RAPD MARKERS UTILIZATION ON THE FORMATION OR MAINTENANCE OF CONSERVATION NUCLEI OF LIVESTOCK SPECIES

USO DE MARCADORES RAPD EN LA FORMACIÓN O MANTENIMIENTO DE NÚCLEOS DE CONSERVACIÓN DE ANIMALES DOMÉSTICOS

Egito, A.A.*[1], B. Fuck[2], A.L. Spritze[2], R.R. Oliveira[3], C. McManus[2], A.S. Mariante[1], M.N. Ribeiro[3], M.S.M. Albuquerque[1], S.R. Paiva[1], S.T.R. Castro[1] and S.A. Santos[4]

[1]Embrapa Recursos Genéticos e Biotecnologia. PqEB – Final W5 Norte. Brasília-DF. Brasil.
[2]Universidade de Brasília. Faculdade de Agronomia e Veterinária. Brasília-DF. Brasil.
[3]Universidade Rural de Pernambuco. Recife-PE. Brasil.
[4]Embrapa Pantanal. Caixa Postal 109. Corumbá-MS. Brasil.
*E-mail: egito@cenargen.embrapa.br.

SUMMARY

Genetic characterization is an essential issue in the conservation and breeding programs of any country. In these work we report the use of RAPD markers in population genetic study of three breeds of different species that are in Brazilian genetic conservation program. The objective was to analyze the genetic similarity of individuals of those populations breeds in order to contribute to *ex situ* and *in situ* conservation. The selection of animals with least genetic similarity can help to conserve the maximum variability within a population, and can even optimize the choice of breeders for *ex situ* conservation programs. These studies were carried out in the Animal Genetics Laboratory (AGL) at Embrapa's Center for Genetic Resources and Biotechnology (CENARGEN). The native populations studied include: the Pantaneiro horse (*in situ* conservation nuclei), the bovine Crioulo Lageano (*in situ* conservation nuclei) and the Moxotó goat (five populations of these breed distributed in Northeast states). Using RAPD markers, obtained for each specie, we generated a Jaccard coefficient similarity matrix by NTSYS-pc v. 2.0 program. By the comparison between pairs of individuals we could chose those that were more similar between itself and those that were more distant. Using those matrix we suggested three kinds of procedure that could be used for helping the conservation of those breeds: the choice of the males to be semen donors - those less similar - avoiding duplication of samples (animals with high genetic similarity); the indication of preferential crossings aiming at the maintenance of the maximum genetic variability and the indication of animals for discarding - those that had high similarity - the breeder chose one of them for the discard. This kind of analysis could be done between two populations of the same breed in order to contribute to the genetic sample

Arch. Zootec. 54: 277-281. 2005.

exchange between different nuclei. We must remember that these recommendations are based exclusively on genetic data and must lead in account the phenotypic characteristics of each animal, not having however to be considered separately.

RESUMEN

La caracterización genética es una materia esencial en los programas de conservación y de cría de cualquier país. En este trabajo informamos del uso de marcadores RAPD en el estudio de genética de poblaciones de tres razas de diferentes especies incluidas en los programas de conservación en Brasil. El objetivo fue analizar la similitud genética individual de aquellas poblaciones raciales para contribuir a su conservación *ex situ* e *in situ*. La selección de animales con menor similitud genética puede ayudar a conservar el máximo de variabilidad dentro de poblaciones y puede optimizar el trabajo de los criadores para los programas de conservación *ex situ*. Estos trabajos fueron realizados en el Laboratorio de Genética Animal (AGL) del Centro para los Recursos Genéticos y la Biotecnología (CENARGEN) de EMBRAPA. Las poblaciones nativas incluidas fueron: caballo Pantaneiro (núcleo de conservación *in situ*), el bovino Criollo Lageano (núcleo de conservación *in situ*), y la cabra Moxotó (cinco poblaciones de esta raza distribuidas en los estados del Nordeste). Usando marcadores RAPD, obtenidos para cada especie, generamos una matriz de coeficientes de similitud de Jaccard, mediante un programa NTSYS-pc v.2.0. Mediante la comparación entre pares de individuos podríamos escoger aquellos que fueron más similares entre ellos y aquellos que fueron más distantes. Usando estas matrices sugerimos tres tipos de procedimientos que podrían ser usados para apoyar la conservación de estas razas: la elección de machos para donantes de semen (animales con alta similitud genética); la indicación de apareamientos preferenciales buscando el mantenimiento de la máxi-

ma variabilidad genética y la indicación de animales a descartar -aquellos que tienen la máxima similitud- los criadores escogen uno de ellos para descartar. Este tipo de análisis podría realizarse entre dos poblaciones de la misma raza para contribuir al intercambio de individuos entre diferentes núcleos. Debemos recordar que estas recomendaciones están basadas exclusivamente en datos genéticos y debemos tener en cuenta las características fenotípicas de cada animal, no teniendo, de cualquier manera que ser consideradas separadamente.

