GENETIC VARIABILITY OF BRAZILIAN BUFFALOES: FROM THE INTRODUCTION TO THE MOLECULAR MARKERS

[1]MARQUES, J. R. F.; ALBUQUERQUE, ~~EGITO~~, M. S. M.; A. A.; MARIANTE, A. S.; COSTA, M. R.

[1]Embrapa Amazônia Oriental, Trav. Dr. Enéas Pinheiro, s/n,
CEP. 66.095-100 – Belém PA. E-mail: marques@cpatu.embrapa.br

ABSTRACT

The genetic variability reflects the differences among the organisms, which occur due to variations in the environment and/or alterations in the genotype of the individuals, in this case, being transmitted to future generations. The use of molecular markers has contributed a lot to the development and enhancement of these concepts and also for the verification of the proposed models. It also permits the determination of the genetic value of an animal as well, with high precision, even before the expression of its phenotype. The production of an animal is the result of its genetic value (genotype) with the characteristics of the environment. The domestic buffaloes in the world are classified according to many authors as pertaining to the *Bubalus bubalis* species, with two subspecies, *Bubalus bubalis bubalis* – the river buffalo and *Bubalus bubalis kerabao* – the swamp buffalo. In general, the species *Bubalus bubalis*, known worldwide as water buffalo, is described with three subspecies: a) *Bubalus bubalis*, variety *bubalis* – is the domestic buffaloes, presenting karyotype 2 = 50 chromosomes, called river buffalo, including the herd of India, Pakistan, China, Turkey and many European and American countries, even the Italian ones, which also belong to this subspecies; in Brazil it is represented by the breeds Jafarabadi, Mediterraneo and Murrah, all known by Brazilian Association of Buffaloes Breeders - BABB; b) *Bubalus bubalis*, variety *kerebao* – the buffalo found in Malaysia, Indonesia, Philippines, Ceylon, Thailand and others countries, presenting karyotype 2 = 48 chromosomes; in Brazil it is represented by the breed Carabao. It is known in the world literature as swamp buffalo; and c) *Bubalus bubalis*, variety *fulvus* – the native buffalo of the Northeast region of India, especially from Assam, living generally in the wild or semi-domesticated. It is an animal of smaller size when compared to the previous subspecies. Its tone of color is brownish or reddish, having a resemblance to the Baio type from Brazil, without having any scientific proof. The Brazilian buffaloes are four breeds officially known by the BABB are: Carabao, Jafarabadi, Mediterraneo and Murrah. The Baio type can be added to them, probably belonging to the group Murrah, with a small number of animals all over the country nowadays. At the end of the last century many import transactions of buffaloes were made for our country, although the number of animals were small, mainly in the Murrah and Jafarabadi breeds. Molecular markers is a very important tools for elucidate this aspect. But, there aren't many references with molecular markers in buffaloes, there are relevant works involving the morphologic, biochemical and molecular markers, comparing different species as in the case of the domestic Buffaloes. This way, the genetic markers can really shorten the paths to be followed, especially at this first moment with the similarity information among the herd and/or animals to be used as parents in the future generations. In order to determine which crossbreeding will have better responses and which animals inside a herd are genetically superior. The molecular markers associated with the productive characteristics, will also be the most secure indicators to improve the buffaloes breeding programs.

Key words: breedrs, buffalo, genetic variability, molecular markers

INTRODUCTION

The genetic variability reflects the differences among the organisms, which occur due to variations in the environment and/or alterations in the genotype of the individuals, in this case, being transmitted to future generations. It has a fundamental role to the evolution of the species, since the

natural selection acts in the differences that exist among the individuals of a population, allowing the breeding success of those that are more adapted to the environment where they live. Therefore, the bigger the variability in the population, the greater is the chance of perpetuation and/or survival, as the success in enhancement programs.

This way, any strategy of handling domestic animals or the preservation of natural populations would be inefficient without genetic information regarding the existing allele variability, among and inside these populations (13). Nevertheless it is important to know the distribution of the variability, the genetic structure of the populations and their breeding efficiency, since the genetic variability is correlated with the reproductive system.

The production of an animal, for example, is the result of its genetic value (genotype) with the characteristics of the environment where it lives, as follows: the diet, climatic variations, sanitary handling, among others. However, there are differences in even in animals with the same gene group inside a species. These differences might occur due to little changes/mutations at DNA level or more specifically, in the nucleotide sequence, which constitutes the genetic information or the genetic inheritance of each individual.

The genetic diversity is a concept that has been applied in two different ways: the first refers to the richness of present species inside an ecosystem (22, 42) and the second refers to the level of heterozygosity of a population obtained from the allele frequencies (31). This value corresponds to the expected quantity of heterozygous and shows a level of genetic variation of a population in a certain species.

Besides the heterozygosity other diversity indexes have been used to evaluate, characterize and compare the levels of genetic variation in the populations, as the percentage of polymorphic loci and average number of allele per locus (2).

Gregor Mendel, in 1865, marked the beginning of the use of morphologic characteristics in order to observe the transmission mode of the characters. In the case of animal genetics, the traditional enhancement with the methodology of genetic evaluation of the population, through the methods of the quantitative analysis, produced a significant advance in the selection process, impelling the gains of animal production to an outstanding level. It became more evident after the performance tests, by comparing the productive data of an animal or its progeny and/or its collaterals. In this case, it is extremely important to have reliable data obtained from the productions of the own animal and/or its relatives, which in some cases might demand too much effort and time.

In the studies of the populations' genetics where most of the work are based on concepts taken from experimental observations, theoretical models were developed (6) for the analysis and interpretation of the results. However, due to the development of biochemical techniques and molecular genetics, the relation between the biological and genetic diversity is being now better explored and investigated.

