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## Effects of Inbreeding on Phenotypic Traits in the Hispano-Arabian Horse

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## INTRODUCTION

Throughout history, mating between related individuals has been perceived as a direct means of consolidating desired traits. In individuals belonging to the equine or canine species, this becomes especially relevant, given the marked importance attributed to ani-

#### SUMMARY

Our study investigates the influence of genetic parameters related to inbreeding in the Hispano-Arabian horse, focusing on coat color patterns. A total of 11,010 horses born between 1900 and 2019 were analyzed. Fourteen coat colors were studied: Chestnut/Sorrel, Black, Bay, Dun, Grey, Isabelline, Overo, Palomino, Cremello, Pearl, Piebald, Roan, Smokey Cream, and White. These coats were categorized into two groups: pure coats (Chestnut/ Sorrel, Bay and Black) and diluted coats (Dun, Grey, Isabelline, Cremello, Overo, Palomino, Pearl, Piebald, Roan, Smokey Cream, and White). Using Bayesian analysis, we determined that inbreeding levels are higher in pure coat colors compared to diluted ones. The highest average inbreeding value was found in the bay coat (4.5%), while the lowest values were observed in white and piebald coats (0%). These findings highlight the need for careful management of breeding programs to preserve genetic diversity and minimize inbreeding in the Hispano-Arabian horse.

## Efectos de la endogamia en los rasgos fenotípicos en el caballo Hispano-Árabe

## RESUMEN

Nuestro estudio investiga la influencia de los parámetros genéticos relacionados con la endogamia en el caballo Hispano-Árabe, centrándose en los patrones de color de capa. Se analizaron un total de 11,010 caballos nacidos entre 1900 y 2019. Se estudiaron catorce patrones de capa: Castaño, Negro, Alazán, Bayo, Tordo, Isabelino, Overo, Palomino, Cremello, Perla, Pío, Ruano, Crema Ahumado y Blanco. Estos pelajes se categorizaron en dos grupos: pelajes puros (Castaño, Alazán y Negro) y pelajes diluidos (Bayo, Tordo, Isabelino, Cremello, Overo, Palomino, Perla, Pío, Ruano, Crema Ahumado y Blanco). Mediante análisis bayesiano, determinamos que los niveles de endogamia son mayores en los colores de pelaje puro en comparación con los diluidos. El valor promedio de endogamia más alto se encontró en el pelaje castaño (4.5%), mientras que los valores más bajos se observaron en los pelajes blanco y pío (0%). Estos hallazgos subrayan la necesidad de una gestión cuidadosa de los programas de cría para preservar la diversidad genética y minimizar la endogamia en el caballo Hispano-Árabe.

> mals based on the popularity of their ancestors. Thus, the selection of individuals for certain popular traits can exert strong pressure on the genetic diversity present in a population. However, inbreeding can act as a double-edged sword, negatively impacting the genetic health of these populations, leading to higher mortality rates, decreased fertility, and reduced adaptability to

the environment (Falconer et al. 1976). A high level of homozygosity reduces favorable genetic combinations that condition the proper development of individuals (Sinha et al. 1975). For this reason, the primary goal of conservation breeding is the protection of genetic diversity, as supported by the Food and Agriculture Organization of the United Nations (FAO) through the implementation of the Domestic Animal Diversity Information System (DAD-IS) tool (FAO, 2019).

The Hispano-Arabian horse is a composite equine breed formed from the crossbreeding of two pure equine breeds (the Purebred Spanish Horse and the Purebred Arabian Horse), classified as an indigenous breed in danger of extinction. Its origin dates back to the 1800s. Since 2008, the management of the conservation and genetic improvement program has been the responsibility of the Unión Española de Ganaderos de Pura Raza Hispano-Árabe (ÚEGHá), along with the AGR-218 research group. All data has been collected in accordance with the studbook registration standards of the Ministry of Agriculture, as per APA Order 2129/2008 (Official Catalogue of Spanish Breeds). In addition to the specific requirements that individuals must meet to be included in the studbook, as outlined in the breeding program, the association has systematically recorded coat color patterns in these individuals. The aim of this study is to evaluate the influence of selective breeding based on coat color on the levels of inbreeding in the Hispano-Arabian horse.

