Genetic Diversity of the N'Dama Breed in Mali Using SSR Markers

Drissa Konaté^{1,*}, Diakaridia Traoré^{1,2}, Sognan Dao¹, Rokiatou Fané¹, Oumar Ouattara¹, Ramata Diop¹, Amadou Hamadoun Babana¹

¹Department of Biology, Faculty of Sciences & Techniques, University of Sciences, Techniques and Technologies, Bamako, Mali

²National Center for Artificial Insemination, Bamako, Mali

Email address:

konatedrissa80@gmail.com (D. Konaté), dtraore@laborem-biotech.com (D. Traoré), sognandao@gmail.com (S. Dao), oumarouattara2012@gmail.com (O. Ouattara), diopramata@yahoo.com (R. Diop), ahababana@laborem-biotech.com (A. H. Babana)

*Corresponding author

To cite this article:

Drissa Konaté, Diakaridia Traoré, Sognan Dao, Rokiatou Fané, Oumar Ouattara, Ramata Diop, Amadou Hamadoun Babana. Genetic Diversity of the N'Dama Breed in Mali Using SSR Markers. *International Journal of Animal Science and Technology*.

Vol. 2, No. 3, 2018, pp. 30-35. doi: 10.11648/j.ijast.20180203.11

Received: September 22, 2018; Accepted: October 15, 2018; Published: November 13, 2018

Abstract: The N'Dama race, trypanotolerant and well adapted to the climatic conditions of Mali, is threatened of disappearance by the introduction of other genes by means of the artificial insemination with the exotic races or zebus. In order to adopt preservation and conservation strategies, it is important to study the genetic characteristics of the race across the country. In this study, carried out on the race in its cradle (Yanfolila district, Sikasso region), the genetic diversity of 119 N'Dama race, from the Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock, was evaluated with 9 microsatellite markers (SSR). A total of 60 alleles were obtained. The number of alleles varied from 2 (BM 1824) to 12 (INRA 37) with an average of 6.67 per locus. The PIC ranked from 0.39 (BM 1824) to 0.9183 (INRA 37) with an average of 0.6605. Genetic diversity ranged from 0.4293 (BM 1824) to 0.9228 (INRA) with an average of 0.6908. The 119 N'Dama races were classified into two groups I and J according to the genetic similarities revealed by the 9 SSR markers using the UPGMA method. Group J was formed with the majority (85%) of individuals and composed of four (4) clusters J₁, J₂, J₃ and J₄. The 69 Nd, 71 Nd and 72 Nd individuals showed strong dissimilar compared to other individuals in group J and formed cluster J₁. Moreover, one cluster, with 15% of individuals, was belonged to Group I. The results of this study will contribute to the application of molecular tools and strengthen strategies for conservation, preservation and genetic improvement of the N'Dama race in Mali.

Keywords: Genetic Diversity, N'Dama, SSR Marker, Mali

1. Introduction

N'Dama is a rustic breed and well adapted to the southern humid environment of Mali [1]. Beyond its trypanotolerant nature and resistance to high humidity, this breed is more tolerant to hunger and thirst. N'Dama oxen are well adapted to hitching up in agriculture and less demanding in terms of health care [1]. These characters make it more interesting with most of the livestock householders and those in charge of animal product development [2]. With the increasing demand for animal products (milk and meat), breeders tend to introduce other genes through artificial insemination with exotic breeds or by simple interbreeding with zebus due to

increasing demand for animal products (milk and meat) [1; 3]. This situation contributes to miscegenation of the N'Dama and can lead to a loss of genes of interest with decreasing of N'Dama number in the environment since crosses are more sensitive to environmental factors with expensive health care [1]. Therefore, genetic diversity study will be one of the strategies management N'Dama herd for conservation and also select the best genotypes for breeding program. Several genetic diversity studies have been conducted on cattle breeds across the world [4-7]. In Africa, Goudarzi *et al.* [7] studied genetic variability of 201 individuals of the Somba cattle race from Benin and Togo with 33 microsatellite markers whereas, Gororo *et al.* [28] study's was about 50

