



Genetic relationship between the Nordestino horse and national and international horse breeds

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ABSTRACT. Knowledge of genetic diversity and relationships between breeds is very important for conservation programs. Hair samples were collected from 393 individual Nordestino horses and genotyped using 14 microsatellite markers in order to investigate the genetic relationship between this breed and 66 international horse breeds. There was high allelic diversity and inbreeding coefficient within population values were not significant, which was probably due to crossbreeding. Despite the Nordestino horse population being in Hardy-Weinberg equilibrium, a global deficit of heterozygotes was observed. This may represent

evidence of repeated use of the same stallions for breeding, which is consistent with the high number of castrated males found. Campolina, Mangalarga Marchador, and Mangalarga were the Brazilian horse breeds most closely related to the Nordestino horse, which is a reflection of recent introgressions. Among Iberian horse breeds, the Sorraia breed appears to have had an important influence on the genetics of the Nordestino horse. Those results provide important information that can guide future conservation programs.

Key words: Conservation programs; Genetic formation; Genetic variability; Horse

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO, 2007), the horse is the most endangered species worldwide and is among the domestic mammalian species with the highest proportion of endangered breeds (23%). The first horses arrived during the colonization of Brazil, and were mainly Iberian Peninsula horses. These breeds adapted to the conditions in Brazil and gave rise to the Nordestino horse. Each breed represents an essential reservoir of genes, which can support future human demands resulting from abrupt climate change (FAO, 2007). In this context, the Nordestino horse is an important national breed that was shaped in the semiarid Brazilian Northeast. This breed is important for local culture and has great economic, historic, and social significance for small livestock breeders. The Nordestino horse is characterized by its small size, small head, strong hooves, deep frog, dark skin, and work ability in semiarid regions of the Northeast, mainly within the Caatinga biome (Melo, 2011). These adaptations allow this breed to survive the conditions of the semiarid Brazilian Northeast. The Nordestino Horse Breed Association closed in the early 1990's (Melo, 2011). As a result, the official record service of the Nordestino horse was disabled, most animals were slaughtered, and stallions were castrated. Nordestino horses have been mated with other breeds in haphazard crossbreedings (Costa et al., 2001), which may have led to variation in the appearance and loss of important genes such as those associated with adaptive capacity to drought condition genes. Therefore, the aim of this study was to investigate the genetic relationship between the Nordestino horse and national and international horse breeds.

MATERIAL AND METHODS

A total of 393 hair root samples of the typical Nordestino horse were collected from the semiarid ecoregions of Pernambuco, Piauí, and Bahia States, Brazil. These places contain remnant populations of the Nordestino horse. The animals found in Pernambuco have lived with few management practices and horses from Piauí and Bahia States have lived in ultraextensive systems. Because the remaining animals were raised under wild conditions and are located in distant places geographically, it was necessary to determine whether these represent genetically different populations or are members of the same population (Pires et al., 2014).

DNA extraction was carried out at the Laboratory of Genetics in the Animal Science Department at the Veterinary School (LGEV) of the Federal University of Minas Gerais (UFMG), Brazil, according to the method described by Coelho et al. (2004). PCR mixtures were prepared with 5.0 μ L Phusion Flash Master Mix Enzyme, 1.0 μ L ultrapure water, 3.0 μ L primer mix, and 1.0 μ L extracted DNA. The genotyping panel consisted of 14 microsatellite markers: AHT4 and AHT5 (Binns et al., 1995), ASB2 and ASB17 (Breen et al., 1997), HTG4 and HTG6 (Ellegren et al., 1992), HMS3, HMS6, and HMS7 (Guérin et al., 1994), ASB23 (Irvin et al., 1998), HTG7 and HTG10 (Marklund et al., 1994), LEX33 (Coogle et al., 1996) and VHL20 (van Haeringen et al., 1994). Three multiplex panels were used for PCR: one with an annealing temperature of 60°C (AHT4, AHT5, ASB17, ASB23, HMS6, HMS7, HTG4, and VHL20), one with an annealing temperature of 56°C (ASB2, HMS3, and HTG10), and one with an annealing temperature of 60°C (LEX33, HTG6, and HTG7). The same annealing temperature was used in two multiplex panels because LEX33, HTG6, and HTG7 were not being used routinely in the laboratory. For microsatellite amplification using the three multiplex panels, the following conditions were applied: activation at 98°C for 10 s, followed by 34 cycles of 95°C for 45 s (denaturation step), 56° or 60°C (depending on the multiplex panel) for 30 s (annealing), and 72°C for 30 s (extension), and a final extension step of 60 min at 72°C. Capillary electrophoresis was performed using 0.3 μ L LIZ™ (standard molecular weight), 8.7 μ L Formamide Hi-Di (Applied Biosystems), and 1 μ L panel mixture from three PCR products. Capillary electrophoresis was performed in the ABI3130 genetic analyzer (Applied Biosystems). Fragment sizes were confirmed with the GeneMapper v.4.0 software (Applied Biosystems, 2005). The samples were genotyped at LGEV/UFMG, Minas Gerais, Brazil.