#### MATERIAL AND METHODS

The historic pedigree records of Há, PRE and PRá breeds were considered in this study comprising a database consisting of 207,100 horses. We analyzed the complete pedigree of the Hispano-Arabian horse breed (11,010 animals), of which 6,742 were mares and 4,268 were stallions born between 1900 and 2019. Therefore, the studied population can be considered representative of the entire Hispano-Arabian horse population. This data was collected and provided thanks to the "Spanish Union of Purebred Hispano-Arabian Horses Breeders (UEGHá)".

Genetic analysis was conducted on the historical dataset comprising all animals (dead and living) and on a filtered dataset containing only living animals to evaluate the evolution and trends described by

diversity and population structure parameters.

Generation intervals and the mean age of parents when their offspring were born were calculated for the four gametic pathways (stallion to colt and filly and mare to colt and filly) across coat colour possibilities. The stallion/mare ratio was calculated considering the percentage of mares and stallions with breeding progeny and the number of breeding animals selected.

The calculation of individual inbreeding coefficients for each individual in the population was performed using the software Endog version 4.8 (Gutierrez el al. 2005) and CFC version 1.0 (Sargolzaei et al. 2006), and also consisted of estimating inbreeding trends over time. To ensure a correct calculation of these genetic diversity parameters, information from the studbooks of the Purebred Spanish Horse and Purebred Arabian Horse breeds was considered. The tool for Bayesian inference of ANOVA in IBM SPSS Statistics software version 25 (Corp. 2017) was used to detect differences in average inbreeding among the different coat colors present in the Hispano-Arabian horse population registered in the studbook

## RESULTS

The level of inbreeding in the Hispano-Arabian horse breed is 2.89% for the historical population and 2.85% for the current population. The maximum inbreeding coefficients are 49.61% for both the historical and current populations. The number of equivalent generations is  $6.15 \pm 3.04$  for the historical population and  $6.34 \pm 2.96$  for the reference population , which demonstrates a good pedigree completeness index. However, it should be noted that the inbreeding level was higher when the historical population was considered, due to the impossibility of avoiding mating between related individuals as a result of the very low number of individuals born during that period.

Table I presents Bayesian Inference of ANOVA for Detecting Differences in Individual Inbreeding Based on Coat Color in the Hispano-Arabian Horse Breed where there are significant differences in individual inbreeding between the different coat colors were observed.

The posterior distribution statistical parameters for the average inbreeding value based on coat color were analyzed and presents in Table II and continuations. with the highest result observed in the bay coat colour (4.5%) and the lowest in the white and piebald coats (0%). However, the largest difference between the upper and lower limits was observed in those with the lowest mean values in the piebald and white coats (0.071 and -0.071, respectively).

#### DISCUSSION

The genetic diversity and inbreeding aspects of the Spanish Pure Breed (PRE) and Arabian Pure Breed (PRá) horse breeds have been shaped by historical practices, genetic bottlenecks, and breeding policies. Despite the significant genetic contribution from PRá horses (with approximately 70% PRá and 30% PRE genetic influence), the diversity of coat colors primarily originates from the PRE lineage, as reported by Stachurska (2007) and Marín et al. (2021).

The PRE breed has experienced multiple genetic bottlenecks since its isolation in the 1880s (Beuchat 2020), which were exacerbated by events such as the Spanish Civil War and the World Wars, leading to drastic population declines. In an effort to recover the breed, policies were enacted that banned crossbreeding and exportation. These measures proved effective after 1966 (Navas 2019), and even more so after the end of the Francoist regime in 1975 (Ferrari et al. 1990). However, these restrictive breeding policies led to a narrow focus on specific coat colors, particularly grey, bay, and black, causing a significant loss of genetic di

 Table I. Bayesian Inference of ANOVA for Detecting Differences in Individual Inbreeding Based on Coat

 Color in the Hispano-Arabian Horse Breed (Inferencia Bayesiana de ANOVA para detectar diferencias en la consanguinidad

 individual dependiendo del color de la capa en la raza equina Hispano-Árabe).