The use of molecular markers has contributed a lot to the development and enhancement of these concepts and also for the verification of the proposed models. It also permits the determination of the genetic value of an animal as well, with high precision, even before the expression of its phenotype, which has, according to literature (38), allowed significant advance in the knowledge of the genetic and biology of organisms, providing tools that help the manipulation of the genotypes. This way, desired results can be obtained (superior genotypes) in genetic enhancement in a shorter time. For example: the allele B of α- casein of the milk is associated with a greater concentration of protein in the milk. Then, the selection of animals with this allele will result in a more profitable milk to be used in cheese production. The great differential is that by using DNA markers it will be possible to verify if the animal will synthesize the referred allele before the milk production starts as well as indicate if the animal is homo or heterozygous for a specific characteristic in question. It is important to attempt to the fact that in the case of the heterozygous, the presence of recessive allele can be detected by the study of progeny or by the analysis of DNA. Taking it all into consideration there is a great saving in time during the process of selection, permitting a true revolution in the real genetic progress, since it changes even the concept of interval of generations, which is very difficult in the case of long life-term animals as the buffaloes.

At a later time, systems of molecular markers based on the PCR (Polymerase Chain Reaction), became available (28, 37). PCR is a technique of lots of resources that allows the enzyme synthesis *in vitro* of millions of copies of a specific DNA segment, using a polymerase DNA.

DNA markers like RAPD, microsatellites and AFLP have been used in the evaluation of genetic diversity. A great advance in the area of molecular markers happened after the technique known as RAPD (Random Amplified Polymorphic DNA), developed independently by two groups of researchers (43). In this method shorter primers of unrestricted sequence are used to direct the amplification of regions in the genome of the individual. RAPD allows us to obtain a number virtually unlimited of molecular markers, covering all the organism genome and can be used in the study of genetics and enhancement as well as in determining genomic fingerprints (29). Its target sequence is unknown (Ferreira & Gratapaglia, 1996), contrary to others that require information about the sequence of the target DNA, for the drawing of specific primers, what allows the performance of studies of genetic analysis in many species (44).

The polymorphism generated could have been caused by a single change of base, which prevents a stable association with the primer by changes that either alter the size (little insertions or deletions) or prevent the amplification of fragments (insertions that separate the sites in more than 3000 bp, inversions, deletions) (40).

This technique can detect many polymorphisms; it is simple, easy, fast and needs small quantities of genomic DNA (17); it doesn't use radioactive material, being the electrophoresis made in agarose gel and the products colored with bromide of etidio. The polymorphisms are inherited in mendelian pattern and can be generated in many species (9, 17, 43), without the need of changing the technique (Williams, et al., 1990; Welsh et al., 1993; Fairbanks et al., 1991) and without the knowledge of the informative DNA sequence; they can be used as genetic markers as well (9, 43).

RAPD is considered a "dominant" marker. A segment that is homozygous for the presence of the band can't be distinguished from a heterozygous, that is, when observing the presence of the band in the gel, it is not possible to determine if it was originated from one or two copies of the amplified sequence. Besides, the presence of the fragment is dominant when related to the absence. Many loci can be evaluated simultaneously, but little information about each locus is obtained, since RAPD obtains only one allele *per* locus (10).

Due to problems in the purity of DNA caused by different extractions, it is necessary the optimization of the quantity of DNA used in the different experiments so that these ones can be reproducible and present good amplifications (5, 45) or, yet the problems could have been caused by the competition among the amplifiable sites.

Although the RAPD technique results in a series of advantages since it presents a wonderful simplicity and speed, it is very sensitive to little modifications in the components concentration of the reaction, being able to produce alterations in the pattern of the obtained markers. Because of that, it is recommended a careful optimization of the experimental conditions in order to obtain reproducible and reliable amplifications.

On the other hand, microsatellites, also called SSRs (Simple Sequence Repeats) or STRs (Short Tandem Repeats) (21), are parts of the genome made by the repetition of a short and simple sequence of nucleotides, which can be of duo, triple or tetra nucleotides (39, 41). Polymorphism of DNA is based on these short sequences of repetition in tandem, where each repetitive unity has one to six nucleotides (11). These regions frequently show wide multi-allele variations in these numbers of repeated copies, constituting themselves into highly informative genetic markers. The polymorphisms are generated by the number of repetitions of a determined sequence, being the length of the allele determined by PCR. Primers that flank the repetitive sequence are used to do so. The differences in the number of these sequences can be easily distinguishable and their variations are inherited as alleles of a simple genetic locus (4).

The main characteristics of the microsatellites are: high frequency, wide dispersion by the genome, high mutation rate that they are subjected to, their codominance and mendelian segregation. Being very frequent and randomly distributed, they allow a complete coverage of any eucharyot genome. They are also easy to be identified, of single locus, able of being automatized, and presenting adequate

mutation rate to be used as genetic markers. They have been widely used in intensive mapping studies in domestic species of animals (4,15) and according to such characteristics (Ron, 1993), the microsatellites are too close to be the ideal markers for the genetic analysis.

The microsatellites markers offer a high resolution discrimination between highly related populations, inside of the same species (4), being also able to be used in close animal species, by the use of heterologous primers, as in the case of bovine and buffaloes species (4,26).

The great limitation of the technology of microsatellites is the enormous quantity of work necessary for the prior development of the markers. During the process of obtaining the pairs of primers that amplify repetitive regions of microsatellites, steps of abundant characterization of different repetitive motives (motifs) are necessary, libraries construction, sequencing of DNA and wide triage (selection) of the built primers, aiming to identify more informative loci. However, once they're developed, these primers can rapidly become available to the scientific community (12).