Sanga cattle race from Zimbabwe using 16 SSRs. In Senegal, N'diaye *et al.* [5] evaluated the genetic diversity of 120 cattle composed of four races from Senegal including N'Dama race using 12 SSR markers. In Mali, there is little information on the genetic diversity of our indigenous races, especially N'Dama. Simple sequence repeats (SSR) markers are commonly used in molecular genetic studies such as genetic linkage mapping [8] and population structures [9] due to their reproducibility, polymorphism and dominance [10; 11]. Thirty SSR markers have been recommended by the International Society of Animal Genetics (ISAG) and the Food and Agriculture Organization (FAO) for the genetic characterization of cattle [12]. In this study, we evaluated the genetic diversity of 119 individuals of N'Dama in Madina Diassa ranch using 9 microsatellite markers per PCR.

2. Material and Methods

2.1. Herd Management

The herd of Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock at Yanfolila in the Sikasso region was composed of two batches of 241 individuals in total. The first batch, was adapted by ONDY (Operation N'Dama Yanfolila) and the second was introduced few years later by PROGEBE (Regional Project for Sustainable Management of Endemic Ruminant Livestock in West Africa) respectively in Yanfolila, Kita and Kenieba environments. The animals were split up to two separated parks with two male parents each. All animals were kept together except calves less than 3 months old. The mating monitoring was done by technicians and shepherds of the Center. Sometimes, undesirables mating were done by young male which have not been kept in the herd as male parents. Most of the animals in the center have been purchased and had a very homogeneous shade color with dominant tawny.

2.2. Blood Sample Collection

Sampling was done in April 2015 on the Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock. Four milliliters (4 ml) of blood were collected in EDTA tubes from 119 individuals randomly selected. Samples were tagged, stored in ice and sent to the Laboratory of Microbiology and Microbial Biotechnology Research (LaboREM-Biotech) of the Faculty of Science and Technology (FST) for molecular analysis.

2.3. Extraction of Genomic DNA

Genomic DNA of N'Dama was extracted from whole blood with the Promega ReliaPrep™ Blood Extraction Kit gDNA Miniprep System. The concentration of the DNA was determined using the Eppendorf Spectrophotometer. DNA samples were diluted into 20 ng/μl with Water Nuclease Free VWR and stored at -20°C.

2.4. DNA Amplification PCR

DNA samples were amplified with 9 primer pairs of SSR markers (Table 1). The PCR was performed using a reactive mixture of 25 μl composed of Promega PCR kit ingredient (8.5 μl of pure water, 12.5 μl of Go Taq Green Master Mix 2X, 1 μl of forward primer 100 pmol and 1 μl of 100 pmol reverse primer) and 2 μl of 20 ng/μl genomic DNA. The mixture was partitioned between PCR 8-Strip tubes with Strip. Amplification was performed with the thermocycler TECHNE-PRIME according to the following program: initial denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, hybridization varying between 51-57°C, elongation at 72°C for 1 mn 45s, final elongation at 72°C for 10 min and storage at 4°C. Denaturation, hybridization, and elongation constituted one cycle repeated at 35 times.

Table 1. Sequences and Repetitive Patterns of SSR Primers.

Name	Chrs		Primers	References
ILSTS005	10	Forward	GGAAGCAATGAAATCTATAGCC	[13]
		Reverse	TGTTCTGTGAGTTTGTAAAGC	
INRA063	18	Forward	ATTTGCACAAGCTAAATCTAACC	[14]
		Reverse	AAACCACAGAAATGCTTGAAG	
MM12	9	Forward	CAAGACAGGTGTTTCAATCT	[15]
		Reverse	ATCGACTCTGGGGATGATGT	
BM1824	1	Forward	GAGCAAGGTGTTTTTCCAATC	[16]
		Reverse	CATTCTCCAAGTCTTCCTTG	
ILSTS011	14	Forward	GCTTGCTACATGGAAAGTGC	[13]
		Reverse	CTAAAATGCAGAGCCCTACC	
TGLA 122	21	Forward	CCCTCCTCCAGGTAAATC AGC	[17]
		Reverse	AATCACATGGCAAATAAGTACATAC	
TGLA 53	16	Forward	GCTTTCAGAAATAGTTTGCATTCA	[17]
		Reverse	ATCTTCACATGATATTACAGCAGA	
INRA37	11	Forward	GATCCTGCTTATATTTAACCAC	[14]
		Reverse	AAAATTCCATGGAGAGAGAAAC	
INRA172	26	Forward	CCACTTCCCTGTATCCTCCTCT	[18]
		Reverse	GGTGCTCCCATTGTGTAGAC	