A data bank containing genotype information on 66 horse breeds was used to compare the Nordestino horse sequences and to determine the ancestry of this breed. The Przewalski horse was used as an outgroup. Standard measures of diversity from these breeds can be viewed in the report by Cothran et al. (2011) and Conant et al. (2012). Genetic variability was assessed by estimates of allele number per locus (N_A), effective number of alleles per locus (N_E), observed (H_o), and unbiased expected heterozygosities (UH_E), which were estimated per locus using GenAlex 6.4 (Peakall and Smouse, 2006). Heterozygote deficiency and deviations from Hardy-Weinberg equilibrium (HWE) were estimated using GenePop v.4.1.1 (Raymond and Rousset, 1995; Rousset, 2008) with Markov Chain parameters (10,000 dememorization, 20 batches, and 5000 iterations per batch). The F_{IS} (inbreeding coefficient within population) parameter was obtained with 1000 bootstrap values and 95% confidence interval by GENETIX v.4.04 (Belkhir et al., 2003). F_{ST} (genetic differentiation between populations) and gene differentiation coefficient (G_{ST}) parameters were estimated among the Nordestino horse breed, 10 South American breeds (five Brazilian horse breeds: Mangalarga, Mangalarga Marchador, Campolina, Pantaneiro, and Brazilian Criollo; and five others: Peruvian Paso, Puerto Rican Paso Fino, Columbian Paso Fino, Chilean Criollo, and Venezuelan Criollo) and Iberian breeds (Andalusian, Lusitano, Garrano, and Sorraia). The F_{ST} parameter was estimated with GenePop v.4.1.1 (Raymond and Rousset, 1995; Rousset, 2008) and G_{ST} using GENETIX v.4.04 (Belkhir et al., 2003). Analysis of molecular variance (AMOVA) was carried out with GenAlex 6.4 (Peakall and Smouse, 2006) and was conducted with 999 permutations. Chord genetic distance (Cavalli-Sforza and Edwards, 1967) among Nordestino horse and the other 66 horse breeds was used to build restricted maximum likelihood trees. A majority-rule consensus tree was

generated with PHYLIP v.3.69 (Felsenstein, 1989). The Archaeopteryx v. 0.957 beta software (Han and Zmasek, 2009) displayed a consensus tree graphically to infer admixture among Nordestino horse and the other 66 horse breeds.

RESULTS

A total of 129 alleles were observed with an average of 9.214 alleles (Table 1). Average values of H_O and UH_E were 0.755 and 0.767, respectively. Allelic diversity ranged from 0.589 (HTG7) to 0.862 (HTG10). HMS6 and LEX33 loci were not in HWE. However, global analysis showed that the population was in HWE (Table 1). No F_{IS} values were found to be significant; however, a global deficit of heterozygosity was observed ($P = 0.0092$). Among microsatellite markers, HMS3 ($P = 0.0001$), ASB2 ($P = 0.0256$), and HTG6 ($P = 0.0206$) had heterozygote deficits.

Table 1. Genetic composition of remnant populations of the Nordestino horse.

Loci	N	N_A	N_E	H_O	UH_E	HWE	F_{IS}
AHT4	393	9	5.138	0.840	0.806	NS	-0.041 ^{NS}
AHT5	393	9	4.596	0.771	0.783	NS	0.016 ^{NS}
ASB17	393	16	5.528	0.822	0.820	NS	-0.002 ^{NS}
ASB2	393	12	5.719	0.807	0.826	NS	0.024 ^{NS}
ASB23	393	11	5.813	0.830	0.829	NS	-0.001 ^{NS}
HMS3	393	7	5.215	0.751	0.809	NS	0.073 ^{NS}
HMS6	393	7	3.064	0.695	0.675	*	-0.030 ^{NS}
HMS7	393	8	3.733	0.697	0.733	NS	0.049 ^{NS}
HTG10	393	10	7.191	0.830	0.862	NS	0.038 ^{NS}
HTG4	393	7	3.659	0.743	0.728	NS	-0.021 ^{NS}
HTG6	393	7	2.639	0.573	0.622	NS	0.080 ^{NS}
HTG7	393	5	2.431	0.590	0.589	NS	-0.002 ^{NS}
LEX33	393	10	4.967	0.791	0.800	**	0.010 ^{NS}
VHL20	393	11	6.930	0.832	0.857	NS	0.029 ^{NS}
Total	393	129	-	-	-	-	-
Means	-	9.214	4.759	0.755	0.767	NS	0.016 ^{NS}
SE	-	0.743	0.397	0.023	0.023	-	0.010