The molecular markers have their main role as auxiliary tools in animal genetic enhancement; especially in the assisted selection by markers, in which the characteristics are difficult to be measured, as well as in those with low inheritability, which will have considerable gains in a near future. It can determine or not the success of the undertaking in the market, because it will be possible to study the similarity among breeds/populations; set breeding programs; select genetically superior animals, and also set markers related to specific characteristics that are economically important.

GENETIC VARIABILITY OF BUFFALOES FROM BRAZIL

In order to adequately set the distribution of a specific characteristic in a population, it is necessary to know its average and its variance or variability (Figure 1). It is understood as variability the dispersion around the average of a specific characteristic.

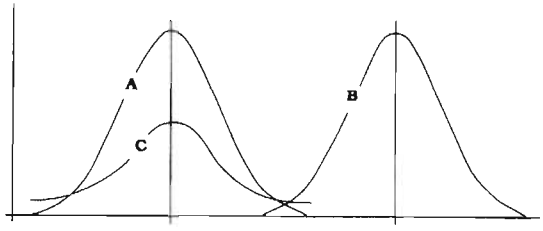

Figure. 1 – Normal curve representing different populations (A, B and C), showing the variation in dispersion around the average.

The variability is measured by the variance (V) that can be shown by the formula:

$$\frac{\Sigma(X - X)^2}{n - 1}$$ where:

$\Sigma(X - X)^2$ = deviation of the characteristic around average;

n = number of observation.

The representation PHENOTYPE = GENOTYPE + ENVIRONMENT shows, in practical terms, all the essence of the variability and its interactions with the environment, which are impossible of being dissociated. Therefore, if the environment was equal, all the variability would be from genetic or genotypic origin (VG); if the genotypes were equal, all the variability would be environmental (VE). In practice, it is very difficult to happen, and in fact, the (VT) is equals the result of adding (VG) and (VE), that is:

VT = VG + VE

If the genotype was constant, as in the case of twins or clones, then:

VT = VE

Nevertheless, what happens in fact is that the quantitative characteristics are the result of the action of many different genic loci, which relate among themselves in the following ways:
a) ADDING - with two variations: 1- There is no great difference among the genotypes and 2- There are gradations between the extremes; In this case, there aren't dominants or recessives, it counts the value of each gene which is transmitted to future generations. It is important to highlight that in the adding case, Transgression might happen, that is, when breeding alike individuals, there are possibilities of obtaining extreme descendants, for example: two mulattos can have white or black offspring. Still in this case, when there isn't dominance and the environment doesn't suffer alterations, the average of descendants is the same of the parents.
b) DOMINANCE – There are expressive dominant genes and recessive ones. The answer to the crossing of homozygous will be AA, Aa and aa, not being transmitted to future generations;
c) INTERACTION AMONG GENES – There are two cases to be considered: 1- when there is epistasis, that is: allele of different loci or two or more loci contribute to the same phenotype and 2- when there is OVER DOMINANCE or SUPER DOMINANCE (same locus allele), being the heterozygous different from the homozygous and superior to it.
It is difficult, or even almost impossible to exist the situation of GENOTYPE + EXCELLENT ENVIRONMENT + PERFECT INTERACTIONS and, as consequence of the relationship among the genes, that is, of the adding effect, dominance and interactions, the genotypic variance (**VG**) can be subdivided into:
Va – Variance caused by the adding effects of the genes
Vd – Variance caused by the dominance effects among the genes
Vi – Variance caused by the interaction effects among the genes
These aspects of variability is better explicit by the origin of Brazilian buffaloes breeds, however the studies about this are scarce. When analyzing the origin, the imports that formed the primary herds and the work that has already been done so far, it is possible to infer important data and tendencies for the evaluation of variability and consequently, indicate the paths for genetic enhancement programs of the buffaloes in Brazil.
The domestic buffaloes in the world are classified according to many authors as pertaining to the *Bubalus bubalis* species, also called water buffalo, with two subspecies, *Bubalus bubalis bubalis* – the river buffalo and *Bubalus bubalis kerabao* – the swamp buffalo. Such classification was elaborated based on cytogenetic, biochemical and taxonomy studies (8, 14, 23). The "African buffaloes", which are in fact Cape Buffaloes as they belong to the *Syncerus caffer* species, subdivided into two subspecies, *S. caffer caffer* and *S. caffer nanus*, are wild animals that still live in big herds in the African meadows. In the same way, the animals of the group *Anoa* that live only in Sulawesi islands, Indonesia, are also called buffaloes due to their resemblance to domestic buffaloes. However, they belong to a different species: *Anoa depressicornis*, with three subspecies: *Anoa depressicornis depressicornis* (from wetlands like the Amazon plains), *Anoa d. ferqusoni* (from mountainous areas) and *Anoa d. quarlesi* (from "quarles") (Dolan, 1965). The classification of the group *Anoa* as water buffalo is controversial according to authors who have been studying the species (8, 14).
The facts about the origin of Brazilian buffaloes, the imports that happened at many moments and their zoologic classification were commented by literature (23) and in general, the species *Bubalus bubalis*, known worldwide as water buffalo, is described with three subspecies: a) *Bubalus bubalis*, variety *bubalis* – is the domestic buffalo or Indian, presenting karyotype 2 = 50 chromosomes, called river buffalo, including the herd of India, Pakistan, China, Turkey and many European and American countries, even the Italian ones, which also belong to this subspecies; in Brazil it is represented by the breeds Jafarabadi, Mediterraneo and Murrah, all known by Brazilian Association of Buffaloes Breeders - BABB; b) *Bubalus bubalis*, variety *kerebao* – the buffalo found in Malaysia, Indonesia, The Philippines, Ceylon, Thailand, etc., presenting karyotype 2 = 48 chromosomes; in Brazil it is represented by the breed Carabao or Rosilho (term not in use anymore; pretty much used in the past in Marajó island, referring to the color of its coat). It is known in the world literature as swamp buffalo; and c) *Bubalus bubalis*, variety *fulvus* – the native buffalo of the

Northeast region of India, especially from Assam, living generally in the wild or semi-domesticated. It is an animal of smaller size when compared to the previous subspecies. Its tone of color is brownish or reddish, having a resemblance to the Baio type from Brazil, without having any scientific proof.
In India there is a great diversity of domestic Buffaloes gathered in five big groups, according to the Table 1, below.

