

SHORT COMMUNICATION

Genetic variability in *Bracco Italiano* dog breed assessed by pedigree data

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Abstract

The Bracco Italiano is one of the oldest pointing dog breed, used for hunting ever since the Renaissance time. The complete electronic record of the breed was downloaded from the ENCI database [whole population (WP) = 24,613 animals registered since 1970 to 2011] with the aim to estimate genetic variability in Bracco Italiano dog breed using pedigree records. Up to 97% of the individuals had registered parents and 86% registered grandfathers. Average generation interval was 4.68 ± 0.545 for stallions and 4.08 ± 0.321 year for dams. Reference population (RP) was defined as the population of interest that include living reproductive animals approaching the last three generations and include 9006 dogs of which 34% were inbreds. The number of ancestors was 564 in WP and 188 in RP. while the effective number of ancestors was 46 and 34 respectively. To explain 50% of the genetic variability, a total of 18 and 9 ancestors enough, respectively in the WP and RP. The average inbreeding coefficient in the RP resulted 6.7% while the average increase in inbreeding was estimated to be 1.29% (Ne=38.86). Nevertheless a regular monitoring of genetic variability of the population is important and must be adopted, in order to avoid the danger of an excessive increase of inbreeding in the future, which would result in significant inbreeding depression and in significant loss of genetic variation.

Introduction

Many dog breeds are characterized by reduced genetic diversity related to small numbers of founders, and to the popular sires, whose allelic pool is over represented in subsequent generations. Moreover the mating between close relatives is frequently used leading to high rates of inbreeding with a consequent increase of some genetic diseases (Ubbink et al., 1992), mortality of puppies (Van der Beek et al., 1999) and inbreeding depression (Leroy, 2011; Shinkarenko et al., 2010), which is described as the decline in performances of inbred animals, particularly in the areas of reproduction and of health (Urfer. 2009; Oliehoek et al., 2009). For these reasons genetic typifying is an important preliminary step in any safeguard biodiversity program. The genetic variability of a population can be estimated from genealogical data (Cecchi et al., 2009; Grazewska, 2007; Leroy et al., 2006; Leroy et al., 2009; Maki et al., 2001) or using the short tandem repeat (STR) molecular markers (Kim et al., 2001; Koskinen et al., 2000; Leroy et al., 2009). At the present several researches used SNPs (Quignon et al., 2007; vonHoldt et al., 2010; Wayne and vonHoldt, 2012) because of the ease in genotyping a biallelic marker and the ability to multiplex thousands of markers in one reaction (Parker, 2012). The Bracco Italiano is one of the oldest pointing dog breed, used for hunting ever since the Renaissance time. The late 19th-early 20th century saw a decline in numbers but due to the enthusiasm of breeders the Bracco was saved from extinction. The definitive breed standard was approved by ENCI in February 1949 and nine months later, in November, the Società Amatori Bracco Italiano (Association of Italian Bracco Amateurs, SABI) was founded. At the present the studies on the genetic characterization by microsatellites of the Bracco Italiano breed, using ISAG panel as suggested by the international ISAG canine comparison test (Ciampolini et al., 2012), showed a low genetic variability of the breed (Ciampolini et al., 2011).

The aim of this study was to analyse the genetic variability of the Bracco Italiano dog breed in terms of inbreeding and computing a number of genealogical parameters that could integrate the information obtained from the analysis of molecular data and to identify strategies to conserve or restore the genetic variability of the breed.

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Materials and methods

The complete electronic record of the breed was downloaded from the ENCI database. Animals were born between 1970 and 2011 and all dogs constitutes the whole population (WP) containing all founders, ancestors, and their offsprings. The population is divided in: the base of population (BP) defined as individuals with one or both unknown parents, the reference population (RP) defined as the population of interest that include living reproductive animals approaching the last three generations. The effective population size of the RP was performed using the methodology described by Gutierrez *et al.*, (2009) and using the regression-based estimates.

The following demographic and genetic parameters have been calculated, using the program ENDOG v4.8 (Gutiérrez and Goyache, 2005): i) the effective number of founders (fe) the absolute number of founders (f) and the effective number of ancestors (fa); ii) the Inbreeding coefficients (F); the number of inbred animals and average inbreeding coefficient for each traced generation; iii) the pedigree completeness: the number of full traced generations, the maximum number of generations traced and the equivalent complete gen-





erations; iv) the generation intervals.

