

ORIGINAL ARTICLE

Pedigree-Based Assessment of Inbreeding Effects on Conformation and Performance Traits in the Peruvian Paso Horse: Implications for Sustainable Genetic Improvement

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ABSTRACT The Peruvian Paso Horse (PPH) is a breed distinguished by its unique four-beat lateral gait. Nowadays, it faces significant genetic challenges related to declining genetic diversity and inbreeding depression, which may substantially affect conformation and performance traits. This study aimed to analyze inbreeding depression in PPH, focusing on its effects on gluteal temperature and morphometric and functional traits. A total of 35 traits were evaluated in 148 animals using pedigree records from 1945 to 2023 provided by the National Association of Breeders and Owners of PPH. Bayesian animal models were employed to estimate heritability and estimated breeding values (EBVs), accounting for fixed effects, including sex, breeder, gait velocity, and age. The findings provide suggestive evidence that both the inbreeding coefficient (F) and its rate of change (ΔF) could exert measurable effects on several traits, although these findings should be interpreted as exploratory. Pedigree analyses revealed that both parents were recorded for over 94% of the animals, indicating a good pedigree depth. Certain historical periods were identified as having reduced ancestral diversity, highlighting the importance of monitoring genetic diversity to prevent population bottlenecks in the future. Inbreeding was associated with reductions in key traits, such as withers height and scapulo-humeral angle. Furthermore, ΔF suggests a more immediate and pronounced effect on specific traits. Correlations between EBVs calculated with and without inclusion of inbreeding as a covariate were significant for certain traits, suggesting that inbreeding introduces estimation bias. Preliminary analyses suggest a tendency for inbreeding to be associated with variations not only in linear body measurements but also in anatomical angles, potentially reflecting the influence of pleiotropic genes affecting multiple morphological traits. Moreover, functional and physiological traits appear to be particularly sensitive to inbreeding effects, underscoring the need for strategic genetic management of this breed.

Keywords: Peruvian Horse; Inbreeding Depression; Genetic Diversity; Breeding Value

INTRODUCTION

The Peruvian Paso horse (PPH) is a breed distinguished by its unique four-beat lateral gait, originating from horses brought to Peru during the Spanish conquest, which began in 1532. Today, this breed holds considerable international relevance, with active breed associations established in Argentina, Panama, Ecuador, Canada, and the United States, among others (Vilela et al., 2025; Larrea Izurieta et al., 2022). However, it faces significant genetic challenges related to declining genetic diversity and inbreeding depression, which may substantially affect its conformation and performance traits.

Inbreeding depression refers to the reduction in biological fitness and phenotypic performance resulting from mating between related individuals, leading to increased homozygosity and the expression of recessive deleterious alleles (Leroy, 2014; Keller & Waller, 2002). Although inbreeding has historically been employed to fix desirable traits, it is now critically important to monitor its levels to preserve genetic variability and ensure sustainable genetic improvement of the breed (Larrea Izurieta et al., 2022; Karlau et al., 2025).

There remains a notable gap in understanding the specific impact of inbreeding depression in the PPH. Although studies have been conducted on other breeds, such as the Old Kladruber, Purebred Spanish Horse, and Campolina, their findings are not directly transferable due to genetic and selection differences (Bussiman et al., 2018; Gutiérrez et al., 2008; Vostry et al., 2021). In Ecuadorian PPH populations, Larrea Izurieta et al. (2022) reported average inbreeding coefficients as high as 8.11% and effective population sizes below 50, whereas Montenegro et al. (2018) found values of 5.44%. These figures suggest a loss of genetic variability attributable to historical bottlenecks and genetic drift. Nevertheless, the quantitative effect of inbreeding on productive or functional traits in the PPH has not yet been assessed. This study aimed to address this gap by analyzing the impact of inbreeding on genetic variability and morphometric, physiological, and functional traits.

Mitigating inbreeding depression is essential for the sustainable development of the PPH. Linear regression mod-

els, widely used in quantitative genetics, relate inbreeding coefficients to phenotypic traits while incorporating environmental covariates, thereby improving estimation accuracy by isolating genetic effects (Cortes *et al.*, 2024; Chaves *et al.*, 2011). The rate of inbreeding (ΔF) is preferably employed over the absolute inbreeding coefficient (F), as it provides a more dynamic and precise indicator for managing genetic diversity (Caballero *et al.*, 2021; Gómez-Raya *et al.*, 2015). The impact of inbreeding can also be evaluated through its effect on estimated breeding values (EBVs), which reflect the genetic merit of an individual and its relatives (Lowman, 2004). Comparing EBVs calculated with and without adjustment for inbreeding enables the quantification of its negative effect on performance (Becker *et al.*, 2016; Townsend & Jamieson, 2013). Incorporating correlations between adjusted and unadjusted EBVs enhances the understanding of inbreeding depression and informs mitigation strategies within breeding programs.

By evaluating the levels of inbreeding and their phenotypic consequences through advanced genetic modeling, this study will provide critical insights for breeders and geneticists. This will facilitate the development of strategies to preserve genetic diversity, enhance performance, and maintain the quality of the PPH, while also serving as a model for the conservation and improvement of other equine breeds. Therefore, this study aimed to 1) quantify the rate of inbreeding accumulation in registered PPH individuals, 2) evaluate its effect on gluteal temperature and morphometric and functional traits, and 3) determine the correlation between EBVs estimated with and without adjustment for inbreeding.

MATERIAL AND METHODS

Animals and pedigree

All data and measurements for each animal were collected between October 2021 and March 2023. All animal handling and procedures were conducted in strict accordance with animal welfare principles, ensuring that no harm was caused. Written informed consent was obtained from all the owners before data collection.

This study compiled genealogical records of all registered PPH from 1945 to the year 2023, provided by the Asociación Nacional de Criadores y Propietarios del Caballo Peruano de Paso (ANCPCPP). A systematic and thorough review of the database was performed using a custom-designed template in .xlsx format. Individual records, including those of each animal sire and dam, were extracted and compiled into a pedigree file for subsequent analysis to estimate the individual inbreeding coefficients F and individual accumulation ΔF . Animals whose parents were recorded with duplicate names, lacked registration numbers, or presented identification errors were excluded from the final dataset. A total of 40,993 animals were included in the final pedigree file. A total of 151 animals were phenotyped, and ENDOG v4.8 software was used to cal-

culate individual F and individual ΔF (Gutiérrez & Goyache, 2005). The annual increase in the rate of inbreeding was estimated as the mean and standard deviation, along with the average number of equivalent complete generations per year.

Traits

A total of 35 traits were evaluated according to Vilela Velarde *et al.*, 2025 (see Table 1 for full descriptions). Animals for which certain traits could not be measured were excluded from the corresponding models; consequently, the sample size varied across analyses.

Measurements

Withers height (WH), croup height (CRH), sub-pectoral height (SH), and mid-dorsal height (MDH) were measured using an aluminum hippometer with a levelling device (NC Equine, UK). Thoracic girth (TG), metacarpal circumference (MC), metatarsal circumference (MT), forelimb pastern perimeter (FPP), and hindlimb pastern perimeter (HPP) were recorded using a flexible measuring tape (SUMVIBE model, Tape 3M-3Y, USA). Angular measurements, including the back inclination angle (BIA), hock angle (HA), coxofemoral angle (CFA), scapulohumeral angle (SHA), femorotibial angle (FTA), and brachial angle (BA), were obtained using a digital goniometer (GemRed Model 82305, USA). All angular measurements were taken three times for each animal, and the arithmetic mean was used for subsequent analyses. Linear measurements, including upper arm length (UL), chest width (CHW), femur length (FL), metacarpal length (MCL), metatarsal length (MTL), croup length (CL), croup width (CW), neck length (NL), body length (BL), and head length (HL), were recorded using a 5-meter retractable tape measure (Stanley Black & Decker, USA). Forelimb (FPL) and hindlimb pastern lengths (HPL) were measured using a digital electronic caliper (Ubermann, Germany).

Functional traits, overreach (OVR), extension (EXT), term (TER), acuteness (ACS), and vertical acceleration (RMS) were obtained via video recording of each animal performing the paso llano gait, led by a handler over a distance of 50 m at speeds between 2.5 and 4.0 m/s. Recordings were made using a smartphone camera (Motorola Edge 30 Fusion, USA) with a resolution of 1920 × 1080 pixels at 60 fps. The camera was mounted on a professional tripod (Benro T980, USA) positioned 1.3 m above ground level, spatially aligned along the X, Y, and Z axes, and placed perpendicular to the animal's direction of motion at approximately 12 m. Lateral motion was recorded for all traits except for TER, for which frontal recording was used (see Figure 2 in Vilela Velarde *et al.*, 2025, for a graphic definition of the "term" trait).

