Exploring genetic diversity in an Italian horse native breed to develop strategies for preservation and management

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Abstract

Genetic diversity is a key factor for both adaptation and response to selection. The loss of genetic diversity causes a decrease in individual fitness, and it has a dramatic negative effect on the population lifespan in the long term. This study aimed in exploring the genetic diversity at pedigree level of the Bardigiano horse breed, which is a native breed from Italy shaped for living in rural areas. In the 1977, the Bardigiano studbook was founded to preserve the breed and for improving its use for riding and draft purposes. Pedigree data contained 9,469 horses, of which 3,416 were alive. Demographic and genetic parameters were estimated on sub-populations to evaluate potential genetic diversity differences among breeding and non-breeding animals, and animals showing different breeding values (EBVs) for an index combining 18 conformation traits. Throughout the studied period, inbreeding steadily increased, reaching in the last birth year cohort a mean value equal to 0.10. The rate of inbreeding per generation, assuming a mean generation interval of 8.74 years, was equal to 1.64%. Moreover, significant differences on both average relatedness and inbreeding among horses with high and low EBVs were shown. Our study unravelled the state of genetic diversity in the Bardigiano breed, highlighting that breeding strategies for optimising the contribution of breeding animals in the future generations are needed to ensure long term survival of the Bardigiano horse breed.

Keywords

Genetic diversity, pedigree, Bardigiano horse breed, conservation, breeding strategies

Introduction

The Bardigiano horse breed is an Italian native population with excellent resilience, well adapted to roughage diet, harsh climate and pasture conditions, and with an old breed history. Already in the 19th Century the Bardigiano played an important role in human society, as it was used for transportation in agriculture and for meat production (Libro Genealogico Cavallo Bardigiano, 2018). In the early 20th Century, the Bardigiano breed underwent introgression, mainly with two native Italian breeds: Haflinger and Maremmano. After II World War, the number of Bardigiano horses decreased dramatically, with only 5 stallions and 150 mares surviving the War (Di Stasio, Perrotta, Blasi, & Lisa, 2008). To overcome the decrease in population size, the Bardigiano studbook was founded in 1977. The main aim of the studbook is to preserve the Bardigiano distinctive features while improving its use for riding and draft purposes. The current population counts 3,416 Bardigiano horses; since introgression with other breeds is not allowed anymore, it is classified as a closed population. Bardigiano horses have been bred in several Italian areas, mainly in the North of Italy. Few breeders are also present outside Italy, such as in Germany, Switzerland and Hungary. Up to date, the Bardigiano breed is classified as a small native population, no longer at risk of extinction following FAO legislations (Sherf, 2000). However, due to the relatively small size and the closed status of the current population, it is extremely important to implement breeding strategies that avoid the risk of permanent loss of genetic material. Genetic diversity is a key factor for both adaptation and response to selection. The loss of genetic diversity not only causes a decrease in individual fitness, but it also has a dramatic negative effect on the population life span in the long term. Inbreeding generally reduces performance, health and fertility at individual level (Hill & Mackay, 2004; Sairanen, Nivola, Katila, Virtala, & Ojala, 2009), thus, it should be monitored. Bottlenecks, limited introgression and limited number of individuals can radically affect the rate of genetic diversity. To prevent the negative effects of the loss of genetic diversity, the Food and Agricultural Organisation (FAO) set a maximum inbreeding rate of 1% per generation (Sherf, 2000). Pedigree analysis can be considered as a useful tool to study genetic diversity in a population and to detect changes that occur during breed formation, generation to generation (Bokor et al., 2013). Inbreeding coefficients and average relationships over time have been used to evaluate how genetic diversity evolved during breed formation (Hill and Mackay, 2004). Demographic and genetic parameters, based on population structure by origin and based on founders and ancestors contributions, have also been used to monitor the changes in a population over a short period of time (Boichard, Maignel, & Verrier, 1997). These principles have been applied to characterize the genetic diversity in several horse breeds, including both sport and isolated breeds (Bussiman et al., 2018; Duru, 2017; Giontella, Pieramati, Silvestrelli, & Sarti, 2018; Hamann & Distl, 2008; Hasler et al., 2011; Kjöllerström, Gama, & Oom, 2015; Onogi, Shirai, & Amano, 2017; Schurink, Arts, & Ducro, 2012; Vicente, Carolino, & Gama, 2012; Vostrá-Vydrová, Vostrý, Hofmanová, Krupa, & Zavadilová, 2016; Vostry, Vostra-Vydrova, Hofmanova, Vesela, & Majzlik, 2015). Those estimates can be in turn used to optimise breeding strategies to avoid loss of genetic diversity and enhance breeding programs. Analyses at pedigree level are effective tools to unravel the state of genetic diversity, especially when studying small and under-development populations with often limited economic resources, causing the unavailability of more advanced technologies as genomic data. Exploring genetic diversity at pedigree level does not need extra economic resources, since from already-available data it is possible to identify indicators of genetic variability. The aim of this study was to investigate the genetic diversity of the Bardigiano breed at pedigree level to: 1) assess past and current trends in the genetic structure, 2) check the up-to-date genetic variability, and 3) provide a starting point to optimise the breeding program.