INTRODUCTION

The proposal for conservation of endangered livestock populations is being the cryopreservation of gametes and zygotes of several donators and *in situ* conservation nuclei (live animals in reproducing herds). Taking into account that the main objective is to store a representative sample of the population variability, its fundamental to evaluate the genetic variation of the stocked individuals and to maintain the maximum variability as possible in the conservation herd.

Genetic characterization, contrary to phenotypic characterization, is totally free of environmental influences, thereby making the data generated more accurate. The use of molecular markers can aid decisions relating to choice of breeds/populations to be conserved, when there is a shortage of resources, as well as estimating the genetic variability of species, breeds and populations (Egito *et al.*, 1999). Thus being the techniques for the analysis of the genetic variability are an essential ingredient for the programs of rational conservation and improvement, once

they must be based on the combination of the phenotypic and genetic data (Hetzel and Drinkwater, 1992).

The calculation of pairwise genetic similarities indices between individuals may help to choose those animals that possess a lesser genetic similarity and might be a value tool to select the future donators of germplasm for the Brazilian Animal Germplasm Bank or to indicate the preferential mating of less similar animals with intention to keep the maximum variability on the nuclei (Egito *et al.,* 2001).

In these work we report the use of RAPD markers in population genetic study of three breeds of different species that are in the Brazilian Genetic Conservation Program. Using Jaccard's coefficient we analyzed the genetic similarity of individuals, in pairs, in order to contribute to *ex situ* and *in situ* conservation.

MATERIAL AND METHODS

Similarity studies in breeds/species conservations

The studies were carried out in the Animal Genetics Laboratory (AGL) at Embrapa's Center for Genetic Resources and Biotechnology (CENARGEN) and had been part of a program of posgraduation that exist in partnership between the Embrapa and some Brazilian Universities.

The native populations studied include: the Pantaneiro horse (*in situ* conservation nuclei, Embrapa Pantanal, Corumbá, MS, Brazil), the bovine Crioulo Lageano (*in situ* conservation nuclei, Canoas Farm, Ponte Alta, SC, Brazil) and the Moxotó goat (seven populations of these breed distributed in Pernambuco, Paraiba and Rio Grande do Norte, Brazilian northeast States).

Genomic DNA extraction from blood cells of individuals was based on Miller *et al*. (1988) with slight modifications.

In Pantaneiro horse a total of 13 primers were selected from 146 decamer oligonucleotide primers and used to amplify DNA from 48 animals. These primers amplified a total of 44 polymorphic bands.

In the Crioulo Lageano herd, from a total of 210 animals studied, we selected the youngest animals (80 animals that were borne from 1996 to 2000) to analyze the pairwise similarity or dissimilarity in order to propose a breeding scheme for the nuclei. They were amplified with 43 primers selected from a total of 120 oligonucleotides that generated 77 RAPD markers.

Seven populations (N= 264 animals) of Moxotó goat were analyzed separately one from the other with the objective to help in crossbreeding inside each population and for the formation of conservation's nuclei of these breeds. The DNA from these animals was amplified with 16 primers that generated 56 polymorphic bands.

RAPD-PCR reactions were carried out in a final volume of 13 μl, with 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 3.5 mM MgCl$_2$; 200 μM of each dNTP; 8 percent of 2.5 mg/ml of BSA; 0.4 μM of the arbitrary primer; 1.5 U Taq DNA polymerase (Phoneutria Biotechnology & Services) and 9 ηg DNA. Reactions were performed in a MJ Research (PTC100) thermocycler under 40 cycles of 15 seconds duration at 95ºC, 30 seconds at 35ºC, 1 minute

at 72ºC and at the end of the cycle 7 minutes at 72ºC, for complete extension of the amplified products.

