Genetic characterization of the Bracco Italiano (Italian Hound) breed: first results on 22 STRs from the ISAG Canine Comparison Test

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We wish to thank the President and the Board of Directors of the SABI for having actively promoted the present project among the Society's members.

INTRODUCTION

The Italian Bracco is one of the oldest pointing dog breed, used for hunting ever since the Renaissance time. The breed has been officially registered by ENCI (the Italian Cynological Club) in 1949, when the definitive standard was established. The SABI (Società Amatori Bracco Italiano) is financing a research project aimed at investigating the demographic, genetic and genealogical structure of the breed (the BIGG study). As a part of the project, the complete electronic record of the breed, including 20,499 animals born between 1970 and 2007, was downloaded from the ENCI database. In the present work, we show an assessment of the genetic variability determined for 22 STRs typed in a sample of 33 unrelated Italian hounds, here called "Bracchi", and a sample of 43 dogs from an admixture of other breeds ("Other Dogs"); the latter were included in two consecutive ISAG "Canine Comparison Tests".

ANIMAL SAMPLING

Blood specimens were collected in the course of several meetings organized by the Bracco Italiano breed club. Individual ID codes of all sampled subjects were traced in the breed database, and the coefficient of relationship was determined for all possible animal pairs. When a coefficient of exceeded the value of 0.2, one of the two animals was excluded. Thus, the final sample included only subjects with coefficient of relationship < 0.2 with any other animal.

	N_a		
Locus	**B.I.**	**O.D.**	**Diff.**
AHTk253	5	6	-1
AHT121	6	11	-5
FH2054	6	9	-3
*CXX*279	6	9	-3
INRA21	6	6	0
AHTk211	4	6	-2
REN54P11	5	10	-5
REN162C04	3	9	-6
AHTh260	5	10	-5
AHTh171	7	11	-4
REN105LO3	4	10	-6
AHTh130	5	11	-6
REN169O18	6	13	-7
REN64E19	4	12	-8
REN169D01	7	13	-6
FH2848	6	8	-2
AHT137	8	10	-2
REN247M23	5	12	-7
INU005	4	9	-5
INU030	3	8	-5
INU055	4	7	-3
Average	**5.2**	**9.5**	**-4.3**

Tab. 1

	B.I			O.D.		
	H_{obs}	H_{exp}	Diff.	H_{obs}	H_{exp}	Diff.
	0,455	0,579	-0,12	0,488	0,674	-0,19
	0,667	0,728	-0,06	0,605	0,891	-0,29
	0,727	0,720	0,01	0,791	0,853	-0,06
	0,576	0,712	-0,14	0,744	0,805	-0,06
	0,727	0,786	-0,06	0,605	0,760	-0,16
	0,636	0,511	0,13	0,698	0,784	-0,09
	0,576	0,723	-0,15	0,581	0,857	-0,28
	0,667	0,638	0,03	0,512	0,778	-0,27
	0,364	0,586	-0,22	0,674	0,840	-0,17
	0,697	0,767	-0,07	0,791	0,855	-0,06
	0,303	0,406	-0,10	0,628	0,832	-0,20
	0,545	0,635	-0,09	0,605	0,841	-0,24
	0,697	0,790	-0,09	0,651	0,849	-0,20
	0,515	0,569	-0,05	0,535	0,881	-0,35
	0,576	0,806	-0,23	0,651	0,868	-0,22
	0,515	0,698	-0,18	0,535	0,844	-0,31
	0,727	0,652	0,08	0,698	0,862	-0,16
	0,606	0,607	0,00	0,442	0,831	-0,39
	0,606	0,671	-0,06	0,465	0,752	-0,29
	0,515	0,535	-0,02	0,605	0,770	-0,16
	0,455	0,519	-0,06	0,721	0,813	-0,09
	0,579	**0,649**	**-0,071**	**0,620**	**0,821**	**-0,201**

Tab. 2

HWE P-value		
Locus	B.I.	O.D.
AHTk253	0,099	0,014
AHT121	0,091	0,000
FH2054	0,115	0,179
*CXX*279	0,096	0,032
INRA21	0,202	0,135
AHTk211	0,432	0,237
REN54P11	0,002	0,000
REN162C04	0,485	0,001
AHTh260	0,000	0,040
AHTh171	0,155	0,010
REN105LO3	0,013	0,000
AHTh130	0,448	0,001
REN169O18	0,034	0,001
REN64E19	0,624	0,000
REN169D01	0,000	0,000
FH2848	0,000	0,000
AHT137	0,631	0,001
REN247M23	0,131	0,000
INU005	0,556	0,000
INU030	0,744	0,010
INU055	0,521	0,174