Material and Methods

Data available and quality control

The data were provided by the breeding association of the Bardigiano horse breed (Libro Genealogico Cavallo Bardigiano, Associazione Regionale Allevatori dell'Emilia Romagna, Parma, Italy). The quality control (QC) of the data was performed on: inconsistency of dam and sire registration number, birth date and sex identification. After QC of the data, the herd-book contained 9,469 horses (TP) which 3,416 were currently alive: 2,575 females (75.38%) and 841 males (24.62%).

Reference populations

Horses currently alive were defined as reference population (RP), and their pedigrees were traced back to the earliest recorded ancestors. The first ancestor recorded was traced back to 1939 and the youngest horses included in the study were born in 2017. In addition to the RP, we distinguished breeding mares and stallions from the reference population to analyse the current breeding population (BP). To build the BP, two filters on age and number of offspring were applied: in the case of mares, we included only female horses younger than 20 years old with at least 1 offspring; in the case of stallions, we included only male horses younger than 20 years old with at least 5 offspring. The minimum number of offspring was designed considering the distribution of progeny in the two genders separately. Since differences in the selection strategies are expected between genetically superior and inferior animals, thus causing differences in their genetic variability, two additional reference populations considering breeding values (EBVs) were defined. The EBVs are indexes of 18 combined conformation traits, such as: height at wither, neck position, shoulder length and leg correctness (PERCHè CE NE SONO SOLO 4, CONVIENE ELENCARLI TUTTI?). Percentile classes of EBVs were calculated from the 3,416 currently alive horses and the 1st percentile class and the 4th percentile class were considered as the two reference populations for inferior (IP) and superior (SP) horses respectively (Table 1).

Population structure & breeding strategy

To characterize the Bardigiano population structure, several demographic parameters were analysed: number of registered horses, number of sires and dams per birth year and the total number of offspring per sire and dam. The geographical area of origin was known for 7,251 horses, thus the population structure by province of origin was also considered. The term geographical area of origin is referred to the province of origin, which is the Italian administrative division between municipality and region. The F-statistics, F_{st}, F_{is} and F_{it}, were used to assess if sub-populations genetic differences were present between and within areas (Caballero, 2000). The following parameters were calculated within geographical area of breeding: number of individuals born, percentage of individuals with sire born in the same area, percentage of individuals with sire born in a different one. The geographical areas of breeding by municipalities were further classified considering their altitude to assess whether the Bardigiano breeding has been more popular in mountains, hills or low lands. The classification in altitude type was made following the guidelines from the Italian National Statistical Institute (ISTAT, 2018); where mountains are defined as lands higher than 700 meters from sea-level, hills: from 300 to 700 meters from sea-level and low lands: lower than 300 meters from sea-level. To classify the altitude type within province of breeding, the municipality of origin was used to assess the altitude classification. Thus, per each province, three classes of altitude were calculated. The completeness of pedigree information was investigated by using the equivalent complete generation (CGE), which is computed as the sum of $(1/2)^n$, where n is the number of generations between individuals and each known ancestor (Maignel, Boichard, &