Parameters	Sum of Squares	Degrees of Freedom	Mean Square	F	Sig.	Bayes Factor
Between groups	0.235	13	0,018	6.801	0.01	0.003
Within groups	29.194	10988	0,003			
Total	29.429	11001				

Table II. Posterior Distribution Statistics for Average Inbreeding Based on Coat Color in the Hispano-Arab ian Horse Breed (Estadísticos de la distribución posterior para la consanguinidad media dependiendo del color de la capa en la raza equina Hispano-Árabe).

Parameter		Posterior	95% Credibility Interval		
Coat colour	Mode	Mean	Variance	Lower Limit	Upper limit
Bay	0.030	0.030	0.000	0.028	0.032
Black	0.037	0.037	0.000	0.032	0.043
Chesnut/Sorrel					
	0.034	0.034	0.000	0.031	0.036
Dun	0.045	0.045	0.000	0.037	0.054
Grey	0.025	0.025	0.000	0.023	0.026

 Table II (cont.). Posterior Distribution Statistics for Average Inbreeding Based on Coat Color in the Hispano-Arab Horse Breed (Estadísticos de la distribución posterior para la consanguinidad media dependiendo del color de la capa en la raza equina Hispano-Árabe).

Parameter	Posterior			95% Credibility Interval		
Coat colour -	Mode	Mean	Variance	Lower Limit	Upper limit	
Isabelline	0.042	0.042	0.000	0.032	0.051	
Overo	0.023	0.023	0.000	-0.019	0.064	
Palomino	0.010	0.010	0.000	-0.026	0.045	
Pearl	0.024	0.024	0.000	0.010	0.038	
Piebald	0.000	0.000	0.001	-0.071	0.071	

Table II (cont.). Posterior Distribution Statistics for Average Inbreeding Based on Coat Color in the Hispano-Arab Horse Breed (Estadísticos de la distribución posterior para la consanguinidad media dependiendo del color de la capa en la raza equina Hispano-Árabe).

Parameter	Posterior			95% Credibility Interval		
Coat colour	Mode	Mean	Variance	Lower Limit	Upper limit	
Roan	0.026	0.026	0.000	0.005	0.048	
Smoky Cream	0.018	0.018	0.003	-0.083	0.119	
White	0.000	0.000	0.001	-0.071	0.071	

versity. By the end of World War II, approximately 75% of PRE horses carried the G allele associated with the grey coat, underscoring the complex breeding history, which also included misclassification of coat colors (Rico Mansilla 1990).

The prevalence of homozygous grey individuals, which can only produce grey foals, complicates the recovery of other coat colors and may negatively affect the breed's genetic diversity and functionality. The lifting of the 30-year ban on registering various coat colors in 2003 led to a gradual increase in the number of chestnut and sorrel horses, reflecting breeders' attempts to diversify after years of restriction. Breeders began preserving previously banned colors in the 1980s, anticipating changes and enabling DNA-based

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parentage verification for registration (Rico Mansilla 1990; Grilz-Seger et al. 2017).

Genetic analysis of the PRE population reveals a clear structure among coat color subgroups, with distinct clustering patterns associated with specific genes, such as SLC45A2/MATP, as suggested by Sevane et al. (2019) and Holl et al. (2019). Despite some genetic differentiation, both PRE and PRá horses share considerable genetic diversity. However, the study of the Pedigree Completeness of Index (PCI) shows that the first five generations of these breeds exhibit very high PCI values, indicating the potential for genetic erosion, as supported by Stachurska (2007) and Marín et al. (2021).

PCI values drop below 60% for coat color clusters like pearl, cremello, and isabelline from the fourth generation onward, suggesting that historical restrictions and incomplete genealogical records may have limited genetic diversity. Moreover, while some genetic erosion has been observed, it remains minimal for diluted coat colors, which are subjected to higher selection pressures.