Reference anatomical landmarks were marked with 4 × 4 cm adhesive tapes to facilitate motion tracking. All videos were stored in MP4 format and processed using

Kinovea software (version 0.9.5; Free Software Foundation, Inc., Boston, MA, USA; <http://www.kinovea.org/>). Stride-level records for OVR, EXT, TER, and ACS, measured 3 to 5 times (once per stride), were retained for the Bayesian animal models, and a permanent environmental effect was included to account for repeated measurements within the individuals. Animal-level means were used only for descriptive summaries and correlation analyses.

Vertical acceleration was recorded continuously throughout the lateral displacement of each animal, and measurements were captured every 15 ms.

A 10-cm adhesive tape strip, placed parallel to the ground on the ribcage side facing the camera, served as the spatial calibration reference. All frames recorded during the paso llano gait were processed using a two-pass Butterworth filter to reduce image noise. Vertical acceleration values (m/s²) were averaged using the root mean square (RMS) formula as follows:

$$\text{RMS} = \sqrt{\sum \frac{a_i^2}{n}}$$

where a_i is the individual vertical acceleration value and n is the number of observations during the gait cycle.

The gluteal temperature difference (GTD) and maximum gluteal temperature difference (GTD_X) were measured using an infrared thermal camera HTI-19 (Dongguan Xintai Instrument Co., Ltd., China) with a resolution of 320 × 240 pixels, 300k px, and an emissivity coefficient of 0.95. The camera was positioned 20 cm from the animal's surface, in accordance with the manufacturer's guidelines. Temperatures (°C) were recorded before and after the performance of the paso llano gait under identical environmental and solar positioning conditions. The final temperature difference was used for the statistical analysis.

Regression analysis

To assess the effect of inbreeding on animal performance, 151 stallions and mares were phenotyped based on 35 morphometric and functional traits and gluteal temperature traits. Records from animals with questionable data or missing measurements were excluded from the analyses on a trait-by-trait basis. Of these, between 88 and 148 animals (depending on trait completeness) were analyzed using a linear regression model with specific model structures applied according to the trait type.

The following models were applied for morphometric traits (1) and functional traits (2):

$$Y_{ijklm} = \mu + E_i + S_j + C_k + F_l b_1 + e_{ijklm} \quad (1)$$

$$Y_{ijklmn} = \mu + E_i + S_j + C_k + F_l b_1 + V_m b_2 + e_{ijklmn} \quad (2)$$

where Y_{ijklmn} represents the measured trait, μ represents the overall mean, E_i = age, S_j = sex, C_k = breeder, F_l = inbreeding coefficient or rate of inbreeding (ΔF), V_m = covariate of gait velocity, and e_{ijklmn} = residual effect. The b coefficients represent the covariability coefficients for each trait. Linear regression analyses were conducted using JASP (version 0.19; Amsterdam, Netherlands). The significance level for the analyses was set at 0.05. Given the evaluation of 35 traits across two inbreeding metrics (F and ΔF), resulting in approximately 70 regression tests, we also applied the Benjamini-Hochberg procedure to control the false discovery rate (FDR) at $q < 0.10$ (Benjamini & Hochberg, 1995). This threshold balances the identification of biologically relevant signals while limiting the expected proportion of false positives among the declared significant results. Both unadjusted P -values and FDR-adjusted q -values are reported in Table 3. Results with $q < 0.10$ are interpreted as robust to multiple testing; results with $0.05 \leq P < 0.10$ but $q \geq 0.10$ are considered suggestive and warrant validation in independent cohorts. Effect sizes (regression coefficients) and their 95% confidence intervals are emphasized alongside statistical significance to support the biological interpretation.

Genetic and statistical analysis

To estimate the heritability values for the traits, two models were considered using Bayesian linear mixed models implemented in the MCMCglmm package (Hadfield, 2010) in R (R Core Team, 2024). From the 148 animals analyzed, between 84 and 144 were retained for analysis after excluding individuals with pedigree inconsistencies. The number of animals used for each trait is presented in Table 5. For repeated-record traits, each stride-level observation was included as a separate record. The number of repeated observations per animal ranged from 3 to 5, and the permanent environmental effect accounted for non-genetic between-individual variation shared across repeated records (Model A). For all other traits, the permanent environmental effect was omitted (Model B). Model A is expressed as:

$$y = X\beta + Z_1a + Z_2pe + e$$

where y is the vector of phenotypic observations, β is the vector of fixed effects (sex, age, breeder, velocity and inbreeding), $a \sim N(0, A\sigma_a^2)$ is the vector of additive genetic effects, with σ_a^2 denoting the additive genetic variance and A the numerator relationship matrix derived from the pedigree, $pe \sim N(0, I\sigma_{pe}^2)$ is the permanent environmental effect with variance σ_{pe}^2 , and $e \sim N(0, I\sigma_e^2)$ is the residual error with variance σ_e^2 , X is the incidence matrix relating fixed effects to observations, Z_1 is the incidence matrix relating additive genetic effects to observations, and Z_2 is the incidence matrix relating permanent environmental effects to observations.

Candidate fixed effects included age, sex, and breeder for all traits, whereas gait velocity was considered only for functional traits, where biologically appropriate. Age and gait velocity were fitted as continuous covariates, whereas sex and breeder were fitted as categorical fixed effects. The number of breeder levels included in each trait-specific model was adjusted as needed. Breeder categories represented by fewer than three phenotyped animals were combined into a single category. The numerator relationship matrix was constructed from the complete cleaned pedigree of 40,993 registered animals, including phenotyped animals and non-phenotyped ancestors. Heritability was computed as

$$h^2 = \frac{\sigma_a^2}{(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)}$$

Model B is identical to Model A, except that the *pe* effect was not considered, and heritability was computed as

$$h^2 = \frac{\sigma_a^2}{(\sigma_a^2 + \sigma_e^2)}$$

All the traits were modeled using Gaussian error distributions. Continuous traits were analyzed on their original scale.

A stepwise model selection procedure was applied to determine the optimal set of fixed effects. Candidate models were sequentially built by adding fixed effects (sex, age, breeder, velocity), and the model with the lowest Deviance Information Criterion (DIC) was selected as the best-fitting model for inference. The candidate fixed-effect structures and their DIC values are listed in Table 4. When the Δ DIC between competing models was < 2 , the simpler model was retained. Bayesian animal models were fitted for all evaluated traits to estimate genetic parameters and EBVs. These models were not restricted to traits showing significant associations with *F* or ΔF in the regression analyses because heritability estimation and inbreeding-effect testing address different analytical questions. For each trait, the fixed-effect structure selected for the baseline model was retained, and either *F* or ΔF was added as an additional continuous fixed covariate. Because fixed-effect selection was performed separately for each trait, model-selection uncertainty may have affected the variance component and EBV estimates. Therefore, the estimates should be interpreted cautiously, particularly when competing models show similar DIC values. Heritability estimates were reported from the model with the lowest DIC for each trait. Weakly informative inverse-gamma priors ($V=1$, $v=1$) were assigned to all variance components. The exact MCMCglmm prior specification was as follows: prior = list(R = list(V = 1, v = 1), G = list(G1 = list(V = 1, v = 1), G2 = list(V = 1, v = 1))), where G1, G2, and R correspond to the additive genetic, permanent environmental,

and residual variance components, respectively. Although traits were measured on different scales, the parameterization with $V=1$ and $v=1$ was chosen because it acts as a boundary-avoiding prior (Hadfield, 2010). This specification results in a highly right-skewed inverse-gamma distribution with a long right tail, which places minimal prior mass away from zero and ensures that the posterior variance estimates are predominantly driven by the data likelihood rather than the prior specification, regardless of the trait's original scale. Furthermore, to empirically verify this, we conducted a prior sensitivity analysis on a subset of traits with different scales ($V=1$ and $v=0.002$ or $V=1$ and $v=2$). The results confirmed that the posterior estimates were similar and not driven by the prior specification. Markov Chain Monte Carlo (MCMC) chains were run for 600,000 iterations, with a burn-in of 100,000 and a thinning interval of 100, yielding 5,000 posterior samples for inference. MCMC convergence was assessed using the effective sample size ($ESS > 200$), trace plots, posterior density plots, and autocorrelation diagnostics for all variance components and fixed effects. The OVR model did not initially meet the predefined ESS threshold. Consequently, it was rerun with extended MCMC chains (NITT = 2,300,000; BURNIN = 300,000; THIN = 100), which resulted in an ESS value exceeding 200, thereby meeting our convergence criteria. Posterior means and posterior standard deviations are reported for heritability, additive genetic variance, permanent environmental variance, residual variance, and repeatability (Table 5).

