SHORT COMMUNICATION

Genetic variability of the Bracco Italiano dog breed based on microsatellite polymorphism

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Abstract

The Bracco Italiano is one of the oldest breeds of Italian pointing dogs, used for hunting ever since Renaissance times. After the Second World War it was included among the breeds officially recorded by the ENCI (the Italian Cynological Club), and since 1970 more than 23,000 animals have been registered; there are currently approximately 750 births per year. In this paper, we present the breed characterization of the population at the molecular level using 21 STR markers from the panels recommended for the 2006, 2008 and 2010 ISAG canine comparison test. Number of alleles, allele frequencies, deviations from Hardy-Weinberg proportions, linkage disequilibrium among *loci*, genetic similarity, genetic distances and molecular co-ancestry-based parameters were calculated. The number of alleles ranged from 3 to 9 (mean 6.43) whereas the expected heterozygosity ranged from 0.44 to 0.81 (mean 0.64). There was a high genetic similarity within the whole population (0.455) showing the great homogeneity of the sampled animals, as confirmed also by the small kinship distance (0.336), by the high values of the self molecular coancestry (0.703) and of the inbreeding coefficient (0.406). These results suggest the need for a careful genetic management of the population in order to avoid the risk of an excessive increase in the inbreeding level which would result in significant inbreeding depression and in significant loss of genetic variation.

Introduction

The Bracco Italiano is one of the oldest pointing dog breeds, used for hunting since Renaissance times. Fourteenth century paintings show hunting scenes with dogs similar to the present-day Bracco. It was originally used as a *net dog* breed to locate quails (partridges and francolins) which were then captured in nets. Historical records report that some of these dogs were sent from Tuscany to the court of the kings of France in the 15th century (De Giuliani, 2006). At the end of the 19th century, the breed suffered a period of decline due to an incorrect selection criteria which resulted in the animals being too heavy, lymphatic, and slow. However, the breed was revitalized after the Second World War when a club of supporters brought the breed back to its original characteristics through a prudent selection strategy. The breed was officially registered by the ENCI (the Italian Cynological Club) in 1949, when the definitive standard was established. The Italian Bracco belongs to Group 7 (Pointing Dogs) of the ENCI and the first animals were recorded in 1970. Since that time, more than 23,000 animals have been recorded and more than 750 puppies are registered every year. Since 1970, there have been more than 4,700 farmers, more than 1500 stallions and more than 1,900 bitches.

Today, the Bracco Italiano is increasingly important all over the world for hunting.

The traditional approach of evaluating the genetic variation present in a population is to estimate the mean coefficient of inbreeding from genealogical data. This method has been extensively used in dog breeds (Grazewska, 2007; Leroy *et al*., 2006; Leroy *et al*., 2009a; Maki *et al*., 2001). However, it is well known that it may result in erroneous estimates because of incomplete records and/or pedigree errors. More recently, the considerable advances in molecular genetics have provided a convenient way for characterizing the genetic structure of populations. The genetic structure of the domestic dog has been investigated using mitochondrial DNA (Tsuda *et al*., 1997; Vilà *et al*., 1997; Vilà *et al*., 1999), or microsatellite markers (Kim *et al*., 2001; Koskinen *et al*., 2000; Irion *et al*., 2003; Leroy *et al*., 2009b; Parker *et al*., 2004), or both (Parra *et al*., 2008).

The aim of the present study was to investigate the extent of genetic variation characterizing the Bracco Italiano breed using microsatellites. Our objectives were to determine the values of the main parameters describing the genetic health of the breed, and to ascertain whether specific management strategies should be adopted to avoid the increasing genetic impoverishment that would result from the limited effective population size.

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Key words: Genetic variability, Bracco Italiano dog breed, Microsatellite markers.

Acknowledgments: the authors are very grateful to Dr. G. Grecchi and the Board of Directors of the Società Amatori Bracco Italiano for having actively promoted the present project among the Society's members.

Received for publication: 16 November 2010.
Revision received: 23 September 2011.
Accepted for publication: 23 September 2011.

Italian Journal of Animal Science 2011; 10:e59
doi:10.4081/ijas.2011.e59

Materials and methods

Animals

The study was performed using 72 unrelated Bracco Italiano dogs. Data were collected at meetings organized by the Bracco Italiano breed club; first-degree and second-degree relatives were excluded; the final sample included animals from 44 different farms (breeders) scattered throughout Italy.

Genomic and statistical analysis

Genomic DNA was extracted from 5 mL of peripheral blood samples and DNA was isolated using the Genelute blood genomic DNA kit (Sigma-Aldrich, Milano, Italy).

