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

Robertata Ciampolini,1 Francesco Cecchi,1 Assunta Bramante,1 Fabio Casetti,2 Silvano Presciuttini3
1Dipartimento di Patologia Animale Profilassi ed Igiene degli Alimenti, Università di Pisa, Italy
2Società Amatori Bracco Italiano, Mirabello di Senna Lodigiana (LO), Italy
3Dipartimento di Scienze Fisiologiche, Università di Pisa, Italy

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 originally 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.

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 H2O. The PCR reaction was carried out on a Gene Amp PCR System 2700 thermal cycler (Applied) 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 Analyser (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 loci were performed using the ARLEQUIN package (Excoffier et al., 2005). Genetic similarities between animals were computed at the population level using the Molkin program (Gutiérrez et al., 2005): molecular coancestry coefficients (Caballero and Toro, 2002), kinship distance, and F-statistics (Wright, 1978). The following parameters were computed at the population level using the Molkin program.

### Results and discussion

All 21 loci were polymorphic and had a total of 135 alleles ranging from 3 (INU030) to 9 (AHTH171). Among the loci studied, the population was found to be in Hardy-Weinberg equilibrium at all the following loci: AHTK211, FH2054, INRA21, INU055 and REN169018. On the other hand, six microsatellites showed significant deviation (AHTH260, CXX279, REN165L03, REN54P11, REN162C04; P<0.01; AHTH130; P<0.05). Mean observed and expected heterozygosities 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 (Fis=0.061±0.024). Similar values were reported by Morera et al. (1997) (Fis=0.085) and by Jordana et al. (1992) in a group of 10 Spanish dog breeds (Fis=0.040). Such moderate values of Fis 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 Fis 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 1. Number of alleles, polymorphism information content (PIC), observed and expected heterozygosity, and departure from Hardy-Weinberg equilibrium for the 21 microsatellite loci.

| Microsatellite marker | No. of alleles | PIC     | Observed | Heterozygosity Expected | P     |
|-----------------------|----------------|---------|----------|------------------------|-------|
| AHT121                | 7              | 0.675   | 0.649    | 0.719                  | 0.198 |
| AHT137                | 8              | 0.602   | 0.740    | 0.643                  | 0.274 |
| AHT1H30               | 6              | 0.602   | 0.597    | 0.668                  | 0.033 |
| AHT1H171              | 9              | 0.693   | 0.675    | 0.743                  | 0.133 |
| AHT1H260              | 7              | 0.418   | 0.364    | 0.444                  | 0.000 |
| AHTK211               | 5              | 0.449   | 0.597    | 0.528                  | 0.410 |
| AHTK253               | 6              | 0.527   | 0.451    | 0.574                  | 0.244 |
| CXX279                | 6              | 0.627   | 0.597    | 0.665                  | 0.008 |
| FH2054                | 8              | 0.690   | 0.714    | 0.740                  | 0.112 |
| FH2048                | 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 |
| REN165L03             | 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 |
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 (s_m=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 (Marietta 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.

References

Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.
Caballeró, A., Toro, M.A., 2002. Analysis of genetic diversity for the management of conserved subdivided populations. Conserv. Genet. 3:289-299.
Ciampolini, R., Cecchi, F., Ciani, E., Mazzanti, E., 2008. Three local cattle breeds from Tuscany (Italy): genetic diversity and similarity. Page 63 (abstr.), Session 8, in Proc. 59th Annual Meet. EAAP Vilnius, Lithuania.
Ciampolini, R., Cecchi, F., Mazzanti, E., Ciani, E., Tacredi, M., De Sanctis, B., 2007. The genetic variability analysis of the Amiata donkey breed by molecular data. Ital. J. Anim. Sci. 6(Suppl.1):78-80.
Ciampolini, R., Cetica, V., Ciani, E., Mazzanti, E., Fosella, X., Marroni, F., Biagetti, M., Sebastiani, C., Papa, P., Filippini, G., Ciani, D., Prescuitti, S., 2006. Statistical analysis of individual assignment tests among four cattle breeds using fifteen STR loci. J. Anim. Sci. 84:11-19.
Ciampolini, R., Moazami-Goudarzi, K., Vaiman, D., Dillmann, C., Mazzanti, E., Fouille, J. L., Leveziel, H., Ciani, D., 1999. Individual multilocus genotypes using microsatellite polymorphism to permit the analysis of the genetic variability within and between Italian beef cattle breeds. J. Anim. Sci. 77:2329-2338.
Ciani, E., Cecchi, F., Bramante, A., Castellana, E., D’Andrea, M., Pasquariello, R., Pane, F., Albenzio, M., Ciampolini, R., 2010. Molecular coancestry and classical genetic distances depict different patterns of relationships among sheep breeds from Southern Italy. Proc. 9th World Congr. on Genetics Applied on Livestock Production, Leipzig, Germany, pp 3-47.
Dalvit, C., De Marchi, M., Zanetti, E., Cassandro, M., 2009. Genetic variation and population structure of Italian native sheep breeds undergoing in situ conservation. J. Anim. Sci. 87:3837-3844.
Dalvit, C., Sacca, E., Cassandro, M., Petrose, P., Plasentier, E., 2008. Genetic diversity and variability in Alpine sheep breeds. Small Ruminant Res. 80:45-51.
D’Angelo, F., Albenzio, M., Sevi, A., Ciampolini, R., Cecchi, F., Ciani, E., Muscio, A., 2009. Genetic variability of the Gentile di Puglia sheep breed on microsatellite polymorphism. J. Anim. Sci. 87:1025-1029.
D’Angelo, F., Ciani, E., Sevi, A., Albenzio, M., Ciampolini, R., Ciani, D., 2006. The genetic variability of the Podolica cattle breed from the Gargano area. Preliminary results. Ital. J. Anim. Sci. 5:79-85.
De Giuliari, C., 2006. Enciclopedia dei Bracchi. De Vecchi Ed., Milano, Italy.
Excolfier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.
Fredholm, M., Wntero, A.K., 1995. Variation of short tandem repeats within and between species belonging to the Canidae family. Mamm. Genome 6:11-18.
Grazewska, I., 2007. Genetic diversity in Polish hounds estimated by pedigree analysis. Livest. Sci. 113:296-301.
Guo, X., Elston, R.C., 1999. Linkage information content polymorphic genetic markers. Hum. Hered. 49:112-118.
Gutiérrez, J.P., Rojo, L.J., Alvarez, I., Goyache, F., 2005. Mollkin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. J. Hered. 96:718-721.
Irion, D.N., Shaffer, A.L., Famula, T.R., Eggleson, M.L., Huges, S.S., Pederson, N.V., 2003. Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. J. Hered. 94:367-374.
ISAG/FAO, 2004. Secondary Guidelines for Development of National Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Recommended Micro-satellite Markers. Recommendations of joint ISAG/FAO Standing Committee. Available from: http://dad.fao.org/en/referlibrary/guidelinemaker.marker
Jordana, J., Piedrafta, J., Sanchez, A., Puig, P., 1992. Comparative F statistics analysis of the genetic structure of ten Spanish dog breeds. J. Hered. 83:367-374.
Kim, K.S., Tanabe, Y., Park, C.K., Ha, J.H., 2001. Genetic variability in East Asian dogs
using microsatellite loci analysis. J. Hered. 92:398-403.

