

Genetic variability in Mediterranean buffalos evaluated by pedigree analysis

Variabilidade genética na raça bubalina Mediterrânea avaliada por meio de análise de pedigree

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ABSTRACT

The objective of this research was to study the population genetic structure of four herds of Mediterranean buffaloes in Brazil. It was used pedigree data from 6,588 buffaloes of Mediterranean breed born from 1980 to 2002. Of the total number of animals studied, 60.5, 15.3 and 2.1% had a pedigree in the first, second and third ascendancy, respectively. The effective number of herds that provided breeding males was 1.60 for parents, 1.16 for grandparents and 1.00 for great-grandparents. There were 923 founder animals and only 71 effective founders. The effective number of ancestors explaining the genetic variability of the population was 71 and only 30 ancestors accounted for 50% of the genetic variability of the population. The average relatedness coefficient (AR) between individuals and inbreeding (F) of the population were estimated at 0.37 and 0.34% respectively. The average estimate of generation interval was 8.71±2.85 years. The variability of the current population is the result of a few ancestors, who are mostly also founders showing that the population was developed from a narrow genetic base which characterizes the occurrence of founder effect.

Key words: *Bubalus bubalis*, inbreeding, population genetic structure, founders, ancestors.

RESUMO

O objetivo deste trabalho foi estudar a estrutura genética populacional de bubalinos da raça Mediterrânea, em quatro rebanhos, no Brasil. Foram utilizados dados de pedigree

de 6.588 bubalinos da raça Mediterrânea nascidos no período de 1980 a 2002. Do total de animais estudados, 60,5; 15,3 e 2,1% possuíam pedigree na primeira, segunda e terceira ascendência, respectivamente. O número efetivo de rebanhos que forneceram machos reprodutores foi de 1,60 para pais; 1,16 para avôs e 1,00 para bisavôs. O número de animais fundadores foi 923 e o número efetivo de fundadores foi apenas 71. O número efetivo de ancestrais que explicaram a variabilidade genética da população foi de 71 e somente 30 ancestrais explicaram 50% da variabilidade genética da população. Os coeficientes médios de relação (CR) entre os indivíduos da população e de endogamia (F) foram estimados em 0,37 e 0,34%, respectivamente. A estimativa média do intervalo de gerações foi de 8,71±2,85 anos. A variabilidade da população atual é fruto da contribuição de poucos ancestrais, que são, na sua maioria, também fundadores, evidenciando que a população se desenvolveu a partir de uma base genética estreita, o que caracteriza a ocorrência do efeito fundador.

Palavras-chave: *Bubalus bubalis*, endogamia, estrutura genética populacional, fundadores, ancestrais.

INTRODUCTION

Four buffalo breeds are officially recognized by the Brazilian Association of Buffalo Breeders: Mediterranean, Murrah, Jaffarabadi (river buffalo) and Carabao (water buffalo). All breeds are of Asian origin.

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Out of a global population of greater than 194 million individuals (FAO, 2010), recent estimates suggest that there are about 1.2 million buffalos distributed across all Brazilian states (IBGE, 2010).

In spite of the world buffalo population has increased by approximately 18 million over the past 10 years, showing annual increase about 1.13% (KUMAR & SINGH, 2010), in Brazil the buffalo population has undergone major expansion, perhaps surprisingly, with higher growth than cattle population in recent years (MALHADO et al., 2007).

According to VAZ et al. (2003), buffalo breeding increased in places where cattle breeds do not have the same productivity, and this could be related mainly to the rusticity of the animals, well adapted to low fertility soils and swampy areas. On the other hand, buffalo breeding programs are almost absent in Brazil and the few existing initiatives in this direction are associated with the tenacity of a small number of researchers and breeders.

SANTANA JR et al. (2011) and MALHADO et al. (2012) studying Murrah breed in Brazil reported problems in population structure, mainly small effective size, bottle-neck and low genetic variability. In spite of few parameters estimated by MALHADO et al. (2008) and MARCONDES et al. (2010), the information about the population structure of Mediterranean breed raised in Brazil is scarce.

Studies based on pedigree information shows some disadvantages such as low completeness of genealogical information and pedigree errors, which are common in animals of production (GELDERMANN et al., 1986; RON et al., 1986). On the other hand, pedigree information is an important tool to study the genetic structure of the populations, having the advantage of simplicity and low cost compared to the use of molecular markers (MALHADO et al., 2009). Thus, the objective of this research was to study the population genetic structure of Mediterranean buffaloes in Brazil, generating information that will guide expansion and/or genetic improvement programs of the breed.