Inbreeding remains a concern, particularly due to the use of overbred individuals. Current genetic diversity issues mainly stem from the short-term loss of genetic contributions from founding horses, rather than the long-term effects of inbreeding. Inbreeding rates above 1-3% per generation (McManus et al. 2013) can rapidly fix harmful recessive genes, potentially compromising population vitality. Conversely, rates below 1% suggest that some populations may be purging deleterious genes, promoting genetic health, as suggested by Rolf (2014) and Iglesias et al. (2021).

Population structuring can help mitigate extinction risks, as demonstrated by the low numbers of equivalent subpopulations within horse breeds (Gutiérrez et al. 2009). However, this structuring can also lead to decreased effective population sizes and increased inbreeding rates (Ministerio de Agricultura 1997). The study found that effective population size estimates based on coancestry are more accurate, reflecting ongoing non-random mating practices associated with specific coat colors (Druml et al. 2009).

Historical breeding practices and sociopolitical influences have shaped the current genetic structure, leading to the overrepresentation of certain coat colors. Despite efforts to enhance diversity, challenges remain, especially with the risk of inbreeding and potential health issues linked to coat color. To ensure the long-term sustainability of these breeds, careful management strategies focusing on genetic conservation and reducing inbreeding rates are crucial. The findings emphasize the need for breeders to balance aesthetic preferences for coat colors with the health and viability of horse populations, safeguarding the rich genetic heritage of PRE and PRá horses.

#### CONCLUSIONS

The results of this study indicate that the level of inbreeding in the Hispano-Arabian horse breed has been gradually decreasing over time, reflecting an improvement in genetic management practices. This decline in inbreeding is further supported by the increase in the number of equivalent generations, which points to a well-maintained pedigree completeness index. Additionally, the study highlights significant differences in inbreeding levels based on coat color, with pure coat colors, such as Chestnut/Sorrel, Bay and Black, showing higher inbreeding indices compared to diluted coat colors. These findings suggest that selective breeding for certain coat colors may have contributed to the elevated inbreeding levels in pure-colored horses. The results emphasize the importance of incorporating genetic diversity considerations into breeding programs, especially when selecting for physical traits like coat color, to ensure the long-term sustainability and genetic health of the Hispano-Arabian horse breed.

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#### BIBLIOGRAPHY

- Falconer, D.S.; Mackay, T.F.C. Introduction to Quantitative Genetics. Longman Scientific and Technical: Harlow, Essex, UK., 1996; pp. 317-318.
- Sinha, S.K.; Khanna, R. Physiological, biochemical, and genetic basis of heterosis. In Advances in Agronomy, Elsevier: 1975; Vol. 27, pp. 123-174.
- FAO. Domestic Animal Diversity Information System (DAD-IS). 2019.
- Gutiérrez, J.a.; Goyache, F. A note on ENDOG: a computer program for analysing pedigree information. Journal of Animal Breeding and genetics 2005, 122, 172-176.
- Sargolzaei, M.; Iwaisaki, H.; Colleau, J. CFC: a tool for monitoring genetic diversity. In Proceedings of 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Minas Gerais, Brazil, 13-18 August; pp. 27-28.
- Corp., I. IBM SPSS Statistics for Windows, 25.0; IBM Corp: Armonk, NY, 2017.
- Stachurska, A.; Ussing, A.P. Coat colour versus performance in the horse (Equus caballus). Pol. J. Natur. Sc. 2007, 22, 43–49.
- Marín Navas, C.; Delgado Bermejo, J.V.; McLean, A.K.; León Jurado, J.M.; Rodriguez de la Borbolla y Ruiberriz de Torres, A.; Navas González, F.J. Discriminant Canonical Analysis of the contribution of Spanish

and Arabian purebred horses to the genetic diversity and population structure of Hispano-Arabian horses. Animals 2021, 11, 269.