Verrier, 1996). The maximum number of fully traced generations was also considered for each individual. Generation intervals (GI) were calculated for the following pathways: sire to son, sire to daughter, dam to son and dam to daughter by using the average age of parents at the birth of their offspring.

Genetic variability

Both the effective number of founders (f_e) and the effective number of ancestors (f_a) were calculated as the minimum number of founders and ancestors explaining the observed genetic diversity in the reference population, respectively (Lacy, 1989). To assess if the population experienced bottlenecks, the ratio between f_e and f_a was calculated: when the ratio is close to unity, the population is stable in terms of the numbers of effectively contributing animals. In contrast, if the effective number of founders is larger than the effective number of ancestors this means that some ancestors have played a major role in population formation (Boichard et al., 1997). The founder genome equivalent (f_{ge}), which is defined as the effective number of founders with non-random loss of founder alleles describing the observed genetic variability, was calculated in the two population clusters concerning EBVs percentile class (SP and IP) and compared to the result obtained from the RP.

The individual inbreeding coefficient (F), as the probability that an individual has two identical alleles by descendant, was computed following Meuwissen and Luo, 1992. The rate of inbred Bardigiano horses within generation was calculated: if a horse showed an inbreeding coefficient higher than 0.05, then it was considered as inbred. The threshold of F ($F > 0.05$) was chosen considering the distribution of the individual inbreeding coefficients in the RP. The average relationship (AR) defines the mean relationship of each individual with the remaining of the population and it can thus be interpreted as a representation of the animal contribution in the whole pedigree. The trends of F and AR coefficients throughout the analysed generations were evaluated with the GLM procedure in SAS (SAS Institute 9.4). The rate of the increase in inbreeding (ΔF) was also calculated per generation, as well as the AR. Average F and AR were calculated per each breeding province divided by altitude type (low lands, hills and mountains). The effective population size (N_e) was estimated as follow: a) computing the regression coefficient of the individual inbreeding coefficient in the TP over: i) the number of full generations traced; ii) the maximum number of generations traced; and iii) the equivalent complete generations, and considering the corresponding regression coefficient as the increase in inbreeding between two generations, b) as individual increase in inbreeding in the population $N_e = \frac{1}{2\Delta F}$ for TP, RP and BP. The ENDOG v4.8 software was used to carry out the above mentioned analyses (Gutierrez & Goyache, 2005).

The degree of non-random mating practiced by breeders was assessed by comparing the F and the AR among sires and dams belonging to the breeding population to the results obtained from the reference population. Differences in the number of sires building the superior and inferior populations (SP and IP), the inbreeding coefficient and the average relationship coefficients between those two sub-populations were tested by two sample t-tests in SAS 9.4.