N = sample size; SE = standard error; N_A = number of alleles per locus; N_E = effective number of alleles; H_O and UH_E = observed and unbiased expected heterozygosity; HWE = Hardy-Weinberg equilibrium (NS = not significant; * $P < 0.05$; ** $P < 0.01$); F_{IS} = inbreeding coefficient value.

The genetic difference (G_{ST}) was explained in 15.8% between Nordestino, Mangalarga, Mangalarga Marchador, Campolina, Pantaneiro, Brazilian Criollo, Peruvian Paso, Puerto Rican Paso Fino, Columbian Paso Fino, Chilean Criollo, Venezuelan Criollo, Andalusian, Lusitano, Garrano, and Sorraia horse breeds. AMOVA results also showed that 84.2% of the total variation was from differences within the populations. The average F_{ST} value among those 15 breeds was 0.088, which indicated that 8.8% of the total genetic variability could be assigned to differences among Brazilian, South American, and Iberian (Andalusian, Lusitano, Garrano, and Sorraia) horse breeds. The Nordestino horse is part of the South American group of horse breeds, closest to Campolina, Mangalarga Marchador, and Mangalarga (Figure 1). Some bootstrap values in the dendrogram tree were low. Cothran and Luís (2005) confirmed that trees containing normal horse breeds have low bootstrap values; however, the group formation was consistent. Bootstrap values correspond to the number of times that a group was formed in 1000 bootstraps and can establish the most suitable arrangement.

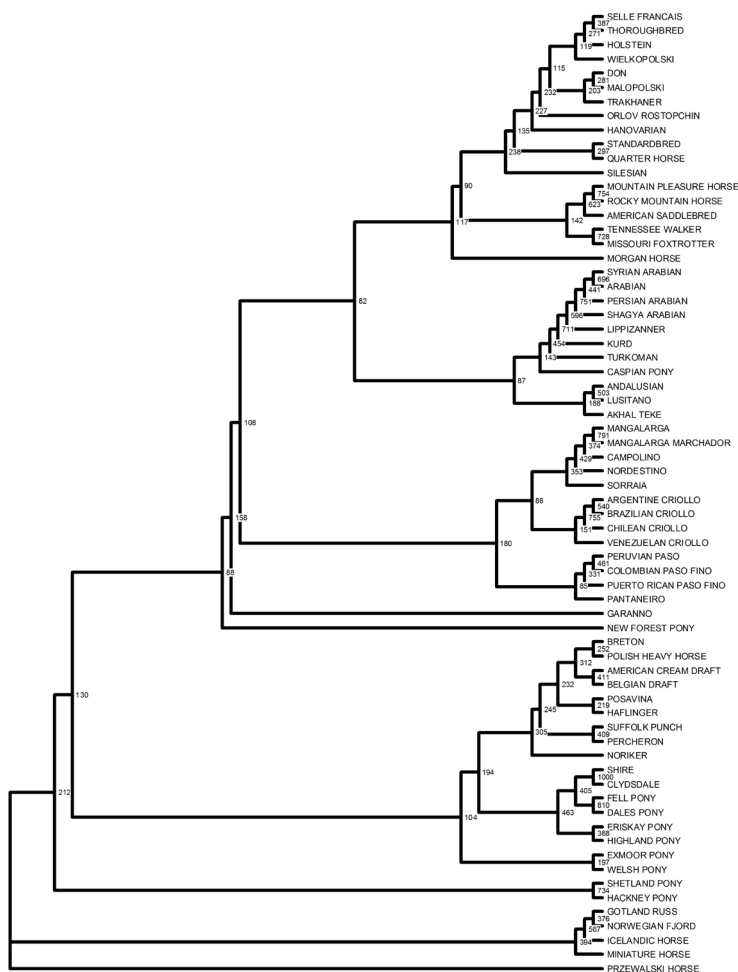


Figure 1. Consensus tree of 1000 bootstraps based on Chord genetic distance using REML method among the Nordestino horse and 66 other horse breeds (Przewalski data as outgroup).