The fragment data were entered in a computer file as a (0/1) binary matrix. Data were analyzed using NTSYS-pc, version 2.0 (Exeter Software, Setauket, N.Y.). Similarities between individuals were estimated using Jaccard coefficients of similarity (Sneath & Sokal, 1973) and the resulting pairwise similarities were expressed as distance matrices.

RESULTS AND DISCUSSION

By comparing the indices of genetic similarity obtained by the Coefficient of Jaccard one could choose those individuals that possess a lesser genetic similarity for the formation of conservation nuclei or to improve the genetic variability of other populations (exchange of animals). By this way, from the generated matrices of similarity (population x population) it was possible to indicate the pairs of less similar individuals or the closest individuals of each population.

With the purpose to suggest possible discarding and crossbreeding, a comparison of these indices was made to identify the pairs of individuals with bigger or lower similarity between them. The identification was made (i) only between the males, (ii) only between the females and (iii) between males and females.

Using the matrices and these strategy we suggested three kinds of procedure that could be used for helping the conservation of those breeds: the choice of the males to be semen donators - those less similar - avoiding duplication of samples (animals with high genetic similarity); the indication of preferential crossings aiming at the maintenance of the maximum genetic variability and the indication of animals for discarding - those that had high similarity - the breeder chose one of them for the discard.

In Pantaneiro horse was possible to identify six animals that possibly have some degree of miscegenation because they did not clustered together (dendrogram done, with UPGMA method, based on similarity matrix) with animals from their own breed. Analyzing the similarity matrix we suggest 14 preferential crossbreeding and could observe 11 males and 9 females were very similar between their sex group. In Crioulo Lageano breed we could suggest 10 preferential crossbreeding (pairs with lesser similarity - less then 50 percent), 5 semen donators and 35 animals that were very similar to other from the herd. In Motoxó breed the preferential crossbreeding (N=10) and animal discarding (N=8) were indicated according with the variability observed in each population. These appointments can take into account when a discard program or a donator chooses are necessary.

The suggestions indicated in these studies are based exclusively on the genetic data generated by RAPD markers, and its only purpose consists in the maintenance of the biggest possible genetic variability of those populations. They are destined to contribute for the elaboration of the plan of discarding and reproduction of the herd. They do not have however to

be considered separately from others analysis. The phenotypic and productive characteristics of each animal must to taken into account to choose what animal must be retained in the population or what will be the germplasm donator.

CONCLUSIONS

The selection of animals, based on DNA markers, with least genetic similarity can help to conserve the maximum variability within a population, and can even optimize the choice of breeders for *ex situ* conservation programs.

These results can be used to help in the choice of animals to be utilized in *ex situ* conservation so that the germplasm bank may contain the maximum genetic variation which exists in these populations, avoiding duplication of samples (animals with high genetic similarity). Since most of the *in situ* populations are small, low genetic variance might be expected. Therefore these estimates will be useful in crossbreeding and genetic sample exchange between different nuclei.

This kind of analysis could be done between two populations of the same breed in order to contribute to the genetic sample exchange between different nuclei helping to avoid the increasing endogamy in herds with a small number of individuals.

REFERENCES

Egito, A.A. do, M. do S.M. Albuquerque and A. da S. Mariante. 1999. Situação atual da caracterização genética animal na Embrapa Recursos Genéticos e Biotecnologia. In: II Simpósio de Recursos Genéticos para América Latina e Caribe - Sirgealc. Brasília. Proceedings... Brasília. Cd-ROM.

Egito, A.A. do, M. do S.M. Albuquerque, A. da S. Mariante, C. Mcmanus, J.R.F. Marques, U.P.G. Abreu and G.M.S. Serrano. 2001. Caracterização genética de raças naturalizadas. In: Simpósio de Recursos Genéticos para América Latina e Caribe, 2001, Londrina-PR Proceedings...: Instituto Agronômico do Paraná - IAPAR. v. 3: 121-126.

Hetzel, D.J.S. and R.D. Drinkwater. 1992. The use of DNA technologies for the conservation and improvement of animal genetic resources. In: Hodges, J. The management of global animal genetic resources. Proceedings of an FAO Expert Consultation. Rome, Italy, pp. 309.

Miller, S.A., D.D. Dykes and H.F. Polesky. 1988. A Simple Salting Out Procedure for Extracting DNA from Human Nucleated Cells. *Nucleic Acids Research,* 16: 1215.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy. The principles and practice of numerical classification. Ed. W.H. Freeman and company. San Francisco. USA, 573 p.