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Organizational structure of the Mangalarga herd and the influence of geographic distribution and different coats on the genetic diversity

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ABSTRACT - The objective was to describe the organizational structure of the Mangalarga herd and evaluate the influence of geographic distribution and different coats on the population structure and genetic diversity. Genealogic data of horses born between 1919 and 2018 were submitted to the software ENDOG 4.8, corresponding to the total population (TP), and those born between 2009 and 2018, representing the reference population (RP). Wright's fixation index, inbreeding coefficient (F), and average relatedness (AR) were estimated considering each Brazilian state as a subpopulation and, afterwards, the different coat colors as subpopulations. The origin and use of breeding animals were used as parameters to classify the populations in the different states within the strata: Nucleus, Multiplier, Commercial, and Isolated. Considering Brazilian states as subpopulations, the mean value estimated for F_{IS} in the RP was 0.0091, F_{IT} of 0.0107, F_{ST} of 0.0016, F of 5.81%, and AR of 3.81%. In the RP, the sorrel coat accounted for 87.58% of the genetic contribution. Considering different coat colors as subpopulations, the mean value estimated for F_{IS} in the RP was 0.0103, F_{IT} of 0.0107, F_{ST} of 0.0004, F of 3.53%, and AR of 3.29%. In the RP, the breed is present in 14 states. Of those, 53.33% were classified as Multiplier herds and 46.67% as Commercial herds. The organizational structure of the Mangalarga breed is not pyramid shaped and, even with the greater genetic contribution of the state of São Paulo and of the sorrel coat, the inbreeding coefficient is under control.

Keywords: biodiversity, equine, inbreeding

1. Introduction

The Mangalarga breed was formed from the search for horses with a comfortable gait that are capable of hunting and handling cattle. Due to the sports ability and specific gait, the breed expanded among breeders in the Brazilian state of São Paulo, who spread it across that state and neighboring ones (Junqueira, 2004). In 1934, the Associação Brasileira de Criadores de Cavalos da Raça Mangalarga (Brazilian Association of Mangalarga Horse Breeders; ABCCRM) was founded as the expansion of the breed led to the need of standardizing the herd.

According to Almeida et al. (2021), up until 2018, Mangalarga breeding farms had been identified in 23 of the 26 Brazilian states plus the Distrito Federal. The state of São Paulo holds the largest herd of the breed (75.71% of the animals), followed by Minas Gerais (11.29%) and Bahia (4.64%). It is visible that only these two states of the Southeast region host 87% of the registered herd. In addition, those authors observed that, irrespective of the state, the sorrel coat is predominant, present in 72.7% of the herd. The concentration of Mangalarga horses in the state of São Paulo and the high frequency of a single coat color in the herd raises questions on the genetic diversity of the population.

Genetic diversity studies have been very important in breed improvement programs and conservation as they provide information on the identification of genetically superior animals and on inbreeding, thus contributing to decision-making regarding genetics (Assis et al., 2018). Among the tools employed to study genetic diversity is F statistics (Wright, 1978), which uses inbreeding coefficients to describe the partition of the intra- and interpopulation genetic variation.

Animal genetic improvement programs usually have an organizational structure of the herds in three strata, forming a pyramid-shaped arrangement. The genetic provision chain begins at the top of the pyramid, in the Nucleus herd, where intensive selection of economically important characteristics takes place. Next are the Multiplier herds, and on the base are commercial animals, which consist in the final product (Guimarães et al., 2017). Despite the importance of the matter, studies on the structure and gene flow direction between the different strata in horse breeding programs are scarce.

The objective was to describe the organizational structure of the Mangalarga herd and evaluate the influence of geographic distribution and different coats on the population structure and genetic diversity of the breed.

2. Material and methods

2.1. Pedigree data

Information from the database of the genealogic registration service at ABCCRM of horses born between 1919 and 2018 in Brazil was used, yielding a file with data on 206,426 animals.

The study was carried out considering the total population (TP) and the reference population (RP), defined as the group of animals registered with date of birth between 2009 and 2018 (n = 20,539). That group made up the most recent generation, according to the mean generation interval calculated for the breed of 9.43 years.

2.2. Statistical analyses

The pedigree data were submitted to the software ENDOG 4.8 (Gutiérrez and Goyache, 2005).