EBVs were calculated as the posterior means of the additive genetic effects for each phenotyped individual (Models A or B, depending on the trait structure). The distribution of EBV prediction accuracies is provided in Supplementary Material 1. Posterior standard deviations, 95% credible intervals, posterior median, and accuracy for individual EBVs are provided in Supplementary Material 2 to reflect uncertainty in genetic merit estimates. All EBVs were derived from the same MCMC chains used for heritability estimation, ensuring consistency in the inference. Two additional models extended the baseline by including either i) individual *F* or ii) ΔF as a linear fixed effect. These models assumed linear effects of *F* or ΔF on each trait, and while nonlinear effects were not evaluated, they should be considered in future studies with larger sample sizes. *F* and ΔF were included as continuous covariates (expressed as percentages ranging from 0 to 100) and fitted in separate models to avoid potential collinearity and assess their individual effects on EBV estimates. To assess the sensitivity of EBVs to inbreeding adjustment, EBVs from baseline and inbreeding-adjusted models were compared. Pearson's correlation coefficients, Lin's concordance correlation coefficients (CCC), and Spearman's rank correlation coefficients were computed to assess the association, agreement, and ranking consistency among EBV estimates, as follows: (a) EBVs from the baseline model and those adjusted for *F*, and (b) EBVs from the baseline model and those adjusted

for ΔF . EBV comparisons were first performed across all phenotyped animals for each trait (Supplementary Material 3, global correlations). Additional sensitivity analyses were restricted to the top and bottom 20 animals ranked by the baseline EBV. These extreme-group comparisons were treated as exploratory because correlations within small groups may be biased by range restriction. Lin's CCC was interpreted as a measure of agreement between EBV sets, while rank-based metrics were used to evaluate potential changes in selection ranking. To test whether the correlation between EBV and EBV_F significantly differed from that between EBV and EBV_ΔF, Steiger's z-test for dependent overlapping correlations was implemented using the appropriate cocor function for correlations sharing one variable in R (Steiger, 1980; Diedenhofen & Musch, 2015). *P*-values from Steiger's tests were adjusted using the Benjamini-Hochberg procedure across traits within each EBV stratum. The null hypothesis was that the correlation between baseline EBVs and *F*-adjusted EBVs equals the correlation between baseline EBVs and ΔF -adjusted EBVs

[$H_0: r(\text{EBV}, \text{EBV}_F) = r(\text{EBV}, \text{EBV}_{\Delta F})$]. EBV comparisons were performed for 35 traits in each stratum. The number of traits exceeding the predefined concordance thresholds was reported separately for the top-20 and bottom-20 groups. The significance level was set at $\alpha = 0.05$. Significant Steiger tests were interpreted as evidence that *F*- and ΔF -adjusted EBVs differ in their similarity to baseline EBVs, not as evidence that one adjustment is biologically superior.

RESULTS

Descriptive Analysis of Morphometric, Physiological, and Functional Traits

Thirty-five traits were analyzed in the PPHs studied (148 valid records; 3 discarded), including measurements of angles, body length, and perimeters. Table 1 summarizes the descriptive statistics and normality (Anderson-Darling test): most traits were normally distributed ($P > 0.05$), with a few exceptions ($P < 0.001$).

Table 1.
Descriptive statistical analysis for the 35 variables studied.

Trait ^a	Observations	Mean	SD	Minimum	Maximum	CV(%)	<i>P</i> -value Anderson-Darling
WH	148	145.08	2.98	137.00	153.00	2.05	0.273
CRH	148	146.05	3.59	132.00	155.00	2.46	0.089
SH	148	75.04	6.57	67.00	148.50	8.76	0.175
MDH	148	137.59	3.62	128.90	149.00	2.63	0.348
CHW	148	34.02	2.31	29.00	39.00	6.78	0.252
TG	148	176.93	7.27	155.00	198.00	4.11	0.378
BL	148	153.58	5.65	140.00	168.00	3.68	0.800
MC	148	17.53	0.85	15.00	20.00	4.85	<0.001
MT	148	18.68	0.86	17.00	21.00	4.63	<0.001
BIA	148	63.15	4.30	49.33	77.00	6.82	0.087
HA	148	139.12	3.92	129.67	154.67	2.82	0.097
SHA	148	91.66	3.06	81.33	103.33	3.34	<0.001
FTA	148	124.25	5.07	116.67	139.33	4.08	<0.001
BA	148	123.06	5.55	110.67	158.83	4.51	<0.001
CFA	148	91.66	2.86	85.00	104.33	3.12	<0.001
FPL	148	11.27	1.37	7.96	23.00	12.19	<0.001
HPL	148	11.26	1.24	7.81	21.00	11.04	<0.001
NL	148	60.57	3.83	48.00	69.00	6.32	0.517
UL	148	34.83	2.48	29.00	53.00	7.11	0.021
FL	148	35.91	2.51	29.00	44.00	7.00	0.229
FPP	148	16.70	0.90	14.00	21.00	5.40	<0.001
HPP	148	17.58	0.82	15.50	21.00	4.67	<0.001
CW	148	34.96	4.41	25.00	56.00	12.62	0.050
CL	148	49.18	3.78	32.00	58.00	7.68	0.062
HW	148	22.40	1.26	20.00	31.00	5.61	<0.001
HL	148	61.47	2.28	56.00	68.00	3.70	0.187

Trait ^a	Observations	Mean	SD	Minimum	Maximum	CV(%)	P-value Anderson-Darling
MCL	148	26.57	1.75	22.00	30.00	6.58	0.017
MTL	148	30.54	1.72	26.00	35.00	5.64	0.061
EXT	134	42.23	4.60	22.07	55.33	10.89	0.361
OVR	136	27.95	20.99	-14.04	72.44	75.09	0.780
TER	137	25.30	5.97	8.93	43.47	23.60	0.858
ACS	134	71.55	6.68	52.03	87.37	9.33	0.938
GTD	88	1.60	2.02	-3.40	9.40	125.98	0.425
GTDX	88	1.05	2.99	-6.60	13.10	286.35	0.066
RMS	135	2.63	0.93	0.85	5.36	35.33	0.888

^aTraits: ACS: Acuteness; BA: Brachial angle; BIA: Back inclination angle; BL: Body length; CFA: Coxofemoral angle; CHW: Chest width; CL: Croup length; CRH: Croup height; CW: Croup width; EXT: Extension; FL: Femur length; FPL: Forelimb pastern length; FPP: Forelimb pastern perimeter; FTA: Femorotibial angle; GTD: Gluteal temperature difference; GTDX: Maximum gluteal temperature difference; HA: Hock angle; HL: Head length; HPL: Hindlimb pastern length; HPP: Hindlimb pastern perimeter; HW: Head width; MC: Metacarpal circumference; MCL: Metacarpal length; MDH: Mid-dorsal height; MT: Metatarsal circumference; MTL: Metatarsal length; NL: Neck length; OVR: Overreach; RMS: Vertical acceleration; SH: Sub-pectoral height; SHA: Scapulohumeral angle; TER: Term; TG: Thoracic girth; UL: Upper arm length; WH: Withers height.

SD: standard deviation, CV: coefficient of variation

Pedigree structure and population genetic parameters

The total population in the pedigree (40,993 animals) comprised 39.2% males and 60.8% females, and in the phenotyped subpopulation (151 individuals), 22.5% were males and 77.5% females (Table 2). In the total population, both parents were recorded in more than 94% of the animals; in the phenotyped group, this figure was 100%, indicating higher pedigree accuracy in this subset. The average inbreeding was 6.74% (total population) and 9.02% (phe-

notyped group), with a similar annual increase (~1.4%). The average relatedness coefficient indicates a high level of genetic relationship. The total population spanned 5.75 generations, whereas the phenotyped group spanned 7.70 generations. A total of 1,758 and 310 founders were identified, respectively; however, only 27 and 17 founders effectively contributed (f_e), and even fewer as the effective number of ancestors (f_a ; 18 and 11), revealing a strong dependence on a small number of individuals in the genetic base.