Popular sires (defined here as >100 recorded offspring) and popular dams (>40 offspring) were identified. The number of inbreds and the average inbreeding coefficient per year were performed using CFC software (Sargolzaei *et al.*, 2006). The distribution of inbreeding level in the whole population were analysed and eight different class level of inbreeding were considered: $0 < F \le 0.05$; $0.05 < F \le 0.10$; $0.10 < F \le 0.15$; $0.15 < F \le 0.20$; $0.20 < F \le 0.25$; $0.25 < F \le 0.30$; $0.30 < F \le 0.35$; $0.35 < F \le 0.40$ (Sargolzaei et al., 2006).

Results and discussion

Demographic parameters

Into the ENCI database, 24,613 dogs has been recorded in the years 1970-2011 (12,191 males and 11,890 females). In the period 1970-1975 only 211 dogs were registered. Starting from 1976 the average value was of 663.5±112.61 dogs per year (the lowest value has been recorded in 1983 with 411 dogs and the highest in 2003 with 897 dogs). In Figure 1, the details of the pedigree quality in *Bracco* Italiano dog breed are shown. The percentage of completeness was computed as about 97% for the parental generation, from 86.5% to 88.4% for grandparent generation and from 75% to 79% for the third-generation of the great-grandparents. The pedigree completeness level was similar both in dam and sire pathway.

Pedigree completeness was also assessed by

Table 1. Numbers of offspring per sire and per dam, numbers of popular sires and popular dams and percentage of sires and dams that are popular.

Number of sires	1783	Number of dams	2916
Sire offspring	1100	Dam offspring	2010
Maximum	191	Maximum	73
Medium	13.5	Medium	8.2
Number of popular sires	18	Number of popular dams	15
% of total sires	1.01	% of total dams	0.51

Table 2. Main genealogical parameters computed for the Bracco Italiano dog pedigree.

Whole population	24,613	
Males	12,191	
Females	11,890	
Inbreed in the whole population	16,832	
Reference population	9006	
Males	4589	
Females	4417	
Inbreed in the reference population	3055	
Base population		
(one or two unknown parents = f_t)	616	

Table 3. Top 9 ancestors contributing to genetic variability of the reference population.

Ancestors	Sex	Year of birth	Genetic variance, %	Progeny
CI027616	M	1979	11.05	78
CI043072	M	1979	8.18	91
CI054207	M	1986	7.53	191
CI013840	M	1970	5.51	108
CI018248	M	1975	4.85	95
CI052431	F	1985	4.77	34
CI053314	M	1985	3.81	33
CI016197	F	1970	3.50	9
CI013252	M	1970	3.38	53

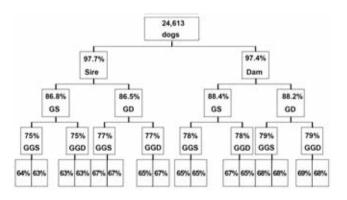


Figure 1. Pedigree completeness level in the whole pedigree data files (GS and GD, grandparents; GGS and GGD, great grandparents).

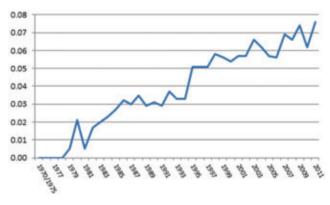


Figure 2. Inbreeding trend for birth year.





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 7.27 and 3.56 respectively while the number of equivalent generations traced (sum, on all the generations of the ancestors, of the ancestors' proportion known to every generation) was 4.70. The generation interval is an important parameter to derive various demographic indexes of the populations. It has been calculated separately for the stallions and the dams and limited to the animals whose progeny is reproduced in turn. The average generation interval for animals born in the period 1980-2011 was 4.68 ± 0.545 for stallions and 4.08±0.321 for dams; no significant differences were found between the two pathways within each parent. These values were similar to that reported on Beauceron dog breed (Leroy et al., 2006), but higher than has been observed for Bolognese (Sabbioni et al., 2008), for Basset fauve de Bretagne, for Bouledogue Français and for Dogue de Bordeaux (Leroy et al., 2006). Our results were lower than has been observed for Lagotto (Sabbioni et al., 2007), for Barbet and for Berger des Pyrénées, and for Braque Saint-Germain, and for Epagneul Breton and for Montagne des Pyrénées (Leroy et al., 2006).

Genealogical data

Sires and dams (Table 1) in total were 1783 and 2916 respectively, with a ratio dams-sires of 1.63. Most dams and sires have just one reg-

istered litter (178 dams and 99 sires). Popular sires were 18 (1.01% of the sires) while popular dams were 15 (0.51% of the dams).

