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Genetic divergence and phenotypic characterization in the Mangalarga Marchador breed

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ABSTRACT - The objective was to verify the existence of lineages in the Mangalarga Marchador breed using microsatellites and to phenotypically characterize the lineages found using linear morphometric measurements taken at the time the animals were registered to be used as a tool for selecting individuals according to each breeder's selection objective. The genotyping database contained 255,257 Mangalarga Marchador horses born between 2005 and 2021. In addition, a database was used with pedigree information on 622,299 animals born between 1952 and 2020 and a database of phenotypes assessed at the time of definitive registration of these animals, containing 303,248 animals born between 1950 and 2018. The databases used came from the Associação Brasileira dos Criadores do Cavalo Mangalarga Marchador. To verify the existence of genetic differences according to the lineages of the breed, a discriminant analysis of principal components (DAPC) and the Kruskal Wallis test were carried out to perform phenotypic characterization to compare the means of the morphometric measurements, both using the R software. The DAPC analysis showed the genetic differentiation of the Angai and Herdade lineages in relation to the others. The Herdade lineage showed greater phenotypic differentiation when compared with the three separate genetic groups in this study, demonstrating that the animals of this lineage are smaller and more gathered and have higher scores for gait and morphology. When compared to the historically described Mangalarga Marchador lineages, the breed does not have so many genetic groups, separating it into just three groups (Angai, Herdade, and General), and when compared to phenotypic characteristics, only the Herdade lineage stood out.

Keywords: equine, genetic improvement, lineages, microsatellites, principal components

1. Introduction

The Mangalarga Marchador (MM) breed is characterized by its elegant size and docile temperament and for being suitable for riding (Casiuch, 2016). It was considered a national heritage by law no. 12.975/2014, to guarantee its preservation and genetic diversity (ABCCMM, 2020).

The Associação Brasileira dos Criadores do Cavalo Mangalarga Marchador ("Brazilian Association of Mangalarga Marchador Horse Breeders", ABCCMM) and the breeders mainly value animals with traits for work on rural properties, horseback riding, hunting, and sports competitions, in addition to comfort, rusticity, docility, and adequate gait (Beck, 1992).

In search of a greater standardization and with an interest in fixing mainly the traits of gait, the families of the south of Minas Gerais began to select the animals according to their own interests and personal preferences, forming "lineages" in the breed with individual traits, using matings, most of the time endogamous (Costa et al., 2005a).

Casiuch (2016) and Bortoni (1991) historically affirm the existence of lineages in the MM breed, but few studies have verified the existence of lineages using information from molecular markers. Thus, there are many questions about these historical claims.

Costa et al. (2005b) and Santos et al. (2019) carried out studies to identify lineages in the breed, but none of them sought genetic differentiation using microsatellite markers type (SSR). However, Souza (2021), even using information from molecular markers, did not observe a separation of the population into different lineages.

The hypothesis of this study is that there are genetically different lineages in the MM breed and these have phenotypic differences in terms of morphology. As we can see, there are few studies within the MM breed aiming at identifying lineages and, consequently, knowing the genetic variability of the breed. Thus, the objectives of this research were to genetically identify possible lineages in the MM breed using SSR and describe the phenotypic characterization of these lineages, so that it can be used as a tool when selecting individuals according to the selection objective of each breeder.

2. Material and Methods

2.1. Database

The databases used in the research were provided by the ABCCMM. The microsatellite genotyping bank contained 255,257 horses born between 2005 and 2021. In addition to this, a database with pedigree information of 622,299 animals born from 1952 to 2020 and a bank of phenotypes evaluated at the time of definitive registration containing information from 303,248 animals born from 1950 to 2018 were also used. The animals came from different properties throughout Brazil.

We used genotyping data from 11 highly polymorphic microsatellite loci standardized by the International Society of Animal Genetics (ISAG), namely AHT4, AHT5, ASB2, ASB17, ASB23, HMS2, HMS3, HMS6, HMS7, HTG4, and VHL20. These SSR used are distributed in eleven different chromosomes of the equine genome (24, 8, 15, 2, 3, 10, 9, 4, 1, 9, and 30, respectively).

2.2. Animal selection criteria

Sampling was performed from the genotyping and pedigree databases. The search for animals belonging to each lineage, according to Casiuch (2016), was carried out by searching in the pedigree database if each animal presented DNA parentage verification and if their parents and/or paternal and maternal grandparents also belonged to the same lineage, that is, if they had the same prefix or suffix, so that in this way the animals remained closed in the lineages until the second and/or third generation, increasing the reliability of the data studied.

In addition, animals that were closed in parents and/or maternal and paternal grandparents of this lineage, but that did not have the name of the same lineage in their suffix/prefix, were also considered from the same lineage.

2.3. Statistical analysis

2.3.1. Genetic differentiation between lineages

First, the database was previously separated into groups according to the lineages described by Casiuch (2016).

To verify the existence of lineages of genetically similar animals in the MM breed from the SSR data, the discriminant analysis with principal components (DAPC) was performed by the adegenet 2.0.0 package (Jombart et al., 2010) of the R software version 4.1.3 (R Core Team, 2022).

To choose the ideal number of principal components (PC) to be used in the analysis, the α -score was calculated using the adegenet package (Jombart et al., 2010) of the R software, whose method uses interpolation to reduce the intensive use of processing, reducing the number of estimated α -scores in the chosen PC range.