Table 1 - Main breeds groups of river buffaloes from Indian.

GROUPS	BREED
Murrah	Murrah, Nili, Ravi and Kundi
Gujarat	Surti, Mehsana and Jafarabadi
Uttar Pradesh	Bhadawari and Tarai
Centre of India	Nagpuri, Pandhirpuri, Manda, Jaragi Kalahadi and Sambalpur
South of Índia	Toda and South Kanara

The Brazilian buffaloes are four breeds officially known by the BABB are: Carabao, Jafarabadi, Mediterraneo and Murrah. The Baio type can be added to them, probably belonging to the group Murrah, with a small number of animals all over the country nowadays. Following, in agreement (24) the characteristics brazilian buffaloes breeds are:
Carabao: It is known as the "living tractor from Orient" .It was one of the first introduced in the country (Chart 02), having the aptitude to produce meat and for traction work. It is a very important work tool in the swampy countries of Asia, especially because of the work developed in the rice plantations. In Brazil today, there are few herd of these animals, being considered a genetic group facing extinction and/or loss of characterization. The largest population of this breed is concentrated in Marajó Island, in the Pará state.
Its characteristics according to the BABB are: head in the front-nasal position, rectilinear skull outline, with rectilinear chamfron or featheredge as well. Horns: long, big and strong, of triangular section, emerging sideways from the head growing in a horizontal position to the outside and then to the back and upward. Its hair is dark gray or reddish, having light or white spots on the paws, in the shape of one to three collars around the neck, right below the jaw and next to the chest, in the shape of round and parallel stripes, plus light tufts on the superior orbital arches, on the commissural lips and on the abdomen. It shows a nervous temperament, but not brave and was introduced in Brazil during the last century, through Marajó island, multiplying itself by uncontrolled breeding, especially with the Mediterranean breed. A herd of this breed, living in the Bank of Animal Germplasm of Eastern Amazon (BAGAM), in Marajó Island, Salvaterra – Pará state, is one of the only pure in the country. The critical number of the primary herd and the uncontrolled breeding, put the genetic group about to be extinct and lose its characterization. It has sizes that go from medium to large and the main body measurements (cm) are: Average weight in adult females of 550kg and males of 750kg; anterior height – 132; posterior height – 130; body length – 158; hindquarter length – 38.33; hindquarter width – 24; Thoracic perimeter – 207 and scrotum circumference – 27. It is an animal to be bred in open areas, flooded and/or swamped, in a extensive system, presenting great capacity of weight gain, being invincible when it comes to moving throughout these areas due to its already adapted anatomy. When breeding with other buffaloes breeds, it shows dominant phenotype related to particularities as: lighter hair in the pastern, near fetlock joint, lips and neck areas, as well as body conformation. It also shows the great heterosis, understood by the precocity of the F1. Even in closer herds one cannot notice big phenotypic variation, without the regularity in the upcoming of hereditary diseases, showing homogeneity in its genetics group.
Jaffarabadi: Because of its size, in the West, it can be considered as a meat producer by the ones who aren't too aware, however, it shows great performance in dairy competitions, as in its origin country, India, where it's possible to find excellent female breeders, with good levels of dairy production. The main inconvenient of this breed is its daily diet, since they are more demanding animals due to their size. It comes originally from the forest of Gir, peninsula of Kathiavar, west of India. It is characterized by the peculiar shape of the head with long horns, fallen heading upward. It is considered to be of a mix aptitude, meat and milk, it is the heaviest of the buffaloes. However, it shows the lowest efficiency of carcass

(around 46%). The hair is black and well defined. In Brazil, there are two very distinct varieties, the buffalo Gir, more delicate and with light bone structure, which used to live in the forest regions, and the Palitana, an animal with a heavier bone structure, having a big cuirass in the front region, being used as a defense instrument given by nature, and put there imposed by the need of protecting themselves from lions, which lived in great numbers all over the Indian prairies. According to BABB, the pattern of Jafarabadi's in Brazil has the following breed characteristics: the head in front-nasal position has an ultra-convex skull outline and rectilinear to sub-convex chamfron or featheredge. Its horns are long, strong and thick, with triangular or oval section, heading back and down with the final curvature going upward and inside, harmoniously placed with the skull outline.

The average weight for males is from 500kg to 1200kg (sometimes reaching 1500kg) and for females from 450kg to 900kg. The hair has strong correlation between the color of the fur and the skin all over the body, being the fur and the skin black. The breed Jafarabadi must be raised in good grazing areas, respecting its size. It shows great variability in the phenotype, having varied gradations in the form, when submitted to breeding between the two varieties. It strongly transmits breeding characteristics, especially the size and the bone structure, giving a special feature to low expressive herds.