Table 2.

Pedigree structure and genetic parameters for the total and phenotyped populations.

Parameter	Total population	Phenotyped population
Number of animals	Total	151
	Males	34
	Females	117
Animals with known parents (%)	With known father	100.00
	With known mother	100.00
Inbreeding	Mean F (%)	9.02
	Mean ΔF (%)	1.41
Average relatedness coefficient (%)	8.37	11.98
Average maximum generations	11.58	16.00
Average complete generations	3.62	4.85
Average equivalent generations	5.75	7.70
Effective population size per equivalent generation	37.46	40.66
Founders	1758	310
Equivalent founders	1661	291
Ancestors contributing to the population	1592	140
Effective number of founders (f_e)	27	17

CONTINUE

Parameter		Total population	Phenotyped population
Effective number of ancestors (f_d)		18	11
Wright's fixation indices	FIS	0.0250	0.0265
	FST	0.0016	0.000082
	FIT	0.0266	0.0266
Generation interval $\pm$ SE		8.90 $\pm$ 0.03	9.04 $\pm$ 0.30
Age at first reproduction (A_p)		9.30	5.28
Regression coefficient (β) of A_p on $F \pm$ SE		-5.20 $\pm$ 0.62	-38.48 $\pm$ 12.57
Regression coefficient (β) of A_p on $\Delta F \pm$ SE		6.54 $\pm$ 2.08	-189.73 $\pm$ 103.56

SE: Standard error.

Wright's fixation indices indicated a genetically homogeneous structure: the Fixation Index within subpopulations (FIS) was 0.025 for the total population and 0.0265 for the phenotyped population, reflecting a slight excess of homozygosity within subpopulations. The Fixation Index among subpopulations (FST) was nearly zero (< 0.002), revealing that almost all genetic variation was due to individual differences, with high gene flow. The Fixation Index for the total population (FIT) (0.0266) confirmed that overall inbreeding was primarily driven by FIS and not by differentiation among subpopulations. The average age at which individuals first became parents (A_p) was 9.30 years (total population) and 5.28 years (phenotyped). The regression of A_p on F showed negative coefficients: $\beta = -5.20$ (total) and $\beta = -38.48$ (phenotyped), suggesting that higher inbreeding is associated with earlier reproduction, particularly in the phenotyped subpopulation. However, given the magnitude of these estimates, a nonlinear model or the inclusion of additional covariates may be necessary to better capture this relationship.

Analysis of inbreeding, temporal trends, and genetic impact

The average inbreeding showed significant fluctuations throughout the evaluation period. The values ranged from 0% to 10.2%, with notable peaks in specific years. Figure 1 displays the annual trends for the inbreeding coefficient F , ΔF and Equivalent Generations (EG) per year. EG reflects the average number of generations separating current individuals from their common ancestors, reaching its highest value in 2022 (8.417). The observed inbreeding peaks suggest periods of intensified mating among related individuals, possibly due to demographic bottlenecks or population size constraints.

Linear regression analysis with respect to inbreeding

The effects of F (inbreeding coefficient) and ΔF (individual rate of inbreeding) on the 35 PPH traits were evaluated (Table 3). F significantly affected SHA, according to the q -values. The regression coefficients (b) ranged from -0.308 to $+0.369$, re-

flecting both inverse and direct associations with inbreeding, showing a moderate association. ΔF significantly influenced SHA, with large standard errors that indicated greater variability. Standardized regression coefficients were included to enable a comparison of the effects across variables measured in different units. These standardized coefficients indicate that although unstandardized coefficients suggest a stronger impact of inbreeding on the brachial angle trait (BA) than on withers height (WH), the standardized coefficients indicate similar or even greater sensitivity in WH, highlighting the importance of using standardized measures to correctly interpret the magnitude of inbreeding effects.

For example, when comparing WH and BA, the unstandardized regression coefficient suggests that F affects BA nearly 1.6 times more than WH, and ΔF affects BA 1.9 times more than it affects WH. However, because these traits are measured in different units, such a direct comparison does not allow for an accurate interpretation. In contrast, when comparing standardized coefficients, it becomes evident that both traits could be affected by F and ΔF almost equally, or in some cases, WH may even be affected by F up to 1.2 times more than BA.

Bayesian animal models and heritability estimates

The model selection process based on DIC resulted in varying combinations of fixed effects for the traits analyzed (Table 4). Morphological traits such as WH and Mid-dorsal height (MDH) required models including age, sex, and breeder effects. In contrast, angular measurements, such as the back inclination angle (BIA) and hock angle (HA), were best explained by simpler models containing only age or sex. All selected models met the convergence criterion of ESS > 200 for the variance components and fixed effects. This includes the OVR model, which achieved convergence after being rerun with the modifications described above. In most cases, functional traits were affected by velocity. The ESS values varied among traits, and models with low ESS were interpreted cautiously or rerun with longer chains. Robustness should be assessed for each variance component rather than inferred from a single trait-level ESS value.

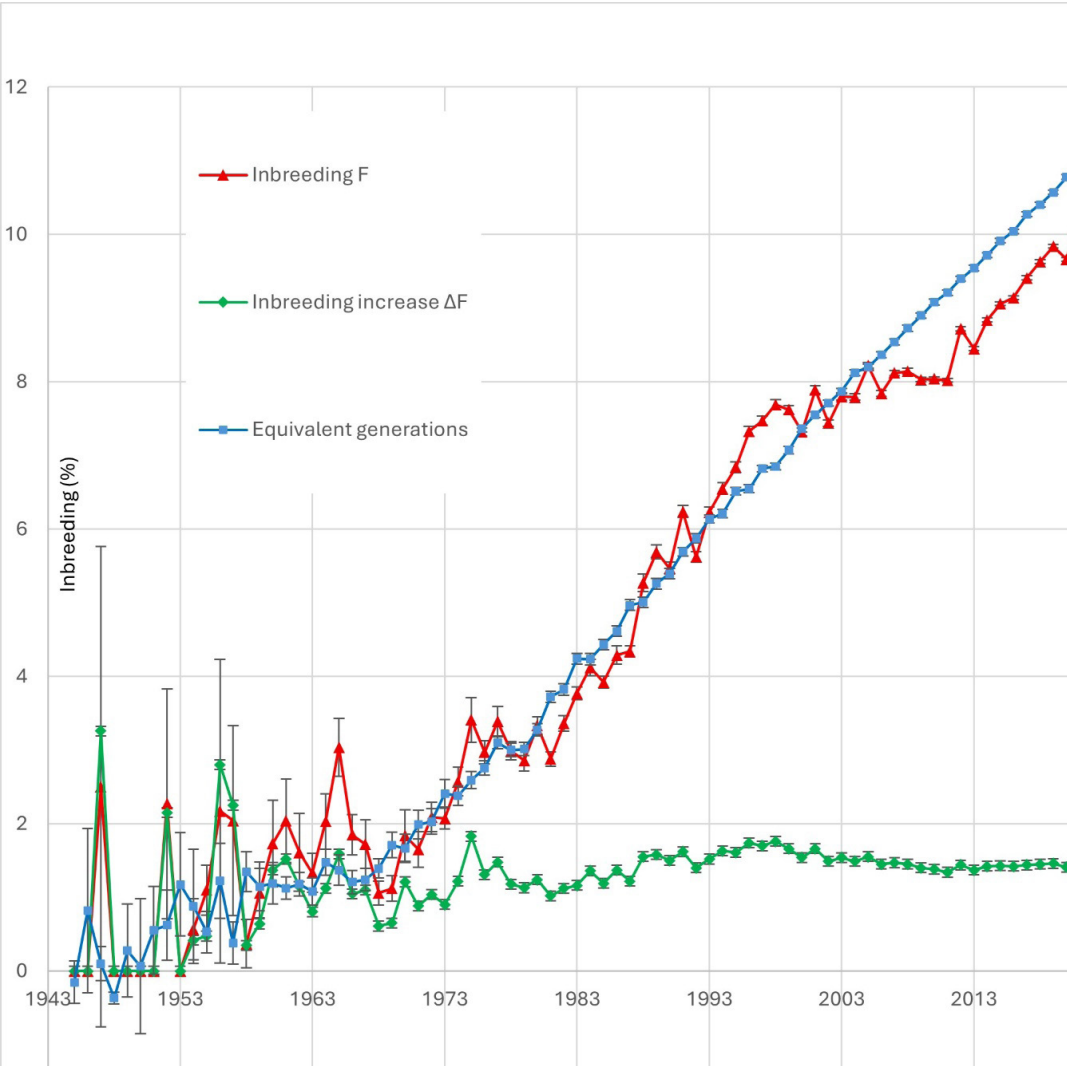


Figure 1. Average inbreeding, average annual inbreeding increase and equivalent generations per year. Error bars indicate the standard error of the mean.