Table 2 summarizes the main genealogical parameters computed by ENDOG. Number of animals in the reference population was 9006 (4589 males and 4417 females), 33.92% of which were inbreds. Number of animals in the base population (one or both unknown parents: absolute founders = f_t) was 616 (2.5%); this low percentage points out the good knowledge of the genetic structure of the breed. The number of ancestors was 564 in WP and 188 in RP, while the effective number of ancestor (fa) was 46 in WP and 24 in RP. To explain 50% of the genetic variability, a total of 18 and 9 ancestors enough, respectively in the WP and RP. These results point out that only a small number of males are used in reproduction. The contribution of the main ancestors to the RP is reported in Table 3. Only 9 dogs explained more than half of the genetic variability within the breed. In terms of number of progeny, the most represented ancestors are the first five sires with 563 offsprings and more than 37% of genetic variance.

Considering the WP the number of effective founders (f_e) was 61.3; the effective number of founders depends on both the total number of founders and the disequilibrium between their expected contributions to the gene pool. The comparison between the effective number of founders (f_e) and the effective number of ancestors (f_a) allows to reveal the decrease in genetic variation in populations that have passed through a bottleneck (Boichard $\it et al.$,

1997): the medium-high value of the ratio f_a/f_e (0.75) would point out the absence of a bottleneck in the history of the Bracco Italiano.

Inbreeding coefficient

Average value of inbreeding (F) and relatedness (AR) in the whole population were 4.10% and 3.51% respectively, while the average value of F and AR in the reference population (RP) were 6.29% and 4.11%. 6.29% is a medium value in comparison at what reported on others breeds. In fact, concerning Italian dog breeds the average coefficient of inbreeding ranged from 2.27% in *Lagotto Romagnolo* (Sabbioni *et al.*, 2008) to 10.81% in Bolognese dog breed (Sabbioni *et al.*, 2007).

A lot of studies have reported inbreeding results on foreign dog breeds. In particular the average coefficient of inbreeding observed in an Italian population of Pit Bull Terrier dogs

Table 4. Distribution of inbreeding coefficients in the whole population.

Range	No.
0.00 < F < 0.05	9849
0.05 < F < 0.10	3931
0.10 < F < 0.15	1821
0.15 < F < 0.20	608
0.20 < F < 0.25	291
0.25 < F < 0.30	260
0.30 < F < 0.35	60
0.35 < F < 0.40	12

Table 5. Population statistic on average inbreeding.

Traced generation	Animals, n	Average F, %	Inbred, %	Average F for inbred, %	Mean AR, %
0	532	0.00	0	0	0
1	1860	0.00	0	0	0.55
2	1737	0.70	4.19	16.55	1.19
3	1502	2.34	19.38	12.05	1.82
4	1562	3.00	43.04	6.96	2.46
5	1814	3.88	61.04	6.35	3.01
6	1738	3.61	73.51	4.91	3.53
7	1789	4.13	85.72	4.82	3.92
8	1738	4.66	94.08	4.95	4.15
9	1762	5.30	97.27	5.45	4.46
10	2017	5.76	99.90	5.77	4.75
11	1917	6.52	100.00	6.52	4.98
12	1784	6.04	99.33	6.08	5.04
13	1828	6.27	98.74	6.35	5.16
14	756	6.21	100.00	6.21	5.33
15	237	8.46	100.00	8.46	5.34
16	40	12.39	100.00	12.39	5.58

AR, average relatedness.





was smaller than Bracco Italiano (3.73%; Ciampolini et al., 2013). The inbreeding coefficients ranged from 1.8% in the Golden Retriever breed to 7.0% in the Kooiker dog breed (Nielen et al., 2001). 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 while in a study on breeds raised in France (Leroy et al., 2006) the average coefficient of inbreeding ranged from 3.3% in Bouledougue Francais to 12.4% in Barbet breed dog. Cole et al. (2004) reports that populations of German Shepherd and Labrador Retriever guide dogs exhibited an average inbreeding level of 26.2% and 22% respectively, while Cecchi et al. (2009) in an Italian colony of dog guide reports values of 0.45% in German Shepherd dogs, 0.38% in Labrador dogs and 0.49% for Golden Retriever dogs. Considering therefore the complete database, 16,832 dogs resulted inbred (8547 males and 8285 females); 9849 dogs of this (58.51% of the inbred), showed an inbreeding value lower 5% while 623 dogs (3.70% of the inbred) showed an inbreeding value higher than 20% (Table 3). Figure 2 shows the trend of inbreeding computed by the year of birth of individual. Due to scarcity of data, average inbreeding is equal to zero for nine years; starting from the beginning of the 1980s, with more genealogical data, inbreeding increases consistently and reaches a maximum peak in 2011 (F=0.076). Starting from the 2003 the F average value was higher 6.25%, i.e. the value resulting from the mating of two animals sharing two grandparents (cousin mating) and all dogs resulted inbred. AR values are always smaller than the corresponding F values and are always below the 5.6% (data not shown).