Chrs: chromosome

2.5. Electrophoresis of Amplified Products

PCR product was loaded into 3% (w / v) agarose MS-4 gel, prepared with TBE 0.5X (Tris, EDTA Acid Borate) and 30 µl of 10% ethidium bromide (1 mg/ml) [19]. The gel was running for 2 hours 30 minutes at 80V and photographed with Gel Documentation System E-BOX VX2 version 15.06.

2.6. Statistical Analyzes

Allele size of each SSR marker was determined in base pairs using the E-Capt software version 15.06. The diversity of N'Dama was assessed based on the number of alleles, frequency of alleles, genetic diversity and the PIC (Polymorphism Information Content). These statistical parameters were calculated according to Dao *et al.* [19]. The matrix of genetic distance was determined using Power Marker software version 3.25 [20]. The phylogenetic tree was constructed using UPGMA (Un-weighted Pair Group

Method with Arithmetic Mean) and edited with MEGA 7.0 [19; 21].

3. Results and Discussion

Molecular characterization showed a strong allelic variability of N'Dama revealing a total of 60 alleles. The number of allele ranged from 2 (BM 1824) to 12 (INRA 37) with an average of 6.67 per locus (Table 2). The highest allele frequency was observed at the locus BM 1824 on chromosome 1 and the lowest at the locus INRA37 on chromosome 11. The PIC ranged from 0.39 (BM 1824) to 0.9183 (INRA 37) with an average of 0.6605. All SSRs had a PIC greater than 0.50 except ILSTS005 and BM1824. The genetic diversity is proportional to PIC, in this study it was ranged from 0.4293 to 0.9228 with an average of 0.6908 for the same SSRs.

Figure 1. PCR product profiles from N'Dama with INRA37 SSR makers on Agarose MS-4 gel. M: size marker (in base pair), N: Negative control, 161 to 129: samples N ' Damas.

Table 2. Genetic diversity and polymorphism information content of N'Dama in Madina Diassa Ranch.

Marker	Number of alleles	allele size (pb)		Allele No	Major allele frequency	Gene diversity	PIC
		Max	Min				
BM 1824	2	223	178	3	0.7311	0.4293	0.39
MM 12	7	443	59	9	0.5882	0.6077	0.5744
TGLA 53	7	241	138	19	0.3949	0.8071	0.7946
ILSTS 011	7	561	80	8	0.2689	0.7925	0.7612
TGLA 122	6	380	144	6	0.3697	0.6805	0.6167
INRA 172	10	300	100	16	0.3361	0.7916	0.765
INRA 063	6	450	103	9	0.5126	0.683	0.6526
INRA 37	12	245	61	33	0.1681	0.9228	0.9183
ILSTS 005	3	232	67	5	0.6807	0.5031	0.4712
Mean	6.67					0.6908	0.6605

PIC: Polymorphism Information Content

Referring to the formula of Botstein *et al.* [22], 98% of SSR markers were highly informative with a PIC greater than 0.5. A similar result was obtained by Kumar *et al.* [23] with 95% of SSRs on the Hallikar breed in India. Kramarenko *et al.* [24] also reported that all the loci of the Red cattle breed population were highly polymorphic. The genetic diversity of

this study is specific to the N'Dama breed of Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock and depends on the breeding environments. However the same value of PIC was observed by Ndiaye *et al.* [5] on the N'Dama of Kolda (Eastern Senegal and Upper Casamance). According to Grema *et al.*