Table 3. Standardized and unstandardized regression coefficients (b) including the independent variables: individual inbreeding coefficient (F) and individual inbreeding rate (ΔF). SE: Standard error of the coefficients with P-value and q-value are shown.

Trait ^a	F					ΔF				
	b		SE	P-value	q-value ^b	b		SE	P-value	q-value ^b
	unstandardized	standardized				unstandardized	standardized			
WH	-0.193	-0.240	0.081	0.018	0.210	-1.052	-0.210	0.493	0.035	0.306
CRH	-0.132	-0.134	0.094	0.177	0.563	-0.613	-0.100	0.573	0.287	0.667
SH	-0.111	-0.164	0.064	0.086	0.301	-0.546	-0.129	0.394	0.168	0.517
MDH	-0.189	-0.193	0.100	0.062	0.268	-0.945	-0.155	0.614	0.126	0.487
CHW	-0.022	-0.035	0.058	0.706	0.824	-0.151	-0.039	0.354	0.670	0.850
TG	-0.160	-0.081	0.163	0.327	0.673	-0.852	-0.069	0.994	0.393	0.724
BL	-0.096	0.150	-0.063	0.522	0.783	-0.392	-0.041	0.916	0.669	0.850

CONTINUE

Trait ^a	<i>F</i>					<i>ΔF</i>				
	<i>b</i> unstandardized	<i>b</i> standardized	SE	<i>P</i> -value	<i>q</i> -value ^b	<i>b</i> unstandardized	<i>b</i> standardized	SE	<i>P</i> -value	<i>q</i> -value ^b
MC	-0.003	-0.011	0.021	0.906	0.955	-0.006	-0.004	0.130	0.966	0.966
MT	-0.022	-0.096	0.024	0.353	0.686	-0.132	-0.090	0.146	0.370	0.719
BIA	0.106	0.092	0.107	0.322	0.673	0.685	0.095	0.651	0.295	0.667
HA	0.074	0.070	0.096	0.443	0.775	0.366	0.560	0.587	0.534	0.822
SHA	-0.236	-0.298	0.074	0.002	0.002	-1.459	-0.296	0.452	0.002	0.070
FTA	0.048	0.035	0.122	0.698	0.698	0.216	0.025	0.745	0.773	0.865
BA	-0.308	-0.207	0.149	0.041	0.268	-2.017	-0.218	0.906	0.028	0.306
CFA	-0.047	-0.064	0.075	0.537	0.783	-0.246	-0.054	0.458	0.593	0.844
FPL	0.042	0.112	0.035	0.228	0.665	0.278	0.119	0.211	0.192	0.517
HPL	0.002	0.006	0.031	0.947	0.955	-0.012	-0.005	0.188	0.951	0.966
NL	0.172	0.165	0.090	0.060	0.268	0.948	0.146	0.552	0.089	0.445
UL	-0.029	-0.043	0.058	0.623	0.823	-0.111	-0.027	0.355	0.756	0.865
FL	-0.016	-0.024	0.054	0.766	0.865	-0.172	-0.041	0.329	0.603	0.844
FPP	0.003	0.013	0.021	0.885	0.955	0.035	0.023	0.131	0.791	0.865
HPP	0.012	0.055	0.021	0.563	0.788	0.130	0.094	0.129	0.315	0.667
CW	-0.105	-0.087	0.106	0.323	0.673	-0.495	-0.066	0.647	0.446	0.781
CL	0.075	0.074	0.076	0.321	0.673	0.613	0.096	0.459	0.185	0.517
HW	-0.061	-0.178	0.033	0.069	0.268	-0.138	-0.150	0.203	0.120	0.487
HL	-0.003	-0.005	0.056	0.955	0.955	0.061	0.016	0.339	0.858	0.910
MCL	-0.091	-0.193	0.036	0.013	0.210	-0.441	-0.150	0.224	0.051	0.357
MTL	-0.034	-0.075	0.041	0.400	0.737	-0.103	-0.036	0.248	0.680	0.850
EXT	0.200	0.087	1.067	0.502	0.783	1.201	0.154	0.805	0.139	0.487
OVR	-0.190	-0.037	0.422	0.653	0.824	-1.575	-0.049	2.561	0.540	0.822
TER	0.116	0.073	0.162	0.475	0.783	0.679	0.069	0.986	0.492	0.820
ACS	0.369	0.203	0.183	0.047	0.268	2.090	0.186	1.118	0.065	0.379
GTD	-0.138	-0.240	0.063	0.034	0.268	-0.825	-0.241	0.368	0.030	0.306
GTDX	-0.107	-0.145	0.100	0.293	0.673	-0.582	-0.133	0.583	0.324	0.667
RMS	0.008	0.035	0.022	0.695	0.824	0.040	0.027	0.131	0.758	0.865

^aTraits: ACS: Acuteness; BA: Brachial angle; BIA: Back inclination angle; BL: Body length; CFA: Coxofemoral angle; CHW: Chest width; CL: Croup length; CRH: Croup height; CW: Croup width; EXT: Extension; FL: Femur length; FPL: Forelimb pastern length; FPP: Forelimb pastern perimeter; FTA: Femorotibial angle; GTD: Gluteal temperature difference; GTDX: Maximum gluteal temperature difference; HA: Hock angle; HL: Head length; HPL: Hindlimb pastern length; HPP: Hindlimb pastern perimeter; HW: Head width; MC: Metacarpal circumference; MCL: Metacarpal length; MDH: Mid-dorsal height; MT: Metatarsal circumference; MTL: Metatarsal length; NL: Neck length; OVR: Overreach; RMS: Vertical acceleration; SH: Sub-pectoral height; SHA: Scapulohumeral angle; TER: Term; TG: Thoracic girth; UL: Upper arm length; WH: Withers height.

^b*q*-values represent Benjamini-Hochberg FDR-adjusted *P*-values across all 35 regression tests per inbreeding metric. Significance thresholds: *q* < 0.05 (significant after FDR correction); *q* < 0.10 (suggestive, hypothesis-generating).

Table 4.

Models with the lowest DIC applied to each trait for the estimation of genetic parameters and their corresponding effective sample size (ESS)

Trait ^a	Age	Sex	Breeder	Velocity	DIC	Δ DIC ^b	ESS
WH	✓	✓	✓		675.04	2.34	336.76
CRH	✓	✓			-76.12	15.59	684.57
SH	✓				348.19	37.99	278.67
MDH	✓	✓	✓		710.57	17.27	779.04
CHW	✓	✓			53.97	81.89	704.14
TG		✓			-145.03	1.15	835.32
BL	✓	✓			877.17	11.10	317.62
MC	✓	✓	✓		-198.87	25.32	601.94
MT	✓	✓	✓		315.30	24.8	418.19
BIA	✓				676.36	27.37	1601.43
HA		✓			730.07	20.36	404.61
SHA		✓			718.79	0.86	861.06
FTA		✓			187.12	3.93	456.69
BA	✓	✓			804.58	20.89	252.20
CFA		✓			671.78	17.82	205.86
FPL	✓	✓			-200.58	5.17	982.05
HPL	✓				-174.65	10.44	876.61
NL	✓	✓			679.55	0.34	425.51
UL	✓	✓			641.43	0.46	826.37
FL		✓			-19.99	15.31	762.18
FPP	✓	✓			109.94	126.27	1184.45
HPP			✓		177.17	2.64	598.29
CW	✓	✓			663.11	77.98	202.43
CL	✓	✓			134.89	31.06	599.80
HW	✓				426.56	1.09	5000.00
HL	✓				506.93	3.81	3724.82
MCL		✓			-196.06	25.86	1077.07
MTL		✓			-177.77	7.22	959.66
EXT				✓	1921.84	0.09	1932.72
OVR				✓	2907.14	0.089	390.46
TER			✓		2149.82	0.16	395.91
ACS				✓	2066.04	0.13	793.73
GTD	✓				250.72	2.08	4577.98
GTDX			✓		244.94	1.70	3433.71
RMS		✓		✓	262.86	1.09	5000.00