To determine whether there is genetic differentiation between the lineages, we used the standardized statistics, F (Fis), which represents the population's coefficient of inbreeding, and D-Jost, which tells us how the population is structured compared to others, using the diveRsity package in the R software. The effective population size was determined using NeEstimator. The coefficient of inbreeding and kinship was determined using the TrioML method in the coancestry program.

2.4. Phenotypic characterization

The phenotypic characterization of each lineage was carried out using the morphometric measurements evaluated at the time of definitive registration, which are: height at withers, height at croup, head length, neck length, back-loin length, croup length, shoulder length, body length, head width, croup width, thoracic perimeter, and shin perimeter, in addition to scoring measures such as gait and morphology.

Descriptive analyzes were performed for each trait by lineage using the SAS (Statistical Analysis System, 2010) program and the Kruskal Wallis test at 5% probability to compare means, using the R software. The Kruskal-Wallis is a non-parametric statistical test that assesses the differences between three or more groups of independent samples on a single non-normally distributed continuous variable.

3. Results

Data from 1,357 MM horses were used, divided into eleven lineages previously described by Casiuch (2016). Lineages that had less than ten animals were discarded from the analyzes (Table 1).

The mean total percentage of missing data according to genotyping in the eleven loci was 8.9% in the studied animals. The locus ASB23 presented 80% of missing data in the Tabatinga lineage, which is considered a high number; however, it was not necessary to remove it from the analysis since the total percentage of missing data of this locus was 21.4%, considering all the lineages (Figure 1).

The confidence level to determine whether there is differentiation between the lineages reaches 100% considering at least six loci (Figure 2). Thus, as eleven loci were used during the research, the reliability of the study is considered high.

Table 1 - Number (n) of animals per lineage and total number of evaluated horses of the Mangalarga Marchador breed

Lineage	n
Angai	161
Bela Cruz	36
Catuni	121
Caxambu	540
Favacho	69
Herdade	141
Narciso	95
Passa Tempo	44
Porto	28
Tabatinga	15
Traituba	107
Total	1357

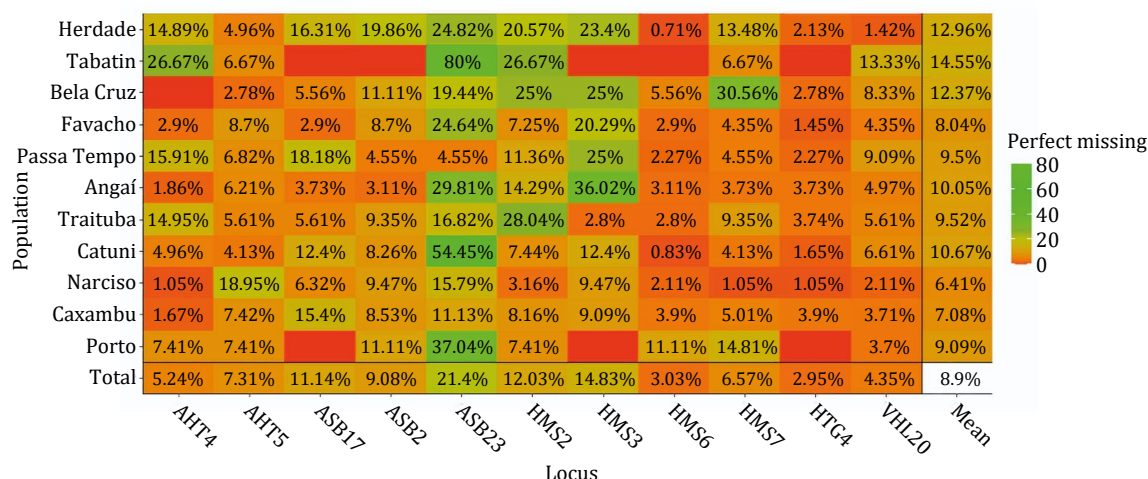


Figure 1 - Percentage of missing data in each lineage according to each studied locus and total mean percentage.