MATERIAL AND METHODS

The data used derived from pedigree information of 6,588 Mediterranean buffaloes, born between 1980 and 2002 in four farms located in the states of Rio Grande do Sul, Parana, Sao Paulo and Bahia. The program Endog (GUTIÉRREZ & GOYACHE, 2005) was used for pedigree analysis and parameter estimation based on the probability of gene origin. Average coefficient of inbreeding, average relatedness coefficient, generation intervals, effective number of

founders and the effective number of ancestors were also estimated. The effective number of founders is the number of animals with an equal contribution that would produce the same genetic variability found in the population. The classical approach in determining the effective number of founders was given by:

$$f_e = 1 / \sum_{k=1}^f q_k^2 . \text{ Where: } f_e = \text{effective number of founders;}$$

q_k = probability that the gene is originated from the founder k .

The effective number of ancestors represents the minimum number of animals (founders or not) needed to explain the complete genetic diversity of the population. The determination of the effective number of ancestors was performed by computing the marginal contribution of each ancestor by:

$$f_a = 1 / \sum_{j=1}^a q_j^2 . \text{ Where: } f_a = \text{effective number of ancestors;}$$

q_j = marginal contribution of an ancestor j (not necessarily founder), i.e., the genetic contribution of an ancestor that is not explained by an ancestor previously chosen.

Inbreeding coefficient (F) was calculated using the algorithm proposed by MEUWISSEN & LUO (1992). The average relatedness coefficient (AR) estimates both inbreeding and individual co-ancestry (GUTIÉRREZ et al., 2003). This parameter was calculated using an algorithm as described in GUTIÉRREZ & GOYACHE (2005).

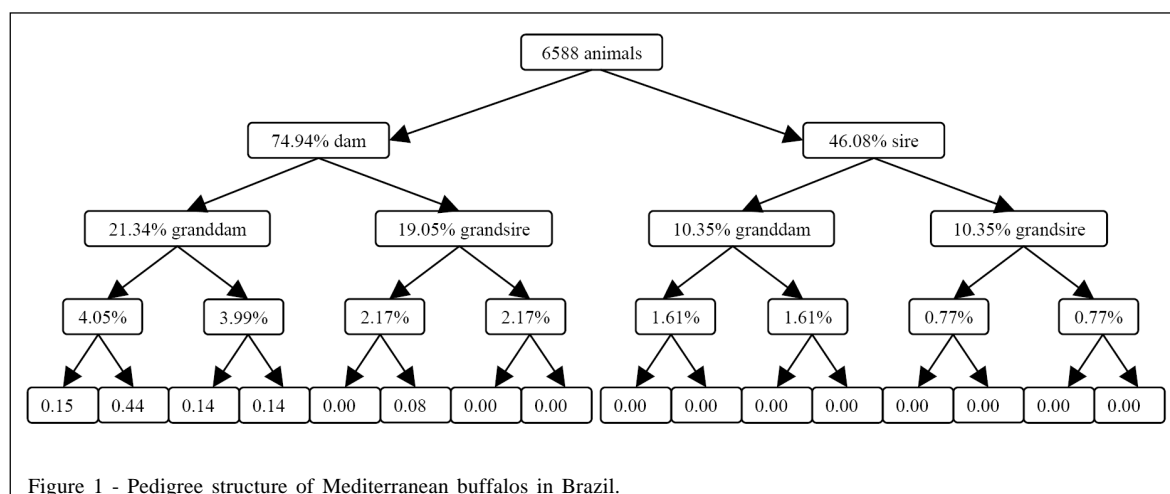
The average generation interval was estimated for the four pathways: sire-son, sire-daughter, dam-son and dam-daughter.

The main founders and/or ancestors were ordered in a decreasing manner based on their CR and contribution values, respectively, by means of PRO SORT of SAS (2002). Animals' ranking of CR and contribution was submitted to correlation analysis using the Spearman correlation of PROC CORR of SAS (2002).

RESULTS AND DISCUSSION

Percentage of known ancestors at the first, second, third and fourth generation were 60.51, 15.27, 2.14 and 0.05% respectively (Figure 1). It is important to point out the large loss of information passing from the first to the second generation; in fact 39.49% of the animals had no known father and mother and 84.73% did not have grandparents in the pedigree.

The effective number of herds providing breeding males was 1.60 for parents, 1.16 for grandparents and 1.00 for great-grandparents



confirming thus, the data of pedigree completeness. This information is important because all the estimated parameters are a consequence of this structure, and the more complete the pedigree the more accurate and reliable are the estimates.

There were 923 founder animals and, considering the half-founders, for which only one parent (2,601 animals) was known, the equivalent number of founder animals was 3,524, accounting to 53.49% of the population analyzed. The effective number of founder animals and ancestors was 71 (2.73% of the equivalent number of founders), and only 30 ancestors accounted for 50% of the population genetic variability (Table 1).