Tab. 3

RESULTS

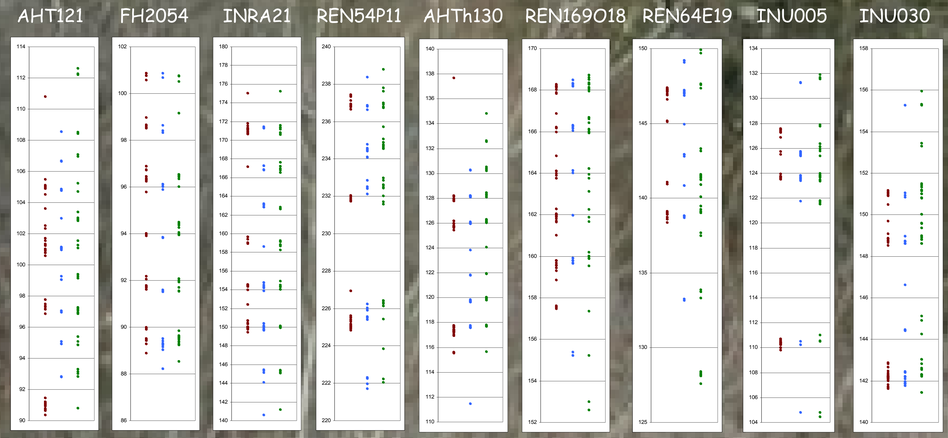
Across the 22 loci, the number of different alleles ranged 3 to 8 (mean 5.2) in the "Bracchi", compared with 6-13 (mean 9.5) in the "Other Dogs" (Tab. 1). This reduction of genetic variability is expected in a single breed when it is compared to an admixture of many different breeds, though a difference of 4.3 allele on average per locus is certainly a remarkable value.

The expected locus heterozygosities ranged 0.41-0.81 (mean 0.65) in the "Bracchi", compared with 0.67-0.89 (mean 0.82) in the "Other Dogs" (Tab. 2), thus confirming a reduction of the average gene diversity (-0.17) in the "Bracchi". Interestingly, the difference between the observed and expected heterozygosities was lower in the "Bracchi" than in the "Other Dogs" (0.65 - 0.58 = 0.07 vs. 0.82 - 0.62 = 0.20), despite the absolute values were lower in the "Bracchi". This result may be interpreted in term of the Wahlund effect, which is expected to be higher in an admixture of different breeds with respect to a single breed, whereas the genetic variability should be lower in the second case.

Analysis of Hardy-Weinberg equilibrium confirmed this notion, as four markers only showed highly significant disequilibrium values (red) in the Bracchi, against 14 markers in the "Other Dogs" (Tab. 3).

GENETIC TYPING

Three multiplexes were worked out (two heptaplexes and one octaplex), which allowed analyzing 22 STR markers from the panels recommended for the 2006 and 2008 ISAG Canine Comparison Test. Allele call was based on a statistical analysis of all the raw data generated by the sequencer (ABI Prism 310), including both the samples provided as a part of the CCT and our B.I. samples. The raw data were graphically plotted to visualize the alignment of the alleles across the different runs, and the allele size in bp was determined using the CCT reference samples as anchor values. The data were analyzed using ARLEQUIN 3.11.



Legend to figure

For each marker, three "lanes" are shown (brown, blue and green). The first refers to the "Bracchi", the others to the two samples of the Comparison test (2006 and 2008, respectively). Each lane includes all the raw data obtained from the sequencer for an entire sample of animals; these graphs consent to appreciate the variability that exists both within and among alleles in each sample, as well as the variability that exists among experiments performed at different times.

Conclusions

The present results show the practicability of a a program aimed at characterizing a particular dog breed by STRs.

Given the genealogical structure of the Bracco Italiano, it is difficult to define a sample of truly "unrelated" animals. However, our final collection of about 80 animals will provide a clear picture of the genetic health of this breed.

A natural extension of this study will be to determine the correlation between the coefficient of relationship calculated from genealogical records for all pairs of animals and their relatedness evaluated by molecular markers.