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Demographic approach on the study of genetic parameters in the dog *Braque Français* type *Pyrénées* Italian population

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ABSTRACT

The complete electronic record of the Braque Français type Pyrénées reared in Italy was downloaded from the ENCI database in order to estimate the genetic variability of the breed. Pedigree records of all active registered animals (reference population = RP) were considered. The whole population (WP = RP and its genealogy) included 1250 dogs (628 males and 622 females). Seven hundred eighty-three dogs were inbred. To explain 50% of the genetic variability, a total of seven ancestors were sufficient in the RP. The average inbreeding coefficient (F) in the RP was 4.3%, while the average inbreeding of the inbred was 5.19%. F was <0.05 in 511 dogs (65.3% of inbred), whereas it was >0.20 in only 13 dogs (1.66% of inbred). F per year ranged from 0.98% for dogs born in 2003 (27 dogs) to 7.05% in 204 dogs born in 2009. Ten traced generations were highlighted. A maximum average inbreeding value (6.62%) was observed in the dogs with 10 traced generations. This research highlighted the good genetic variability of the breed obtained through the efficient management by the Italian Club of Braque Français Type Pyrenees (CIBF), in collaboration with the member breeders and the active cooperation of the French Club. The CIBF has made arrangements with our genetic centre to constantly monitor the genetic variability of the breed in order to prevent excessive increases in inbreeding in the future.

Introduction

In selection, inbreeding is used as a mating method because it enables the characteristics and traits of the best representative of a breed to be fixed. However, this mating method can lead to high rates of inbreeding with resulting risks that particularly threaten small populations and those originating from a small number of ancestors. Several studies have analysed the impact of inbreeding depression on traits linked to reproduction and production or the occurrence of specific diseases (Ubbink et al. 1992; Van der Beek et al. 1999; Mäki et al. 2001; Urfer 2009). Significant, but limited effects of inbreeding depression were found for hip dysplasia in German shepherd dogs and Golden Retrievers (Mäki et al. 2001) and fertility in Irish wolfhounds (Urfer 2009). No effects of inbreeding depression on morphological traits were observed in the Bracco Italiano dog breed (Cecchi et al. 2015).

Several indicators have been used to assess the genetic variability in a population. As well known, it can be estimated from genealogical data (Maki et al. 2001; Leroy et al. 2006; Cecchi et al. 2009; Cecchi et al. 2013; Ciampolini et al. 2013; Ceh & Dovc 2014) or using STR molecular markers (Koskinen et al. 2000; Kim et al. 2001; Ciampolini et al. 2011; Ciampolini et al. 2013; La Manna et al. 2015).

Braque Français are hunting dogs, originating from a very old type of gun dog used for pointing the location of game birds for a hunter. There are two breeds of Braque Français, both from the south of France: the Braque Français, type Gascogne (larger size) and the Braque Français, type Pyrénées (smaller size). They are popular hunting dogs in France, but are seldom seen elsewhere. The original Braque Français type of pointing dog has existed since the fifteenth century. The first breed club was formed in 1850, and the standards for both breeds were written in 1880. Both types belong to group 7 of the ENCI classification. The Italian Club of Braque Français (CIBF) born in 2000, but the ENCI recognised the breed in 2003. The aim of this study was to analyse the genetic variability of the Braque Français, type Pyrénées breed reared in Italy

and to verify the effectiveness of the CIBF management to contain the inbreeding.

Materials and methods

The complete electronic record of the breed was downloaded from the ENCI database. Pedigree records of all living registered animals born between 2003 and 2014 (active population = Reference Population, RP) were considered. The reference population and its genealogy constitute the whole population (WP). The base of the population (BP) is defined as individuals with one or both unknown parents.

The pedigree analysis was performed using ENDOG v4.8 (Gutiérrez & Goyache, 2005). To characterise the depth of the pedigree, the following values were calculated: (a) the average complete generation, computed as the maximum of the average traced generations for each animal; (b) the maximum of the fully traced generations, computed as the value separating the offspring from the farthest generation where the two ancestors of the individual are known; (c) the number of equivalent generations computed as the sum of $(1/2)\ n$, where n is the number of generations separating the individual from each of the known ancestors.