The pedigree quality evaluation considered the proportion of known ancestors per parental generation and the number of equivalent generations known per animal. This was determined as the sum of all known ancestors, computed as (1/2)n, in which n is the number of generations that separate the individual from each known ancestor (Maignel et al., 1996).

To assess the genetic variation of the Mangalarga breed, first, each Brazilian state where Mangalarga horses are registered was considered a subpopulation. A second analysis took into account the different coat colors found in the breed as subpopulations. Information about the Brazilian state where the animal was born and its coat color was extracted from the database.

The genetic contribution of the subpopulations for total diversity was estimated according to Caballero and Toro (2002) using the formula:

$$\bar{f} = \frac{\sum_{i,j=1}^{n} f_{ij} N_i N_j}{N_T^2} = \frac{\sum_{i=1}^{n} f_{ii} N_i}{N_T} - \bar{D} = \sum_{i=1}^{n} \frac{N_i}{N_T} \left[f_{ii} - \frac{\sum_{j=1}^{n} D_{ij} N_j}{N_T} \right] \quad (1)$$

in which f_{ij} is the average of the coancestry pairs between the individuals of subpopulations i and j, including all $N_i \times N_j$ pairs, and f_{ii} is the average coancestry of pairs within subpopulation i, and D_{ij} is the genetic distance (Nei, 1987).

The genetic distance between subpopulations was estimated based on Nei's minimum distance using the following equation:

$$D_{ij} = D_{ij} - [(D_{ii} + D_{jj})/2] \tag{2}$$

The fixation indices or Wright's F-statistics were calculated based on the formulas:

$$F_{IS} = \frac{\bar{F} - \bar{f}}{1 - \bar{f}}$$

$$F_{ST} = \frac{\bar{f} - \tilde{f}}{1 - \tilde{f}} = \frac{\bar{D}}{1 - \tilde{f}}, \text{ and}$$

$$F_{IT} = \frac{\tilde{F} - \tilde{f}}{1 - \tilde{f}} \tag{3}$$

in which $\tilde{f}$ and $\tilde{F}$ are the coancestry medium and the procreation coefficient, respectively, for the entire metapopulation, and $\bar{f}$ is the mean coancestry for the subpopulation, so that [4] $(1 - F_{IT}) = (1 - F_{IS})\,(1 - F_{ST})$, in which $\mathrm{F_{IT}}$ is the fixation index of the total population; $\mathrm{F_{IS}}$ is the fixation index within populations, and $\mathrm{F_{ST}}$ is the fixation index between subpopulations.

The average relatedness (AR), defined as the probability that a randomly chosen allele from the population in the pedigree belongs to a given animal, was calculated as described by Gutiérrez and Goyache (2005).

The coefficient of inbreeding (F) is defined as the probability that an individual has two identical alleles due to ancestry (Wright, 1931) and was calculated as described by Meuwissen and Luo (1992).

To assess the organizational structure of the Mangalarga breed, each Brazilian state where Mangalarga horses are registered was considered a subpopulation. The origin and use of breeding animals were used as parameters to classify the subpopulations into the following strata: Nucleus (herds that only use their own breeding animals and that commercialize them), Multiplier I (herds that use external breeding animals or animals from their own herd, and sell breeding animals), Multiplier II (herds that use only breeding animals purchased from other herds and do not commercialize them), Commercial I (herds that use external and internal breeding animals and do not commercialize them), Commercial II (herds that use internal breeding animals only purchased from other states and do not commercialize them), and Isolated (herds that use their own breeding animals and do not commercialize them).

3. Results

3.1. Pedigree quality evaluation

The number of equivalent generations known per animal progressively increased over time, reaching an average of 2.75 in the TP and 4.88 in the RP.

The mean percentages of ancestors of the TP and RP, respectively, were as follows: 87.25 and 99.89% of known parents, 73.41 and 99.61% of grandparents, 43.12 and 96.01% of great-grandparents, 32.24 and 79.50% great-great-grandparents, and 14.92 and 53.02% of great-great-great-grandparents. Males had a greater amount of ancestral data than females, and the ancestors registered in 2002, 2005, 2008, 2010, 2012, and 2013 had 100% of known parents.

3.2. Genetic diversity considering Brazilian states as subpopulations

The TP of the Mangalarga breed was present in 21 of the 26 Brazilian states plus the Distrito Federal, with no Mangalarga horse registered in only four states of the Northeast region (Ceará, Paraíba, Piauí, and Rio Grande do Norte). The most current population of the breed (RP) was found in only 14 states and the Distrito Federal, not being present in the Northeastern states of Alagoas, Maranhão, Pernambuco, and Sergipe and in three states of the North region (Amapá, Amazonas, and Tocantins).