[25] the level of genetic diversity may be due to historical breed mixture and current breeding practices in the region. The Madina Diassa ranch N'Dama is considered as pure, the genetic information revealed in current study may be used as basic information for conservation and diversity monitoring of the breed. The average PIC observed in this study was lower than that of Barani *et al.* [26] and Hussain *et al.* [4] who worked respectively on 50 unrelated Pulikulam cattle and 11 Pakistani breeds. Similarly, Chaudhari *et al.* [27] obtained an average PIC values of 0.59 and 0.65 for genetic diversity of 45 individuals of Kenkatha and Gaolao breeds using 25 SSRs. These average values of PICs were close to those of current study where only five SSRs were used in common (MM12, INRA63, ILSTS011, ILSTS005, and BM1824). Furthermore, Gororo *et al.* [28] found PIC values close to those of actual study with TGLA53 and TGLA 122 using 16 SSR on three breeds from Zimbabwe. The PIC value of INRA37 reported by Harton and Angus [29] on two breeds was lower than that of current founding. Genetic diversity analysis of 20 Ongole cattle showed PIC of TGLA 122 and INRA063 similar to those of the present study [30]. The total number of allele observed in this study was lower than that of Lirón *et al.* [11] and Moazami-Goudarzi *et al.* [31], who worked respectively on 10 breeds (4 Creole cattle

from Argentina and Bolivia, 4 European bull breeds and 2 American zebu populations) and 11 French cattle breeds. Similarly, 142 alleles were obtained by Montoya *et al.* [29] who characterized five breeds of Colombia with 10 SSR markers. Joshi *et al.* [32] obtained only two alleles with INRA063 from 30 unrelated Nagori cattle. These differences were related to the size of the samples, the breeds as well as the different targeted loci. Teneva *et al.* [33] observed fragments of similar size on 35 Bulgarian cattle with TGLA53, TGLA122 and BM1824. The present study showed different distribution of allelic frequencies at the same loci of different breeds. The finding informations may be used for strategies adoption against introduction of foreign genes [31; 34].

Study classified the 119 N'Dama into two major Groups I and J with high variability within the groups (Figure 2). More than 80% of study materials was under Group J and shared between four (4) clusters (J₁, J₂, J₃ and J₄). Clusters J₃ and J₄ were composed of 31% and 32% individuals, respectively. Individuals in the same cluster showed strong allelic similarity to each other. Moreover, Group I consisted of one cluster had 15% of the genotypes. Individuals 69 Nd, 71 Nd and 72 Nd different to the whole genotype were belonged to cluster J₁ of Group J.

Figure 2. Dendrogram generated from 9 SSR markers amplified 119 N'Dama genotypes based on UPGMA (unweighted pair-group method with averages).

4. Conclusion

Molecular characterization of N'Dama race from Madina Diassa Center is important for preservation, conservation and genetic improvement strategies of cattles. This study on N'Dama race revealed high genetic diversity at the nine (9) target loci. This diversity could be related to the adaptation of the race to the living conditions into the environment and to the interconnection of individuals from different localities. Individuals 69 Nd, 71 Nd and 72 Nd showed high dissimilar and need to be followed up in the field. These data provide basic information to researchers in animal breeding field to follow up the purity of animals' race through marker-assisted selection.

The authors are grateful to the National Committee for Agricultural Research in Mali for its financial support through the West Africa Agricultural Productivity Program 2A (WAAPP-2A). They wish also to thank the Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock of Yanfolila (Mali) for providing genetic materials.

