








Analysis of the pedigree of the Mangalarga breed: Population structure and genetic diversity

Michael dos Santos Maciel¹ , Jorge Eduardo Cavalcante Lucena² , Ana Paula Gomes Pinto³ , Caline Angélica de Menezes Sá Nascimento¹ , Janaina Kelli Gomes Arandas⁴ , Laura Leandro da Rocha⁴ , Juliano Martins Santiago^{3*} 

¹ Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Ciência Animal e Pastagens, Garanhuns, PE, Brasil.

² Universidade Federal do Agreste de Pernambuco, Departamento de Zootecnia, Garanhuns, PE, Brasil.

³ Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, Serra Talhada, PE, Brasil.

⁴ Universidade Federal Rural de Pernambuco, Departamento de Zootecnia, Recife, PE, Brasil.

ABSTRACT - This study used a pedigree analysis to monitor trends in the genetic structure of the Mangalarga breed and identify factors that could impact its genetic variability. For this, genealogical information was used from the Associação Brasileira dos Criadores de Cavalos da Raça Mangalarga, considering as total population (TP) the animals born between 1919 and 2018 (n = 206,426) and as reference population (RP) those born between 2009 and 2018 (n = 20,539), which is the most recent generation according to the average generation interval calculated for the breed of 9.37 years. We evaluated the number of male and female births per year, foals produced by Mangalarga breeding animals, using the ENDOG 4.8, generation interval, proportion of known ancestors per parental generation, number of equivalent generations known per animal, inbreeding coefficient, increase in inbreeding, average relatedness (AR), effective population size, effective number of founders, effective number of ancestors, and founder genome equivalent. The number of equivalent generations known per animal increased over time, reaching an average of 2.75 in TP and 4.88 in RP. The calculated F was 2.26% for TP and 5.57% for RP, while AR was 2.41% for TP and 4.10% for RP. The effective population size was 40.85 for TP and 38.89 for RP. The 206,426 registered Mangalarga horses (TP) were derived from the genetic contribution of 9,011 founders and 8,908 ancestors, whereas those registered in or after 2009 (RP) originated from 2,662 founders and 2,193 ancestors. The effective number of founders represented 1.11 and 1.35% of the total number of founders in TP and RP, respectively. For RP, 11 animals accounted for 29.21% of the genetic pool of the breed. The analysis of population parameters estimated in the present study indicates that the Mangalarga horse population was formed through uneven contributions from founders and ancestors; therefore, genetic management of the breed is required to restrict average inbreeding from increasing over generations.

***Corresponding author:**

jmartinssantiago@yahoo.com.br

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Ines Andretta

Lenira El Faro Zadra

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1. Introduction

The use of horses with a comfortable gait that are suitable for hunting and dealing with cattle is part of the cultural history of the southern portion of the state of Minas Gerais, Brazil. With the arrival

of the Portuguese royal family, horses from the Coudelaria de Áter were introduced into the herd belonging to the Baron of Alfnas on Campo Alegre Farm. The interbreeding of these breeds provided the foundation for the formation of the Mangalarga breed (Prado, 2008; Almeida et al., 2021).

Horses descending from this original herd displayed a strong sports ability and comfortable diagonal gait, which caused the breed to be quickly adopted by breeders in the state of São Paulo and further dispersed to neighbouring states. This expansion of the herd and the appearance of new breeders resulted in increased selection (Prado, 2008; Simões, 2014; Almeida et al., 2021). In 1934, the Associação Brasileira dos Criadores de Cavalo da Raça Mangalarga (ABCCRM; Brazilian Association of Mangalarga Horse Breeders) was created, and once the breed standard was defined, herd uniformisation procedures were initiated (Junqueira, 2004; Prado, 2008; Simões, 2014).

Since the inception of the ABCCRM, different selection criteria have been adopted for the Mangalarga breed. Initially, breeders valued the role of the animals over their conformation and beauty. However, in the 1960s and 1970s, the importation of foreign horse breeds increased, which directed the focus towards enhancing the features of Mangalarga horses to counter competitiveness in the market. This change in breeding selection to emphasise morphology and aesthetic beauty resulted in zootechnical losses (Prado, 2008; Almeida et al., 2021). Currently, the ABCCRM is guiding breeders to select animals based on the concept of a modern work and sport horse (ABCCRM, 2021).

Horse herds are generally small, and selection does not include well-defined economic traits. Furthermore, traits such as gait type, competitive awards and functionality associated with morphology are not measured objectively (Costa et al., 2005). These characteristics make the genetic improvement and population dynamics of horses different from other species of zootechnical interest.

In this context, this study aimed to investigate past and current trends in the genetic structure of the Mangalarga breed based on pedigree analysis and identify factors that could affect the genetic variability of the breed.

