УДК 636.7.082

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INVESTIGATION ON THE GENETIC VARIABILITY OF THE AMERICAN PIT BULL TERRIER DOGS BELONGING TO AN ITALIAN BREEDER USING MICROSATELLITE MARKERS AND GENEALOGICAL DATA

The genetic variability of 18 American Pit Bull Terriers bred in Italy was studied using 21 STR markers from the panels recommended for the 2006, 2008 and 2010 ISAG canine comparison test and the genealogical information. As expected, all statistical analysis showed a reduced genetic variability. It is therefore recommended greater attention in the programming of mating with an increase of gene flow among farmers, which would reduce the average inbreeding in the population and increase genetic variability.

Introduction. The American Pit Bull Terrier arises from the crossing of the white English Terrier and the Old Bulldog from the end of the nineteenth century and the first Pit Bull arrived in Italy in the late '70s; now in Italy there are about 60 regular farms. The American Pit Bull Terrier is not recognized as a breed by the FCI (Federation Cynologique International) or ENCI (National Italian Kennel Club); the UKC (United Kennel Club) and ADBA (American Dog Breeders Association) are the organizations that recognize the Pit Bull in their records and that follow the selection even if maintaining slightly different morpho-character standards.

In the selection the inbreeding is used as a mating method because it allows to fix the characteristics and traits of the best representatives of a breed.

Nevertheless the employment of few reproducers is able to bring to an excessive increase of the inbreeding so the mortality of puppies can significantly increase [1] and a positive correlation was shown between the frequency of some genetic diseases and the average coefficient of inbreeding [2]. Moreover, purebred dogs often have to deal with genetic diseases and more than 400 genetic diseases are registered in this species [3]. The traditional approach for 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 [4–6]; 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 [7, 8], or microsatellite markers [9–12] or both [13].

In this work we wanted to do a real photo of the genetic variability of the Pit Bull Terriers bred in Italy, using the available genealogical information, and the results of molecular analysis performed on 18 dogs belonging to an Italian herd.

Material and methods. *Animals and Genealogical data*. The research was carried out in 2011 in an Italian Pit Bull Terrier dog herd. A total of 18 dogs were studied. Genealogical data and a blood sample of each animal were acquired. The inbreeding coefficients (F), the number of inbreds and average inbreeding coefficient for each traced generation were performed using the program ENDOG v.4.6 [14]. The number of inbreds, the average inbreeding coefficient and the average coancestry in the 18 dogs were performed using CFC software [15]. The distribution of inbreeding level in the whole population were analysed and eight different class level of inbreeding were considered: $0 < F \leq 0.05$; $0.05 < F \leq 0.10$; $0.10 < F \leq 0.15$; $0.15 < F \leq 0.20$; $0.20 < F \leq 0.25$; $0.25 < F \leq 0.30$; $0.30 < F \leq 0.35$; $0.35 < F \leq 0.40$ [20].

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).

Table 1. **Mean inbreeding by maximum generations considering the whole database**

Traced generation	N. animals	Mean F (%)	% inbred	Average for inbred (%)	Average relatedness coefficient (%)
0	260				0.38
1	122				0.70
2	76	1.64	9.21	17.86	1.23
3	65	7.02	41.54	16.90	1.65
4	48	6.48	39.58	19.37	1.77
5	43	9.17	60.47	15.16	2.07
6	40	11.72	75.00	15.63	2.41
7	31	9.54	64.52	14.78	2.24
8	24	10.91	66.67	16.37	2.41
9	20	9.07	90.00	10.07	2.42
10	11	9.54	72.73	13.12	2.88
11	12	7.86	91.67	8.58	2.77
12	8	9.08	87.50	10.38	2.50
13	6	11.15	100.00	11.15	2.73
14	6	5.57	83.33	6.69	2.59
15	3	7.22	100.00	7.22	2.59

The 21 microsatellites investigated belonged to a markers panel proposed from ISAG/FAO, for the «measurements of Domestic Animal Diversity» (ISAG/FAO 2004) and located in 19 chromosomes. Primer sequences for the microsatellites are available at the Web site (http://dad.fao.org/en/refer/library/guidelin/marker.pdf). The 21 microsatellites were amplified in 5 multiplexes PCR reactions.

Amplification of the five 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_2O. 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).

Genetic similarities of animals were investigated by comparing the individual multilocus genotype of each individual with each other [16]. 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. The following parameters were computed at the population level using the program MolKin (v.2.0) [17]: molecular coancestry coefficients [18], kinship distance, and the mean polymorphism information content (PIC).

Exact tests for deviations from the Hardy-Weinberg equilibrium (HWE), and pair-wise linkage disequilibrium among microsatellite loci and F_{IS} value were performed using the ARLEQUIN package [19].