Results

Population structure & breeding strategy

From the ´70s, a gradual increase of the number of registered horses was recorded, with the highest peak in 2009 with 389 horses listed. Since 2010, a reverse bending in the annual number of registered horses has been observed, dropping from 336 in 2010 to 165 in 2016 (complete year). The average number of mares registered throughout the studied period was always significantly higher than males ($p < 0.0001$), being on average more than 3 times more abundant. The number of registered males over the 62 evaluated years ranged between 1 in 1939 to 178 in 2008. Up to 1976, with the only exception of year of birth 1960, all the male horses registered to the studbook were used for breeding. Since the foundation of the studbook in 1977, a steady decrease of the number of males used for breeding was observed, reaching to the point where only 4.24 % of the males born and registered in 2009 had offspring in the following years. The number of sires born in the last birth cohort and used for breeding was equal to 50, which is the 5.9% of the males currently present in the population (**Errore. L'origine riferimento non è stata trovata.**). The most influenced stallion produced 222 offspring; half of the registered stallions produced more than 10 offspring and 13.8% of the sires produced one offspring only. The Number of offspring per breeding mare ranged from 1 to 14, with an average of 2.60 offspring per mare. When considering the 7,251 horses with information available on the geographical area of breeding, we found that the Bardigiano has been bred in 14 areas in Italy, and in three countries outside Italy. Nevertheless, 93.5% of the horses were from four geographical areas only: Parma, Piacenza, Genova and La Spezia, with 55.7% (4,041 horses) belonging to Parma only. Breeding strategies differed between geographical areas of breeding: in the case of Parma, 64% of the stallions used for breeding were local stallions, whereas in Piacenza, Genova and La Spezia, the majority were foreign stallions. The least number of breeding stallions born and used in the area of origin was shown in La Spezia, where only 4% of the horses registered within this area were born from native stallions (**Errore. L'origine riferimento non è stata trovata.**). The F-statistics were calculated to evaluate the within-population genetic differences between Parma, Piacenza, Genova and La Spezia, the values were as follow: F_{st}= 0.0038, F_{is}=0.0941 and F_{it}= 0.0122. From the classification in classes of land-type in respect to altitude (mountains, hills and low lands), it resulted that the majority of Bardigiano horses have been bred in mountain and hilly areas: 3,117 (46%) and 3,234 (48%) horses respectively. The equivalent complete generation (CGE) investigates the completeness of the pedigree information, which resulted equal to 4.0 generations in the TP and 5.2 when considering only alive horses (RP). Percentage of ancestors known per ancestral generation differed between birth year cohorts. The maximum number of known ancestral generations ranged from 15 in the last birth year cohort (2011-2017) to 9 in the first birth year cohort after the foundation of the Bardigiano breeding association (1975-1983). Average equivalent complete generations differed between cohorts of horses born from different birth year cohort, e.g.: 2011-2017 was 6.1, from birth year cohort 1984-1992 was 2.9 and from birth year cohort 1966-1974 was 0.43. As expected, pedigree of horses born in the most recent years was deeper and had a higher quality (**Errore. L'origine riferimento non è stata trovata.**). The average generation interval (GI) calculated from all the pathways was equal to 8.74 years; the GI in the maternal lineages was higher than in the paternal lineages: mother-daughter = 8.67 years, and mother-son = 9.18 years, whereas father-daughter = 8.45 and father-son = 8.65 years.