^aTraits: ACS: Acuteness; BA: Brachial angle; BIA: Back inclination angle; BL: Body length; CFA: Coxofemoral angle; CHW: Chest width; CL: Croup length; CRH: Croup height; CW: Croup width; EXT: Extension; FL: Femur length; FPL: Forelimb pastern length; FPP: Forelimb pastern perimeter; FTA: Femorotibial angle; GTD: Gluteal temperature difference; GTDX: Maximum gluteal temperature difference; HA: Hock angle; HL: Head length; HPL: Hindlimb pastern length; HPP: Hindlimb pastern perimeter; HW: Head width; MC: Metacarpal circumference; MCL: Metacarpal length; MDH: Mid-dorsal height; MT: Metatarsal circumference; MTL: Metatarsal length; NL: Neck length; OVR: Overreach; RMS: Vertical acceleration; SH: Sub-pectoral height; SHA: Scapulohumeral angle; TER: Term; TG: Thoracic girth; UL: Upper arm length; WH: Withers height.

^b Δ DIC = DIC_i – DIC_{min}, where DIC_i is the DIC value of model i and DIC_{min} is the lowest DIC value among all candidate models. Models with Δ DIC < 2 are considered to have substantial support, while Δ DIC > 10 indicates essentially no support.

Table 5 summarizes the genetic parameters estimated for each trait. Posterior mean heritability (h^2) estimates with 95% credible intervals for the evaluated traits grouped by category (Figure 2) varied widely across the dataset, ranging from low values such as 0.10 for extension (EXT) and 0.16 for body length (BL) to very high estimates exceeding 0.90 for thoracic girth (TG) and femorotibial angle (FTA). However, exceptionally high values and those with substantial posterior uncertainty warrant cautious

interpretation because of the limited number of phenotyped animals and the sensitivity of variance components to model specification and priors. Repeatability (R) was calculated only for traits with repeated stride-level records retained in the model and was calculated as $R = (\sigma_a^2 + \sigma_{pe}^2) / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$ (EXT, OVR, TER, and ACS), with values ranging from 0.77 to 0.86. Notably, OVR exhibited the highest additive variance ($V_a=303.94$) but also the largest standard error.

Table 5.

Heritability (h^2), Additive variance (V_a), Residual variance (V_e), Permanent environment variance (V_{pe}) and Repeatability (R) with posterior standard deviations in brackets.

Trait ^a	Observations	h^2	V_a	V_e	R	V_{pe}
WH	144	0.35 (0.29)	4.23 (4.55)	5.85 (2.29)		
CRH	144	0.82 (0.24)	19.76 (7.66)	3.28 (3.61)		
SH	144	0.62 (0.28)	6.25 (3.63)	2.96 (1.78)		
MDH	144	0.52 (0.32)	10.06 (7.93)	7.02 (3.87)		
CHW	144	0.68 (0.26)	5.81 (3.02)	2.22 (1.47)		
TG	144	0.96 (0.10)	92.14 (17.16)	3.63 (6.63)		
BL	144	0.16 (0.24)	7.29 (14.09)	28.24 (7.43)		
MC	144	0.63 (0.16)	0.53 (0.21)	0.28 (0.10)		
MT	144	0.54 (0.18)	0.56 (0.27)	0.42 (0.14)		
BIA	144	0.49 (0.32)	13.69 (11.12)	10.69 (5.53)		
HA	144	0.49 (0.32)	11.78 (9.80)	9.35 (4.83)		
SHA	144	0.23 (0.22)	2.64 (3.16)	7.23 (1.76)		
FTA	144	0.92 (0.13)	42.25 (9.94)	3.20 (4.04)		
BA	144	0.80 (0.28)	44.98 (19.68)	7.95 (9.40)		
CFA	144	0.30 (0.27)	3.25 (3.79)	5.76 (1.94)		
FPL	144	0.79 (0.17)	2.62 (0.83)	0.56 (0.39)		
HPL	144	0.81 (0.14)	2.19 (0.61)	0.45 (0.27)		
NL	144	0.56 (0.33)	12.79 (9.42)	7.15 (4.62)		
UL	144	0.33 (0.18)	2.50 (1.69)	4.66 (1.02)		
FL	144	0.90 (0.10)	9.67 (2.08)	1.00 (0.84)		
FPP	144	0.69 (0.14)	0.83 (0.27)	0.34 (0.13)		
HPP	144	0.63 (0.15)	0.63 (0.23)	0.34 (0.11)		
CW	144	0.75 (0.19)	23.87 (11.86)	5.61 (5.62)		
CL	144	0.37 (0.34)	8.37 (9.55)	9.73 (4.71)		
HW	143	0.65 (0.18)	1.59 (0.65)	0.79 (0.32)		
HL	143	0.85 (0.16)	7.85 (2.23)	1.16 (1.00)		
MCL	143	0.63 (0.16)	0.53 (0.21)	0.28 (0.10)		
MTL	143	0.54 (0.18)	0.56 (0.27)	0.42 (0.14)		
EXT	132	0.10 (0.12)	2.96 (4.47)	5.89 (0.52)	0.77 (0.03)	17.46 (3.26)
OVR	133	0.53 (0.40)	303.94 (248.25)	63.10 (5.46)	0.86 (0.05)	109.79 (120.79)

CONTINUE

Trait ^a	Observations	<i>h</i> ²	<i>V</i> _a	<i>V</i> _e	<i>R</i>	<i>V</i> _{pe}
TER	133	0.37 (0.34)	19.98 (20.48)	9.49 (0.83)	0.77 (0.06)	14.55 (9.95)
ACS	133	0.19 (0.27)	13.79 (23.83)	7.58 (0.66)	0.85 (0.03)	33.16 (12.13)
GTD	84	0.53 (0.29)	3.62 (2.72)	2.50 (1.35)		
GTDX	84	0.61 (0.35)	9.13 (6.48)	3.98 (3.13)		
RMS	128	0.57 (0.18)	0.54 (0.24)	0.36 (0.12)		

^aTraits: ACS: Acuteness; BA: Brachial angle; BIA: Back inclination angle; BL: Body length; CFA: Coxofemoral angle; CHW: Chest width; CL: Croup length; CRH: Croup height; CW: Croup width; EXT: Extension; FL: Femur length; FPL: Forelimb pastern length; FPP: Forelimb pastern perimeter; FTA: Femorotibial angle; GTD: Gluteal temperature difference; GTDX: Maximum gluteal temperature difference; HA: Hock angle; HL: Head length; HPL: Hindlimb pastern length; HPP: Hindlimb pastern perimeter; HW: Head width; MC: Metacarpal circumference; MCL: Metacarpal length; MDH: Mid-dorsal height; MT: Metatarsal circumference; MTL: Metatarsal length; NL: Neck length; OVR: Overreach; RMS: Vertical acceleration; SH: Sub-pectoral height; SHA: Scapulohumeral angle; TER: Term; TG: Thoracic girth; UL: Upper arm length; WH: Withers height.