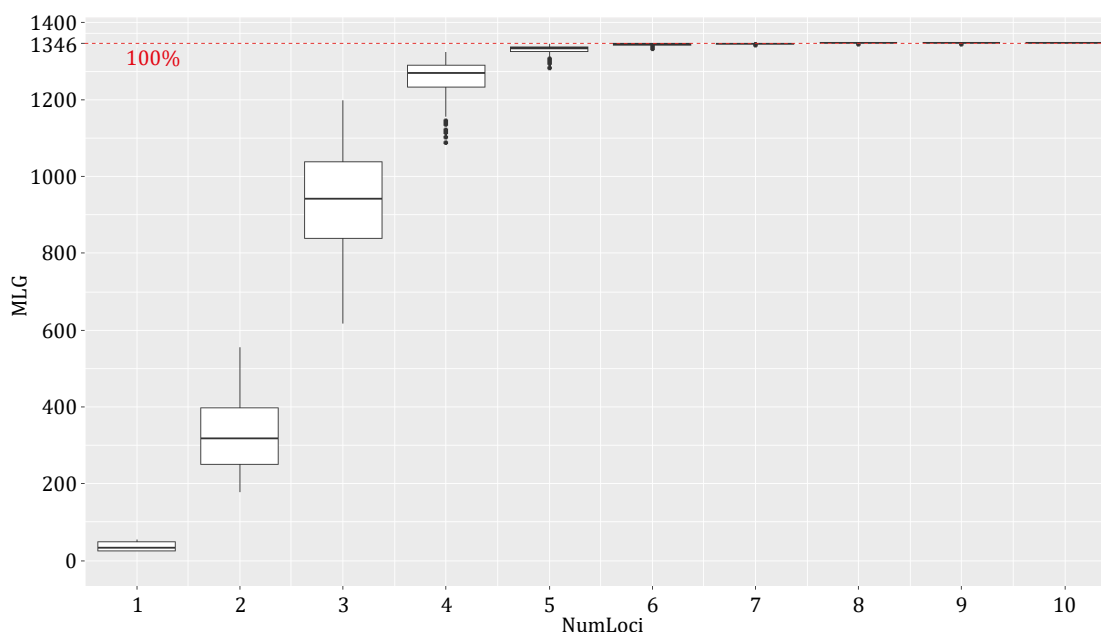


Figure 2 - Reliability level in relation to the number of loci studied in the Mangalarga Marchador breed.

The results of the coefficient of inbreeding (Fis) found in each lineage considering the loci studied obtained a mean of 4.30% in the Herdade, 6.30% in Tabatinga, 7.2% in Bela Cruz, 1.30% in Favacho, 5.4% in Passa Tempo, 4.80% in Angai, 6.30% in Traituba, 1.40% in Catuni, 7.70 in Narciso, 2.00% in Caxambu, and 1.70% in Porto (Table 2).

We observed that only the Favacho and Porto lineages presented more than half of the loci with excess homozygosity, which demonstrates a higher percentage of inbreeding, with a minimum of (−0.056 or 5.6%) and (−0.027 or 2.7%) and a maximum of (0.031 or 3.1%) and (0.150 or 15%), respectively (Table 2).

Table 2 - Fis values for each locus in relation to each lineage studied in the Mangalarga Marchador breed

Lineage	AHT4	AHT5	ASB17	ASB2	ASB23	HMS2	HMS3	HMS6	HMS7	HTG4	VHL20	Overall
Angai	-0.016	-0.082	-0.047	0.009	-0.070	-0.084	-0.096	-0.026	-0.043	-0.081	-0.022	-0.048
Bela Cruz	-0.104	-0.016	-0.026	-0.200	-0.076	-0.011	-0.033	0.000	-0.088	-0.097	-0.088	-0.072
Catuni	0.060	0.016	-0.034	0.086	0.059	-0.030	-0.086	-0.086	-0.065	-0.086	-0.029	-0.014
Caxambu	-0.045	-0.049	0.012	0.039	-0.027	-0.042	-0.074	0.017	-0.049	0.006	-0.006	-0.020
Favacho	0.031	0.014	0.016	0.038	0.004	-0.103	-0.107	0.023	0.015	-0.032	-0.056	-0.013
Herdade	-0.076	0.009	-0.095	-0.086	0.024	-0.007	0.008	-0.019	-0.107	-0.032	-0.069	-0.043
Narciso	-0.135	-0.219	-0.060	0.049	0.234	-0.089	-0.107	-0.312	-0.123	0.063	-0.169	-0.077
Passa Tempo	-0.038	-0.094	-0.011	-0.022	-0.112	-0.147	0.001	-0.017	-0.093	0.003	-0.056	-0.054
Porto	0.011	-0.027	0.150	0.088	0.031	0.025	-0.081	0.072	-0.197	-0.205	-0.033	-0.017
Tabatinga	-0.230	-0.098	-0.126	0.029	0.333	-0.132	0.247	-0.089	-0.107	-0.031	-0.331	-0.063
Traituba	0.031	-0.038	-0.099	-0.025	-0.029	-0.134	-0.134	0.000	-0.108	-0.067	-0.112	-0.063

In addition to Fis, the analysis of the coefficient of inbreeding and kinship was performed by the TrioML method in the coancestry program. Coefficients are given comparatively across study populations. We observed that the Porto lineage is the most inbred of the populations studied (0.15), while the Caxambu lineage is the one with the lowest inbreeding rate (0.07) (Table 3).

Regarding the kinship coefficient, the Tabatinga lineage presents higher values more frequently when compared with the other lineages. The Herdade lineage, on the other hand, presents lower values more frequently (Table 4).

The Bela Cruz, Catuni, Favacho, Herdade, and Caxambu lineages have private alleles in their loci, the latter being the lineage with the most private alleles (36) within the studied population (Table 5).

Considering the mean of all loci and P-value of 0.05, it is demonstrated that the Herdade, Angaí, Traituba, Catuni, Narciso, and Caxambu lineages are not in Hardy-Weinberg equilibrium (HWE). On the other hand, the Tabatinga, Bela Cruz, Favacho, Passa Tempo, and Porto lineages showed HWE values that demonstrate that they are in balance (Table 6).

The effective population size (N_e) was analyzed in each lineage studied, and this analysis takes into account the number of males and females that are effectively reproducing in the herd (Table 7).

The averages found for D-Jost at each locus, whose function is to separate populations according to alleles, are shown in Table 8. Values greater than 0.15 indicate good structuring of that locus in relation to the population studied, and this is what we observed in all 11 loci analyzed in this study, i.e., all loci are well structured (characterized) when compared to each other.