For the TP, the mean estimated value of F_{IS} was 0.0094, ranging from −0.0771 in Amapá to 0.0121 in Goiás, while the mean estimated value for F_{IT} was 0.0106. In the RP, the mean estimated F_{IS} value was 0.0091, ranging from −0.0965 in Rondônia to 0.0135 in São Paulo, while the mean estimated value for F_{IT} was 0.0107.

The state of São Paulo contributed with 66.01% of the genetics of the entire breed in the TP, followed by Minas Gerais (1.28%), Paraná (0.69%), Bahia (0.24%), and Goiás (0.14%), with little contribution by the other states, adding up to 0.20% (Table 1).

Table 1 - Number of individuals and genetic variability parameters for registered herds of the Mangalarga breed per Brazilian states in the total population and reference population

Federative unit	N	%	F (%)	AR (%)	PBSH	PBAH	BBHAF	BABOH
			Total population					
São Paulo	155,029	66.01	2.29	2.46	130,994	24,050	160,440	29,446
Minas Gerais	12,853	1.28	3.05	2.93	3205	9648	6696	3491
Paraná	7699	0.69	1.70	2.30	1194	754	2405	1211
Bahia	6750	0.24	3.09	3.05	1908	4842	2381	473
Goiás	4379	0.14	2.25	1.98	613	3766	1002	389
Mato Grosso	1107	9.10×10^{-2}	1.12	1.14	11	1096	940	929
Mato Grosso do Sul	3147	6.64×10^{-2}	1.93	2.05	366	2781	522	156
Rio de Janeiro	1429	2.69×10^{-2}	2.04	1.82	322	1107	394	72
Distrito Federal	1115	3.69×10^{-3}	2.92	3.18	78	1037	135	57
Alagoas	65	2.93×10^{-3}	3.57	1.95	10	55	10	0
Pará	797	2.31×10^{-3}	2.98	2.71	43	754	172	129
Pernambuco	128	1.46×10^{-3}	6.86	1.79	7	121	7	0
Rondônia	110	1.46×10^{-3}	1.46	2.58	0	110	74	0
Santa Catarina	771	1.02×10^{-3}	1.62	2.54	74	704	7	0
Espírito Santo	43	8.82×10^{-4}	1.94	2.06	0	50	12	12
Sergipe	31	8.76×10^{-4}	0.20	0.50	0	31	0	0
Tocantins	248	8.84×10^{-4}	1.67	2.10	0	248	38	38
Rio Grande do Sul	1333	1.49×10^{-4}	2.51	3.14	200	1133	243	43
			Reference population					
São Paulo	15,543	76.23	5.27	4.05	1907	13,636	2243	336
Paraná	360	3.80	6.07	4.98	31	329	36	5
Minas Gerais	2310	2.10	6.18	4.72	181	2129	565	384
Goiás	385	0.53	4.23	3.32	8	377	8	0
Bahia	954	0.27	5.10	4.32	36	918	42	6
Espírito Santo	27	0.02	2.56	2.18	0	27	12	12
Distrito Federal	149	0.01	7.26	5.00	14	135	14	0
Rio de Janeiro	166	1.95×10^{-2}	3.86	2.30	9	157	14	5
Rondônia	12	1.70×10^{-2}	1.54	2.98	0	12	0	0
Mato Grosso	13	1.19×10^{-2}	6.34	2.05	0	13	0	0
Mato Grosso do Sul	195	7.39×10^{-3}	3.64	2.06	6	189	14	8
Rio Grande do Sul	114	3.69×10^{-3}	5.64	4.66	12	102	50	38
Pará	150	2.47×10^{-3}	7.06	4.91	1	149	1	0
Acre	128	2.43×10^{-3}	7.93	5.27	4	124	4	0
Santa Catarina	27	5.30×10^{-4}	5.40	4.40	0	27	0	0

N - number of individuals registered in the herd; % - genetic contribution; F - inbreeding coefficient; AR - average relatedness; PBSH - number of individuals born in the herd with parents born in the same herd; PBAH - number of individuals born in the herd with parents born in another herd; BBHAF - number of times male breeding animals born in the herd acted as fathers; BABOH - number of times male breeding animals born in the herd acted as breeders in other herds.

