



Paulo Santos Cruz Standard of Fila Brasileiro Breed: codification by DNA markers

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I have always been fascinated about the Fila Brasileiro breed. Born in Governador Valadares city, in the interior of Minas Gerais state, my first contact with these magnificent dogs occurred when I was still a little five years old boy. The second son of two human doctors and living in a big house from a noble neighborhood, I still remember the mixture of fear and admiration that Catucha do Ibituruna used to cause on me. She was a sand color bitch, with strong structure and temperament for home guard, bought by my father from another doctor, the radiologist Dr. Paulo Angotti, owner of the Ibituruna Kennel, certainly one of the best known in the breed. In one of the CAFIB shows held in the city, in 1984, Catucha got the first prize in the puppy category. It was the first stimulation for me to continue, until today, preserving that bloodline and working as an tireless activist in the conservation of this so representative Brazilian native breed: a genetic-historic-cultural inheritance of our country. Fortunately, the Fila Brasileiro breed has also found important collaborators around the world.

CAFIBE, *Club of Fila Brasileiro friends in Spain*, have outstand itself as a defender association of the breed and shall have its exemplar organization, commitment, communication among members and concession of good specific literature access through the bulletins, followed by other clubs with similar objectives. This is why I am honored and satisfied in starting my collaboration to it, writing and interpreting technical-scientific articles which are of great interest for, us dog lovers and breeders. As a veterinary doctor, with deeper and deeper knowledge in animal reproduction and genetics, I will comment extremely important aspects to the preservation of genetic resources, specially through the very novel scientific researches I have been working in with the Fila Brasileiro breed, as part of my PhD studies at the Veterinary School of the Federal University of Minas Gerais, Brazil.

Nowadays, the utilization of molecular techniques to genetic characterization of different species, bloodlines and/or populations is a reality. Besides offering a high precision, they permit the generation of a great number of data that have no influence, neither from the environment nor from the development condition of the animal. Among the markers used with this aim, microsatellites are the outstand ones. They are also known as STR (Sequence Tandem Repeats). These markers have multi-allelic nature, codominant inheritance, abundant and spacious distribution along the genome. The mutation tax of the microsatellites is sufficient high to detect genetic variability even in familiar relationship situation. At the same time, it is low to keep a strong stability from generation to generation

and, this way, they permit the definition of highly informative and stable genetic profiles to discriminate bloodlines and individuals.

All the genetic information of a live organism is kept in its DNA sequences that can be accessed by the microsatellites markers. There is small variation (polymorphisms) when nucleotide sequences of individual's DNA are compared, even in the same specie or breed. The analysis of this variation (election markers) by laboratorial methods that determine the DNA sequences makes possible, among others, the classification of this live organism in its respective breed, the genetic identification for legal purposes and forensic and the identification of genome areas responsible for genetic diseases.

Microsatellites are the reference markers recommended by the *International Society for Animal Genetics* (ISAG) for parentage tests, because of its high specificity and accuracy in determining important polymorphic variables present in the genome of evaluated individuals. It's to say, they permit genetically identify an individual like a fingerprinting in humans. Still, they permit, through the analysis of animal specific DNA points, to identify when it belongs or not to a specific breed. This way, it was essential that a pioneer and very well conduced scientific work could identify the main genetic frequencies in the *Paulo Santos Cruz standard* to the Fila Brasileiro breed, with the aim of establishment of the basis in which the discrimination of dogs, carrying strange genes to this *stem genetic pool*, could be done with the maximum efficiency. So, the amplification and optimization of an orientated conservation and multiplication process for these correctly identified dogs (fitting in the PSC genetic standard) will exclude, from reproduction, the mixbred ones.

In general it is a consensus among Fila breeders that the dog breeds that share (shared) most common characteristics to our breed and that have had contributed in a more emphatic manner to its formation would be the old Mastiff, the old English Bulldog and the Bloodhound. By the other hand, the proportion of these genes, the environmental factors printed by the geographical isolation in Brazilian territory (essential to the selection of the most adapted ones) and the timing of this genetic odyssey would be forever forgotten if there was no commitment of engaged researchers looking for genetic and historical answers. Fortunately, we can say that these researches have already begun and that precise answers are walking on firm steps to our direction.