To estimate the genetic variability the following values were calculated: (a) individual inbreeding coefficient (F) and average relatedness coefficients (AR), which correspond, respectively, to the probability that both alleles, randomly selected, are identical by descent, and the mean relationship of each individual with the rest of the animals of the breed; the inbreeding coefficients (F), the number of inbred animals and average inbreeding coefficient for each traced generation; (b) parameters characterising the concentration of gene origin, such as the effective number of founders (f_e), defined as the number of equally contributing founders (animals without known ancestors) expected to produce the same genetic diversity as in the studied population (Lacy 1989); the absolute number of founders (f_t) defined as the animals with one or both unknown parents (absolute founders) and the effective number of ancestors (f_a) (Boichard et al. 1997) defined as the number of ancestors with known pedigrees or without them (founders), which would equally contribute genes to the reference population gene pool. Therefore, f_a determines the minimum number of ancestors (not necessarily founders) required to explain the whole genetic variability of the population and to account for the loss of genetic variability caused by the unbalanced use of ancestors in reproduction, leading to genetic bottlenecks (Boichard et al. 1997); (c) the increase in inbreeding (ΔF) is calculated for each generation by means of the classical formula $\Delta F = (F_t - F_t - 1)/(1 - F_t - 1)$, where F_t and $F_t \neg 1$ are the average inbreeding at the ith generation. Using ΔF, ENDOG computes the effective population size (N_e) in the WP and in the RP as $N_e = 1/(2\Delta F)$ for each generation with $F_t > F_t - 1$ to roughly characterise the effect of remote and close inbreeding. N_e is defined as the number of breeding animals that would lead to the actual increase in inbreeding if they contributed equally to the next generation.

At the population level, ENDOG computes the generation intervals, defined as the average age of parents at the birth of their progeny kept for reproduction (James 1977) and the average age of parents at the birth of their offspring (used for reproduction or not). Both parameters are computed for the four pathways (father–son, father–daughter, mother–son and mother–daughter).

Popular sires (defined here as >40 recorded offspring) and popular dams (>30 offspring) were identified. The number of inbred and the average inbreeding coefficient per year were performed using CFC software (Sargolzaei et al. 2006). The distributions of inbreeding levels in the whole population were analysed, and eight different class levels of inbreeding were considered: $0 < F \leq 0.05$; $0.05 < F \leq 0.10$; $0.10 < F \leq 0.15$; $0.15 < F \leq 0.20$; $0.20 < F \leq 0.25$; $0.25 < F \leq 0.30$; $0.30 < F \leq 0.35$; $0.35 < F \leq 0.40$ (Sargolzaei et al. 2006).

The authors considered the RP values because they believe that this population can provide more complete data compared to WP population, since the numerical difference between them is low and part of this difference is constituted by the base population (individuals with one or both unknown parents and often with unknown birth data) and by dogs with only one traced generation.

Results and discussion

Demographical parameters

The number of dogs registered to ENCI per year is reported in Figure 1. In Italy, 921 puppies (479 males and 442 females) were registered from 2003 to 2014 (average value: 76.6 ± 40.24 dogs per year). The minimum value was observed in 2005, with 30 registered puppies, while the peak in the number was observed in 2010, with 173 puppies registered to ENCI, when many French dogs, imported to Italy in the previous years, were registered in the Italian herd book (ROI).