The 21 microsatellites investigated belonged to a markers panel proposed by ISAG/FAO, for the measurements of Domestic Animal Diversity (ISAG/FAO, 2004) and located in 19 chromosomes. Primer sequences for the microsatellites are available from *http://dad.fao.org/en/refer/library/guidelin/marker.pdf*. The 21 microsatel-

lites were amplified in 5 multiplex PCR reactions. Amplification of the 5 multiplex was carried out in a total reaction volume of 10 μL consisting of 6.25 μL MasterMix (Qiagen Multiplex PCR kit), 0.1 μL of each primer (10 μM), 1 μL of DNA sample (2 ng/μL) and 1.55 μL of H_20. The PCR reaction was carried out on a Gene Amp PCR System 2700 thermal cycler (Applera) by an initial denaturation at 95° C for 15 min, followed by 47 cycles at 95°C for 30 s, 58°C for 90 s and 72°C for 60 s. The thermal profile ended with a final extension at 60°C for 30 min. Amplicons were separated and detected by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using POP4 and a 36-cm capillary array. Apparent DNA fragment size was analyzed with the internal size standard Genescan 500ROX and GeneMapper Analysis version 4.0 software (Applied Biosystems, Monza, Italy).

Allelic frequencies were estimated by direct counting. Exact tests for deviations from the Hardy-Weinberg equilibrium (HWE) and pairwise linkage disequilibrium among microsatellite *loci* were performed using the ARLEQUIN package (Excoffier *et al*., 2005). Genetic similarities between animals were investigated by comparing the individual multilocus genotype of each individual (Ciampolini *et al*., 1995). Genetic similarity is defined as P=A/2L, where P is the proportion of common alleles (A) in relation to the 2L possibilities (L=number of considered *loci*). The similarities between each pair of individuals were then averaged over the whole population. Genotype assignment test of individuals coming from different farms was performed using the ARLEQUIN package in order to highlight possible genetic sub-structures in the total sample originating from farm membership. The following parameters were computed at the population level using the Molkin program (Gutièrrez *et al*., 2005): molecular coancestry coefficients (Caballero and Toro, 2002), kinship distance, and F-statistics (Wright, 1978). The molecular co-ancestry between 2 individuals, i and j (f_{ij}), is the probability that two randomly sampled alleles from the same *locus* in 2 individuals are identical by state (Caballero and Toro, 2002). The molecular co-ancestry of an individual i with itself is self-coancestry (s_i), which is related to the coefficient of inbreeding of an individual i (F_i) by the formula $F_i = 2s_i - 1$. In turn, the kinship distance (D_k) between 2 individuals i and j is $D_k = [(s_i + s_j)/2] - f_{ij}$ (Caballero and Toro, 2002). MolKin computes within-breed molecular co-ancestry and D_k by simply averaging the corresponding values for all the within-population pairs of individuals.

Table 1. Number of alleles, polymorphism information content (PIC), observed and expected heterozigosity, and departure from Hardy-Weinberg equilibrium for the 21 microsatellite *loci*.

Microsatellite marker	No. of alleles	PIC	Heterozigosity		P
			Observed	Expected	
AHT121	7	0.675	0.649	0.719	0.198
AHT137	8	0.602	0.740	0.643	0.274
AHTH130	6	0.602	0.597	0.668	0.033
AHTh171	9	0.693	0.675	0.743	0.133
AHTH260	7	0.418	0.364	0.444	0.000
AHTk211	5	0.449	0.597	0.528	0.816
AHTK253	6	0.527	0.481	0.574	0.244
CXX279	6	0.627	0.597	0.665	0.008
FH2054	8	0.690	0.714	0.740	0.112
FH2848	8	0.574	0.597	0.601	0.715
INRA21	7	0.728	0.792	0.768	0.359
INU005	6	0.576	0.571	0.642	0.085
INU030	3	0.467	0.506	0.532	0.866
INU055	5	0.408	0.442	0.472	0.676
REN105L03	5	0.513	0.338	0.560	0.000
REN162C04	5	0.591	0.675	0.665	0.009
REN169018	6	0.773	0.727	0.807	0.204
REN169D01	8	0.676	0.675	0.708	0.113
REN247M23	8	0.651	0.675	0.686	0.168
REN54P11	7	0.626	0.532	0.664	0.000
REN64E19	5	0.509	0.519	0.597	0.072

Results and discussion

All 21 *loci* were polymorphic and had a total of 135 alleles ranging from 3 (INU030) to 9 (AHTh171) (Table 1). Among the *loci* studied, the population was found to be in Hardy-Weinberg equilibrium at the following *loci*: AHTK211, FH2054, INRA21, INU055 and REN169018. On the other hand, six microsatellites showed significant deviation (AHTH260, CXX279, REN105L03, REN54P11, REN162C04: $P<0.01$; AHTH130: $P<0.05$). Mean observed and expected heterozigosities were 0.594 and 0.639, respectively; expected heterozygosity values were lowest for AHTH260 (0.444) and highest for REN169018 (0.807). Although different markers were used, the expected heterozygosity of the Bracco Italiano is similar to that of other European dog breeds (0.56, Bedlington Terrier; 0.62 Golden Retriever; 0.64, Pembroke Welsh Corgi; 0.64 German Shepherd; and 0.72 Wirehaired Dachshund) (Koskiner and Bredbacka, 2000).