2. Material and Methods

2.1. Pedigree data

Genealogical information from Mangalarga horses born between 1919 and 2018 in Brazil was obtained from the ABCCRM genealogic registry services database and analysed. After the removal of inconsistent information (wrong sex) and duplicate information, a file containing data on 206,426 animals remained.

The study considered the total population (TP) as well as the reference population (RP), which was defined as the group of animals registered with dates of birth between 2009 and 2018 ($n = 20,539$). The RP encompassed the most recent generation according to the average generation interval calculated for the breed of 9.37 years.

2.2. Pedigree analysis

The ENDOG 4.8 software (Gutiérrez and Goyache, 2005) was used to analyse pedigree information quality and calculate demographic parameters. The number of male and female births per year and number of foals born from Mangalarga breeding animals between 1940 and 2018 were calculated. Due to the small volume of records and/or lack of information, data from animals born between 1919 and 1939 were not considered in this analysis.

The generation interval, which is the average age of the parents at the birth of progeny that are held for breeding (James, 1977), was calculated for the four gametic pathways (father-son, father-daughter, mother-son, and mother-daughter).

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).

The coefficient of inbreeding (F) is defined as the probability that an individual has two identical alleles due to a shared ancestor (Wright, 1931) and was calculated as described by Meuwissen and Luo (1992). The rate of inbreeding (ΔF) was calculated for each generation by means of the formula $\frac{F_t - F_{t-1}}{1 - F_{t-1}}$, in which F_t and F_{t-1} are the average inbreeding at the i -th generation. The values obtained for ΔF were used to calculate the effective population size (N_e) using the equation $N_e = \frac{1}{2\Delta F}$. In addition, N_e was calculated based on individual increase in inbreeding, as suggested by Gutiérrez et al. (2009).

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 gene origin probability was analysed based on the following parameters: effective number of founders (f_e), effective number of ancestors (f_a) (Boichard et al., 1997), and founder genome equivalent (f_g) (Ballou and Lacy, 1995).

The effective number of founders (f_e) was calculated as $(f_e = \frac{1}{\sum_{k=1}^f q_k^2})$, in which q_k is the probability of gene origin of the k ancestor (James, 1972; Lacy, 1989). The effective number of ancestors (f_a) was calculated as $(f_a = \frac{1}{\sum_{j=1}^a q_j^2})$, in which q_j is the marginal contribution of ancestor j , which is an ancestor other than those previously chosen (James, 1972; Lacy, 1989). The number of founder genome equivalent (f_g) was calculated as the inverse of twice the average co-ancestry of the individuals included in the RP (Caballero and Toro, 2000).

3. Results

The ABCCRM database contains records of Mangalarga horse births since 1919. However, the institution itself only began recording births in 1934, when the organisation was founded.

3.1. Demographic analysis

In the 1940s, 435 males and 1,370 females (Figure 1) were registered, and in the 1950s, 327 males and 951 females were registered. From the 1960s to the end of the 1980s, registration issuing progressively increased. In the 1960s, there was an increase of 82.29% in the number of births/registrations of

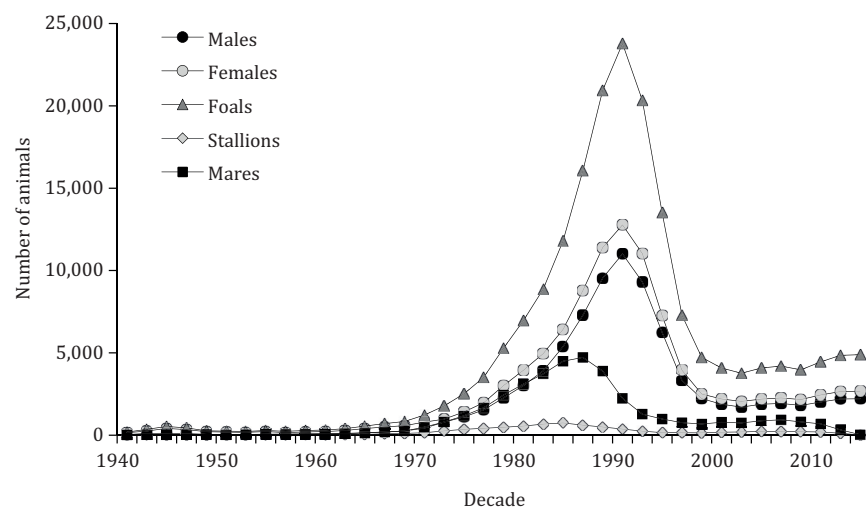


Figure 1 - Number of males, females, breeding animals (stallions and mares), and foals of the Mangalarga breed born/registered between 1940 and 2018.