Table 3 - Coefficient of inbreeding by the TrioML F coancestry method for each lineages of the Mangalarga Marchador breed

Lineage	Means TrioML F
Herdade	0.13
Tabatinga	0.14
Bela Cruz	0.10
Favacho	0.13
Passa Tempo	0.08
Angaí	0.11
Traituba	0.08
Catuni	0.08
Narciso	0.10
Caxambu	0.07
Porto	0.15

Table 4 - Coefficient of kinship by the TrioML coancestry method among lineages of the Mangalarga Marchador breed

Lineage	Herdade	Tabatin	Bela Cruz	Favacho	Passa Tempo	Angaí	Traituba	Catuni	Narciso	Caxambu
Herdade										
Tabatinga	0.13									
Bela Cruz	0.13	0.14								
Favacho	0.06	0.15	0.13							
Passa Tempo	0.08	0.17	0.12	0.12						
Angaí	0.10	0.12	0.11	0.09	0.10					
Traituba	0.07	0.09	0.09	0.12	0.08	0.08				
Catuni	0.08	0.09	0.09	0.11	0.09	0.07	0.10			
Narciso	0.08	0.17	0.08	0.10	0.14	0.12	0.08	0.07		
Caxambu	0.09	0.07	0.09	0.09	0.08	0.07	0.09	0.10	0.09	
Porto	0.14	0.13	0.10	0.09	0.09	0.12	0.07	0.09	0.14	0.11

Table 5 - Private alleles of the lineages Bela Cruz, Catuni, Caxambu, Favacho, and Herdade

Private allele	Bela Cruz	Catuni	Caxambu	Favacho	Herdade
AHT4.38	0	0	1	0	0
AHT4.33	0	0	3	0	0
ASB17.15	0	0	16	0	0
ASB17.26	0	0	1	0	0
ASB17.27	0	0	3	0	0
ASB17.00	0	0	1	0	0
ASB23.23	0	0	1	0	0
HMS2.21	0	0	0	0	1
HMS2.23	4	0	0	0	0
HMS2.00	0	0	2	0	0
HMS3.24	0	0	3	0	0
HMS6.16	0	2	0	0	0
HMS7.15	0	0	0	1	0
HTG4.00	0	0	4	0	0
VHL20.15	0	0	1	0	0

The lineages that presented the highest frequency of D-Jost values above 0.15 were Herdade, Tabatinga, and Angaí (Table 9). These results demonstrate that the differentiation between these three lineages is much more defined when compared with the others in this study.

The D-Jost values compared with the lineages presented in this study show satisfactory values at the 95% confidence interval (Table 10).

According to the DAPC analysis, the ideal number of clusters was defined by the number of lineages analyzed in this study ($K = 11$), and the ideal number of PC retained is 28 to carry out the analysis (Figure 3).

Herdade and Angaí lineages obtained the greatest differentiation in relation to the others. Although they have not completely separated from the quadrant, it is possible to observe a genetic group that is further away and with less interference when compared with the others (Figures 4 and 5). This result confirms the differentiation according to the D-Jost values (Table 9).

After the DAPC, the D-Jost analysis was performed for the three genetic groups observed; Angaí, Herdade, and the lineages that did not separate by DAPC, were considered only one group, called General. When we compared the three groups, we observed that the Herdade and Angaí lineages presented a value greater than 0.15, demonstrating a good structure among them (Table 11).

For the phenotypic characterization study, data from 536 animals were used, which were separated into three groups: Angaí, Herdade, and General. The Kruskal Wallis mean test was carried out, which is a test for non-parametric data, and a significant value of $P = 0.05$ was considered. We observed that the only morphometric measurements that did not differ between the breeds were: head length, neck length, shoulder length, croup length, and loin length (Table 12).

For the most part, the means of morphometric measurements are lower in the Herdade lineage when compared with the others. However, the gait and morphology scores are also higher in the same lineage (Tables 13, 14, and 15).

4. Discussion

Costa et al. (2005a) found in the entire population of the MM breed registered an average of 11.30% for the coefficient of inbreeding, which is considered an acceptable level, corroborating those found

Table 6 - Probability values obtained for the exact test of Hardy-Weinberg equilibrium verified in the lineages of the Mangalarga Marchador breed

Lineage	AHT4	AHT5	ASB17	ASB2	ASB23	HMS2	HMS3	HMS6	HMS7	HTG4	VHL20	Overall
Angai	0.587	0.263	0.934	0.000	0.012	0.973	0.141	0.658	0.728	0.556	0.987	0.000
Bela Cruz	0.985	0.402	0.484	0.275	0.352	0.588	0.951	0.141	0.429	0.818	0.900	0.943
Catuni	0.254	0.352	0.320	0.015	0.176	0.654	0.106	0.457	0.758	0.895	0.001	0.008
Caxambu	0.454	0.073	0.997	0.000	0.000	0.850	0.000	0.853	0.596	0.686	0.509	0.000
Favacho	0.359	0.038	0.268	0.478	0.556	0.568	0.562	0.532	0.442	0.948	0.877	0.604
Herdade	0.337	0.881	0.532	0.000	0.223	0.999	0.867	0.675	7e-04	0.827	0.326	0.000
Narciso	0.827	0.027	0.910	0.575	0.044	0.000	0.155	0.006	0.364	0.000	0.289	0.000
Passa Tempo	0.756	0.005	0.533	0.745	0.421	0.906	0.732	0.786	0.172	0.959	0.133	0.382
Porto	0.276	0.521	0.586	0.021	0.362	0.949	0.622	0.030	0.899	0.878	0.218	0.184
Tabatinga	0.705	0.522	0.494	0.292	0.111	0.927	0.516	0.391	0.062	0.506	0.282	0.269
Traituba	0.016	0.976	0.916	0.261	0.863	0.826	0.041	0.000	0.257	0.351	0.748	0.000