Koskinen, M.T., Bredbacka, P., 2000. Assessment of the population structure of five Finnish dog breed with microsatellites. Anim. Genet. 31:310-317.

Leroy, G., Rognon, X., Varlet, A., Joffrin, C., Verrier, E., 2006. Genetic variabilità in French dog breed assessed by pedigree data. J. Anim. Breed Genet. 123:1-9.

Leroy, G., Terrier, E., Mriaux, J.C., Rognon, X., 2009a. Genetic diversity of dog breeds: within-breed diversity comparing genealogical and molecular data. Anim. Genet. 40:323-332.

Leroy, G., Terrier, E., Mriaux, J.C., Rognon, X., 2009b. Genetic diversity of dog breeds: between-breed diversity, breed assignation and conservation approaches. Anim. Genet. 40:333-343.

Mäki, K., Groen, A.F., Liinamo, A.E., Ojala, M., 2001. Population structure, inbreeding trend and their association with hip and elbow dysplasia in dogs. Anim. Sci. 73:217-228.

Marletta, D., Tupac-Yupanqui, I., Bordonaro, S., Garcia, D., Guastella, A.M., Crisione, A., Cañon, J., Dunner, S., 2006. Analysis of genetic diversity and determination of relationships among western Mediterranean horse breeds using microsatellite markers. J. Anim. Breed. Genet. 123:315-325.

Morera, L., Barba, C.J., Garrido, J.J., Barbancho, M., de Andrés, D.F., 1997. Genetic variation detected by microsatellites in five Spanish dog breeds. J. Hered. 90:654-656.

Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A., Kruglyak, L., 2004. Genetic structure of the purebred domestic dog. Science 304:1160-1164.

Parra, D., Méndez, S., Cañón, J., Dunner, D., 2008. Genetic differentiation in pointing dog breeds inferred from microsatellites and mitochondrial DNA sequences. Anim. Genet. 39:1-7.

Pribánová, M., Horák, P., Schřöffelová, D., Urban, T., Bechynová, R., Musilová, L., 2009. Analysis of genetic variability in the Czech Dachshund population using microsatellite markers. J. Anim. Breed. Genet. 126:311-318.

Tsuda, K., Kikkawa, Y., Yonekawa, H., Tanabe, Y., 1997. Extensive interbreeding occurred among multiple matriarchal ancestors during the domestication of dogs: evidence from inter and intraspecies polymorphisms in the D-loop of mitochondrial DNA between dogs and wolves. Genes and Genetic Systems 72:229-238.

Vilà, C., Maldonado, J.E., Wayne, R.K., 1999. Phylogenetic relationships, evolution and genetic diversity of the domestic dog. J. Heredity 90:71-77.

Vilà, C., Savolainen, P., Maldonado, J.E., Amorim, L.R., Rice, J.E., Honeycutt, R.L., Crandall, K.A., Lundeberg, J., Wayne, R.K., 1997. Multiple and ancient origins of the domestic dog. Science 276:1687-1689.

Wright, S., 1978. Evolution and the genetics of populations. Variability within and among natural populations. University of Chicago Press, Chicago, IL, USA.

Zajc, I., Mellersh, C.S., Sampson, J., 1997. Variability of canine microsatellites within and between different dog breeds. Mamm. Genome 8:182-185.