The five states with the greatest genetic contribution in the TP were also the ones that most contributed genetically to the RP; however, the states of Paraná and Goiás climbed one position in the classification, at the same time Minas Gerais and Bahia dropped one position. Moreover, those five states had a greater genetic contribution to the RP when compared with the contribution to the TP. The state of São Paulo contributed with 76.23% of the genetics of the entire breed, followed by Paraná (3.80%), Minas Gerais (2.10%), Goiás (0.53%), and Bahia (0.27%), with the remaining states contributing with 0.09%.

The mean F value for the TP was 2.40%, ranging from 0.20% in Sergipe to 6.86% in Pernambuco. The highest F values were observed in the Mangalarga herds of Pernambuco (6.86%), Alagoas (3.57%), Bahia (3.09%), and Minas Gerais (3.05%). In the state of São Paulo, which holds the largest herd of the breed in Brazil, F was 2.29%.

The mean F value for the RP was 5.81%, between 1.54% in Rondônia and 7.93% in Acre. The highest F values were observed in the Mangalarga herds of Acre (7.93%), Distrito Federal (7.26%), Pará (7.06%), and Mato Grosso (6.34%). In the state of São Paulo, the F value of the RP was 5.27%.

As for the TP, the states that most contributed to the formation of the breed (São Paulo, Minas Gerais, and Paraná) exhibited AR values between 2.30 and 2.93%. Across all Brazilian states, the average was 2.24%, ranging from 0.50% in Sergipe to 3.18% in the Distrito Federal. In the RP, the mean value of AR was 3.81%, between 2.05% in Mato Grosso and 5.27% in Acre. The states of São Paulo, Minas Gerais, and Paraná had AR values of 4.05, 4.72, and 4.98%, respectively in the RP.

In the TP, the mean F_{ST} value in the herds of Brazilian states was 0.0012, with variation from 5.3×10^{-6} between Amapá and São Paulo to 0.0289, between Amapá and Sergipe. In the RP, the mean F_{ST} value in the herds of Brazilian states was 0.0016, with variation from 6.29×10^{-5} between Santa Catarina and São Paulo to 0.0473 between Mato Grosso and Rondônia.

3.3. Genetic diversity considering different coat colors as subpopulations

The sorrel coat accounted for 78.99% of the genetic contribution to the TP, followed by bay and grey, at 5.52 and 5.28%, respectively. The other coat colors had very low contributions when compared with the aforementioned ones, representing 10.19% of genetic contribution. In the RP, the genetic contribution of the sorrel coat increased (87.58%), while the contribution of the remaining coat colors decreased, except for palomino.

The mean F value for the TP was 1.03%, ranging from 0.21% in the buckskin coat to 2.36% in the sorrel tobiano coat (Table 2). In the RP, the mean F value (3.53%) was higher than in the TP. As in the TP, the lowest F value was also found in the buckskin coat (0.92%), but the highest F in the RP was found for the sorrel coat (5.99%).

The mean AR value for the TP was 1.15%, ranging from 0.18% in the buckskin coat to 2.45% in the sorrel tobiano coat. As observed for F, the mean AR value was also higher in the RP (3.29%) than in the TP. The lowest AR value was also found in the buckskin coat (2.24%), while the highest AR was found for the sorrel coat (4.44%).

The mean F_{IS} value estimated for the TP was 0.0097, ranging from −0.0123 in the sorrel tobiano coat to 0.0099 in the sorrel coat, while the mean estimated value for F_{IT} was 0.0106. In the RP, the mean estimated F_{IS} value was 0.0103, from −0.1089 in the palomino tobiano coat to 0.0116 in the sorrel coat, while the mean estimated value for F_{IT} was 0.0107.

For the TP, the mean F_{ST} value was 0.0006, considering all registered coat colors of the Mangalarga herd, ranging from 2.14×10^{-5} between the black tobiano and sorrel coats and 0.0050, between the black tobiano and sorrel tobiano coats. For the RP, the mean F_{ST} value was 0.0004, considering all coat colors, ranging from 0.0002, between sorrel and palomino tobiano to 0.0522, between palomino tobiano and buckskin tobiano.