Mangalarga horses compared with the 1950s. The increase from the 1960s to 1970s was of 409.79%, and from the 1970s to 1980s, it was of 351.18%.

After 1990, the number of definitive registrations issued declined, growing by only 7.76% compared with the previous decade. In the first decade of the 21st century, the number of definitive registrations issued by the ABCCRM experienced a strong retraction, with a mean reduction of 75.15%, compared with the 1990s, while a reduction of 7.95% was observed in the 2010s, compared with the 2000s.

The number of stallions (7,846) and mares (39,220) represented 9.01 and 32.86% of the TP of males (87,086) and females (119,340), respectively. Those animals produced, on average, 4.45 ± 2.72 foals per mare and 23.66 ± 25.82 foals per stallion, with a mare to stallion ratio of 4.99.

3.2. Generation interval

The average generation interval was 9.37 years for the TP, and females were an average of 0.22 years younger than males (Table 1). The average generation interval for the RP was 10.93 years, with similar ages for males and females.

Table 1 - Generation interval (years) for the four gametic pathways of the Mangalarga breed in the total population and reference population

Pathway	Total population		Reference population	
	N	Mean±SD	N	Mean±SD
Father-son	7,110	9.63±5.26	448	10.73±5.33
Father-daughter	36,451	9.05±4.57	1,433	10.86±5.12
Mother-son	6,340	9.31±4.69	448	10.46±5.07
Mother-daughter	33,076	8.93±4.83	1,431	11.21±6.00
Average interval		9.37±4.75		10.93±5.49

N - number of observations; SD - standard deviation.

3.3. Pedigree completeness

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 TP and RP, respectively, were as follows: known parents (87.25 and 99.89%), grandparents (73.41 and 99.61%), great-grandparents (43.12 and 96.01%), great-great-grandparents (32.24 and 79.50%), and great-great-great-grandparents (14.92 and 53.02%) (Figure 2). Males had a greater amount of ancestral data

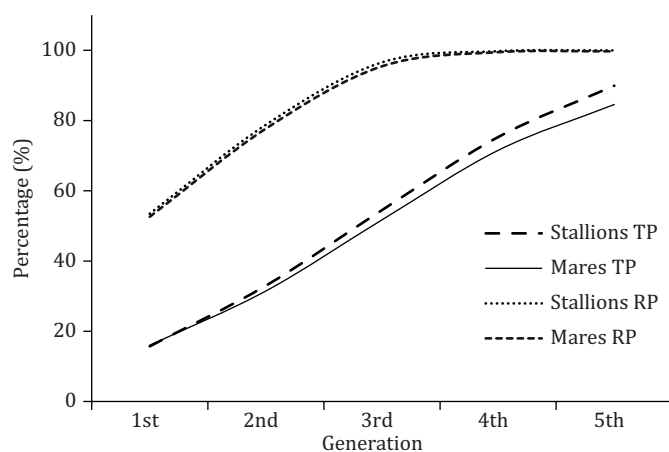


Figure 2 - Pedigree completeness of paternal and maternal lines of the total population (TP) and reference population (RP).

than females, and the ancestors that were registered in 2002, 2005, 2008, 2010, 2012, and 2013 had 100% of the parents known.

3.4. Inbreeding parameters

The mean F value was 2.26% for the TP and 5.57% for the RP (Table 2). The mean F per year of birth progressively increased between 1920 and 2018, reaching a maximum value of 6.03% in 2018 (Figure 3).

The maximum F values were 43.36% for the TP and 40.16% for the RP. Among the TP, 4.89% of the animals had F values different from zero, with the first inbred animal appearing in 1950, with an F of 25%. In the RP, 95.90% of the animals had F values that differed from zero.

Beginning in 1950, the number of inbred animals increased, reaching its highest value in the early 1990s, when 12,075 inbred animals were registered. Between 1998 and 2017, the number of inbred animals oscillated between 3,172 and 4,723 (Figure 4). The mean number of inbred individuals progressively decreased after the appearance of the first case and until the 1970s, whereas from the 1980s onwards, mean inbreeding remained close to 5% in the inbred population.

A total of 6,438 highly inbred matings were identified in the Mangalarga horse population. Of these, 66 (0.03%) occurred between full siblings, 4,594 (2.23%) between half-siblings, and 1,778 (0.86%) between parents and offspring.

The AR was 2.41% for the TP and 4.10% for the RP (Table 2). The N_e was 40.85 for the TP and 38.89 for the RP according to the method of Falconer and MacKay (1996). When calculated based on the individual increase in inbreeding (Gutierrez et al., 2009), the estimated N_e value was 43.77.