The effective number of founders recorded for the Mediterranean buffalo in this study was close to that found by MARCONDES et al. (2010) in study on breeds Murrah and Mediterranean; whose effective number of founder animals and ancestors was 48.

Lower genetic variability were reported in Murrah breed by SANTANA JR. (2011) and MALHADO et al. (2012) when observed a small number of founders (19 and 17, respectively) explaining 50% of the genetic diversity of the population. In a study with Murrah and Mediterranean buffaloes, as well as with their crosses, MARCONDES et al. (2010) found an even lower genetic variability when the first 20 ancestors accounted for 69.10% of all variation in the population.

The effective number of founders was well below the total number of animals belonging to the founder population. This result is due to the fact that few males were used as breeders (83 animals) over the years in relation to the herd basis.

The Spearman correlation (classification) of founder animals and/or ancestors was high (0.95) and

statistically significant ($P < 0.01$). This high degree of association indicates that most of the founder animals are also ancestors, and as the effective number of founders and ancestors is small. Founder effect is clearly evident in the studied population of buffaloes.

The founder effect is due to the creation of a population from a small number of individuals. The main result is the reduction of the effective size, genetic variation within populations, high homozygosity, and consequent loss of alleles by genetic drift. According to CARNEIRO et al. (2006) when using populations with small effective sizes in selection processes, the results can be greatly influenced by genetic drift, which can cause large variations in genetic gains.

In the present study the founder and ancestor of greater importance was the animal 778, a male, with an average relatedness of 3.4%, which explained 41% of the variability of the breed (Table 2). It was observed that the imbalance between the contribution due to the ancestors and founders is related to the large number of descendants left by these animals when compared to the average offspring per breeder in the population (36.58 for males and 4.26 for females). It is noteworthy to point out that of the top ten founders and ancestors only one was female, which contributed with five descendants, while the first founder and male ancestor contributed with 258 descendants.

The contribution of the first ten founders was high (28.86%) which leads to reduction of genetic variability. A more critical situation was found by MARCONDES et al. (2010) in herds of Murrah, Mediterranean and their crosses, in which the first ancestor, male and female, were responsible, respectively, for 9.5% and 9.4% of the total variability and the first five ancestors contributed with 41.8 and

Table 1 - Parameters characterizing the concentration of origin of a gene in the Mediterranean population: effective number of herds supplying breeding males, real and effective number (in parentheses) of founder herds, real and effective number of founder animals and effective number of ancestors.

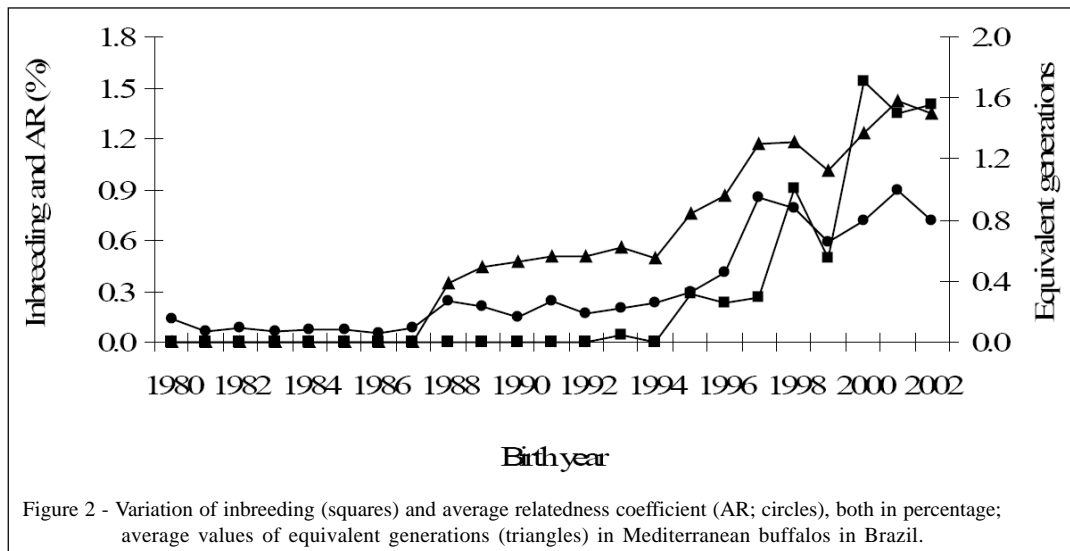
Total population	6588
Number of founder animals in the reference population	923
Effective number of founder animals in the reference population	71
Number of founder animals with one or both unknown parents	3609
Equivalent number of founder animals (one unknown parent = average founder)	2601
Number of animals in the reference population	2979
Number of ancestors in the reference population	915
Effective number of animals in the reference population	71
Real number of founder herds	5
Effective number of founder herds	3
(Effective) number of herds that produce parents	5 (1.60)
(Effective) number of herds that produce grandparents	2 (1.16)
(Effective) number of herds that produce great-grandparents	1 (1)
Number of ancestors that account for 50% of the variability	30