Genetic variability

The effective number of founders (f_e) was equal to 20 and the effective number of ancestors (f_a) was equal to 15 in the TP. When considering only alive animals, both f_e and f_a decreased, resulting equal to 18 and 14, respectively. The ratio between f_e and f_a was equal to 1.30 and to 1.20 in the TP and RP, respectively. The number of ancestors explaining the 50% of the observed genetic diversity in the RP was equal to 8 animals. Conversely, when considering only animals belonging to the SP cluster, the number of ancestors explaining the 50% of the observed genetic diversity decreased to 4. Differences were found on the numbers of founder genome equivalents (f_{ge}) between the two sub-populations defined on percentile class of EBVs: in the case of SP, the number of f_{ge} was 4.6, whereas in the case of IP, f_{ge} was equal to 11.0. The average inbreeding coefficient (F) was equal to 0.06 (S.D. = 0.06) in the whole population (TP), when considering alive animals (RP) F increased to 0.08 (S.D. = 0.06), ranging from 0 to 0.35 within population. Significant differences ($p < 0.05$) in the F between the groups of superior and inferior animals were found: 0.10 ± 0.002 in the SP and 0.06 ± 0.002 in the IP group. Both the F and the rate of inbred animals (RIA) calculated per birth year cohort increased steadily in the RP, reaching in the last cohort an F of 0.10 and a RIA equal to 86.5%. Horses, showing a level of consanguinity equal or higher than 0.25, were found in all the birth year cohorts (Table 2). The rate of inbreeding per generation (ΔF), assuming a mean generation interval of 8.74, was equal to 1.64% in the RP. In contrast, when considering only breeding animals, ΔF increased to 1.93%. Average relatedness (AR) within breeding animals did not significantly differ from the AR in the reference population and it was equal in both populations to 11.0% ($p > 0.05$). In contrast, AR of stallions (13.0%) was higher, if compared to AR of mares (11.0%) ($p < 0.05$). A similar pattern was shown when comparing AR within superior and inferior animals in respect to their EBV ($AR_{SP} = 14.0\%$ and $AR_{IP} = 9.0\%$) ($p < 0.05$). Significant mean differences ($p < 0.05$) were shown for the comparison of F and AR between provinces in all cases except between La Spezia and Genova. Average F and AR, calculated for provinces of breeding nested with altitude type (low lands, hills and mountains), showed higher average inbreeding and relationship among individuals belonging to mountain and hill areas compared to low land locations in all provinces except for the case of La Spezia, however no significant differences were found between altitude types within province. Animals belonging to mountain areas in the province of Piacenza showed the highest average F and AR, being equal to 0.10 and 0.13 respectively (Table 3). The effective population size (N_e) in the TP resulted equal to 60.81, 26.41 and 18.26 when using the increase in breeding by maximum generation, equivalent generation and complete generation, respectively. The N_e calculated in respect to the ΔF was equal to 30.67 in the RP and to 26.32 in the BP. In **Errore. L'origine riferimento non è stata trovata.**, the breeding values (EBVs) of the 66 sires building the BP were plotted against their average relationship in the population. Sires with an AR lower than 13% and with an EBV higher than 50 were showed as rhombus representing the optimal in terms of potential breeding contribution. This result supports the hypothesis that difference in the selection strategies between genetically superior and inferior animals is expected, thus affecting their genetic variability. The 66.0% of the sires showing an EBV higher than average (50) displayed also an AR higher than the average value in the sampled sire population (AR>0.13).

Discussion

The goal of the present study was to investigate the genetic diversity of the Bardigiano breed based on pedigree information to propose breeding strategies for breed management and conservation.

Population structure

The number of Bardigiano horses registered to the studbook showed two separate trends: from the beginning of breed formation to 2009 a steady increase was shown. On the other hand, from 2010 to current days a reverse bending was observed. Similarly, the number of stallions used for breeding gradually decreased. The observed trend might be related to the decrease in the number of Bardigiano horses used for meat production. This reduction might be the consequence of three events. Firstly, the opening up of international markets of equine meat has increased the availability of meat from foreign countries, thus reducing local production. Secondly, due to the Eurozone crisis in the late 2009, a reduction of meat consumption has been generally observed in several European countries. This crisis might have affected horse meat consumption in Italy, thus dropping local horse meat production even further (Na, Minjun, & Wen, 2013). Finally, since the beginning of the 21st century in the equine market the demand of horses for leisure activities has increased deeply. Consequently, the breeding goal of many Bardigiano breeders has changed from meat production to horses shaped for pleasure activities, which have a higher economic value, a longer life span and thus an adverse influence in the number of horses registered per year. In the last 13 years, the number of living animals has been maintained fairly stable; from a previous study it resulted equal to 3,556 animals, which is 140 animals higher than the current population (Sabbioni et al., 2005)**.** Although the Bardigiano is no longer classified as at risk of extinction, its population size is considerably smaller than other Italian native breeds. Currently, the Bardigiano has a registered population of 3,416 individuals which is lower than the Italian Haflinger, Murgese and the Italian Heavy Draft populations: all of them have a population size larger than 5,500 animals (Associazione Italiana Allevatori, 2018). A recent study on the Maremmano horse population showed a registered alive population of 5,705 horses and a breeding population of 1,532, both of them are nearly two times larger than in the Bardigiano breed (Giontella et al., 2018). Similarly, the Bardigiano population size is smaller than international breeds, as an example, the number of Bardigiano horses is roughly half of the current Lusitano population (Vicente et al., 2012). The number of breeding animals in the Bardigiano breed and in the endangered Old Kladruber horse population is very close: 742 and 612, respectively (Vostrá-Vydrová et al., 2016).