Table 2 - Inbreeding coefficient (F, %), effective size (N_e), and average relatedness (AR) of the Mangalarga breed for the total population and reference population

Parameter	Total population (n = 206,426)	Reference population (n = 20,539)
F	2.26	5.37
Animals with F \neq 0	98,866 (47.89%)	19,696 (95.90%)
N_e^1	40.85	38.89
N_e^2	-	43.77
AR	2.41	4.10

¹ Effective size, using the equation: $N_e = \frac{1}{2\Delta F}$

² Effective size, according to Gutierrez et al. (2009).

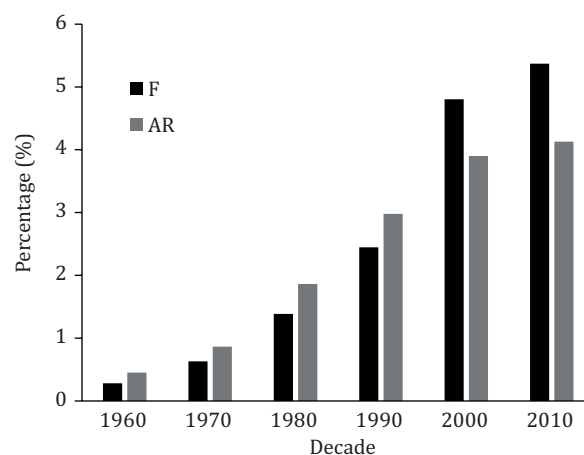


Figure 3 - Inbreeding coefficient (F) and average relatedness (AR) per decade for the Mangalarga breed.

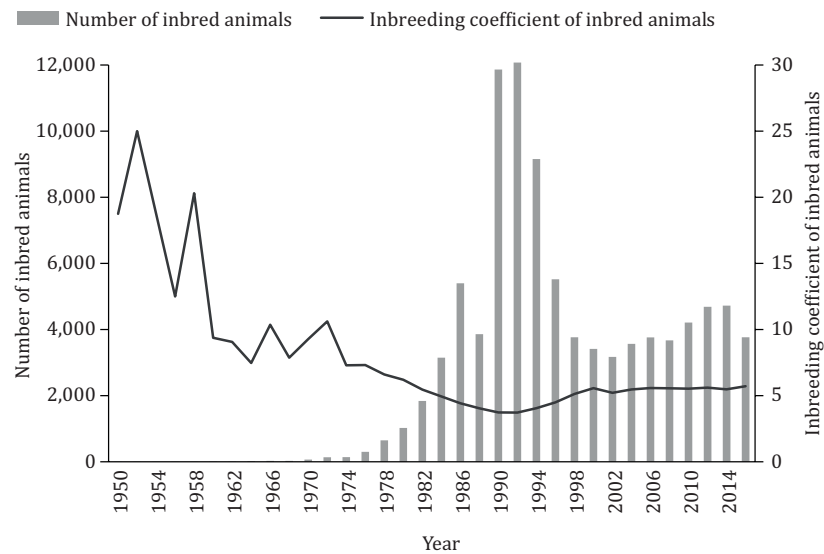


Figure 4 - Number of inbred animals and inbreeding coefficients of inbred animals between 1950 and 2017 for the Mangalarga breed.

3.5. Probability of gene origin

The 206,426 Mangalarga horses registered (TP) originated from the genetic contribution of 9,011 founders and 8,908 ancestors (Table 3). The horses registered in or after 2009 (RP) were derived from the contributions of 2,662 founders and 2193 ancestors.

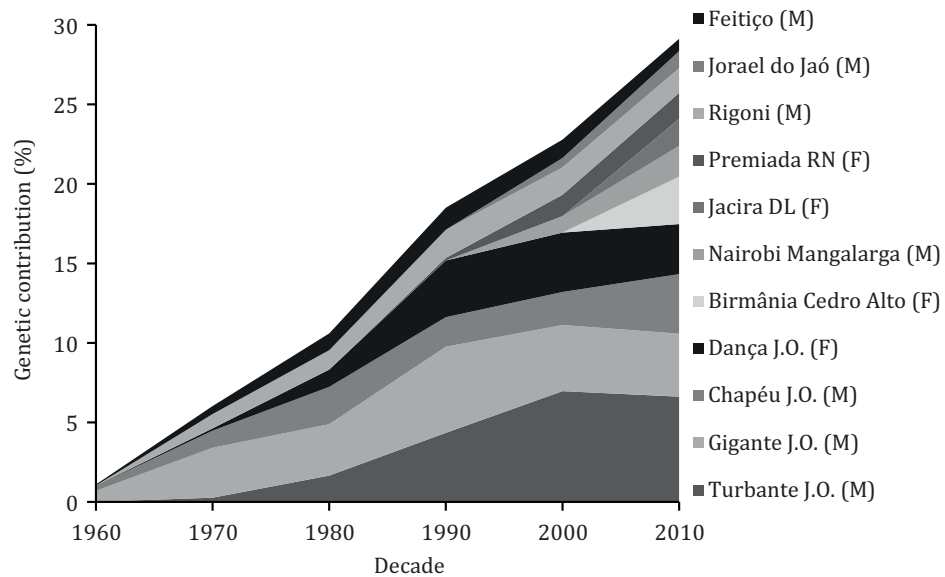
The f_e represented 1.11 and 1.35% of the N_f in the TP and RP, respectively. The f_a was lower than the f_e for both populations analysed. The number of ancestors that contributed to 50 and 90% of the genetic pool represented 0.53 and 20.98% of the N_a of the TP and 0.54 and 12.12% of the RP, respectively. The f_g , which considers all possible losses of founder alleles up to the present generation, had values of 41.23 for the TP and 12.25 for the RP.