The average increase in inbreeding was estimated to be 0.51% per generation for the WP (N_e =98.64) and 1.29% (N_e =38.86) for the RP. The trend of the inbreeding was increasing with rather high value in comparison to that reported in others italian breeds (Sabbioni et al., 2007, Lubas et al., 2008) and foreign breeds (Leroy et al., 2006), but lower than reported on Bolognese dog breed (Sabbioni et al., 2008). The effective population size reveals losses in fitness as well as in genetic variability and 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 16 (Table 4). The percentage of inbred increased with the increasing of the number of traced generations and beginning from the subjects with 10 traced generations, more than 98% of the animals resulted inbred. Average F tended to be always higher than the corresponding AR values. Beginning to the subjects with 5 traced generations the average coefficient of inbreeding was higher than 3.125%. The highest coefficient of inbreeding was observed in the 40 dogs with 16 traced generations (F=0.1239). This value is too high indicating the importance of reducing the inbreeding coefficient through the exchange of breeding animals and avoiding mating between too much related animals.

Conclusions

Inherited diseases have been identified in purebred dogs more and more during recent years, and the management of genetic variability assumed a major importance in dog breeding. The quality of pedigree information of Bracco Italiano dog breed is enough good and although the breed has not an high consistency, the annual registrations to the ENCI database is stable. On the other hand the high percentage of inbred animals for each traced generations and for birth year, and the F and AR values show that the management practices are the main factors explaining the observed results. Often, to produce dogs that met the standard, breeders employed inevitably inbreeding practices with adverse effects for population health, mating together closely related individuals, which damages are accentuated by selectively breeding from small numbers of champion sires. In fact data prove that the abusive representation of some individuals in the whole pedigree can be highly detrimental to maintaining the genetic diversity of the breed. This is supported by the low value of the effective number of ancestors in the RP and that only 9 ancestors explained 50% of total genetic variability of the breed. The rate of increase in the RP is also in the top range of the acceptable level of 0.5-1% recommended by the FAO (1998) guidelines for the livestock populations.

For a long time, pedigrees have been the only data source available for the monitoring of genetic diversity within a given population of dogs. Genealogical analysis provided a comprehensive view of the evolution of genetic variability from the base of the population, particularly if the data are complete and reliable. Infact the limitations of the pedigree analyses are due to the extent of pedigree knowledge, *i.e.* the proportion of registered individuals in

the history of the breed and to the possible existence of pedigree errors, resulting from registration errors or undetected matings. On the other hand molecular data were obtained on a limited number of markers and animals and thus there may be a sampling effect. Today, the molecular approach is becoming even more useful to manage the diversity, because genotyping of markers is becoming less expensive and better correlations were obtained between genealogical and molecular data, which may help in development of viable conservation programs founded on mating practices to avoid inbreeding in dog populations. Some authors (Leroy et al., 2009) highlighted differences between the results obtained with molecular and genealogical estimators explained by the different characteristics of the two approach. On the contrary our results are consistent with estimate obtained from molecular data indicating the robustness of pedigree analyses and the compatibility between these two sources of information. Both methods suggest that strategies for genetic management could be improved in order to avoid the danger of a further increase of inbreeding, which might result in significant inbreeding depression and in significant loss of genetic variation. In particular the most promising approach is to optimise the contribution of the parents to minimize the average kinship. This method may place constraints on breeders limiting the choice of breeding animals to be mated. Outbreeding is a potentially useful tool, especially to introduce diversity or remove inherited disease from a given line. Certainly, the choice of the animals should be made by the Association of the breed (SABI), which must consider the selection goals and the standard of the breed. Moreover, even if this suggestion is difficult to apply, a selection within family should be performed, choosing the best animal within each family and not absolute best animals.

In this way every family will be represented in the next generation avoiding the risk that future breeding animals will be much related and ensuring the maintenance of the genetic diversity.

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