References

- [1] Coulibaly, T & Diallo, L. (2014). Diagnostic de la situation de l'élevage N'Dama dans son berceau de race (cercles de Bougouni et de Yanfolila). p 46.
- [2] Coulomb, J. (1976). La race N'Dama: quelques caractéristiques zootechniques. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, 29(4), 367-380.
- [3] Konaté M. (2014). Etude des effets défavorables de la transhumance sur la gestion des ressources génétiques animales des ruminants Endémiques. Rapport final. p 45.
- [4] Hussain, T., Babar, M. E., Peters, S. O., Wajid, A., Ali, A., Azam, A. & De Donato, M. (2016). Microsatellite Markers Based Genetic Evaluation of Pakistani Cattle Breeds. *Pakistan Journal of Zoology*, 48(6).
- [5] Ndiaye, N. P., Sow, A., Dayo, G. K., Ndiaye, S., Sawadogo, G. J., & Sembène, M. (2015). Genetic diversity and phylogenetic relationships in local cattle breeds of Senegal based on autosomal microsatellite markers. *Veterinary world*, 8(8), 994.
- [6] Kaboré M. (2012). Etude de la diversité génétique des taurins Baoulé du Burkina Faso à l'aide de marqueurs microsatellites. Université d'Ouagadougou Burkina Faso unité de formation et de recherche en Sciences de la Vie et de la Terre (UFR/SVT), CERBA/LABIOGENEUF/SVT, DEA. 86.
- [7] Goudarzi, K. M., Belemsaga, D. M., Ceriotti, G., Laloë, D., Fagbohoun, F., Kouagou, N. T., ... & Touré, S. (2001). Caractérisation de la race bovine Somba à l'aide de marqueurs moléculaires. *Revue d'Élevage et de Médecine Vétérinaire des pays Tropicaux*, 54(2), 129-138.
- [8] Arora, R., Lakhchaura, B. D., Prasad, R. B., Tania, M. S. & Vijh, R. K. (2004). Genetic diversity analysis of two buffalo populations of northern India using microsatellite markers. *Journal of Animal Breeding and Genetics*, 121: 111-118.
- [9] Azhar, P. M., Chakraborty, D., Iqbal, Z., & Malik, A. A. (2018). Microsatellite Markers as a Tool for Characterization of Small Ruminants: A Review. *Int. J. Curr. Microbiol. App. Sci*, 7(1), 1330-1342.
- [10] Portetelle, D., Haezebroeck, V., Mortiaux, F., & Renaville, R. (2000). Traçabilité dans la filière animale. *Biotechnologie, Agronomie, Société et Environnement*, 4(4), 233-240.
- [11] Lirón, J. P., Ripoli, M. V., De Luca, J. C., Peral-García, P., & Giovambattista, G. (2002). Analysis of genetic diversity and population structure in Argentine and Bolivian Creole cattle using five loci related to milk production. *Genetics and Molecular Biology*, 25(4), 413-419.
- [12] FAO (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome.
- [13] Brzeziński, T., & Majid, S. (1993). Quantum group gauge theory on quantum spaces. *Communications in Mathematical Physics*, 157(3), 591-638.
- [14] Vaiman, D., Mercier, D., Moazami-Goudarzi, K., Eggen, A., Ciampolini, R., Lépingle, A., & Lévêziel, H. (1994). A set of 99 cattle microsatellites: characterization, syntenic mapping, and polymorphism. *Mammalian Genome*, 5(5), 288-297.
- [15] Mommens, G., Coppierst, W., Weghe, A., Zeveren, A. V., & Bouquet, Y. (1994). Dinucleotide repeat polymorphism at the bovine MM12E6 and MM8D3 loci. *Animal Genetics*, 25(5), 368-368.
- [16] Bishop, M. D., Kappes, S. M., Keele, J. W., Stone, R. T., Sunden, S. L., Hawkins, G. A., & Yoo, J. (1994). A genetic linkage map for cattle. *Genetics*, 136(2), 619-639.
- [17] Georges M. and Massey J. M. (1992). Polymorphic DNA markers in Bovidae. Patent WO 92/13102 1992. Gębczyński M. and Tomaszewska-Guszkiewicz K. 1987. Genetic variability in the European bison. *Biochemical Systematics and Ecology* 15: 285-288.
- [18] Al-Atiyat, R. M. (2016). Association of allele diversity and polymorphism of microsatellites markers in the tropical goat. *Research Journal of Biotechnology Vol*, 11, 9.
- [19] Dao, S., Timbine, H., Goita, O., & Traore, D. (2018). Genetic Variability Assessment in Irrigated Rice (*Oryza sativa* and *Oryza glaberrima*) by PCR-SSR in Mali. *International Journal of Genetics and Genomics*, 6(2), 22.
- [20] Nei, M., & Takezaki, N. (1983). Estimation of genetic distances and phylogenetic trees from DNA analysis. In *Proceedings of the 5th world congress on genetics applied to livestock production Vol. 21*, pp. 405-412.
- [21] Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33(7), 1870-1874.
- [22] Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics*, 32(3), 314.
- [23] Kumar, S. N., Jayashankar, M. R., Nagaraja, C. S., Govindaiah, M. G., Saravanan, R., & Karthickeyan, S. M. K. (2006). Molecular characterization of Hallikar breed of cattle using microsatellite markers. *Asian Australasian journal of animal sciences*, 19(5), 622.