Table 7 - Effective population size (N_e) in each lineage of the Mangalarga Marchador breed at 0.05 significance level

Lineage	N_e
Herdade	23.3
Tabatinga	124.5
Bela Cruz	23.6
Favacho	23.2
Passa Tempo	29.1
Angaí	41.1
Traituba	24.3
Catuni	41.8
Narciso	49.1
Caxambu	63.9
Porto	22.7

Table 8 - Mean values of D-Jost analysis for each locus studied in the Mangalarga Marchador breed

D-Jost	Mean
AHT4	0.2414
AHT5	0.3000
ASB17	0.2693
ASB2	0.2512
ASB23	0.3421
HMS2	0.2215
HMS3	0.2761
HMS6	0.1634
HMS7	0.2498
HTG4	0.1596
VHL20	0.3008
Global	0.2469

Table 9 - D-Jost values of the relationships between lineages in the Mangalarga Marchador breed

	Herdade	Tabatin	Bela Cruz	Favacho	Passa Tempo	Angaí	Traituba	Catuni	Narciso	Caxambu
Herdade										
Tabatin	0.1829									
Bela Cruz	0.1255	0.1705								
Favacho	0.2517	0.1300	0.1028							
Passa Tempo	0.1877	0.0943	0.1314	0.0937						
Angaí	0.1880	0.1776	0.1443	0.1820	0.1683					
Traituba	0.2272	0.1972	0.1442	0.0788	0.1193	0.1905				
Catuni	0.1607	0.1735	0.1130	0.0688	0.0998	0.1710	0.1130			
Narciso	0.1764	0.0913	0.1718	0.1011	0.0839	0.1240	0.1939	0.1346		
Caxambu	0.1144	0.2150	0.1292	0.1032	0.0898	0.1652	0.1061	0.0608	0.1164	
Porto	0.0726	0.1594	0.1449	0.1736	0.1038	0.0951	0.1731	0.1009	0.0901	0.0754

Table 10 - Confidence interval (CI) of D-Jost values at 95% among lineages in the Mangalarga Marchador breed

DjostEst comparison	BC_Lower_95%CI
Herdade vs. Tabatinga	0.1505
Herdade vs. Bela Cruz	0.0851
Herdade vs. Favacho	0.2213
Herdade vs. Passa Tempo	0.1666
Herdade vs. Angaí	0.1655
Herdade vs. Traituba	0.2056
Herdade vs. Catuni	0.1594
Herdade vs. Narciso	0.1529
Herdade vs. Caxambu	0.1026
Herdade vs. Porto	0.0549
Tabatinga vs. Bela Cruz	0.0989
Tabatinga vs. Favacho	0.0729
Tabatinga vs. Passa Tempo	0.0395
Tabatinga vs. Angaí	0.1388
Tabatinga vs. Traituba	0.1365
Tabatinga vs. Catuni	0.1857
Tabatinga vs. Narciso	0.0279
Tabatinga vs. Caxambu	0.1567
Tabatinga vs. Porto	0.1052
Bela Cruz vs. Favacho	0.0419
Bela Cruz vs. Passa Tempo	0.0940
Bela Cruz vs. Angaí	0.1211
Bela Cruz vs. Traituba	0.1115
Bela Cruz vs. Catuni	0.0829
Bela Cruz vs. Narciso	0.1337
Bela Cruz vs. Caxambu	0.0938
Bela Cruz vs. Porto	0.1064
Favacho vs. Passa Tempo	0.0602
Favacho vs. Angaí	0.1527
Favacho vs. Traituba	0.0393
Favacho vs. Catuni	0.0422
Favacho vs. Narciso	0.0944
Favacho vs. Caxambu	0.0818
Favacho vs. Porto	0.1449
Passa Tempo vs. Angaí	0.1254
Passa Tempo vs. Traituba	0.1269
Passa Tempo vs. Catuni	0.0950
Passa Tempo vs. Narciso	0.0393
Passa Tempo vs. Caxambu	0.1000
Passa Tempo vs. Porto	0.1058
Angaí vs. Traituba	0.1631
Angaí vs. Catuni	0.1677
Angaí vs. Narciso	0.0973
Angaí vs. Caxambu	0.1567
Angaí vs. Porto	0.0804
Traituba vs. Catuni	0.0782
Traituba vs. Narciso	0.1622
Traituba vs. Caxambu	0.0853
Traituba vs. Porto	0.1337
Traituba vs. Narciso	0.1329
Traituba vs. Caxambu	0.0512
Traituba vs. Porto	0.0740
Narciso vs. Caxambu	0.0974
Narciso vs. Porto	0.0558
Caxambu vs. Porto	0.0549