37.0%, indicating large use of few animals. According to these authors, the lower the number of founder animals, the greater the need for monitoring of inbreeding of the studied herd, looking for tools to control it or even the introduction of animals that do not have direct relationship with the principal ancestors identified in the population.

The average relatedness coefficient (AR) between individuals was estimated at 0.37% and the highest individual coefficient was 34.52%. AR was relatively stable from 1980 to 1987, with annual values of less than 0.10% (Figure 2). Between 1988 and 1996 the average relatedness coefficient presented gradual growth ranging from 0.24 to 0.41%. The highest values

Table 2 - Relationship of the most important ten founders and ten ancestors of the population structure of Mediterranean buffaloes. It was described the identification of sire and dam, sex, birth year, the average relatedness coefficient (AR) and the contribution, both in percentage.

Founder	Sire	Dam	Sex	Year of Birth	AR	Offspring
778			M	1982	3.4	258
568			M	1980	1.5	62
586			M	1980	1.5	100
807			M	1985	1.4	117
652			M	1984	1.1	118
16			M	1983	1.0	87
1893			M	1984	0.9	94
804			F	1982	0.9	5
1150			M	1988	0.8	96
359			F	1980	0.8	1
Ancestor					---Contribution---	
778			M	1982	7.41	258
2399	586	359	M	1988	3.37	188
807			M	1985	3.14	117
586			M	1980	3.00	100
652			M	1984	2.33	118
16			M	1983	2.22	87
1893			M	1984	2.00	94
804			F	1982	1.94	5
1150			M	1988	1.81	96
1126			M	1988	1.64	90



were found from 1997 to 2002, when AR presented an average of 0.76% and maximum value of 0.90% in 2001.

The average inbreeding coefficient (F) and the average F only for the inbred animals were 0.34 and 16.24% respectively. It is worth mentioning that 57 animals presented an F coefficient of 25.00% and the highest value found was 37.5% observed in only one animal. The average coefficient of inbreeding from 1980 to 1994 showed a value of zero from 1980 to 1994, except for 1993 (0.04%), followed by an abrupt increase by 2002 reaching the maximum value of 1.54% (Figure 2), the increase of inbreeding and the average relatedness coefficient was directly related to the increase in the number of equivalent generations. The low values of inbreeding should be explained by a lower probability of finding common ancestors with low the pedigree completeness level (MALHADO et al., 2012). Thus, probably the inbreeding coefficient and average relatedness coefficient may have been underestimated due to the small completeness pedigree.

According to QUEIROZ et al. (2000), a better knowledge and control on the genealogy allows a more accurate calculation of F and AR, with consequent increase in their values. Knowledge of more generations of an individual increases the possibility of an important ancestor appearing several times in the pedigree, which may explain this result.

Estimates of the generation interval for the four gamete passages, for the whole pedigree of the Mediterranean buffaloes, were 8.79 ± 2.29 (sire-son), 9.59 ± 2.80 (sire-daughter), 7.42 ± 3.04 (dam-son), 7.94 ± 2.7 years (dam-daughter), with an average interval of 8.71 ± 2.85 years. The generation intervals observed in this study were large but lower than those observed by MALHADO et al. (2008) evaluating the population

structure of Mediterranean buffaloes, when there was an average generation interval of 11.3 ± 4.3 years. SANTANA JR. et al. (2011) reported generation intervals lower than those found in this study, which were 7.38 (sire-son), 7.42 (sire-daughter), 6.60 (dam-son), 6.44 (dam-daughter), with an average interval of 6.89 years.

The buffaloes are recognized for their long productive and reproductive life and the need for herd growth over the years allows some farmers to maintain the reproducing animals in the herd for longer periods, increasing the generation interval (MALHADO et al., 2008). The average generation interval is critical in a screening program, because it is directly related to the annual genetic gain of selected characteristics and to the economic return of the program (CARNEIRO et al., 2009).

CONCLUSION

The variability of the current population is the result of the contribution of few ancestors, who are mostly also founders, evidencing that the population was developed from a narrow genetic base that characterizes the occurrence of founder effects. The small number of founder animals shows the need for reducing of inbreeding in studied herd by the introduction of animals that do not have direct relationship with the main ancestors.

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