- [24] Kramarenko, A. S., Gladyr, E. A., Kramarenko, S. S., Pidpala, T. V., Strikha, L. A., & Zinovieva, N. A. (2018). Genetic diversity and bottleneck analysis of the Red Steppe cattle based on microsatellite markers. *Ukrainian Journal of Ecology*, 8(2), 12-17.
- [25] Grema, M., Traoré, A., Issa, M., Hamani, M., Abdou, M., Soudré, A., & Periasamy, K. (2017). Short tandem repeat (STR) based genetic diversity and relationship of indigenous Niger cattle. *Archives Animal Breeding*, 60(4), 399-408.
- [26] Barani, A., Rahumathulla, P. S., Rajendran, R., Kumarasamy, P., Ganapathi, P., & Radha, P. (2015). Molecular characterization of Pulikulam cattle using microsatellite markers. *Indian Journal of Animal Research*, 49(1), 36-39.
- [27] Chaudhari, M. V., Parmar, S. N. S., Joshi, C. G., Bhong, C. D., Fatima, S., Thakur, M. S., & Thakur, S. S. (2009). Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers. *Animal biodiversity and conservation*, 32(2), 71-76.
- [28] Gororo, E., Makuza, S. M., Chatiza, F. P., Chidzwondo, F., & Sanyika, T. W. (2018). Genetic diversity in Zimbabwean Sanga cattle breeds using microsatellite markers. *South African Journal of Animal Science*, 48(1), 128-141.
- [29] Montoya, A. E., Cerón-Muñoz, M. F., Moreno, M. A., Martínez, E., Corrales, J. D., Tirado, J. F., & Calvo, S. J. (2010). Genetic characterization of the Hartón del Valle, Angus, Brangus, Holstein, and Senepol cattle breeds in Colombia, using ten microsatellite markers. *Revista Colombiana de Ciencias Pecuarias*, 23(3), 283-291.
- [30] Devi, K. S., Gupta, B. R., Vani, S., Asha, U., Kumar, U. R., & Krishna, C. H. (2017). Microsatellite Analysis of Ongole Cattle (*Bos indicus*) Of A. P. *International Journal of Science, Environment and Technology*, Vol.6, 173 – 178.
- [31] Moazami-Goudarzi, K., Vaiman, D., Mercier, D., Grohs, C., Furet, J. P., Leveziel, H., & Martin, P. (1994). Emploi de microsatellites pour l'analyse de la diversité génétique des races bovines françaises: premiers résultats. *Genetics Selection Evolution*, 26(1), S155.
- [32] Joshi, P., Vyas, P., & Kashyap, S. K. (2018). Molecular characterization of Nagori cattle using microsatellite markers. *Journal of Pharmacognosy and Phytochemistry*, 7(2), 3250-3252.
- [33] Teneva, A., Todorovska, E., Tyufekchiev, N., Kozelov, L., Atanassov, A., Foteva, S., & Zlatarev, S. (2005). Molecular characterization of Bulgarian livestock genetic resources: 1. Genetic diversity in Bulgarian grey cattle as revealed by microsatellite markers. *Biotechnology in Animal Husbandry*, 21(5-6-2), 35-42.
- [34] Moore, E. E., Cogbill, T. H., Jurkovich, G. J., Shackford, S. R., Malangoni, M. A., & Champion, H. R. (1995). Organ injury scaling: spleen and liver (1994 revision). *Journal of Trauma and Acute Care Surgery*, 38(3), 323-324.