- Beuchat, C. COI FAQS: Understanding the Coefficient of Inbreeding. Available online: https://www.instituteofcaninebiology. org/blog/ coi-faqs-understanding-the-coefficient-of-inbreeding (accessed on 28 December 2020).
- Navas González, F.J. Structural Organization of Genetic Diversity and Ethofunctional Characterization in the Andalusian Donkey Breed; University of Córdoba: Córdoba, Spain, 2019.
- Ferrari, A.; Ghittino, C. La" desconocida" peste equina. MG Mundo Ganad. 1990, 1, 59–62.
- Rico Mansilla, A. Peste equina en España. MG Mundo Ganad. 1990, 1, 31–38.
- Grilz-Seger, G.; Druml, T.; Neuhauser, B.; Brem, G. Classification and nomenclature of the leopard complex spotting in the Noriker horse breed and its relevance for the breeding for color. Züchtungskunde 2017, 89, 359–374.
- Sevane, N.; Sanz, C.R.; Dunner, S. Explicit evidence for a missense mutation in exon 4 of SLC45A2 gene causing the pearl coat dilution in horses. Anim. Genet. 2019, 50, 275–278.
- Holl, H.M.; Pflug, K.M.; Yates, K.M.; Hoefs-Martin, K.; Shepard, C.; Cook, D.G.; Lafayette, C.; Brooks, S.A. A candidate gene approach identifies variants in SLC45A2 that explain dilute phenotypes, pearl and sunshine, in compound heterozygote horses. Anim. Genet. 2019, 50, 271–274.
- McManus, C.; Santos, S.A.; Dallago, B.S.L.; Paiva, S.R.; Martins, R.F.S.; Braccini Neto, J.; Marques, P.R.; Abreu, U.G.P.d. Evaluation of conservation program for the Pantaneiro horse in Brazil. Rev. Bras. Zootec. 2013, 42, 404–413.
- Rolf, M. Color Patterns in Crossbred Beef Cattle; Oklahoma Cooperative Extension Service: Oklahoma State University, Stillwater, OK, USA, 2014.
- Iglesias Pastrana, C.; Navas González, F.J.; Ruiz Aguilera, M.J.; Dávila García, J.A.; Delgado Bermejo, J.V.; Abelló, M.T. Whitenaped mangabeys' viable insurance population within European Zoo Network. Sci. Rep. 2021, 11, 674.

- Gutiérrez, J.P.; Cervantes, I.; Goyache, F. Improving the estimation of realized effective population sizes in farm animals. J. Anim. Breed. Genet. 2009, 126, 327–332.
- Ministerio De Agricultura Pesca Y Alimentación. Real Decreto 1682/1997, de 7 de noviembre, por el que se actualiza el Catálogo Oficial de Razas de Ganado de España. BOE » núm. 279, de 21 de noviembre de 1997; Real Decreto 1682/1997; Ministerio De Agricultura Pesca Y Alimentación: Madrid, Spain, 1997; pp. 34205–34207.
- Druml, T.; Baumung, R.; Sölkner, J. Pedigree analysis in the Austrian Noriker draught horse: Genetic diversity and the impact of breeding for coat colour on population structure. J. Anim. Breed. Genet. 2009, 126, 348–356.
- Navas, F.J.; Jordana, J.; León, J.M.; Barba, C.; Delgado, J.V. A model to infer the demographic structure evolution of endangered donkey populations. Animal 2017, 11, 2129–2138.
- Alderson, G. A system to maximize the maintenance of genetic variability in small populations. In Genetic Conservation of Domestic Livestock; Alderson, L.J., Bodó, I., Eds.; CAB International: Oxford, UK, 1990; Volume 2, pp. 18–29.
- FAO. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans: Management of small populations at risk. Initiative for Domestic Animal Diversity; FAO: Rome, Italy, 1998.
- Fernández, J.; Villanueva, B.; Pong-Wong, R.; Toro, M.Á. Efficiency of the Use of Pedigree and Molecular Marker Information in Conservation Programs. Genetics 2005, 170, 1313–1321.
- Fernández, J.; Toro, M.; Caballero, A. Management of subdivided populations in conservation programs: Development of a novel dynamic system. Genetics 2008, 179, 683–692.
- Oldenbroek, K. Utilisation and Conservation of Farm Animal Genetic Resources; Wageningen Academic Publishers: Wageningen, The Netherlands, 2007.
- Villanueva, B.; Pong-Wong, R.; Woolliams, J.; Avendaño, S. Managing genetic resources in selected and conserved populations. BSAS Occas. Publ. 2004, 30, 113–132.