Breeding strategy

Selection intensity differed between mares and stallions: 26.3% of the females have been used for breeding and this figure becomes 7.8% for males. The proportion of breeding stallions and mares resulted equal to 1 stallion every 10 mares. In European horse breeds for sport performances this proportion is considerable smaller if compared to what we found in the Bardigiano breed: in the Hanoverian population is equal to 20 mares each stallion and 1 stallion every 60 mares in the Dutch harness population (Hamann & Distl, 2008; Schurink et al., 2012). Artificial insemination (AI) improves stallion reproduction efficiency, increasing intensely the number of mated mares obtainable per stallion. Nowadays, AI is broadly used in the majority of European horse breeds for sport performances, whereas in the Bardigiano AI has not been implemented yet. Thus, the absence of AI in the Bardigiano might be the reason why we observed both a lower mares/stallion ratio and a limited average number of offspring per stallion. The number of sires born in the last birth cohort and used for breeding was equal to 50, which is the 5.9% of the current total of male Bardigiano horses. Once a year the pedigree database is updated regarding male horses that undergo surgery for castration via owner declaration. Thus, this portion might be slightly higher as some of the male horses considered as potential breeding animals in reality have been gelded. Breeding strategies differed among areas where Bardigiano horses are commonly bred. Similarly to what previously stated, as the use of AI has not been implemented yet in this breed, the exchange of genetic material becomes uneven and challenging between more distant areas. The higher inbreeding and higher relationship among individuals belonging to the cluster mountain area support this hypothesis. In addition, the F-statistics analyses revealed higher F_{st} and F_{is} than what found in the Maremmano breed, showing more genetic separation between areas of breeding and higher average inbreeding within area of breeding (Giontella et al., 2018). To allow for a more uniform genetic flow between areas, and to reduce the risk of genetic drift and inbreeding within local area, we suggest the application of AI in this breed. However, if AI is introduced, breeding strategies for balancing stallion's contributions are needed to reduce the risk of critical application of only the most popular stallions. In recent years, strategies to optimise the contribution of breeding animals have been implemented. Optimal contribution selection (OCS) was applied in the Norwegian and the North-Swedish cold-blooded trotter, and the importance of annual mating quota per stallion was highlighted to monitor stallion´s contribution in the population (Olsen, Meuwissen, & Klemetsdal, 2013). Hence, especially if AI is introduced in the Bardigiano breed, OCS might be an effective tool to balance stallion's contributions. Mating strategies to control future inbreeding were considered in the indigenous Swiss horse breed, where they combined the best linear unbiased prediction (BLUP) methodology with OCS theory (Hasler et al., 2011). Thus, OCS together with BLUP might be used to monitor the Bardigiano genetic diversity while allowing for response to selection for economically relevant traits. Even though in breeds potentially under endangered status the most urgent matter is to conserve their genetic diversity, their probability to survive is also highly dependent on their economic value. Thus, by improving economically relevant traits, such as conformation, gaits and attitude traits via the breeding program, the profitability of Bardigiano horses increases, hereafter reducing the risk of extinction. Therefore, the simultaneous implementation of a selection program, which optimises the selection of breeding candidates both in term of conservation and breeding values, is suggested to enhance the demand of Bardigiano horses in the equine sector. The generation interval in the Bardigiano horse population was equal to 8.74 years. In draft horse breeds the generation interval varies between 7.0 to 7.9 years for French Comtois draught horse population and for the Austrian Noriker draught horse, respectively (Druml, Baumung, & Sölkner, 2009; Verrier et al., 2010). Horse breeds for sport purposes showed generally a longer generation interval: 10.3 for the Lusitano breed, 10.1 for the Andalusian horse population and 10 years for the Hanoverian horses (Hamann & Distl, 2008; Valera, Molina, Gutiérrez, Gómez, & Goyache, 2005; Vicente et al., 2012). The Dutch harness horse population presented a generation interval akin to what we found in the Bardigiano, being equal to 8.6 years (Schurink et al., 2012). Differences in generation interval among breeds might be related to the final destination of the breed. Draught horse breeds mainly have a shorter generation interval, as this horse type is commonly used for meat production and it is rarely used in competitions. In contrast, since horses belonging to sport breeds attend competitions postponing the reproduction activity, they tend to show longer generation intervals. The generation interval in the Bardigiano population fits in between sport and draft breed-types.