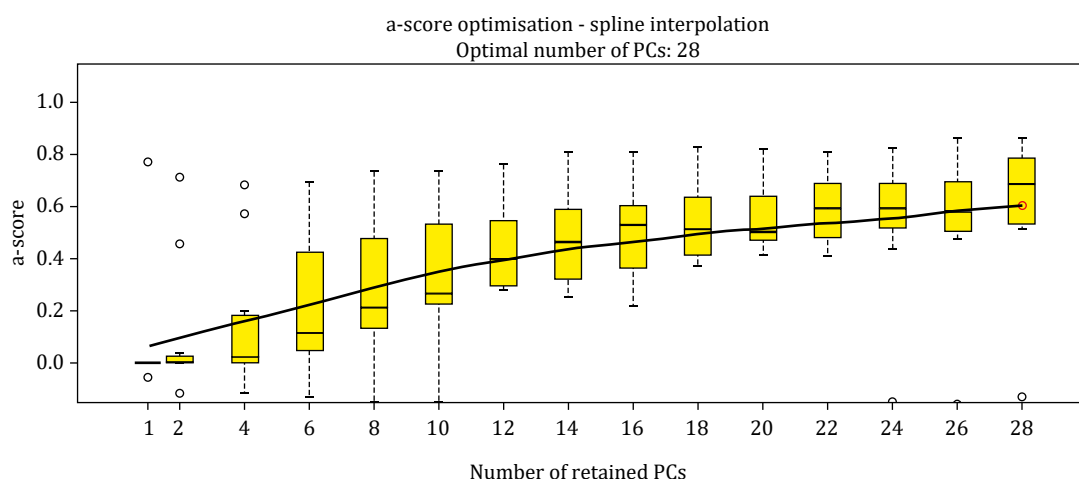


Figure 3 - Optimal number of principal components (PC) to be retained through the α -score.

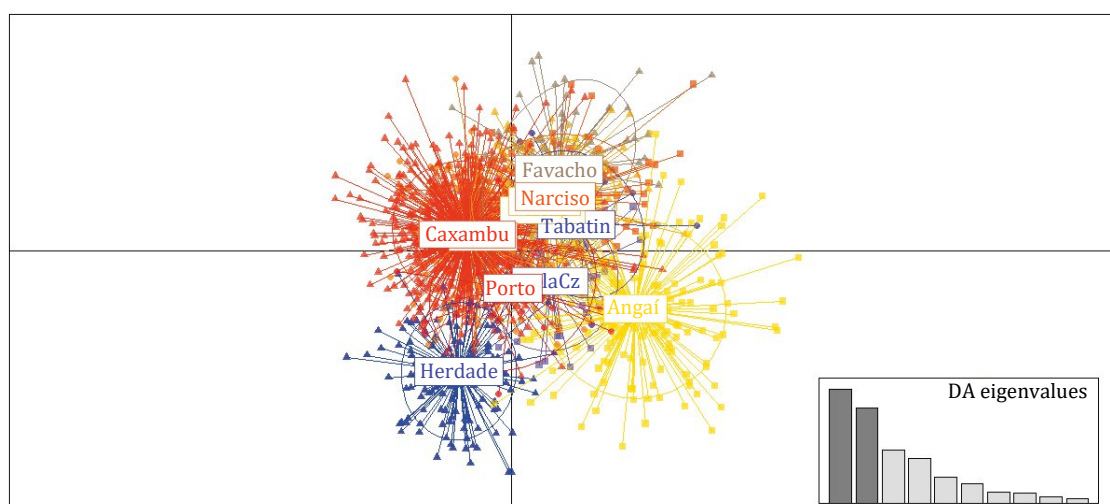


Figure 4 - Scatterplot of the discriminant analysis with principal components (DAPC) using eleven microsatellite markers in eleven lineages of Mangalarga Marchador horses.

in this study, demonstrating that breeders avoid mating between related animals, even within the same lineage.

Baena (2020), using the same molecular markers of this study, observed the F_{is} mean in the MM breed of -0.0195 ± 0.0254 , ranging from 0.0232 (ATH5) to 0.1490 (LEX03). In the present study, although the minimum value was observed in the same marker 0.009 (AHT5), the maximum value was 0.333 (ASB23).

Laat (2001) observed the variation of the average coefficient of inbreeding in the Campolina breed between 0.0129 and 0.0659, within the subpopulations per generation. Andalusian horses had an average inbreeding of 0.0117 (Valera et al., 1997). Other breeds that showed F_{is} values were Thoroughbred English (0.0102), French Trotter (0.0186), Arabian (0.0308), Anglo-Arabian (0.0117), and French Seal (0.007) (Moureaux et al., 1996).

According to studies on horses, the F_{is} values are low, demonstrating that breeders do not perform much mating between related animals, thus maintaining genetic variability in their populations.