In the TP, nine animals (seven males and two females) accounted for 21.58% of the genetic pool of the breed. In the RP, 11 animals (seven males and four females) accounted for 29.21% of the breed variability.

Table 3 - Genetic contribution of founders and ancestors of the Mangalarga breed

Parameter	Total population	Reference population
Number of founders (N_f)	9,011	2,662
Number of ancestors (N_a)	8,908	2,193
Effective number of founders (f_e)	100.31	36
Effective number of ancestors (f_a)	44	18
Founder genome equivalents (f_g)	41.56	12.25
$f_e:f_a$ ratio	2.28	2.0
$f_g:f_e$ ratio	0.41	0.34
$f_g:N_f$ ratio	0.0046	0.0046
Ancestors that contributed with 50% of the genetic pool	48	12
Ancestors that contributed with 90% of the genetic pool	1,869	266
Genetic conservation index (GCI)	1.72	2.09

Between 1944 and 1969, prior to the use of artificial insemination and embryo transfer, the animals that most contributed to the Mangalarga breed genetics were born. Among them was the stallion Turbante JO, which changed the direction of the Mangalarga herd by producing 1686 offspring and contributing 3.64% of the genetic variability of the TP and 6.64% of the RP (Figure 5). Due to the considerable success of that animal, its father (Gigante JO) and siblings were widely used for breeding. Therefore, the stallion Gigante JO (114 offspring) became the ancestor with the greatest influence on the TP, contributing 5.18%, and the animal with the second largest genetic contribution to the RP (3.95%).



M - male; F - female.

Figure 5 - Evolution of the genetic contributions of the 11 main ancestors of the reference population between 1960 and 2018.

4. Discussion

4.1. Demographic analysis

The foundation of the ABCCRM in 1934 and the ability to register animals in an open book contributed to an increase in the number of animals with the purpose of rapidly expanding the breed. In the first decades of the genealogic registry service, the increase in the Mangalarga herd showed that producers had accepted the breed. This increase contributed to greater genetic variability, which resulted in future genetic gains and an expansion of the breed in the market (Almeida et al., 2021).

The growth of the Mangalarga herd that began in the 1960s reflected the general expansion of Brazilian horse breeding at that time. Costa et al. (2005) and Baena et al. (2020) observed an increase in the number of births of Mangalarga Marchador horses in the 1960s, similar to that of Mendes et al. (2018) for the Campolina breed.

The reduction in the Mangalarga herd beginning in the 1990s may be related to the Brazilian economic crisis that occurred early in that decade. The country went through a period of high inflation, low economic growth rates, poor income distribution, and increased foreign debt (Antunes, 2001). In the 1990s, the size of other horse herds reared in Brazil also decreased, such as

those of the Quarter Horse (Faria et al., 2018), Campolina (Procópio et al., 2003; Mendes et al., 2018), Mangalarga Marchador (Baena et al., 2020), Brasileiro de Hipismo (Medeiros et al., 2014), and others (Lima et al., 2006; Lima and Cintra, 2016).

Moreover, the reduction of the herd in the 1990s could also be associated with the intense selection by breeders, which attracted producers of other breeds to Mangalarga horses. This led to the incorporation of Mangalarga animals in other associations, with the purpose of genetically contributing to the improvement of herds, justifying the retraction of the Mangalarga population (Almeida et al., 2021). The incorporation of animals of the Mangalarga breed by other associations in the 1990s, especially the ABCCMM, would have its greatest effect in the subsequent decade, when a 75.15% decline in the herd of the breed was recorded.

4.2. Generation interval

According to Pereira (2008), the average generation interval for horses is 8 to 12 years. Longer generation intervals are generally avoided in animal breeding programs as they reduce the genetic gain per unit of time (Malhado et al., 2008). However, from a conservation genetics perspective, long generation intervals are desirable because this arrangement results in a lower loss of genetic variability per year and helps guarantee the higher participation of a given animal in the population genetic constitution (Veroneze et al., 2014). The efficiency of this strategy depends on the reproductive management of the herd, which must be carried out to avoid inbred matings (Futuyma, 1992; Barros et al., 2011).