Table 2 - Number of individuals registered in the herd (N), genetic contribution (%), inbreeding coefficient (F), and average relatedness (AR) of the registered herds of the Mangalarga breed split by coats in the total population and reference population

Coat	N	%	F (%)	AR (%)
		Total population		
Sorrel	57,449	78.99	2.01	2.28
Bay	9572	5.52	0.49	0.61
Grey	7195	5.28	0.98	1.16
Red roan	1319	4.56	0.71	0.88
Black	2549	1.60	1.07	1.32
Buckskin	1362	0.90	0.21	0.18
Palomino	1461	0.90	1.35	1.57
Black tobiano	1056	0.75	1.88	1.96
Bay tobiano	1280	0.61	0.82	1.12
Grey tobiano	373	0.48	0.45	0.73
Sorrel tobiano	1135	0.35	2.36	2.45
Grullo	124	0.02	0.38	0.30
Buckskin tobiano	104	0.01	0.74	0.40
		Reference population		
Sorrel	2895	87.58	5.99	4.44
Grey	204	3.21	5.35	4.09
Red roan	9	2.66	2.28	2.33
Bay	122	2.54	4.98	3.34
Black	138	1.47	4.37	3.83
Palomino	123	1.02	3.36	3.43
Black tobiano	371	0.39	2.84	2.64
Buckskin	10	0.35	0.92	2.24
Grey tobiano	23	0.30	2.27	3.27
Bay tobiano	207	0.24	2.05	2.67
Sorrel tobiano	295	0.24	4.39	3.93

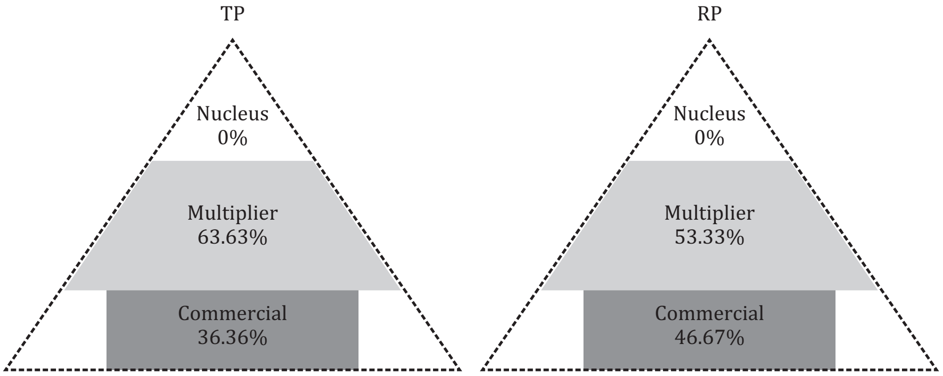
3.4. Organizational structure of the Mangalarga breed

In the TP, among the 21 states where the breed was present plus the Distrito Federal, 63.63% of the herds were classified as Multipliers and 36.36%, as Commercial (Figure 1). In the RP, 53.33% of the herds were classified as Multipliers and 46.67% as Commercial.

In the TP, 11 states (Bahia, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Paraná, São Paulo, Distrito Federal, Pará, Rio de Janeiro, and Rio Grande do Sul) were classified as Multiplier I. Only three states (Espírito Santo, Maranhão, and Tocantins) were classified as Multiplier II herds (Table 3). Four Commercial herds were categorized as Commercial I (Acre, Alagoas, Pernambuco, and Santa Catarina) and the other four were classified as Commercial II (Amapá, Amazonas, Rondônia, and Sergipe).

In the RP, seven states remained as Multiplier I herds (Bahia, Minas Gerais, Mato Grosso do Sul, Paraná, São Paulo, Rio de Janeiro, and Rio Grande do Sul) and only the state of Espírito Santo maintained its herd classification as Multiplier II. The state of Acre kept its herd in the Commercial I category, along with the states of Pará, Goiás, and the Distrito Federal. The herd in Rondônia remained as Commercial II and was joined by Santa Catarina and Mato Grosso.

No state was identified as a Nucleus or Isolated herd in the TP or in the RP.



The dashed line represents the pyramid-shaped structure and the solid geometric shapes, the structure found in the present study.

Figure 1 - Organizational structure of the genetic improvement of the Mangalarga breed for the total population (TP) and reference population (RP).