Seventeen microsatellites out of 21 showed heterozygote deficiency. On average, there was a significant deficit of heterozygotes ($F_{IS}=0.061\pm0.024$). Similar values were reported by Morera *et al*. (1997) ($F_{IS}=0.085$) and by Jordana *et al*. (1992) in a group of 10 Spanish dog breeds ($F_{IS}=0.040$). Such moderate values of F_{IS} can easily be explained by non-random mating or population subdivision, or even by mating between relatives. Alternatively, some null alleles could be present that cause apparent heterozygote deficit (Ciampolini *et al*., 2006). However, the F_{IS} values were rather homogeneous among *loci*, and this evidence points against such an explanation. The mean number of alleles per *locus* was 6.43 (SD=1.47). Although a comparison with other breeds can be biased due to the different marker sets used by different authors, it may be noted that this value is near the upper range of the published values for other breeds: Greyhound, 2.5 alleles/*locus*; Labrador Retriever 3.3; German Shepherd, 3.3 (Zajc *et al*., 1997); Flat-coated Retriever, 4.5; Dachshund, 5.6 (Fredholm and Wintero, 1995); Andalusian Hound, 6.25; Spanish Greyhound 6.5; Maneto, 7.0 (Morera *et al*., 1997); Czech Dachshunds, 7.6 alleles/*locus* (Pribánová *et al*., 2009); and 12 East Asian dog breeds, 7.75 (Kim *et al*., 2001).

The mean polymorphism information content (PIC) was 0.589 with a range of 0.408 (INU055) and 0.773 (REN169018). This parameter was originally introduced by Botstein *et al*. (1980). It refers to the value of a marker for detecting polymorphism within a

Table 2. Genetic similarities within the population, the self molecular co-ancestry (s_i), the average inbreeding (F_i), the mean molecular coancestry (f_{ii}), the kinship distances (D_k), and the F_{IS} value.

Genetic similarities	0.455
Self-molecular coancestry, s_i	0.703
Average inbreeding, F_i	0.406
Mean molecular co-ancestry, f_{ij}	0.337
Kinship distance, D_k	0.336
F_{IS}	0.060

population, depending on the number of detectable alleles and the distribution of their frequency, and has been proved to be a general measure of how informative a marker is (Guo and Elston, 1999). The higher the PIC value, the more informative a marker is. In the present study, microsatellites INU055, AHTH260, AHTK211 and INOU30 appeared to be only moderately informative (<0.50), whereas the other microsatellite *loci* studied were highly informative.

Genetic similarity within the population (0.455) represented a rather low genetic variability (Table 2). This value is higher than those reported in other species such as cattle (0.281, D'Angelo *et al*., 2006; 0.374-0.420, Ciampolini *et al*., 2008) and sheep (0.318-0.370, D'Angelo *et al*., 2009), but lower than that reported on an endangered donkey breed (0.489; Ciampolini *et al*., 2007).

With the exception of the values reported on Amiata donkey breed (Ciampolini *et al*., 2007) the values observed in our study for the mean molecular coancestry (f_{ii}=0.337), for the average individual self-coancestry (s_i= 0.703) and for the inbreeding coefficient (F_i=0.406) were clearly greater than that reported in literature in other species such as cattle (Ciampolini *et al*., 2008), sheep (Dalvit *et al*., 2008; Dalvit *et al*., 2009; D'Angelo *et al*., 2009; Ciani *et al*., 2010) and horse (Marletta *et al*., 2006) while the kinship distance (D_k=0.336) was smaller than that reported in literature. The observed values highlight that the low level of genetic variation has arisen as a possible consequence of mating among relatives. It is well known that the high level of inbreeding due to farm management. In fact, breeders often use this mating method with the aim of enhancing desirable traits.

Conclusions

Our findings highlight the finding that the Bracco Italiano dog breed has a low genetic variability and careful genetic management of the reproductive schemes is needed in order to avoid the risk of an excessive increase in the inbreeding level. This would result in significant inbreeding depression and in significant loss of genetic variation.

A potential strategy to reduce inbreeding would be to identify, through molecular analysis, males and females with the lowest molecular co-ancestry and use these individuals for reproduction.

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