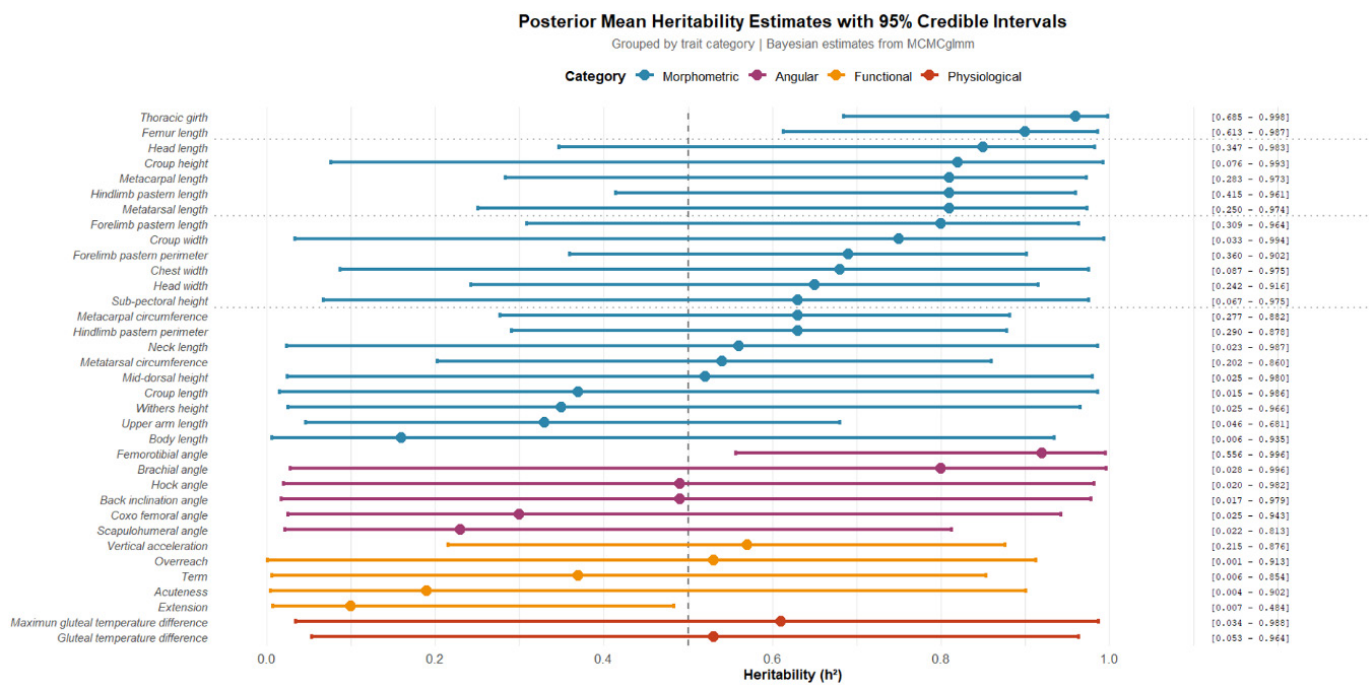


Figure 2. Posterior mean heritability (*h*²) estimates with 95% credible intervals for the evaluated traits, grouped by category. Morphometric, Angular, Functional, and Physiological traits are represented by blue, purple, orange, and red colors, respectively. Points indicate the posterior mean, and horizontal bars represent the 95% credible intervals. The vertical dashed line indicates a reference value of *h*² = 0.5. Numerical values of the 95% credible intervals are shown on the right side of the plot.

Effect of inbreeding on EBVs

Tables 6 and 7 show the correlations between EBVs estimated with and without the inclusion of inbreeding in the top 20 and bottom 20 groups, respectively. Figure 3 presents a comparison of the CCCs between the two groups. Correlations close to 1 indicate little influence of F or ΔF . The correlation analyses revealed that EBVs estimated with and without accounting for inbreeding (F or ΔF) were highly concordant across most traits (Pearson's $r \geq 0.90$, Spearman's $r \geq 0.90$, and Lin's CCC ≥ 0.90 for

28/35 traits in the top 20 animals, and 23/35 in the bottom 20), indicating minimal rank changes; however, significant differences ($P < 0.05$ in Steiger's test) were detected for 16 traits (e.g., EXT, sub-pectoral height [SH], neck length [NL], head width [HW], MCL), where inclusion of ΔF generally produced EBVs more similar to the baseline EBVs than inclusion of F . Lower correlations among the bottom 20 animals suggest greater sensitivity of EBV rankings in this subset, although this result may be affected by small sample size and selection of extreme animals.

Table 6.

Correlations between EBVs estimated for the top 20 animals, considering the inbreeding coefficient (F) and the individual rate of inbreeding (ΔF), compared to EBVs estimated without accounting for inbreeding. Lin's between EBV vs EBV F and EBV vs EBV ΔF with P -value are shown. Test z Steiger with P -value are shown.

Trait ^a	F				ΔF				Steiger's ^b	
	r	rho	CCC	P-value Lin	r	rho	CCC	P-value Lin	z	P
WH	0.86	0.83	0.77	<0.001	0.87	0.84	0.77	<0.001	-0.86	0.52
CRH	0.96	0.91	0.95	<0.001	0.97	0.92	0.96	<0.001	-3.03	<0.01
SH	0.90	0.68	0.79	<0.001	0.94	0.74	0.87	<0.001	-3.46	<0.01
MDH	0.98	0.94	0.96	<0.001	0.98	0.93	0.97	<0.001	-1.38	0.23
CHW	1.00	0.99	0.99	<0.001	0.99	0.99	0.99	<0.001	1.40	0.23
TG	1.00	0.99	0.99	<0.001	1.00	0.98	0.99	<0.001	-0.65	0.52
BL	1.00	0.99	1.00	<0.001	1.00	0.99	0.99	<0.001	3.27	<0.01
MC	1.00	0.99	1.00	<0.001	1.00	1.00	1.00	<0.001	5.19	<0.01
MT	0.98	0.92	0.95	<0.001	0.98	0.93	0.96	<0.001	-1.95	0.09
BIA	1.00	0.98	1.00	<0.001	0.99	0.97	0.99	<0.001	3.63	<0.01
HA	1.00	0.99	1.00	<0.001	1.00	0.96	0.99	<0.001	5.47	<0.01
SHA	0.95	0.9	0.89	<0.001	0.95	0.92	0.90	<0.001	-1.83	0.11
FTA	1.00	0.99	1.00	<0.001	1.00	0.99	0.99	<0.001	4.36	<0.01
BA	0.98	0.94	0.97	<0.001	0.99	0.94	0.97	<0.001	-1.04	0.43
CFA	0.99	0.97	0.98	<0.001	0.99	0.96	0.99	<0.001	-3.08	<0.01
FPL	1.00	0.99	1.00	<0.001	1.00	0.96	0.99	<0.001	6.82	<0.01
HPL	0.99	0.86	0.98	<0.001	0.99	0.88	0.98	<0.001	-1.63	0.14
NL	0.97	0.96	0.87	<0.001	0.98	0.97	0.91	<0.001	-2.89	<0.01
UL	1.00	0.99	1.00	<0.001	1.00	0.99	1.00	<0.001	-6.62	<0.01
FL	1.00	0.97	1.00	<0.001	1.00	0.96	1.00	<0.001	4.82	<0.01
FPP	1.00	0.99	1.00	<0.001	1.00	0.98	1.00	<0.001	5.87	<0.01
HPP	1.00	0.98	1.00	<0.001	1.00	0.99	1.00	<0.001	-7.81	<0.01
CW	1.00	0.98	0.99	<0.001	1.00	0.98	0.99	<0.001	3.49	<0.01
CL	0.96	0.94	0.93	<0.001	0.95	0.91	0.91	<0.001	2.73	0.02
HW	0.99	0.86	0.98	<0.001	1.00	0.92	0.99	<0.001	-4.44	<0.01
HL	1.00	0.99	1.00	<0.001	1.00	0.99	1.00	<0.001	-5.23	<0.01

CONTINUE

Trait ^a	<i>F</i>				ΔF				Steiger's ^b	
	r	rho	CCC	P-value Lin	r	rho	CCC	P-value Lin	z	P
MCL	0.90	0.85	0.86	<0.001	0.94	0.91	0.91	<0.001	-3.53	<0.01
MTL	0.99	0.91	0.99	<0.001	0.99	0.91	0.99	<0.001	-0.98	0.43
EXT	0.83	0.78	0.62	0.019	0.88	0.85	0.72	0.002	-3.38	<0.01
OVR	0.99	0.97	0.97	<0.001	0.99	0.97	0.98	<0.001	-1.97	0.09
TER	1.00	0.99	1.00	<0.001	1.00	0.99	1.00	<0.001	0.78	0.52
ACS	0.99	0.91	0.98	<0.001	1.00	0.93	0.99	<0.001	-3.94	<0.01
GTD	0.90	0.85	0.87	<0.001	0.89	0.85	0.85	<0.001	1.41	0.23
GTDX	0.99	0.97	0.99	<0.001	0.99	0.98	0.99	<0.001	-2.18	0.05
RMS	1.00	0.99	1.00	<0.001	0.99	0.99	0.99	<0.001	4.11	<0.01

CCC: Concordance correlation coefficient, r: Pearson correlations, Rho: Spearman correlations. All correlations are significant with $P < 0.05$

^aTraits: ACS: Acuteness; BA: Brachial angle; BIA: Back inclination angle; BL: Body length; CFA: Coxofemoral angle; CHW: Chest width; CL: Croup length; CRH: Croup height; CW: Croup width; EXT: Extension; FL: Femur length; FPL: Forelimb pastern length; FPP: Forelimb pastern perimeter; FTA: Femorotibial angle; GTD: Gluteal temperature difference; GTDX: Maximum gluteal temperature difference; HA: Hock angle; HL: Head length; HPL: Hindlimb pastern length; HPP: Hindlimb pastern perimeter; HW: Head width; MC: Metacarpal circumference; MCL: Metacarpal length; MDH: Mid-dorsal height; MT: Metatarsal circumference; MTL: Metatarsal length; NL: Neck length; OVR: Overreach; RMS: Vertical acceleration; SH: Sub-pectoral height; SHA: Scapulohumeral angle; TER: Term; TG: Thoracic girth; UL: Upper arm length; WH: Withers height.