Mediterranean: Originally from Italy, it is a breed with double aptitude (milk and meat), although the breeders use the Brazilian ones more often for meat production. Theses animals have medium size and are rather compact. Because of their origin, they are also known as black or Italian buffalo, imported throughout times, from Italy to Marajó island, and later to many parts of the country. They have an intermediate appearance between the Murrah and the Jaffarabadi, also with black hair. The Mediterraneo breed pattern in Brazil, according to BABB, is: head in the front-nasal position, with convex skull outline and rectilinear to sub-concave chamfron or featheredge. The average weight for females is 550kg and for males 750kg. They have hair with strong correlation between the color of the fur and the skin all over the body, being the fur and the skin black. The black color goes all the way to the horns, hooves, nasal-mirror and apparent mucous membrane. It is a "walking" breed, adapting well to extensive conditions, as well as to the more controlled breeders, where it becomes a great dairy producer. It has great phenotypic variability, showing good adaptation to natural selection process, from many introduced genetic groups many centuries ago in the Paludine regions of south Italy. The primary herd in Brazil had a considerable number of animals, showed by the innumerous import transactions made by Para's breeders, especially giving a variability that is often evident in the body conformation and dairy production, that is, those which are results of strong interactions between the genotype and the environment. The latest introductions from Italy, based on known germplasma, will create an amplification in the genetic variability of the Brazilian herd base, even allowing the measurement of the production through the genetic progress of the productive characteristics, especially meat and milk.

Murrah: This breed comes originally from the North of India. It is a breed with double aptitude (milk and meat), too. In Brazil it was uniformly distributed through many regions, especially in the more intensive breeding and /or controlled systems, adapted to the quieter mood of these animals. The name Murrah means snail in Hindu, clearly referring to the animal's horns. It has animals with medium and compact conformation. The head is light and short spirally horns that curve into rings at the level of the skull. They are thorough animals with good digestive capacity, very important characteristics for the dairy producers. Its breed pattern in Brazil, according to BABB, is: head in the front-nasal position, with rectilinear skull outline or slightly sub-convex and rectilinear to sub-concave chamfron or featheredge. The average weight for males is from 450kg to 900kg and for females from 350kg to 700kg. They have hair with strong correlation between the color of the fur and the skin all over the body, being the fur and the skin black. The black color goes all the way to the horns, hooves, nasal-mirror and apparent mucous membrane. The extremity of the tail can be white, black or mixed. It is a quiet breed, adapting very well to the more controlled and intensive conditions, being a great dairy producer. In the closer herds, it has a better body morphology conformation, but with little variability of form. On the other hand it has great phenotypic variability in the productive aspects, showing great environmental interference as well. The primary herd in Brazil has a narrow base, such fact is demonstrated by the few import transactions made, however, in many cases it was expanded by the introduction of genes of the Mediterranean breed, which gives a variability that many times is clear in the body conformation. When closed, the genetic group Murrah

presents many congenital problems (atresia, paralyzation, spasms, etc.) resulting from endogamy, being the direct reflect of the little variability that exists in the genetic base of the Brazilian Murrah herd. Some new introductions, called "new option", tend to increase this base and consequently the variability of the breed. The origin of this germplasma is unknown, making a more deep and concrete analysis of the future generations more difficult.

Baio Type: It is not considered a breed. There are only a few specimen in Brazil. Some authors refer to this genetic group as animals from Assam, India, belonging to the subspecies *Fulvus,* without any scientific proof for such idea. It was introduced in Brazil in 1961/62 by the Leão Farm, Alagoas. Today, there are few Baios in Brazil, being the herd of BAGAM/EMBRAPA, Marajó Island, one of the only existing in the country. In Brazil, there is little information about this group, being Pará the only probable state to have information about the performance of these animals. They are catalogued by the FAO/UNEP among the groups facing extinction, being kept in preservation programs. The adult males have an average weight of 750kg and the females 550kg, with average length and height of 139cm and 133cm respectively. They have brownish color, baia hair and have double aptitude (milk and meat). Their breed pattern can be summarized like this: light head and rectilinear to slightly concave and median outline; median to large body; median to big and large hindquarters; median and strong limbs.

The import and the entering of Buffaloes

At the end of the last century many import transactions were made, according to the Table 2.

Table 2 – Summary of importation transactions of buffaloes to Brazil

YEAR	***ORIGIN***	BREED	PLACE	***FARM***	BREED
1895	Itália	Mediterrâneo	Marajó	Dunas	V. C. Miranda
1900	Trynnidad	Carabao	Marajó	Dunas	V. C. Miranda
1902	Itália	Mediterrâneo	Marajó	-	V. C. Miranda / B. Lobato
1906		Mediterrâneo		Dunas	V. C. Miranda
1907	Índia / Hamburg	Murrah / Baio	Usina Leão-AL	-	Leão Irmãos S.A.
1908	Europa/ Búfalos Indianos	n.d.	South - Paraná	-	Casa Herm. Stoltz & Cia, Karl Hauge Beck, Hamburgo *
1907-1918	Itália	Mediterrâneo	Almeirim - PA	Arumandub a	José Júlio de Andrade
1918	Índia	Jafarabadi	Uberaba- MG	-	Virmondes Martins Borges
1920	tália	Mediterrâneo	S. Paulo	Sta. Marília	Conde Fco Matarazzo
1921	Índia	Jafarabadi Murrah	Sta. Rita de Cássia	Cidreira	Antenor Azevedo / Moacyr de Melo Azevedo
1948	Itália	Mediterrâneo	S.M. Arcanjo - SP	-	Umberto Yemma
1961/1962	Índia	Murrah ** / Mediterrâneo	Londrina and Uberaba	-	Celso Garcia / Cid / Torres Homem R. da Cunha / Rubens de Andrade Carvalho / Veríssimo Costa Jr.

RESULTS WITH GENETIC MARKERS

Although there aren't many references in this area about buffaloes, there are relevant works involving the morphologic, biochemical and molecular markers, comparing different species as in the case of the domestic Buffaloes, the African Syncerus caffer and the Indonesian Anoas.

Using protein markers, studied 334 Buffaloes of the breeds Murrah, Jafarabadi, Mediterraneo and half-breeds, from the region of Vale do Ribeira-SP(20). The locus of the albumin was very informative, suggesting that the allele Alb (A) is probably a breed marker, since it didn't appear in the animals of Murrah pattern. In the grouping analysis two main clusters were observed: one of them including all the populations of Murrah pattern and the other with Jaffarabadi and

Mediterraneo pattern. The results confirm the importance of using protein markers in the study of variability, what is essential for genetic enhancement programs.