In Figure 2, the details of the pedigree quality in Braque Francais dog were shown. The percentage of completeness of the pedigree was computed as about
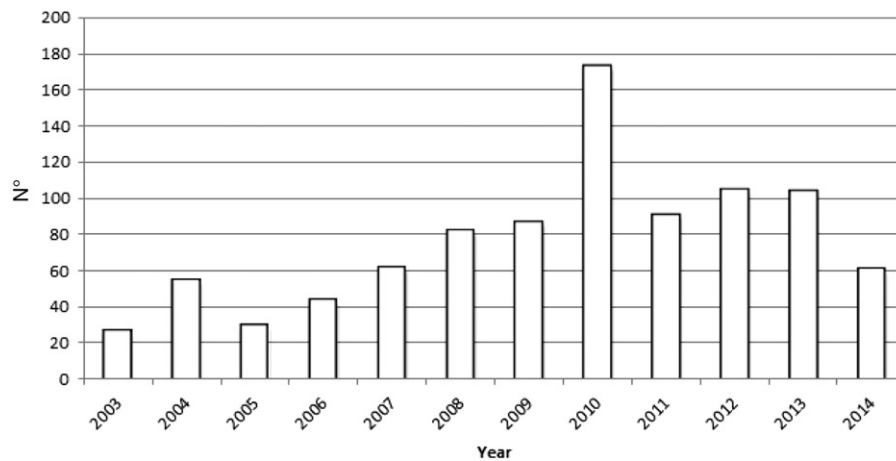
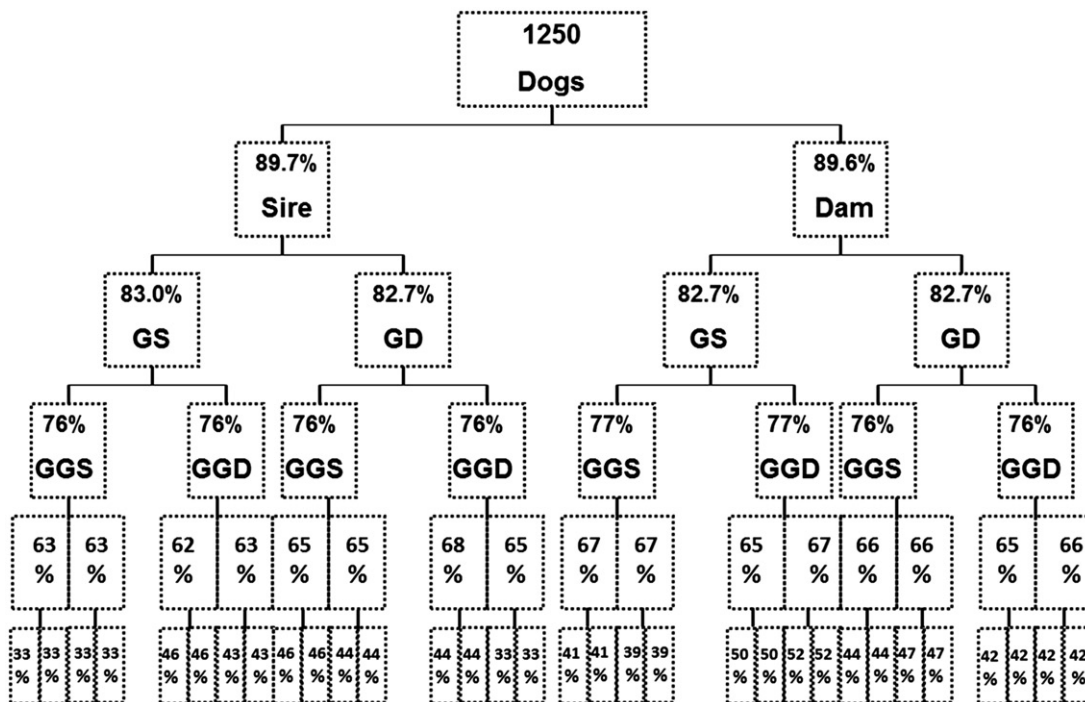Figure 1. Number of registered dogs from 2003 to 2014.

Figure 2. Pedigree completeness level in the whole pedigree data files (GS and GD = grandparents; GGS and GGD = great grandparents).

90% for the parental generation, from 82.7% to 83% for the grandparent generation and from 76% to 77% for the third-generation of the great-grandparents. The pedigree completeness level was similar both in the dam and in the sire pathways.

The pedigree completeness was also assessed by calculating the mean maximum generations, the mean complete and the number of equivalent generations traced. The mean maximum generations and the mean complete generations were 5 and 3.12, respectively, while the number of equivalent generation traced

(sum, on all the generations of the ancestors, of the ancestors' proportion known to every generation) was 3.81. All these values are lower than those observed for Bracco Italiano (Cecchi et al. 2013), due to the different histories and numbers of these two breeds in Italy.

The generation interval is an important parameter to derive various demographic indexes of the populations. In the RP, it was 6.19 ± 0.543 and 5.52 ± 0.513 for the two pathways father–son and father–daughter, respectively, while it was 5.85 ± 0.631 and 4.75 ± 0.486

Table 1. Main genealogical parameters computed for the *Bracco Francese* dog pedigree.