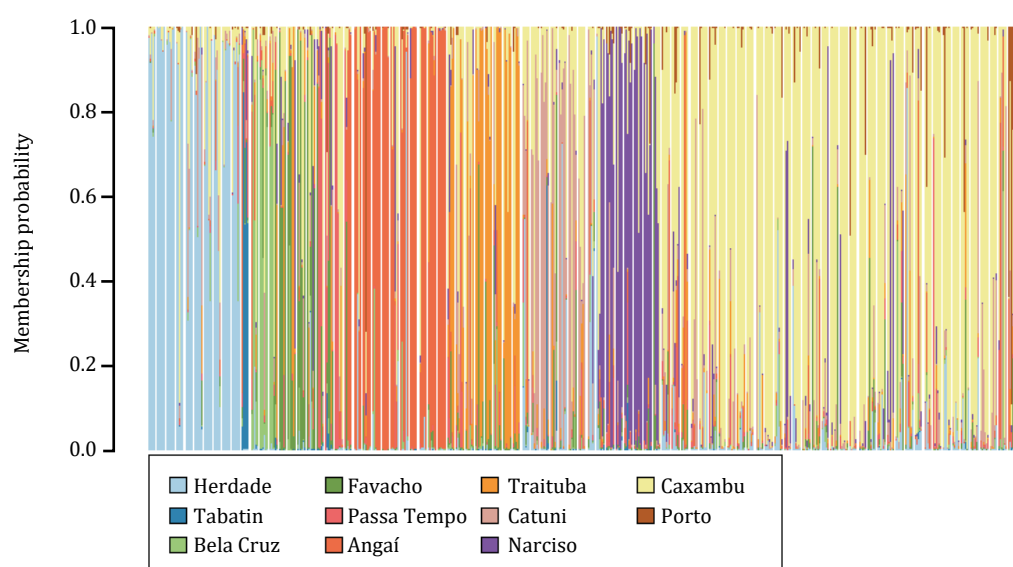


Figure 5 - Compoplot of the discriminant analysis with principal components (DAPC) using eleven microsatellite markers in eleven lineages of Mangalarga Marchador horses.

Table 11 - D-Jost values of the relationships between group genetic separated post DAPC

D-Jost	Herdade	General
Herdade	-	0.124
General	0.124	-
Angai	0.188	0.137

DAPC - discriminant analysis of principal components.

Silva Filho et al. (2007), in a study with the Marajoara, Puruca, Thoroughbred English, MM, Brasileiro de Hipismo, and Quarter Horse breeds observed that the five studied breeds were not in HWE. These same authors observed significant deviations in the loci HSM7, HTG4, and VHL20 in 79 MM animals from a single stud farm, and these loci were also analyzed in the current study.

Assis et al. (2009), considering other microsatellite markers, observed significant deviations in the HWE for the loci ASB3, LEX019, LEX031, TKY321, and VIASH39 when they analyzed each different population, but when analyzed together, they observed deviations only in the loci ASB3 and LEX031, in addition to observing that the value of zero was obtained in the global HWE test.

On the other hand, Silva et al. (2012) analyzed the genetic variability in five horse breeds naturalized in Brazil—Campeira, Lavradeira, Pantaneira, MM, and the Baixadeiro genetic group—, comparing with the exotic breed Purebred Arabe and concluded that the breeds were not in equilibrium. Thus, according to several studies presented, most of the equine breeds and populations studied were not in HWE, demonstrating that there is an evolution in these populations, that is, that there may be selection, migration, or mutation in these populations.

The values observed for N_e , are lower than those observed by Costa et al. (2005a) in the same breed. This difference may be due to the higher percentage of inbreeding in the populations observed in the present study.

No studies were found using the D-Jost parameter to differentiate between populations in horses. However, in fish, several studies used this parameter to determine whether there are differences between populations. The mean value considering the existence of differences between populations was 0.07 for Okazaki et al. (2017) and 0.14 for Landínez-García et al. (2020). Studies with lions and

Table 12 - Kruskal Wallis test values in comparison to the lineages according to the morphometric measurements of the animals of the Mangalarga Marchador breed

Lineage	Morphometric measurement											
	HW	HC	HL	NL	SL	BL	CL	BLL	HeW	HW	TC	SC
Angai × General	0.21	0.42	0.80	1.00	1.00	0.01*	0.97	1.00	0.04*	0.02*	0.15	0.51
Angai × Herdade	1.0e-06*	1.0e-06*	0.07	0.43	0.79	1.0e-02*	0.26	0.61	2.6e-09*	1.00	1.00	0.07
Herdade × General	1.8e-06*	3.7e-07*	0.21	0.84	0.80	0.20	0.87	0.50	1.9e-10*	2.5e-02*	0.04*	3.9e-03*

HW - height at withers; HC - height at croup; HL - head length; NL - neck length; SL - shoulder length; BL - body length; CL - croup length; BLL - back loin length; HeW - head width; HW - hip width; TC - thoracic circumference; SC - shin circumference; GAIT - gait score; MORP - morphology score.

* Significant values.

Table 13 - Descriptive statistics of morphometric measurements (in meters) in the Angaí lineage

Variable	n	Mean	Min	Max	CV	SD
HW	67	1.47	1.40	1.54	2.27	0.03
HC	67	1.46	1.38	1.53	2.32	0.03
HL	67	0.56	0.50	0.64	3.58	0.02
NL	67	0.61	0.55	0.65	3.91	0.02
BLL	67	0.48	0.41	0.54	5.57	0.03
CL	67	0.51	0.44	0.55	5.40	0.03
SL	67	0.51	0.43	0.57	4.86	0.02
BL	67	1.50	1.39	1.58	2.26	0.03
HeW	67	0.20	0.18	0.22	4.93	0.01
HW	67	0.50	0.43	0.54	4.99	0.02
TC	67	1.70	1.57	1.85	3.12	0.05
SC	67	0.18	0.16	0.20	3.81	0.01
GAIT	65	70.00	60.00	85.00	6.09	4.29
MORP	65	70.00	60.00	81.00	6.06	4.22

HW - height at withers; HC - height at croup; HL - head length; NL - neck length; SL - shoulder length; BL - body length; CL - croup length; BLL - back loin length; HeW - head width; HW - hip width; TC - thoracic circumference; SC - shin circumference; GAIT - gait score; MORP - morphology score; CV - coefficient of variation; SD - standard deviation.