Compared zebu animals Gir and Nelore with the Murrah herd of Brazil(7). For this study blood samples were genotyped in the loci of k-casein and of β-lactoglobulin, being identified the variants A and B in both loci, after amplification of DNA by the polymerase chain reaction (PCR), followed by the digestion by the restriction enzymes Hind III and Hph I. Genetic variation was detected in the bovine breeds, however, no polymorphism in the buffaloes breed.

Analyzing the gene cytochrome B of the mitochondrial DNA (mtDNA), (19) studied the genetic variability of buffalo groups from the East and Southeast of Asia. In this study water Buffaloes, swamp Buffaloes and three subspecies of the group *Anoa depressicornis from Indonesia* were used, (*A. depressicornis* of low lands; *A. ferqusoni* from mountainous areas *and A. quarlesi* from "quarles"). After the analysis the authors concluded that each of the big groups has one specific mtDNA, being the genetic divergence between the river and swamp Buffaloes of 2,67%; the genetic divergence between the water (river and swamp) and Anoa Buffaloes of 3,3%. Inside the Anoa group the genetic divergences were 1,2% between the *Anoa depressicornis ferqusoni X Anoa depressicornis quarlesi;* 3,6 % between the *Anoa depressicornis depressicornis X Anoa depressicornis ferqusoni* and 3,3% between *Anoa depressicornis quarlesi X Anoa depressicornis quarlesi.* The authors found evidences to confirm the taxonomic classification of an Asian buffalo group, with four lineages, that is: river Buffaloes, swamp Buffaloes, Anoa from flooded lands and Anoa from the mountains with the quarles. They also said that the genetic divergence among these four groups was lower than the one found in genus and subgenus level, inside the subfamily Bovinae. These results show that the mtDNA of water and Anoa Buffaloes didn't diverge in genus level, in contrast with some proposals of taxonomic classification. The estimations of genetic distance and the diversity or variability of the species are used as parameters for the characterization of populations. In order to do that, the molecular biology have been proving tools that make the study of genetic variability possible in regions of codifying and non-codifying DNA. The polymorphic systems, determined by different methodologies, are classified in locus type I and type II (32). In this last group, the simple sequence repeats (SSR) also called microsatellites are highlighted (21), which are consisted by small repeated sequences in tandem. These amplified loci via PCR were also called "STMS – Sequence Tagged Microsatellite Sites" that is sites of microsatellites marked by sequence and constitute the most polymorphic class of molecular markers available nowadays. The acronym SSRP (Simple Sequence Repeat Polymorphisms) is also commonly found in literature (10).

Regarding the microsatellites, (3, 18) stand out that up to the present moment specific markers for the buffaloes species haven't been developed, however, a great number of loci of microsatellites have been characterized in bovines and used in many works with Buffaloes. The same way, (33) were analyzed the genetic diversity in fragmented Syncerus populations in the south of Africa, through the variation of seven loci microsatellites using isolated primers to *Bos Taurus.*

The literature, (27), tested a great number of primers microsatellites developed for bovines and determined the parameters of amplification in ewes and in river and swamp Buffaloes. From the 80 loci tested 56 showed polymorphic for buffaloes.

Were, studied the applicability of autosome microsatellite markers of bovines for molecular analysis in African Syncerus, concluding that a great number of these markers are polymorphic tc these animals, being applicable in genetic studies of populations (16).

In an analysis, using the technique of RAPD, were analyzed the genetic divergence in four genetic groups: Murrah, Mediterraneo, Carabao e Baio Type (1). By the generated dendogram from the analysis of 33 polymorphic markers, it was observed that: a) the Carabao animals gather separately from the others, suggesting that this can really be considered as a different subspecies and b) the Murrah, Mediterraneo and Baio Type animals gather in a large cluster what might indicate that they belong to the same subspecies *Bubalus bubalus bubalis.*

In India, (34) while working with Mehsana Buffaloes, made genetic analysis with DNA microsatellites markers, using seven markers for the characterization of the breed. The number of alleles per loci varied from four to seven and the average heterozygosity of 0.40 to 0.92.
The literature (25) while evaluating the productive potential and the adaptability of buffalo populations in different environments, estimated the genetic diversity of Italian and Greek herds based on the frequency of nine loci polymorphic microsatellites: CSSM43, detected alleles per locus varied from two (D21S4) to thirteen (CSSM47). The frequency of allelic distribution was similar in the two populations, which have alike alleles, of high frequency in all the loci, except in CSSM47 and CSSM60. The average diversity of the loci was 0.60 what increased according to the number of alleles. The average heterozygosity observed was 0.167 and 0.177 in the Italian and Greek buffalo populations, respectively. Finally, the degree of differentiation between the two groups was moderate, being estimated in 0.021 ± 0.009.
One hundred and eight microsatellite primer pairs, originally identified from cattle, were evaluated by literature (30) for their applicability in river buffaloes. Eighty-one primer pairs (75%) amplified discrete products, and of these, 61 pairs (56%) gave polymorphic band patterns on a panel of 25 buffaloes. The main number of alleles per polymorphic marker was 4.5 ± 0.20, and the mean heterozygosity per polymorphic marker was 0.66 ± 0.02. Successful genotyping of buffaloes using cattle specific primer suggests that the latter can be a valuable resource for genome analysis in bubaline species.