The molecular coancestry between two individuals, i and j (f_{ij}), is the probability that two randomly sampled alleles from the same locus in two individuals are identical by state [18]. The molecular coancestry 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 (Dk) between two individuals i and j is $D_k = [(s_i + s_j)/2] - f_{ij}$ [18]. MolKin computes within-breed molecular coancestry and D_k by simply averaging the corresponding values for all the within − population pairs of individuals.

Results and discussion. Analysis of the pedigrees of the 18 analyzed dogs shows that the complete database results in 775 dogs (346 males and 429 females), 204 of which were inbreds.

The mean F in the whole database was 3.73 %, that it was a medium value in comparison at what reported on others breeds (3, 5, 20, 21).

The value of 3.73 % higher than 3.125 %, which is the value resulting from the mating of two animals sharing a single grandparent, seemed to suggest a close relatedness among the animals. In fact the coefficient of inbreeding is less than 5 % in 44 dogs, whereas it is more than 20 % (with values that exceeds 40 %) in 55 dogs. Values higher than 40 % correspond to the closest inbreeding, when breeding of brothers with sisters or parents with descendants takes places in several successive generations.

Table 1 shows, in the whole database, the evolution of the average coefficient of inbreeding and the average relatedness coefficient within the different generations. As we can see, the depth of the pedigree was equal to 15 generations.

The inbreeding for each traced generation was high in all generations, with peaks around 11 % in dogs belonging to the 6^{th} and 13^{th} generation, while the average relatedness coefficient was around 2.50 % value starting from the dogs with 6 traced generation.

The percentage of inbreds has an increasing trend with the values greater than 80 % from the dogs with 9 generations traced. Of course the average inbreeding of inbred individuals is higher than the average inbreeding per generation, with a range from 6.69 % in subjects with 14 traced generations up to a maximum of 19.37 % in dogs with 4 traced generations.

Considering instead the 18 dogs the average coancestry and the average inbreeding amounted to 15.10 and 6.84 % respectively.

The value 0.151 is a quite high value, but it is due to the fact that all subjects are related.

The inbreeding coefficients were higher than 6.25 %, i.e. the value resulting from the mating of two animals sharing two grandparents (cousin mating). This is a critical maximum that is not ex-

Table 2. **Number of alleles, locus-by-locus observed (Ho) and expected (He) heterozygosity, PIC and F_{IS} value for the 21 analyzed STR loci**

Marker	N° alleles	Ho	He	P-value	PIC (%)	F_{IS}
AHT121	10	0.722	0.768	0.065	64.13	0.060
CXX279	4	0.722	0.711	0.763	67.13	−0.015
REN105L03	3	0.379	0.402	0.618	34.34	0.057
REN54P11	6	0.716	0.737	0.618	68.10	0.028
ATHk253	4	0.638	0.656	0.127	58.77	0.027
AHTk211	6	0.741	0.884	0.081	69.91	0.162
AHTh171	3	0.511	0.551	0.109	43.82	0.073
INU030	4	0.548	0.533	1.000	44.72	−0.028
INU055	5	0.316	0.571	0.718	34.24	0.447
AHT137	9	0.838	0.829	0.191	81.90	−0.011
FH2848	6	0.727	0.847	0.173	68.18	0.142
REN247M23	3	0.528	0.541	0.074	44.88	0.024
REN169018	6	0.590	0.606	0.327	52.52	0.026
REN169D01	5	0.638	0.676	0.572	58.94	0.056
INRA21	5	0.586	0.615	0.836	53.16	0.047
INU005	5	0.590	0.582	0.800	53.77	−0.014
AHTH130	7	0.722	0.751	0.836	69.15	0.039
REN162C04	6	0.712	0.739	0.512	66.77	0.037
REN64E19	5	0.636	0.661	0.191	57.56	0.038
AHTH260	11	0.781	0.849	0.000	76.50	0.080
FH2054	8	0.802	0.839	0.000	77.40	0.044
Average value	5.42	0.640	0.683			

* Hardy-Weinberg exact test P-value < 0.01.

ceeded when mating principles are applied on many farms.

With the exception of 3 dogs, all sampled subjects were inbred; of these last, 7 dogs have a coefficient acceptable (less than 5 %), while 5 dogs have a high coefficient of inbreeding (between 12.27 and 25.52 %).