Whole population (WP)	n	1250
Males	"	628
Females	"	622
Sires	"	197
Sires offspring average value	"	6.30
Popular Sires	"	4
"	%	2.03
Dams	n	259
Dam offspring average value	"	4.80
Popular Dam	"	3
"	%	1.16
Inbreed in the WP	n	783
Reference population (RP)	"	921
Males	"	479
Females	"	442
Inbreed in the RP	"	772
Base population (one or two unknown parents = f_t)(BP)	"	129

Table 2. Top 7 ancestors contributing to genetic variability of the RP.

	Ancestors	Sex	Year of birth	Genetic variance (%)	Progeny
No.1	LO01130412	F	1998	12.98	30
No.2	ES9916070	M	1995	9.43	7
No.3	LO0543349	F	1999	9.07	10
No.4	LO0749890	M	2004	5.66	40
No.5	LO9953189	M	1998	5.32	2
No.6	LO08126314	M	2005	5.07	6
No.7	LO0844885	M	2007	4.20	66

for the two pathways mother–son and mother–daughter, respectively. The average value was 5.47 ± 0.234, which was similar to that reported for the Braque Saint-German breed (Leroy et al. 2006), but higher than observed for the Bracco Italiano (Cecchi et al. 2013), and for many other dog breeds such as Lagotto (Sabbioni et al. 2007), Bolognese (Sabbioni et al. 2008), Basset fauve de Bretagne, Bouledogue Français, Dogue de Bordeaux, Barbet, Berger des Pyrénées, Beauceron, Epagneul Breton and Montagne des Pyrénées (Leroy et al. 2006). The age of parents at the birth of their first progeny was lower than the respective generation intervals with values ranging from 4.23 ± 0.111 for the pathway mother–son to 4.63 ± 0.129 for the pathway father–daughter (4.41 ± 0.061 as average value).

Genealogical data

A total number of 197 and 259 sires and dams, respectively, were observed, with a ratio of dams-sires of 1.31 (Table 1). Most dams and sires had just one registered litter (128 dams and 92 sires). Popular sires were four (2.03% of the sires), while popular dams were three (1.16% of the dams). The mean number of puppies for each sire was low (6.30) suggesting that many sires were used only once. This is due to the good management of the breed by the CIBF who encouraged farmers not to make excessive use of stallions in order to contain any inbreeding, given the reduced consistency and the young age of the breed in Italy. In Table 1, the main genealogical parameters computed by ENDOG are also reported. The whole population (WP) consisted of 1250 animals (628 males and 622 females), 783 of which were inbred. The number of animals in the reference population (RP) was 921 (479 males and 442 females), 83.82% of which were inbred. The group of 479 males of the RP comprised 81.21%

inbred animals, and 86.65% of the group of 442 females of the RP were inbred. The average inbreeding coefficient in both sexes was similar. A total of 155 full-sib groups (average family size = 6.03) were computed in the WP with a range of between 2 and 24.

The number of animals in the base population (one or both unknown parents: absolute founders = f_t) was 129 (10.32%). Considering the WP, the number of effective founders (f_e) was 32. The number of ancestors was 117 in WP and 63 in RP, while the effective number of ancestors (f_a) was 24 in WP and 19 in RP. To explain 50% of the genetic variability, a total of 9 and 7 ancestors were sufficient in the WP and RP, respectively.

As is well known, the effective number of founders depends on both the total number of founders and the imbalance between their expected contributions to the gene pool. A comparison between the effective number of founders (f_e) and the effective number of ancestors (f_a) reveals the decrease in genetic variation in populations that have passed through a bottleneck (Boichard et al. 1997). The medium value of the ratio f_a/f_e 0.75 (24/32) highlights a balanced use of animals for breeding and an absence of a bottleneck in the history of the Braque Français. The effective number of founders (f_e) found in this research was about half that reported in the Bracco Italiano breed (32 vs. 61.3). Although it is difficult to draw comparisons with other breeds reared in the same country or in different countries, because of their different history and demographic structure and consistency, the estimates of the effective number of founders (f_e) and ancestors (f_a) of the Français type Pyrénées reared in Italy were in most cases similar to those reported by other authors (Cole et al. 2004).

Table 2 reports the contribution of the main ancestors to the RP. Just seven dogs were able to account for more than half of the genetic variability within the breed. The most represented ancestors were the first three (two females and one male) with more than 31% of genetic variance. The first female ancestor was one of the three popular dams observed; in addition, the males numbered four and seven were popular sires.