CONCLUSIONS

The molecular markers are the tools for every assisted selection at the moment and cannot be dissociated from the genetic enhancement programs, being important to help answering the frequently asked questions of the breeders, that is: How to improve the herd?; which breed should I use? Or, Is it worth to crossbreed the buffaloes breeds? It is important to say that before anything, the breeder must provide adequate diet and appropriated sanitary handling, so that the genotype or the hereditariness of the animals are expressed in the form of milk or meat.
In practical terms, you should only use in the genetic enhancement programs the animals that have the best productivity indicators, providing satisfactory genetic gains, that is, the DEP's and or PTA's for the most important characteristics. How can you do that in practical terms? - using the Summary of Bubaline Bulls, according to literature (35) from UNESP – Botucatu/BABB, the breeder can choose animals with no family relations and/or with a little connection and inseminate the females or obtain young descendants of the selected animals, following the indications of the Summary.
This way, the genetic markers can really shorten the paths to be followed, especially at this first moment with the similarity information among the herd and/or animals to be used as parents in the future generations. In order to determine which crossbreeding will have better responses and which animals inside a herd are genetically superior. The molecular markers associated with the productive characteristics, will also be the most secure indicators to improve the buffaloes breeding programs.

REFERENCES

(1) ALBUQUERQUE, M. S. M., MARQUES, J. R. F., GASPAROTTO, C. R., MARTINEZ, A. N., EGITO, A (2000). A.Preliminary evaluation of genetic distance among four buffalo populations conserved on Marajó island (State of Pará - Brazil) In: **Proc.** GLOBAL CONFERENCE ON CONSERVATION OF DOMESTIC ANIMAL GENETIC RESOURCES, 5.Brasília, CENARGEN/EMBRAPA.
(2) ALFENAS, A.C.; PETERS, I. ; BRUNE, W.; PASSADOR, G.C (1991) **Eletroforese de Proteínas e Isoenzimas de Fungos e Essências Florestais**. Viçosa, UFV, 242p.
(3) BARENDSE, W., VAIMAN, D.; KEMP, S. J. et. Al (1990). **A medium-density genetic linkage map of the bovine genome**. Mammalian Genome 8, 21-8. 1997. BECKMANN, J. S.; SOLLER, M. Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellites sites. Biotechnology 8, 930-932.

(4) BARKER, J. S. F (1994). A global protocol for determining genetic distances among domestic livestock breeds. In: Wolrd Congress on Genetics Applied to Livestock Production, 5: 501-508. **Proceedings**...Guelf.

(5) CAETANO-ANOLLÉS, G.; BASSAN, B.;GRESSHOFF, P.M (1991). **DNA amplification fingerprint using very short arbitrary oligonucleotide primers**. Bio/technology 9:553-7.

(6) CROW, J.F.; KIMURA, M (1970).**An introduction to population genetics theory**.Harper International Edition, New York. 591p.

(7) DEL LAMA, S. N.; ZAGO, M. A (1996). Identification of the κ-casein and β-lactoglobulin genotypes in Brazilian *Bos indicus* and *Bubalus bubalis* populations. **Braz. J. of Genetics**. 19(1):73-77.

(8) DOLAN, J. M (1965). Breeding of the lowland anoa, Bubalus (Anoa) depressicornis in the San Diego Zological Garden. **Zoological Gaug.** 30:241-8.

(9) FAIRBANKS, D.J.; ANDERSEN, W.R.; MAUGHAM, P.J (1991). **Analyses for Biological resource characterization: a laboratory manual**. Provo: Brigham Young University. 27p.

(10) FERREIRA, M.E.; GRATTAPAGLIA, D (1996). **Introdução ao uso de marcadores moleculares em análise genética**. Brasília: EMBRAPA-CENARGEN, 220p.

(11) FRIES, R (1993). **Mapping the bovine genome: methodological aspects and strategy.** Animal Genetics, 24:111- 116.

(12) GAIOTTO, F.A (2001).**Inferências sobre herança quantitativa e estrutura genética em populações naturais de Euterpe edulis Mart**. Utilizando marcadores microssatélites. Piracicaba,122p. Tese (doutorado)- ESALQ.

(13) GRATTAPAGLIA, D.; CIAMPI, A. Y.; GAIOTTO, F.A (1997). **DNA technologies for forest tree breeding and conservation. In: Recent advances in biotechnology for tree conservation and management**, Florianópolis, Brazil p.50-61.

(14) GROVES, C. P (1969). Systematics of the Anoa (Mammalia, Bovidae). **Zoological Museum of the Univ. of Amsterdan**.17 (7):1-12.

(15) HETZEL, J (1993). **Livestock genome research on march**. *Nature* Genetics 4 : 327-328.

(16) HOOFT, W. F. van; HANOTTE, O.; WENINK, P. W.; GROEN, A. F.; SUGIMOTO, Y.; PRINS, H. H. T.; TEALE, A (1999). Applicability of bovine microsatellite markers for population genetic studies on African buffalo (*Syncerus caffer*).**Animal Genetics**, 30: 214-220.

(17) HU, J.; QUIROS, C. F (1991). Identification of broccoli and cauliflower cultivar with RAPD markers. **P. Cell Reports**. 10: 505-511.

(18) KAPPES S.M.,; KEELE J.W.; STONE R.T (1997). A second-generation linkage map of the bovine genome. **Genome Research.** 7: 235-49.

(19) KIKAKAWA, Y.; YONEKAWA, H.; SUZUKI, H.; AMANO, T (1997). Analysis of genetic diversity of domestic water buffaloes and anoas based on variations in the mothoconfrial gene for cytochrome b. **Animal Genetics**, 28:195-201.

(20) LARA, M.A.C.,OLIVEIRA.J.S., BUFARAH,G., CONTEL, E.P.B (1999). Caracterização de rebanhos bubalinos (*Bubalus bubalis*) através de polimorfismos protéicos. REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 36, 1999. Porto alegre. **Anais**.Porto Alegre: SBZ, 1999. In: Reunião da sociedade Brasileira de Zootecnia, 1999, Porto Alegre. **Anais** da XXXVI Reunião Anual da Sociedade Brasileira de Zootecnia. Porto Alegre: Sociedade Brasileira de Zootecnia.