Concerning molecular data, all 21 loci were polymorphic and had a total of 103 alleles ranging from 3 (REN105L03, AHTh171 and REN247M23) to 11 (AHTh206) (Table 2). The mean number of alleles per locus was 5.42 (SD = 2.01) and the effective allele number was 3.21 (SD = 1.87). 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 observed by Shinkarenko et al. [11] on the American Pit Bull Terrier, 4.0 alleles/locus as mean value, and on other breeds: Greyhound 2.5 alleles/locus, Labrador Retriever 3.3 alleles/locus, German Shepherd 3.3 alleles/locus [22], Flat-coated Retriever 4.5 alleles/locus, Dachshund 5.6 alleles/locus [23], Andalusian Hound 6.2 alleles/locus, Bracco Italiano 6.4 alleles/locus [12], Spanish Greyhound 6.5 alleles/locus, Maneto 7.0 alleles/locus [24], Czech Dachshunds, 7.6 alleles/locus [25], and twelve East Asian breeds dogs 7.75 alleles/locus [9].

Mean observed heterozigosity was 0.640 ± 0.133 and it was lowest for INU055 (0.316) and highest for AHT137 (0.838) (Table 2).

Seventeen microsatellites out of 21 showed heterozygote deficiency. On average, there was a insignificant deficit of heterozygotes (F_{IS} = 0.063 ± ± 0.013); similar values were reported by Ciampolini et al. (F_{IS} = 0.061) [12], by Morera et al. (F_{IS} = = 0.085) [24] and by Jordana et al. [26] on 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 [34]; however, the F_{IS} values were rather homogeneous among loci, and this evidence points against such an explanation.

The mean polymorphism information content (PIC) was 0.599 with a range of 0.342 (INU055) and 0.819 (AHT137). This parameter was originally introduced by Botstein et al. [28]. It refers to the value of a marker for detecting polymorphism within a 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 [29]; the higher the PIC value, more informative a marker is. In the present study, microsatellites INU055, REN105L03, AHTh171, and INU030 appeared to be only moderately informative (less than 0.50), whereas the other microsatellite loci studied were highly informative. Even in the work conducted by Ciampolini et al. [12] markers INU055 and INU030 appeared to be the least informative.

Genetic similarity within the population (0.412) represented a rather low genetic variability. This value is higher than those reported on other species such as cattle (0.281 [30]; 0.374–0.420 [31]) and sheep (0.318–0.370 [32]), but lower than that reported on Bracco Italiano dog breed (0.455 [12]) and on an endangered donkey breed (0.489 [33]).

With the exception of the values reported on Bracco Italiano dog breed [12] and on Amiata donkey breed [33] the values observed in our study for the mean molecular coancestry (f_{ii} = 0.348), and for the inbreeding coefficient (F_i = 0.357) were clearly greater than that reported in literature on other species such as cattle [31], sheep [32, 34] and horse [35] while the kinship distance (D_k = = 0.330) was smaller than data reported in literature. The observed values highlight that the low level of genetic variation have arisen as a possible consequence of mating among relatives. It's well known that the management of the farms is the reason for high level of inbreeding; in fact breeders often use this mating method with the aim of enhancing desirable traits.

The author consider that 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.

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ИЗУЧЕНИЕ ГЕНЕТИЧЕСКОЙ ИЗМЕНЧИВОСТИ АМЕРИКАНСКИХ ПИТБУЛЬТЕРЬЕРОВ, ВЫВЕДЕННЫХ В ИТАЛИИ С ИСПОЛЬЗОВАНИЕМ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ И ГЕНЕАЛОГИЧЕСКИХ ДАННЫХ

Генетическая изменчивость 18 американских питбультерьеров, выведенных в Италии, была изучена с использованием 21 STR маркеров из панелей, рекомендованных Международной ассоциацией генетики животных (ISAG canine) (2006, 2008, 2010). Как и ожидалось, все статистические анализы под-

твердили невысокую генетическую изменчивость. Поэтому желательно уделять больше внимания планированию скрещиваний с увеличением потока генов, чтобы уменьшить средний инбридинг в популяции и увеличить генетическую изменчивость.

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ВИВЧЕННЯ ГЕНЕТИЧНОЇ МІНЛИВОСТІ АМЕРИКАНСЬКИХ ПІТБУЛЬТЕР'ЄРІВ, ВИВЕДЕНИХ В ІТАЛІЇ З ВИКОРИСТАННЯМ МІКРОСАТЕЛІТНИХ МАРКЕРІВ І ГЕНЕАЛОГІЧНИХ ДАНИХ

Генетична мінливість 18 американських пітбультер'єрів, виведених в Італії, була вивчена з використанням 21 STR маркерів з панелей, рекомендованих Міжнародною асоціацією генетики тварин (ISAG canine) (2006, 2008 і 2010). Як і очікувалося, всі статистичні аналізи підтвердили невисоку генетичну мінливість. Тому бажано приділяти більше уваги плануванню схрещувань із збільшенням потоку генів, щоб зменшити середній інбридинг в популяції і збільшити генетичну мінливість.

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Received 15.03.12