Table 3 - Classification of the Mangalarga breed herds grouped by Brazilian states for the total population and reference population

Type of herd	UEB	UOB	SB	NH	PMP
		Total population			
Nucleus	No	Yes	Yes	0	0
Multiplier I	Yes	Yes	Yes	11	28.85
Multiplier II	Yes	No	Yes	3	100
Commercial I	Yes	Yes	No	4	92.02
Commercial II	Yes	No	No	4	100
Closed	No	Yes	No	0	0
		Reference population			
Nucleus	No	Yes	Yes	0	0
Multiplier I	Yes	Yes	Yes	8	18.61
Multiplier II	Yes	No	Yes	1	100
Commercial I	Yes	Yes	No	4	96.67
Commercial II	Yes	No	No	3	100
Closed	No	Yes	No	0	0

UEB - use external breeders; UOB - use own breeding animals, SB - sell breeding animals; NH - number of herds; PMP - percentage of male breeding animals purchased (%).
Total population - Multiplier I: Bahia, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Paraná, São Paulo, Distrito Federal, Pará, Rio de Janeiro, Rio Grande do Sul. Multiplier II: Espírito Santo, Maranhão, Tocantins. Commercial I: Acre, Alagoas, Pernambuco, Santa Catarina. Commercial II: Amapá, Amazonas, Rondônia, Sergipe.
Reference population - Multiplier I: São Paulo, Minas Gerais, Rio Grande do Sul, Bahia, Mato Grosso do Sul, Paraná, Rio de Janeiro. Multiplier II: Espírito Santo. Commercial I: Acre, Pará, Distrito Federal, Goiás. Commercial II: Mato Grosso, Rondônia, Santa Catarina.

4. Discussion

4.1. Pedigree quality evaluation

The present study observed a progressive increase in ancestry information over time, with the highest percentage of identified parents in the most recent generation.

The use of pedigrees with low completeness may reduce the accuracy of the results (Cervantes et al., 2009) and generate imprecise estimates of genetic and population parameters, thus limiting genetic progress.

4.2. Organizational structure of the Mangalarga breed

In the genetic improvement of horses, the organizational structure does not have three strata in a pyramid shape, unlike what occurs in some species of zootechnical interest such as pigs and poultry. In the equine productive chain, the athlete or work animal itself is the target of genetic selection, whereas genetic improvement in other species takes place in the Nucleus herd (Lima and Cintra, 2016). Therefore, the horses used in reproduction are the ones that stand out in competition or in labor, i.e., the Multipliers and Commercial ones, with no Nucleus herds.

The lack of a Nucleus herd has also been observed in studies with other horse breeds. Valera et al. (2005) used data from 75,389 individuals from the studbook of the Andalusian horse and found no Nucleus or closed herds for the breed. Moreover, 42.6% of the herds were classified as Multipliers and 57.4% as Commercial herds. McManus et al. (2013) studied a population of 10,441 Pantaneiro horses registered since the foundation of the association of breeders, between 1972 and 2009, and observed that 56.3% of the herds were classified as Multipliers and 43.7% as Commercial, with no Nucleus or closed herds.

The existence of Nucleus herds is important as it allows for the development of strategies and the use of modern methodologies of genetic evaluation, which enables more accurate identification of genetically superior animals (Rego Neto et al., 2014). There exist open Nucleus and closed Nucleus herds. In open Nucleus herds, gene flow is bidirectional, occurring from the upper to the lower strata and, in case improved genotypes are identified in the Multiplier or Commercial herds, they can be selected to be used in the Nucleus herd. In closed Nucleus herds, gene flow is unidirectional, always from the upper to the lower strata.

In multiplying herds, Mangalarga breeders aim at the production of animals to take part in morphofunctional contests given the valuation of award-winning individuals. Thus, genetic selection takes place in this stratum.

The lack of closed herds of the Mangalarga breed is a positive aspect as this type of herd lacks gene flow, which may compromise the genetic variability of the population and, consequently, potentialize the onset of negative consequences of inbreeding (Rego Neto et al., 2014).

Multiplier I herds comprised the states featuring the largest effective number of animals. Those are likely more structured and, therefore, are able to have genetic quality both to use their own breeding animals and to sell them, which reduces the need for external breeding animals. In the TP and RP, only 28.85% and 18.61% of the breeding animals in this category were purchased from other herds, respectively.

Commercial I herds employ internal and external breeding animals. However, the fact that 92.02% of the breeding animals in the TP and 96.67% in the RP are purchased reveals the preference is for genetics coming from outside the herd. The greater use of external breeding animals in the states classified as Commercial I may be related to the small number of animals in those herds when compared with the states classified as Multiplier I. The use of external breeding animals enables gene flow between subpopulations, thus preventing subdivisions (structuring). Moreover, the existence of gene flow may contribute to the maintenance of inbreeding at acceptable levels in the overall population.