(21) LITT, M.; LUTY,J.A (1989). A hypervariable microsatellites revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. **America Journal of Human Genetics**, 44: P.398-401.

(22) LUDWING, J.A.; REYNOLDS, J.F (1988). **Statistical ecology**. John Wiley & Sons, N.Y. 337p.

(23) MARQUES, J.R.F., JORGE, W., RAMOS, A.A. (1991). Cytogenetics of domestic buffaloes (*Bubalus bubalis L.*). **Ciência e Cultura**. São Paulo: SBG - SBPC, 43(516): 230 - 235.

(24) MARQUES, J. R. F (2000). BÚFALOS; **O produtor Pergunta a Embrapa responde**. Brasília: Embrapa Amazônia Oriental/SCT. 176 p. (Coleção 500 perguntas 500 respostas).

(25) MOILI, B.; GEORGOUDIS, A.; NAPOLITANO, F.; CATILLO, G.; LUCIOLI, S.; LIGDA, Ch.; BOYAZOGLU (2001). Genetic diversity between Italian and Greek buffalo populations. **Agri**, 29:31-40.

(26) MOORE S. S.; SARGEANT L. L.;KING, T.J.;MATTICK, J. S.;GEORGES, M.& HETZEL, D. J. S (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. **Genomics** 10: 654-660.

(27) MOORE, S. S.; EVANS, D,; BYRNE, K.; BARKER, J. S. F.; TAN, S. G.; VANKAN, D.; HETZEL, D. J. S (1995). A set of polymorphic DNA microsatellites useful in swamp and river buffalo (Bubalus bubalis). **Animal Genetics** 26: 355-359.

(28) MULLIS, K. & FALOONA, F (1987). Specific synthesis of DNA *in vitro* via a polimerase catalyzed chain reaction. **Methods in Enzymology**, 55: 335-350.

(29) MUTHALI, M.; FOR-LLOYD, B. V.; NEWBURY, H. J (1992). The random amplification of polymorphic DNA fingerprinting plants. *PCR* **Methodos and Applications**, 1: 274-276.

(30) NAVANI, N.; JAIN, P. K.; GUPTA, S.; SISODIA, B. S.; KUMAR, S (2002). A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*). **Animal Genetics**, 33:149-54.

(31) NEI, M (1973). Analysis of gene diversity in subdivided populations. **Proceedings** of the National Academy of Sciences of the United States of America, 70(12): 3321-3323.

(32) O` BRIEN, S. J. *et. Al* (1993). Anchored reference loci for comparative genome mapping in mammals. **Nature Genetics**. 3: 103-112.

(33) O` RYAN, C., HARLEY, E. H., BRUFORD, M. W., BEAUMONT, M>, WAYNE, R. K; CHERRY M. I (1998). Microsatellite analysis of genetic diversity in fragmented South African buffalo populations. **Animal Conservation**. 1,85-94.

(34) PUNDIR, R. K.; SAHANA, G.; NAVANI, N. K.; JAIN, P.K.; SINGH, D. V.; KUMAR, S.; DAVE, A. S (2000). Characterization of Mehsana buffaloes in india. **Agri**. 28:53-62.

(35) RAMOS, A. A.; BERGMANN, J. A. G (2002). PROMEBUL; **Sumário de touros bubalinos**. Botucatu, FMVB/UNESP. 21p.

(36) RON, M.; BAND, M.: WYLER, A. *et. Al* (1993). Unequivocal determination of sire allele origin for multiallelic microsatellites when only the sire and progeny are genotyped. **Animal Genetics**, 24: 171-176.

(37) SAIKI, R. K.; GELFAND, D. H.; STOEFFEL, S.; SHCARF, S. J.; HIGUCHI, G.; HORN, G. T.; MULLIS, K.; & ERLICH, H. A (1988). *Primer*-directed enzymatic amplification of DNA with a thermostable DNA polomerase. **Science**, 239: 487-491.

(38) SOUZA, A.P (2001). **Biologia molecular aplicada ao melhoramento**. In: NASS, L.L.; VALOIS, A.C.C.; VALADARES-INGLIS, M.C. (Ed.) Recursos genéticos & melhoramento. Cap. 2: 939-965.

(39) TAUTZ, D.; TRICK, M.; DOVER, G.A (1986). Cryptic simplicity in DNA is a major source of genetic variation. **Nature**. 322: p. 652-656.

(40) WAUGH, R.; POWELL, W (1992). Using RAPD markers in crop improvement. TIBTECH. 10: 186-191.

(41) WEBER, J.L.; MAY, P.E (1989). Abundant class of human DNA polymorphisms which can be typed using polymerase chain reaction. **American Journal of Human Genetics**. 44: 388-396.

(42) WOLDA, H (1981). Similarity indices, sample size and diversity. **Oecologia**, 50: 296-302.

(43) WELSH, J.; PERUCHO, M.; PEINADO, M.; RALPH, D.; Mc CLELLAND, M (1993). Fingerprinting of DNA and RNA using arbitrarily primed PCR. In: PCR II – A practical approach. Eds. Taylor, G.; McPhearson, M.; Hames, D. IRL Press, Oxford, New York.

(44) WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI, J. A.; TINGEY, S. V (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Research**, 18: 6531-6535.

(45) WILLIAMS, J. G. K.; HANAFEY, M. K. RAFALSKI, J. A (1993). TINGEY, S. V. – Genetic analysis using random amplified polymorphic DNA markers. **Methods in Enzymology**, 218: 704-740.