With the exception of ancestor N° 3, all the other ancestors were related with AR values ranging from 0.019 to 0.500. Ancestor N° 2 was closely related to ancestor N° 5 (AR = 0.500), to ancestor N° 4 (AR = 0.250) and to ancestor N° 7 (AR = 0.187).

The number of ancestors, the effective number of ancestors (f_a) and the low number of ancestors explaining 50% of the genetic variability and the close relationships among the top seven ancestors highlighted that only a small number of males were used in reproduction. The reasons for this low number of ancestors and the high relatedness highlighted among them must be investigated in terms of the history of the breed in Italy. In fact, when the CIBF was set up in 2000, the breed was in the hands of only 3–4 breeders. Only a few animals, with a very good morphological or functional evaluation, were used intensively. Only later, with the help of the French Club, and the introduction in Italy of animals from France between 2003 and 2009, the CIBF was able to better implement the genetic management of the breed.

Inbreeding and relationship coefficients

The average values of inbreeding (F) and relatedness (AR) in the whole population (WP) were 3.29% and 7.10%, respectively, while the average values of F and AR in the reference population (RP) were 4.35% and 10.67%, respectively. In this research, the value found for AR is high compared to findings in the Bracco Italiano breed, while the inbreeding coefficient is a medium value compared to what has been reported for other breeds.

Many studies have reported inbreeding results in dog breeds and their analyses show that the mean coefficient of inbreeding differs for different breeds and within the same breed in different countries. The average coefficient of inbreeding observed in the Braque Français for the RP was similar to findings by Voges and Distl (2009) for Bavarian Mountain Hounds (4.5%).

Our value was different from those reported by other authors. In particular, it was smaller than those reported in some different Italian (Cecchi et al. 2013; Sabbioni et al. 2007), and foreign breeds (Nielen et al. 2001; Cole et al. 2004), whereas it was higher than those reported for Golden Retrievers (Nielen et al. 2001), Italian population of Pit Bull Terriers (Ciampolini et al. 2013) and in Lagotto Romagnolo (Sabbioni et al. 2008).

In a study on breeds raised in Finland (Mäki et al. 2001), the average coefficient of inbreeding of dogs born in 1998 ranged from 2.3% in the German shepherd breed to 5.1% in the Finnish hound breed. In a study on breeds raised in France (Leroy et al. 2006),

the average coefficient of inbreeding ranged from 3.3% in Bouledougue Français to 12.4% in the Barbet breed.

Considering the complete database, 783 dogs were inbred. Five hundred eleven dogs of this group (65.26% of the inbred) showed an inbreeding value lower than 5%, while 13 dogs (1.66% of the inbred) showed an inbreeding value higher than 20%. Seventy-six highly inbred matings were computed: four matings (0.32%) between full sibs, seven matings (0.56%) between parent–offspring and 65 matings (5.20%) between half sibs.

Figure 3 shows the inbreeding trend computed by the year of birth of RP dogs. As can be observed, values were nonlinear. The maximum inbreeding value was observed in 2009 ($F = 0.070$); however, the dogs born in 2013 and 2014 had low inbreeding values ($F = 0.024$ in 2013 and 0.018 in 2014). The reason why the rate of inbreeding was reduced in this period was because some dogs imported from France between 2003 and 2009 were registered in the Italian ROI, they had been used by the Italian breeders for in order to introduce "new blood" and reduce inbreeding, following the advice of the CIBF. The average increase in inbreeding was estimated to be 0.67% per generation for the WP ($N_e = 74.63$), and 1.18% ($N_e = 42.42$) for the RP. The average increase in inbreeding was similar to that observed in Bracco Italiano (1.29% and $N_e = 38.86$), but was rather high in comparison to findings in some other Italian (Sabbioni et al. 2007) and foreign breeds (Leroy et al. 2006). Our value was lower than reported in the Bolognese breed (Sabbioni et al. 2008). The effective population size reveals losses in both fitness and genetic variability. It is considered as one of the most fundamental parameters that strongly influence the nature and the rate of genetic information transmission across generations, population variability and conservation strategies.