DISCUSSION

The average allelic diversity within the remnant Nordestino populations was similar to that observed in other horse breeds from Brazil and around the world (Serenio et al., 2008; Cothran et al., 2011; Conant et al., 2012; Silva et al. 2012), in which similar microsatellite markers were used. High genetic diversity was observed in the remnant population of the Nordestino horse studied here, according to estimates of genetic composition (Table 1). This finding could be explained through their mating with other horse breeds. Costa et al. (2001) observed an increase in crossbreeding within Nordestino horse populations in recent years. Nevertheless, the Nordestino horse population was in HWE. Silva et al. (2012) found that populations of Campeiro, Lavradeiro, Pantaneiro, Baixadeiro, Mangalarga Marchador, Arab,

and thoroughbred horse breeds were not in HWE. Those authors used 11 microsatellites, six of which were used in the present study (HMS3, HMS6, HMS7, HTG6, HTG7, and VHL20).

F_{IS} values indicated that random mating was more prevalent because no statistical significance was detected. However, a global deficit of heterozygotes was observed, which may indicate the repetitive use of stallions and mares from a small part of the population (in isolated cases). This could also be due to a large number of castrated males (Melo, 2011), which leads to the use of the same stallions. Nevertheless, the heterozygote deficit was not sufficient to make the F_{IS} value significant and affect HWE. If this scenario continues, it is possible that a considerable decline in genetic diversity may occur, placing the Nordestino horse under threat. Under social, economic, and political scenarios, the Nordestino horse could lose its genetic heritage, whereby it is adapted to the Brazilian semiarid environment. The Nordestino horse breed may undergo a gradual decline in numbers, mainly because it is no longer an officially registered animal, leading to a lack of interest in the breed and the current practice of castrating males.

Garrano, Sorraia, Andalusian, and other Iberian horse breeds were the main contributors to the genetic formation of Brazilian horses (Hendricks, 2007). This is consistent because the colonizers brought Barb horses from the Iberian Peninsula to Brazil. Historically, horses from Argentina and Paraguay arrived in Brazilian territory with Jesuits, who passed through other South American countries. Those historical accounts support the notion that South American horse breeds are members of the same group. Among the Iberian horse breeds used in this study, the Sorraia breed was closest to the Nordestino horse (Figure 1). However, the results should be treated with caution, because the Sorraia breed possessed low levels of allelic diversity and considerable inbreeding (Luís et al., 2007).

The Sorraia horse has similar phenotypic characteristics to the Nordestino horse - both are small animals, have a low slope of the hip and tail-head insertion, and are mediolineous (body balance), with an ability to saddle and provide light traction (Associação Internacional de Criadores do Cavalo do Sorraia, 2006; Melo, 2011). According to Hendricks (2007), the Sorraia horses are able to withstand extreme climates and survive with little foraging while at the same time maintaining their health. The Nordestino horse is able to tolerate the heat and semiarid conditions, takes long walks under strong sun and hard soil conditions, survives on small feedings, and retains performance for a long time (Melo, 2011). Therefore, the Sorraia breed had a significant influence on Nordestino horse formation, and the use of microsatellite markers confirmed the relationship between Nordestino and Sorraia horse breeds.

It was clear that introgressions were common in the Nordestino horse during recent years, mainly with Campolina, Mangalarga Marchador, and Mangalarga horses. In some areas where the Nordestino horse is found (mainly in Pernambuco State), Campolina, Mangalarga Marchador, and Mangalarga studs can be found in close proximity, facilitating crossbreeding.

Older horsemen and cowboys who worked with the Nordestino horse reported that some of these workers in the past had attempted to increase the height of the Nordestino by crossing it with the Campolina. The wither height of the Nordestino horse was subject to criticism because it is a small horse. Even today, this may be a factor contributing to a disinterest in the support of this horse. Even so, the Nordestino is a small horse and different breeds exist for each production system. It is unlikely that a huge horse would perform with the same efficiency as the Nordestino horse runs in a hot, semiarid, and low foliage environment without losing any of its adaptive characteristics. Additional crossing may have occurred with

the Mangalarga Marchador horses. In the past, there was an interest to introduce gait “marcha” in the Nordestino horse. This historical evidence supports the Nordestino horse being closest to the Campolina and Mangalarga Marchador breeds within the tree.

Conflicts of interest

The authors declare no conflict of interest.

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