Genetic variability

Pedigree quality and depth appeared sufficient to calculate genetic diversity parameters. It is known that the absolute level of inbreeding is highly dependent on the quality of the data available (Schurink et al., 2012; Sørensen, Sørensen, Baumung, Borchersen, & Berg, 2008). Hence, inbreeding rate appears to be a more appropriate parameter to assess genetic variability across time within a breed. The average inbreeding in the reference population (RP) was 33.3% higher than in the whole population, thus showing a possible increase in the inbreeding over time. This assumption is confirmed by the rate of inbreeding per generation (ΔF), which resulted equal to 1.64% in RP. The Food and Agriculture Organization (FAO) stated that the value of ΔF should not exceed 1% to avoid substantial loss of genetic material over time (Sherf, 2000); unfortunately, this limit is not respected in the Bardigiano breed. In endangered horse populations we found similar ΔFs: in the French Boulonnais draught horse population ΔF was equal to 1.38% and in the Old Kladruber horse population was equal to 1% (Verrier et al., 2010; Vostrá-Vydrová et al., 2016). Although the Bardigiano is not included in the list of endangered breeds, it currently shows a critical increase in the inbreeding in the last generations. From the comparison between clusters of EBVs, differences in the average relatedness were found between superior and inferior animals: on average, horses with high breeding values were also more related to each other. Future inbreeding might be kept under control by assuring that parents of future breeding animals are not closely related. Thus, including the average relationship as an extra parameter of selection in the breeding program might be an effective solution to avoid high relatedness among matings, thus reducing the inbreeding in the coming generations.

Conclusion

Our study unravelled the state of genetic diversity in the Bardigiano breed, highlighting that further research is needed to optimise the contribution of breeding animals in the upcoming generations. To ensure long term genetic diversity within the Bardigiano breeding population, strategies to optimise mating schemes are needed. One possible solution to monitor the future inbreeding and, in turn, the genetic diversity in the population, is to implement efficient selection strategies. Optimal Contribution Selection might be an effective tool to increase genetic gain while controlling the rate of inbreeding, thus, enabling the maintenance of genetic diversity in the Bardigiano population. Since using animals with lowest AR for breeding can minimise the inbreeding in the coming generations, by balancing the contribution of the founders in the population, we suggest accounting for the average relationship in the breeding program.

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Declaration of interest

The authors have no conflict of interest to declare.

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Tables

Table 1 Description of the data available in the entire pedigree database (TP), in the reference population (RP), in the breeding population (BP), in the superior population (SP) and in the inferior population (IP).