Generation interval results similar to those of the present study were found by Mota et al. (2006) for the Mangalarga breed (9.49 years). In addition, studies with other breeds had relatively similar results: 10.2 years for the Brasileiro de Hipismo (Dias et al., 2000), 8.0 years for the Thoroughbred (Langlois, 1976), 8.3 years for the Campolina (Procópio et al., 2003) and 10.43 years for the Mangalarga Marchador (Baena et al., 2020). These breeds are all considered saddle-type horses, which are generally required to prove their sports qualities in competitions before being used in reproduction.

In draft horse breeds, shorter generation intervals were observed, such as 4.5 years for the Breton (Parés, 1995). As the specimens of those breeds do not usually require equestrian sport testing, they join the reproduction group earlier. In addition, the goals of specific breeders and the technological investments applied to herds strongly influence the variation of the generation interval.

The reduction in generation interval differences between males and females of the Mangalarga breed corroborates the findings of Baena et al. (2020) with Mangalarga Marchador and those of Faria et al. (2018) with Quarter Horses.

Greater accessibility combined with the use of reproductive technologies in the latest Mangalarga generation (RP) has allowed for the use of awarded animals in morphofunctional judgments for reproduction, particularly those of embryo donors. This justifies the higher generation interval values in the RP compared with those in the TP. In addition, the change in breeding objectives of current Brazilian horse breeding could factor into the increase in these values. According to Lima and Cintra (2016), horse breeding for agricultural and livestock activities in Brazil is decreasing, while sport and leisure breeding practices are increasing.

4.3. Pedigree completeness

Pedigree completeness concerns the amount of genealogical information available and the quality of this information (Giontella et al., 2020). The use of pedigrees with low completeness may generate imprecise estimates of genetic and population parameters, thus limiting genetic progress (Cervantes et al., 2009).

The present study found that the volume of genealogic information was the greatest in the most recent generations, which may be explained by the history of the Mangalarga breed. The foundation of the ABCCRM in 1934 and the definition of a standard for the breed induced the genealogic registration of animals and the herd uniformisation process. Initially, open-book registration of animals was available

(i.e. registration with no ancestral information required). Nine years after the foundation of the ABCCRM, the book was closed so that only individuals of known origin were registered (Simões, 2014). This contributed to the higher number of known generations in the pedigrees of the RP.

Once the definitive genealogic registration is issued, the morphological and functional traits of the Mangalarga horse are assessed using a chart, and the animal is scored at the end of the process. The minimum score required for females to be registered in the ABCCRM is less restrictive than that for males because breeder associations are normally more concerned with the quality of males introduced into the herd, as they produce a larger number of descendants and disseminate their genes more widely in the herd.

The lower score required to register females could have resulted in a higher number of females with unknown genealogy registered in the open book than males. This would explain the reduced amount of information on the maternal line of the pedigree compared with that of the paternal line.

4.4. Inbreeding parameters

Inbreeding parameters are strongly influenced by factors such as the size of the population, pedigree completeness level, and breeding policy (Gutiérrez et al., 2003). Since F values below 5% are considered low and values above 25% are considered high (Vicente et al., 2012), the values observed in the present study for the TP (2.26%) and RP (5.57%) are reasonable, which suggests that some inbreeding control actions were used with the Mangalarga breed. To ensure that inbreeding level remains under control in the population, mating of close relatives should be monitored.

Similar results were found by Giontella et al. (2019) when analysing pedigree data of Maremmano horses from Italy (F of 2.94% for the TP and 3.55% for the current population). In contrast, Baena et al. (2020), Faria et al. (2018), and Maciel et al. (2014) observed lower F values than those of Mangalarga horses for Mangalarga Marchador (1.04% for the TP and 1.10% for the RP), Quarter Horses (1.07% for the TP and 1.56% for the RP), and Criollo (0.88% for the TP). Higher values were found by Vicente et al. (2012) in a population of Lusitano horses (F of 9.92% for the TP and 11.34% for the RP).

Although the number of inbred animals in the Mangalarga breed increased until the early 1990s and 95.90% of the RP consisted of animals with F values that differed from zero, the mean inbreeding of the inbred individuals progressively decreased, followed by stabilisation to approximately 5%. This is because the chance of identifying common ancestors increases with the pedigree completeness level; however, those ancestors are more often from distant generations (Gutiérrez et al., 2003).

An efficient way to control inbreeding in the long term is to give preference to animals with low AR since they share a low percentage of genes with the remaining population. According to Goyache et al. (2010), an animal with a low AR and high F may be employed in reproduction without harming population variability.