4.3. Genetic diversity considering Brazilian states as subpopulations

The F_{IS} is employed to measure the reduction in heterozygosis of an individual due to non-random mating in the subpopulation. In the present study, the mean F_{IS} values found both for the TP and the RP was very close to zero, suggesting there is no excess homozygosis in the subpopulations and that the inbreeding rate is low.

Since F_{IT} measures overall inbreeding within a breed, the F_{IT} values observed for the TP and RP of the Mangalarga breed of 0.0106 and 0.0107, respectively, indicate that the inbreeding rate in the overall population is low. Those results corroborate the F values of 2.40 and 5.81% observed for the TP and RP, respectively.

Maciel et al. (2014), when studying the Brazilian Crioulo horse breed, found similar values as those in the present study for all fixation indices: F_{ST} of 0.0005, F_{IS} of 0.0037, and F_{IT} of 0.0042. The values observed by McManus et al. (2013) for the Pantaneiro horse breed were also very close to those found by Maciel et al. (2014) and by this study, with F_{IT} values of −0.0002 and F_{IS} of −0.0027.

Valera et al. (2005) studied a lineage of the Andalusian horse breed and found F_{ST} of 2.6×10^{-5} for a population of 75,389 individuals. The result suggested no differentiation between the Carthusian lineage and the remaining of the population assessed.

Although the Mangalarga breed originated in the state of Minas Gerais, its development mostly took place in the state of São Paulo, which justifies its greater genetic contribution observed in this study. Moreover, the largest Brazilian herd of the breed is found in São Paulo state, within the TP, while the states of Minas Gerais and Paraná hold the second and third largest herds. In other words, the genetic contribution is directly related to the size of the herd in each state as those are precisely the three states that most contributed genetically to the breed. Geographically, São Paulo, Minas Gerais, and Paraná are very close states, which has likely also impacted this result.

The mean F values found for the TP (2.40%) and RP (5.81%) of the Mangalarga breed were low, below the critical value of 10%, at which deleterious effects of inbreeding depression may occur (Paiva et al., 2011; Toro et al., 2011; Tino et al., 2020). In the TP, the F value found was higher than the average observed in national breeds such as Mangalarga Marchador (1.02%; Baena et al., 2020) and Campolina (1.3%; Procópio et al., 2003) and in horses of the American Quarter Horse breed reared in Brazil (1.07%; Faria et al., 2018). On the other hand, the F of the Mangalarga breed was lower than those found in some exotic breeds such as Maremmano (2.90%; Giontella et al., 2019), Andalusian (7.51%; Poyato-Bonilla et al., 2020), and Thoroughbred (12.5%; Cunningham, 1991). The lower F of national breeds when compared with those two exotic breeds may be because the national ones are considered breeds in formation that until recently allowed or still allow the possibility of registering animals of unknown genealogy (open book).

It was expected that the larger the number of animals in a herd (state), the lower F would be as that population would have greater genetic variability. However, that was not the case as Minas Gerais and Bahia, which have the second and third largest herds in the RP, respectively, and were among the states with the highest F value. In Minas Gerais, the state where the Mangalarga breed originated, breeders prioritize individuals of the region for reproduction as they have the functional characteristics most valued in that state instead of the standard established by the ABCCRM (2023). That may justify the greater inbreeding rate of that population when compared with the herds in other states.

The increase in mean F values in the RP in relation to the TP was expected due to the increase in pedigree depth.

According to Ghafouri-Kesbi (2012), high AR values limit the efficacy of selection programs as they indicate that most individuals carry similar alleles, i.e., allele variation is low. Therefore, the AR may be used to manage populations by selecting for reproduction the animals that share the fewest alleles with the population. In the RP, states with the highest AR values, such as Acre (5.27%) and the Distrito Federal (5.00%), are geographically more distant from the states that most contribute genetically to the breed. That may hinder the acquisition of new breeding animals to renew the breeding stock, consequently increasing the sharing of alleles among individuals.

The lowest AR values may be because the herds use external breeding animals, i.e., breeders are constantly purchasing animals and renewing the genetics of the population. That may have been the case of the state of Mato Grosso, classified as a Commercial II herd and exhibiting the lowest AR value (2.05%).