Table 14 - Descriptive statistics of morphometric measurements (in meters) in the General group

Variable	n	Mean	Min	Max	CV	SD
HW	409	1.47	1.40	1.56	2.36	0.03
HC	409	1.45	1.36	1.55	2.29	0.03
HL	409	0.56	0.50	0.62	3.10	0.02
NL	409	0.61	0.52	0.70	4.76	0.03
BLL	409	0.47	0.40	0.56	6.55	0.03
CL	409	0.51	0.41	0.57	4.85	0.02
SL	409	0.51	0.44	0.60	5.05	0.02
BL	409	1.48	1.37	1.59	2.72	0.04
HeW	409	0.20	0.17	0.24	5.74	0.01
HW	409	0.49	0.40	0.55	4.40	0.02
TC	409	1.69	1.53	1.86	3.29	0.05
SC	409	0.18	0.16	0.21	4.58	0.01
GAIT	398	71.00	27.00	90.00	9.83	6.99
MORP	398	71.00	55.00	91.00	9.39	6.64

HW - height at withers; HC - height at croup; HL - head length; NL - neck length; SL - shoulder length; BL - body length; CL - croup length; BLL - back loin length; HeW - head width; HW - hip width; TC - thoracic circumference; SC - shin circumference; GAIT - gait score; MORP - morphology score; CV - coefficient of variation; SD - standard deviation.

bovines from southern Russia considered the determinant D-Jost value equal to 0.15. Thus, in the present study, a value of 0.15 was considered as the minimum to indicate that there is differentiation between populations.

Santos et al. (2019) investigated the formation of family structures in the MM breed through SNP genotyping, in an attempt to identify lineages according to the type of behavior of the animals. But despite finding formation of groups in the population, they did not verify the existence of lineages linked to the type of gait. Souza (2021) also working with the MM breed, analyzed lineage differentiations using 13 microsatellites, but with the methodology used, the author did not observe genetic differences between them.

Costa et al. (2005b) carried out a study to identify the formation of lineages in the MM breed and observed the existence of five distinct genetic groups in the population as representatives of the breed, being the lineages Herdade, Providência, Abaíba, Tabatinga, and Angaí. Three of these lineages

Table 15 - Descriptive statistics of morphometric measurements (in meters) in the Herdade lineage

Variable	n	Mean	Min	Max	CV	SD
HW	60	1.44	1.40	1.57	2.61	0.04
HC	60	1.43	1.38	1.53	2.33	0.03
HL	60	0.56	0.52	0.58	2.46	0.01
NL	60	0.61	0.53	0.68	3.96	0.02
BLL	60	0.48	0.40	0.56	6.23	0.03
CL	60	0.50	0.43	0.56	4.53	0.02
SL	60	0.51	0.44	0.57	5.03	0.02
BL	60	1.47	1.39	1.64	3.13	0.045
HeW	60	0.19	0.17	0.21	6.51	0.01
HW	60	0.50	0.45	0.54	3.79	0.02
TC	60	1.70	1.60	1.80	2.19	0.04
SC	60	0.18	0.17	0.20	3.07	0.00
GAIT	59	77.00	65.00	85.00	5.52	4.24
MORP	59	74.00	65.00	82.00	5.44	4.04

HW - height at withers; HC - height at croup; HL - head length; NL - neck length; SL - shoulder length; BL - body length; CL - croup length; BLL - back loin length; HeW - head width; HW - hip width; TC - thoracic circumference; SC - shin circumference; GAIT - gait score; MORP - morphology score; CV - coefficient of variation; SD - standard deviation.

had the greatest genetic differentiation observed in the present study, demonstrating that they are indeed distinct subpopulations within the MM breed. Marchiori et al. (2019) sought to genetically separate the racing and working lineages of the Quarter Horse breed, and found genetic differences between these lineages. Thus, observing studies seeking to identify lineages in the MM breed, we noticed the absence of work with genetic differentiation of populations, even though this method is of great importance and is the most used in several breeds.

For the phenotypic differentiation and with the performance of the test, it was observed that the Herdade lineage presented greater differentiation in relation to the other lineages. Compared with Angaí, Herdade presented six different morphometric measurements, whereas when compared with General, it presented eight different measurements. Only for neck, head, shoulder, croup, and back-loin length measurements, there was no difference among the three groups. When we compare the differences between Angaí and General, we observe only three different measurements between them.

Analyzing the descriptive statistics, we can conclude that the Herdade lineage has lower height at withers, height at croup, body and croup length, and head width when compared with the other two groups (Angaí and General). However, it presents a score of gait and morphology with higher values, proving to be a lineage with smaller animals but with better gait and morphology.

5. Conclusions

In the present study, the population of Mangalarga Marchador presented three groups with genetic differentiation by SSR: Herdade and Angaí lineages and a single group with all the other nine lineages (General). Only the Herdade lineage showed differences between their morphological measurements taken at the time of the animals' registration, showing animals with lower withers and rump heights and body length. Animals of the Herdade lineage are horses with better morphology and gait, allowing the breeder to look for animals for reproduction.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Araujo, B. P. G. and Meirelles, S. L. C. **Data curation:** Araujo, B. P. G. **Formal analysis:** Araujo, B. P. G. and Perazza, C. A. **Methodology:** Araujo, B. P. G. and Perazza, C. A. **Project administration:** Moura, R. S. and Meirelles, S. L. C. **Resources:** Araujo, B. P. G. **Supervision:** Meirelles, S. L. C. **Validation:** Araujo, B. P. G. **Writing – original draft:** Araujo, B. P. G. **Writing – review & editing:** Araujo, B. P. G.; Perazza, C. A. and Meirelles, S. L. C.

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