Values of AR which are lower than those in the present study were found by Vicente et al. (2012) for the Lusitano (0.1164% for the TP and 0.1354% for the RP) and Faria et al. (2018) for the Quarter Horse breed (1.16% for the TP and 1.20% for the RP). In contrast, higher AR values were observed by Giontella et al. (2019) with the Maremmano breed (5.16% for the TP and 6.13% for the RP). All those studies found an increase in AR in the RP when compared with the TP. However, none of these increases was as large as that observed in the present study (2.41% for the TP and 4.10% for the RP).

The first identified inbred Mangalarga horse was born in 1950, and the lack of recorded inbreeding in the 1930s and 1940s was related to herds consisting of animals with no pedigree information. The 1950s and 1960s reflected the beginning of the upward growth in F and AR curves in the Mangalarga herd when the nine individuals that provided the greatest genetic contribution to the TP of the breed were born (1944 to 1969). These animals were born in the initial phase of curve growth and they had a strong influence during the following decades, which showed an acceleration in F and AR curves.

The 1980s are considered the golden era of Brazilian horse breeding when local breeding increased the horse herds in the country (Almeida et al., 2021). The Mangalarga breed was part of this growth, because when the population began expanding, breeders focused on increasing their numbers of the animal biotypes that prevailed in morphofunctional contests. Thus, valuations of champion individuals and their ancestors and descendants, along with increased access to reproductive biotechniques caused these animals to be utilised to a greater extent for reproduction, which contributed to the increase in F and AR over that period.

In the 2000s and 2010s, a reduction of greater than 70% of the number of horses with definitive registrations in the ABCCRM occurred (Almeida et al., 2021) and, despite this, a slower growth rate of F and AR curves was observed. This may be related to the greater control of mating and changes in ABCCRM guidelines, such as the inclusion of evaluation criteria of animals according to their coats and the development of genetic rescue projects.

Although a breed standard has been established, selection goals can change over time to emphasise morphological and functional traits, which are deemed more efficient for the herd or to meet conceptual and market changes. In the 2000s, the ABCCRM guidelines changed, and the focus turned to animals with different traits than those previously selected. This change led to the redirection of selection goals and criteria, and the choice of breeding animals that were not included in selection programmes of Mangalarga herds, which might have contributed to the slower growth rate of F and AR curves.

According to Almeida et al. (2021), the sorrel coat prevailed among registered animals, with 88.9% for the period 2000–2010. This prevalence was also noted in competitions, whereby most champion horses had that type of coat.

Exotic or less common coats in a breed may result in commercial growth and the incorporation of new breeders who are attracted by the unique feature. Therefore, in 2004, the ABCCRM introduced separate judging and awarding for tobiano, palomino, bay, buckskin, grey, and black/black bay coats to attract more users and new breeders who had shown less interest in the breed due to their single colour and the high competitiveness of horses with chestnut coats in morphofunctional judgements. This change allowed horses of other coats to win more competitions, as they no longer had to compete with the chestnut-coated horses, resulting in additional use of these animals for reproduction.

Because of that decision, Almeida et al. (2021) observed a reduction of 16% in the frequency of the chestnut coat and a marked increase in the frequency of other coats such as tobiano (12.8%). This greater use of horses with other coat colours for breeding may have contributed to the slower growth rate of F and AR curves which began in the 2000s. Corroborating this, Bartolomè et al. (2010) observed that the progressive increase in minority coats in the Spanish Purebred Horse breed was profitable for genetic diversity.

Another factor that may have contributed to the slower growth rate of F and AR curves was the development of rescue projects for the breed, such as *Projeto Raízes*, which began in 2000 (ABCCRM, 2020) aiming at rescuing Mangalarga mares from breeding farms of traditional lineages that were not being used for reproduction. These rescues occurred because of new ABCCRM guidelines, which valued the functional and the gait quality traits of the animals to a greater extent. Therefore, mares with the requisite qualities were introduced into herds for reproduction.

Regarding N_e , studies on other horse breeds found higher N_e values than those of the Mangalarga breed. Faria et al. (2018) recorded N_e values of 195 in the TP and 164 in the RP for the Quarter Horse breed, Giontella et al. (2019) observed an N_e of 71.2 in a recent population of the Maremmano breed, while Vicente et al. (2012) found an N_e of 41.24 in the RP of the Lusitano breed.

4.5. Probability of gene origin

According to Boichard et al. (1997), an analysis of gene origin probability provides information on the loss of genetic diversity due to bottlenecks and genetic drift in a population. In addition, those

authors indicated that the parameters derived from gene origin probabilities are less sensitive than inbreeding parameters to the pedigree completeness level.