The F_{ST} is used to measure the reduction in heterozygosity of the subpopulations that form the metapopulation. Thus, F_{ST} values below 0.05, as observed for the TP and RP, suggest that the populations analyzed have similar allele frequency, i.e., low genetic differentiation (Costa et al., 2009). Such result may be explained by the gene flow between states. Moreover, the high contribution of São Paulo to the genetic pool of the breed (76.23%) suggests the use of the same genetics in different states.

4.4. Genetic diversity considering different coats as subpopulations

According to Almeida et al. (2021), the buckskin and bay coats prevailed in the Mangalarga breed until the 1960s, possibly due to the high frequency of those coat colors in the individuals that formed the breed. The buckskin and bay coats require the presence of the dominant alleles of the genes black, agouti, and dilution (Rezende and Costa, 2019). Therefore, after that decade, the increase in use of stallions and mares recessive for those genes may explain the increase and current predominance of the sorrel coat in the Mangalarga breed, which is 100% recessive for those genes.

Furthermore, the frequency of the sorrel coat in the Mangalarga breed is 88.9% in the total population (Almeida et al., 2021), which means the superior animals have a good chance of having this coat color and, thus, disseminating that genetic material as the animals that stand out are often used in reproduction.

A higher frequency of the sorrel coat was also observed by Bastos et al. (2017) in the Quarter Horse breed during a survey on stallions of the breed used in *vaquejada* competitions in the Northeast region of Brazil. In the Campolina breed, Mendes et al. (2019) also observed a high frequency of the sorrel coat in the herd, coming second only to the buckskin coat.

As observed for Brazilian states, the F value found for the coat colors (0.96%) was also below what was considered critical by Paiva et al. (2011), Toro et al. (2011), and Tino et al. (2020).

The buckskin coat, which had the lowest inbreeding value in both populations studied in the present research, was more frequent in the horses used in the formation of the Mangalarga breed. At that time, the animals were registered in an open book, i.e., they were incorporated into the breed after a phenotypical evaluation with no verification of genealogy. Therefore, the lack of genealogic data may have led to lower inbreeding in the animals of that coat.

The mean AR value observed (1.07%) was considered low (Muniz et al., 2012), which is interesting as high values limit the efficacy of breeding programs as that means most individuals carry similar alleles, i.e., allelic variation is low (Ghafouri-Kesbi, 2012).

The highest AR values observed for the animals of tobiano coat may be associated with the formation of that coat color as the breeders who aim to obtain tobiano animals employ homozygote horses in reproduction to have 100% chance of tobiano foals being born. In addition, the population of tobiano animals in the Mangalarga breed is lower (Almeida et al., 2021).

Another factor that may have influenced the AR values of tobiano horses observed in the study was the fostering decision by the ABCCRM board of directors that, starting in 2004, instituted discrete judging and awards for animals of tobiano, palomino, bay, buckskin, grey, and black/zaina coats. After the judging split, those few tobiano animals started being widely used (Almeida et al., 2021).

The F_{IS} value observed in both populations showed that most coat colors exhibited a trend towards reducing heterozygosity. Almeida et al. (2021) mentioned that separately judging coat colors was positive to increasing the relevance of different coats within the breed, but may be contributing to the reduction in heterozygosity within each coat color as the animals that reached first place in those competitions started being used more in reproduction. As the populations of each coat color are small, the impact on inbreeding is high.

Since the F_{IT} measures overall inbreeding within a breed, the F_{IT} values observed for the coat colors in the TP and RP of the Mangalarga breed indicate that the inbreeding rate in the overall population is low.

The F_{ST} result, which indicated low genetic differentiation among the subpopulations, may have been because it is the same genetic group. Moreover, the Mangalarga breed features the crossing of animals with different coat colors, which means the different coats share alleles.

The results found can be used in the genetic management of the Mangalarga breed to help in decision-making and in choosing conservation strategies, as well as in genetic improvement programs.

5. Conclusions

Even with the greater genetic contribution of the state of São Paulo and of the sorrel coat, the inbreeding coefficient of the global population does not reach values that compromise the conservation of the genetic heritage and evolution of the breed. In addition, there is no genetic differentiation between subpopulations comprising Brazilian states or different coat colors. The organizational structure for the genetic improvement of the Mangalarga breed is not pyramid shaped, featuring only Multiplier and Commercial herds.

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Conflict of interest

The authors declare no conflict of interest.

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