The maximum number of traced generations was 10 (Table 3). The percentage of inbred increased with an increase in the number of traced generations, reaching about 93% in the subjects with 10 traced generations. Beginning with the subjects with seven traced generations, the average coefficient of inbreeding was higher than 3.125%. The highest coefficient of inbreeding was observed in the inbreed dogs with 10 traced generations ($F = 0.071$), which is higher than 6.25%, that is, the value resulting from the mating of two animals sharing two grandparents (cousin mating).

For a long time, pedigrees have been the only data source available for monitoring genetic diversity within a given population of dogs. Genealogical analysis provides a comprehensive view of the evolution of
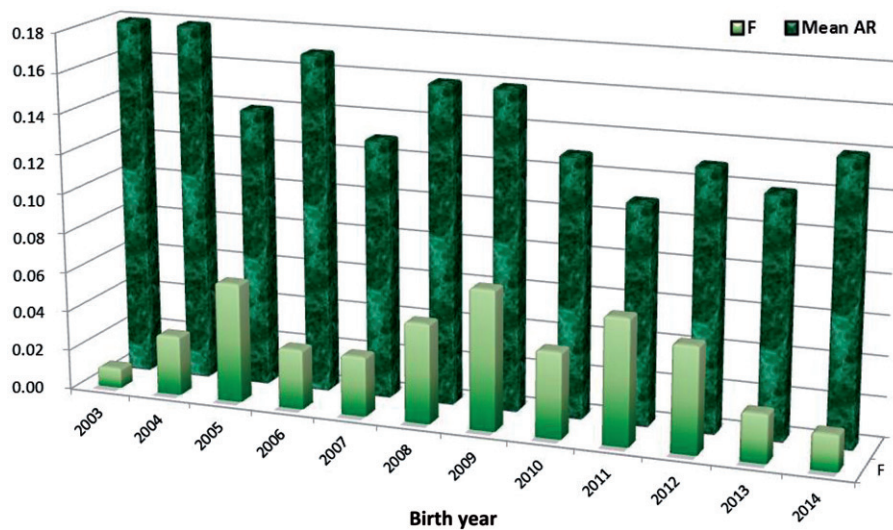
Figure 3. Inbreeding (*F*) and average relatedness (mean AR) trends for birth year. Above: Birth year, not Birth Year

Table 3. Population statistic on average inbreeding.

Traced generation	No. animals	Average *F* (%)	% Inbred	Average *F* for inbred (%)	Mean AR (%)
0	129	0.00	0	0	0
1	75	0.00	0	0	1.77
2	52	0.48	3.85	12.50	2.94
3	40	1.02	10.00	10.16	4.73
4	80	1.78	28.75	6.18	6.73
5	65	2.03	60.00	3.39	7.57
6	122	2.95	77.05	3.82	6.87
7	201	3.61	87.56	4.12	8.45
8	228	5.57	89.47	6.22	9.84
9	185	5.02	92.97	5.40	9.72
10	73	6.62	93.15	7.71	9.53

genetic variability from the base of the population, particularly if the data are complete and reliable. In fact, the limitations of the pedigree analyses are due to the extent of pedigree knowledge, that is, the proportion of registered individuals in the history of the breed and the possible existence of pedigree errors, resulting from registration errors or undetected matings.

The molecular approach is becoming even more useful to manage the diversity, because genotyping of markers is becoming less expensive and better correlations are obtained between genealogical and molecular data. This may help in the development of viable conservation programs based on mating practices to prevent inbreeding in dog populations.

Conclusions

This research highlights that the average inbreeding of Braque Français type Pyrénées is in line with findings observed in other breeds. One very positive aspect is

that the inbreeding trend has not increased over the years, unlike in other breeds.

This was possible not only because the CIBF has always remained closely linked to the French Club, but also thanks to over 100 breeder members of the CIBF who have adopted the guidelines and recommendations of the Technical Committee of the Italian Club for mating. The high level of expertise and sensitivity of the breeders is also key in understanding the importance of containing inbreeding in order to safeguard the breed.

The research will continue with an investigation into the genetic variability at the genomic level through new SNP markers used to check the molecular health and genetics of the breed, which will complement the analysis of genealogical data.

From the results of this research, carried out in collaboration with the CIBF, future arrangements have been made to ensure the constant and continuous monitoring of the genetic variability of the breed in order to continue the current good management of the genetic heritage that CIBF has helped to create and maintain since 2000.

Disclosure statement

The authors report no conflicts of interest. The Authors alone are responsible for the content and writing of this article.

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