Considering RP, despite the high N_f (2,662) and N_a (2,193), the Mangalarga breed exhibited lower effective values for those parameters ($f_e = 36$ and $f_a = 18$) than those observed for many Brazilian breeds, such as Mangalarga Marchador ($N_f = 728$, $N_a = 1572$, $f_e = 225$, and $f_a = 198$) (Baena et al., 2020), Brasileiro de Hipismo ($N_a = 1,736$, $f_e = 222$, and $f_a = 129$) (Medeiros et al., 2014), Pantaneiro ($N_f = 3,331$, $N_a = 3,330$, $f_e = 683$, and $f_a = 618$) (McManus et al., 2013), and the exotic Quarter Horse ($N_f = 4,815$, $N_a = 2,993$, $f_e = 811$, and $f_a = 113$) (Faria et al., 2018).

The discrepancies between N_f and f_e and N_a and f_a indicate an uneven use of founders and ancestors. This likely occurred due to the preference for champion animals in morphofunctional judgments for reproduction, which, allied with the use of reproductive techniques, resulted in a higher number of animals with the same genetic origin. In addition, only 48 and 12 ancestors were responsible for 50% of the genetic pool of the TP and RP, respectively, thereby corroborating this theory.

The values obtained for the $f_e:f_a$ ratio in the TP and RP were both >1 in the present study, which confirms the reduction in diversity due to the occurrence of a bottleneck (Allaby et al., 2019).

An $f_g:f_e$ ratio <1 was observed in the TP and RP, indicating a loss of alleles over time due to genetic drift. The $f_g:N_f$ ratio of 0.0046 for the TP and RP indicates that the most recent population maintained a low proportion of the genetic diversity of the founders.

Founders and ancestors are the first animals of the breed, and the Mangalarga breed followed a paternal line, which attributes the main genetic variability of the herd to the stallion. This strengthens the observation that the information on males is more complete. The presence of two and four females among the animals that most contributed to the breed in the TP and RP, respectively, may indicate that these were the mothers of the males that contributed considerably to the Mangalarga herd.

Females have physiological limitations that only allow them to generate a single foal per year, whereas males can cover tens of mares within a single mounting season, even without the use of reproductive techniques. This justifies the low contribution of females to the genetics of the breed.

The use of reproductive techniques, especially artificial insemination that began in the 1980s in the Mangalarga breed (Severo, 2015), further increased the number of descendants left by a single male. The embryo transfer technique increases the participation of the maternal side in terms of variability (Baena et al., 2020); however, this method was initiated in the Mangalarga herd in the late 1990s; therefore, the embryo transfer technique contributed little to the participation of females in the genetic variability of the breed. Nevertheless, embryo transfers allowed the RP to have twice as many female ancestors with contributions (four females) than those in the TP (two females). These reproductive techniques also affect herd inbreeding.

5. Conclusions

Despite the increase in inbreeding of the Mangalarga breed over time, the conservation of the genetic heritage and breed evolution has not been compromised, which indicates that genetic management is conducted by breeders on their herds. The analysis of gene origin probability revealed a small loss of diversity due to the occurrence of bottlenecks and genetic drift. Although inbreeding is not a current concern, the mating of close relatives must be monitored, to avoid future risks.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Lucena, J. E. C.; Pinto, A. P. G.; Arandas, J. K. G.; Rocha, L. L. and Santiago, J. M. **Data curation:** Maciel, M. S.; Pinto, A. P. G.; Nascimento, C. A. M. S. and Santiago, J. M. **Formal analysis:**

Maciel, M. S.; Lucena, J. E. C.; Pinto, A. P. G.; Nascimento, C. A. M. S.; Arandas, J. K. G.; Rocha, L. L. and Santiago, J. M. **Investigation:** Maciel, M. S.; Lucena, J. E. C.; Pinto, A. P. G.; Nascimento, C. A. M. S.; Arandas, J. K. G.; Rocha, L. L. and Santiago, J. M. **Methodology:** Lucena, J. E. C.; Pinto, A. P. G.; Arandas, J. K. G.; Rocha, L. L. and Santiago, J. M. **Project administration:** Lucena, J. E. C. and Santiago, J. M. **Supervision:** Lucena, J. E. C. and Santiago, J. M. **Validation:** Lucena, J. E. C. and Santiago, J. M. **Visualization:** Maciel, M. S.; Lucena, J. E. C.; Pinto, A. P. G. and Santiago, J. M. **Writing – original draft:** Maciel, M. S.; Lucena, J. E. C.; Pinto, A. P. G. and Santiago, J. M. **Writing – review & editing:** Maciel, M. S.; Lucena, J. E. C.; Pinto, A. P. G. and Santiago, J. M.

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