That was the reason for me, even without grants offered by research fomentation entities, to ask collaboration of traditional breeders and/or the ones compromised with the preservation of pure bloodlines. Part of them conceived material for their dogs' DNA extraction (blood and/or hair bulbs) and paid for the exams. These tests were relatively expensive because of the lack of demand in specialized laboratories. Many other exams were paid by me. Nowadays, after the creation of a demand and better standardization of the tests, with the great support of the *Genetic Laboratory of Veterinary School, at the Federal University of Minas Gerais* (Belo Horizonte, Brazil), we could perform the same analysis for lower prices. This will assure total guarantee and traceability of puppies and registered litters obtained from now. This is already mandatory for the Brazilian horse breed Mangalarga Marchador, as an exigency of the Brazilian Government.

The analysis that have originated the paper presented at the *XXXI International Society for Animal Genetics Conference*, held in Amsterdam on July 20-24th were based on excellent origin and type bloodlines (the article can be read at *ISAG-2008 Proceedings* in ISAG's website and in the *CAFIBE Bulletins* n.8, 2008). The dogs representing these bloodlines were bred or conserved by Brazilian breeders. The main bloodlines analyzed were: *Ibituruna, Tabayara, Alto Quatis, Amparo, Fazenda Mundeó, São José da Lapa, Jaguará, Engenho Velho, Gameleira, Jawa, Caramonã, Príncipe do Oeste, Engenho*. These bloodlines represent significant fraction of the pure Filas available in Brazil nowadays, even finding in some of them parental errors in pedigrees (what is serious and reprehensible but is less harmful to the breed than the introgression of genes from other breeds, said ancestors of the Fila Brasileiro or not).

The paper was recognized and recommended as superior quality by two respected Brazilian Government Entities. For that reason, all the costs for my participation in ISAG-2008 Conference were granted by the *Illustrious Animal Science Pos-Graduation College of the Veterinary School at Minas Gerais Federal University (CPGA / EV-UFMG)* and *Improvement Coordination for Superior Level Researchers (CAPES)*.

As it was demonstrated, the Fila Brasileiro breed has average five alleles per *loci* evaluated by microsatellite markers recommended by ISAG. This number is enough to determine in 99.99% of the cases incorrect pedigrees when both genitors are evaluated and in 100%, the individualization of siblings in the same litter. It became impossible the so known pedigree frauds when presentation of these DNA tests is required for litter registration.

Related to the average endogamy (inbreeding) of the breed, thought to be high due to the utilization of famous sires and dams in many of the evaluated bloodlines, it was determined a 2.9% index. This value, analyzed in association with the average observed heterozygosity ($H_o=0.60$), seems to be relatively low. The reason for it is that, even keeping in the produced litters the characteristics of *Paulo Santos Cruz Standard*, many of the dogs said to be sons and grandsons of great champions are not. It is like we have been working since long ago with pure animals, but with *Initial Register*. There was no selection but multiplication of pure dogs (concluding with success the main objective purposed by the Father of the Breed, Dr. Paulo Santos Cruz, a founder of the *Clube de Aprimoramento do Fila Brasileiro – CAFIB*, in 1978). Looking contradictory, the quality of these litters was only sustained in Brazil and in countries that imported these bloodlines analyzed in our research, because of a high capacity of conserving breeders to select and breed pure animals, even when information in pedigrees did not reflect sometimes the reality. This has significantly damaged the conserving process.

Many other breeders in Brazil and in the whole World did not get success in the task of multiplying pure dogs. They utilized in their breeding (deceived or not) animals from horrible origin, absolutely out of the standard sculpted by Nature and precisely described by the Father of the Breed and, now, also genetically codified. This resulted in significant genetic drift, with introgression of strange genes to the already fixed breed, and also in a significant loose of quality of dogs produced after the endings of the 70's. Many of them were registered as Filas by entities of the official cinology.

Only more detailed genetic studies to be done from now can precisely determine how close our *Paulo Santos Cruz Standard* Fila Brasileiro, bred in different regions of Brazil, is to the other ones bred in our country and around the World. In my trip to Czech Republic, some weeks before the Amsterdam Conference, with the priceless and typical of a *Born Conserver Fileiro* help from my friend Jarda Pörs, I have collected DNA from Filas bred in that country for statistical comparison with our Genetic Data Bank. Soon, we will have material from many other countries too. This is the unmistakable manner to keep the quality control of the *Paulo Santos Cruz Standard* Fila Brasileiro, also known as *Pure, CAFIB Type or Original*.