Parameters	TP	RP	BP	SP	IP
Number of horses	9,469	3,416	742	505	495
Number of males	2,113	841	66	119	35
Number of females	7,356	2,575	677	386	460
Number of horses with no progeny	5,928	2,252	0	236	215
Number of founder horses	1,698	648	35	14	61
Number of matings between full sibs	13	6	0	0	0
Number of matings between half sibs	259	103	16	8	16
Number of matings parent-offspring	162	51	10	2	8

TP = Total population; RP = Reference population; BP= Breeding population; SP = Superior population; IP = Inferior population

Table 2 Average inbreeding coefficient, rate of inbred animals (RIA) and highest inbreeding coefficient calculated for each generation from 1975-1983 to 2011-2017 in the RP.

Birth year cohort	Inbreeding †	RIA % ‡	Highest F §
1975-1983	0.03	20.0	0.25
1984-1992	0.04	29.2	0.25
1993-2001	0.06	56.3	0.32
2002-2010	0.08	77.3	0.34
2011-2017	0.10	86.5	0.35

† Inbreeding: mean inbreeding coefficient calculated per generation. ‡ RIA: rate of inbred Bardigiano horses within generation in percentage, if a horse showed an inbreeding coefficient higher than 0.05, it was considered as inbred. § Highest F: highest individual inbreeding coefficient found within generation.

Table 3 Population structure, inbreeding and relationship among classes of horses born in the most popular areas for the Bardigiano breeding: Genova, Piacenza, Parma and La Spezia, considering the type of land (low land, hill and mountain areas).

Location	N. †	Own Father % †	Foreign Father %†	Mean F ‡	Mean AR ‡	Highest F §	Highest AR §
GE: Low Land	103	4	96	0.03	0.06	0.25	0.15
GE: Hill	204	6	94	0.05	0.09	0.25	0.18
GE: Mountain	548	9	91	0.05	0.10	0.25	0.17
PC: Low Land	116	1	99	0.07	0.10	0.27	0.15
PC: Hill	956	24	76	0.09	0.13	0.35	0.19
PC: Mountain	598	12	88	0.10	0.13	0.21	0.18
PR: Low Land	201	11	89	0.08	0.11	0.27	0.17
PR: Hill	1899	24	76	0.08	0.10	0.31	0.18
PR: Mountain	1941	49	51	0.09	0.12	0.35	0.19
SP: Low Land	15	0	100	0.01	0.04	0.08	0.13
SP: Hill	175	5	95	0.06	0.09	0.22	0.15
SP: Mountain	30	0	100	0.01	0.03	0.01	0.07

† Population structure parameters: N. = number of foals born within location. Own Father = percentage of foals born from stallions belonging to the same area. Foreign Father = percentage of foals born from stallions belonging to different area. ‡ Mean inbreeding (F) and average relationship (AR). § Highest inbreeding (F) and average relationship (AR) within location. GE: Genova, PC: Piacenza, PR: Parma and SP: La Spezia.

Figure legends

Figure 1 Animals registered per year of birth divided by sex: female and male and male with offspring.

Figure 2 Bardigiano demographic distribution and population structure within areas of Italy. Regions of Italy where the 96.5% of the Bardigiano horses have been bred are highlighted. Pie charts represent the percentage of stallion origin used in the breeding program within area. The percentage of breeding stallion born in the same area is represented with a striped pattern, whereas the percentage of foreign breeding stallions is shown as full-fill pattern. GE: Genova, PC: Piacenza, PR: Parma and SP: La Spezia.

Figure 3 Percentage of known ancestors for horses born from 1957 to 2017 for four birth year cohorts where major differences were shown: 1957-1965. 1975-1983. 1984-1992. and 2011-2017. Ancestral generation 1 corresponds to parents, ancestral generation 2 to grandparents, et cetera.

Figure 4 Breeding values plotted against the average relationship of sires to horses in the reference population. The mean breeding value in the sampled sires was equal to 50 and the average relationship to 0.13. The rhombuses represent the 20% best sires in term of optimal contribution.