects of inbreeding in conservation programs, some of which include increasing genetic contributions from significant ancestors by reducing the selection of related individuals in overlapping generations and limiting the use of breeding individuals according to age boundaries (Santana et al. 2016).

The *AR* in the studied population of the autochthonous Busha cattle breed was 3.56% in the total population, while in the reference population it was slightly higher (3.74%). In the Istrian cattle breed, the higher *AR* of 4.00% was recorded, ranging from 3.31% to 4.27% (Ivankovic et al. 2022). Additionally, higher values were observed in the Reggiana breed (5.70%), while the Modenese breed showed values similar to those observed in this study (3.00%) (Schiavo et al. 2022). The preservation of genetic variability within a population is achieved when each parent contributes at least one offspring to future generations. Ideally, these offspring should also have descendants in subsequent generations, thereby maintaining the high genetic diversity, especially in cases of random mating without selection intensity (Wang et al. 2016). A smaller number of breeding animals used in reproduction, coupled with an increase in the number of offspring, significantly affects the dynamics, causing an increase in the inbreeding coefficient and a reduction in the effective population size, as described in the Red Polish cattle breed by Jarnecka et al. (2021). The $N_e$ calculated based on the individual increase in inbreeding for the Busha cattle breed was 131, significantly higher than for the Mertolenga cattle breed (41.7) (Carolino et al. 2020) and for the Istrian cattle breed (91.9) (Ivankovic et al. 2022). Fabbri et al. (2019) recorded significantly lower $N_e$ values in six Italian autochthonous cattle breeds: Calvana (19.7), Mucca Pisana (18.5), Pontremolese (14.6), Sarda (16.6), Sardo Bruna (18.9), and Sardo Modicana (39.8). Wang et al. (2016) noted that the $N_e$ value indicates changes such as genetic drift and enables the planning of strategies to maintain an acceptable value for long-term population conservation.

## CONCLUSION

The completeness and informativeness of the pedigree of the autochthonous Busha cattle breed, considering the number of equivalent complete generations, do not currently provide sufficient data for an accurate assessment of inbreeding-related breeding indicators and the effective population size. This is a consequence of the unsystematic recording of animals in the breed registry. In the studied population, an increase in the inbreeding coefficient was observed in both the total and reference populations, along with a simultaneous increase in the number of animals. Genetic variability parameters, such as effective population size and gene origin probability, indicated an imbalance in founder contributions, and suggested the loss of genetic variability due to genetic drift and bottleneck, which may impact the long-term conservation of the population.

Therefore, assessing the genetic diversity through molecular approaches, including genome and SNP analyses, would substantially enhance our understanding of molecular-level diversity and support the development of effective mating and conservation strategies.

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## Conflict of interest

The authors declare no conflict of interest.

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