^bResults of a comparison of two overlapping correlations based on dependent groups. Comparison between r.jk (EBV, EBV_*F*) and r.jh (EBV, EBV_ ΔF). Difference: r.jk - r.jh. Null hypothesis: r.jk is equal to r.jh. Alternative hypothesis: r.jk is not equal to r.jh (two-sided). *P*-values from Steiger's test were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. Alpha: 0.05

Table 7.

Correlations between EBVs estimated for the bottom 20 animals, considering the inbreeding coefficient (*F*) and the individual rate of inbreeding (ΔF), compared to EBVs estimated without accounting for inbreeding. Lin's between EBV vs EBV_{*F*} and EBV vs EBV_ ΔF with *P*-value are shown. Test z Steiger with *P*-value are shown.

Trait ^a	<i>F</i>				ΔF				Steiger's ^b	
	r	rho	CCC	P-value Lin	r	rho	CCC	P-value Lin	z	P
WH	0.71	0.67	0.56	0.017	0.77	0.73	0.63	0.007	-1.69	0.13
CRH	0.98	0.96	0.98	<0.001	0.99	0.98	0.99	<0.001	-3.58	<0.01
SH	0.97	0.97	0.87	<0.001	0.98	0.97	0.91	<0.001	-2.13	0.05
MDH	0.92	0.89	0.83	<0.001	0.95	0.92	0.90	<0.001	-3.20	<0.01
CHW	1.00	0.98	1.00	<0.001	1.00	0.98	0.99	<0.001	0.88	0.45
TG	0.97	0.94	0.96	<0.001	0.97	0.95	0.97	<0.001	-1.48	0.18
BL	1.00	0.99	1.00	<0.001	1.00	0.99	1.00	<0.001	3.48	<0.01
MC	1.00	1	1.00	<0.001	1.00	1.00	1.00	<0.001	5.60	<0.01
MT	0.92	0.89	0.87	<0.001	0.93	0.88	0.88	<0.001	-0.51	0.68
BIA	1.00	0.99	0.99	<0.001	0.99	0.98	0.99	<0.001	3.68	<0.01
HA	0.98	0.93	0.97	<0.001	0.87	0.73	0.85	<0.001	5.05	<0.01

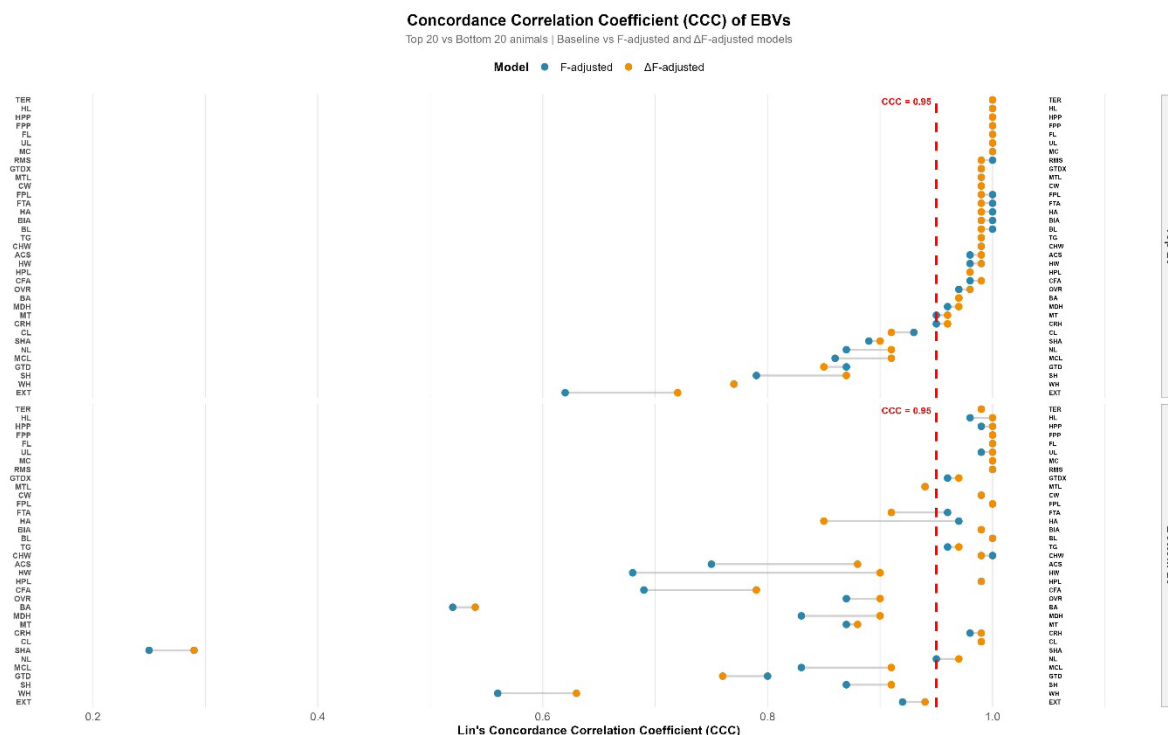
CONTINUE

Trait ^a	F				ΔF				Steiger's ^b	
	r	rho	CCC	P-value Lin	r	rho	CCC	P-value Lin	z	P
SHA	0.61	0.53	0.25	0.382	0.64	0.55	0.29	0.308	-0.94	0.45
FTA	0.98	0.82	0.96	<0.001	0.94	0.74	0.91	<0.001	4.07	<0.01
BA	0.87	0.84	0.52	0.096	0.88	0.86	0.54	0.081	-0.79	0.48
CFA	0.91	0.81	0.69	0.009	0.95	0.91	0.79	<0.001	-3.87	<0.01
FPL	1.00	1	1.00	<0.001	1.00	0.99	1.00	<0.001	6.89	<0.01
HPL	0.99	0.92	0.99	<0.001	0.99	0.92	0.99	<0.001	1.08	0.35
NL	0.98	0.96	0.95	<0.001	0.98	0.97	0.97	<0.001	-1.45	0.18
UL	1.00	0.98	0.99	<0.001	1.00	0.99	1.00	<0.001	-7.08	<0.01
FL	1.00	0.99	1.00	<0.001	1.00	0.98	1.00	<0.001	4.74	<0.01
FPP	1.00	0.99	1.00	<0.001	1.00	0.99	1.00	<0.001	5.38	<0.01
HPP	0.99	0.98	0.99	<0.001	1.00	1.00	1.00	<0.001	-8.12	<0.01
CW	0.99	0.99	0.99	<0.001	0.99	0.98	0.99	<0.001	3.28	<0.01
CL	0.99	0.88	0.99	<0.001	0.99	0.85	0.99	<0.001	2.55	0.02
HW	0.93	0.9	0.68	0.016	0.98	0.95	0.90	<0.001	-4.52	<0.01
HL	0.99	0.99	0.98	<0.001	1.00	0.99	1.00	<0.001	-5.67	<0.01
MCL	0.89	0.84	0.83	<0.001	0.94	0.91	0.91	<0.001	-3.87	<0.01
MTL	0.96	0.92	0.94	<0.001	0.96	0.92	0.94	<0.001	-0.18	0.86
EXT	0.97	0.94	0.92	<0.001	0.97	0.95	0.94	<0.001	-3.10	<0.01
OVR	0.91	0.88	0.87	<0.001	0.93	0.89	0.90	<0.001	-2.84	0.02
TER	1.00	0.98	0.99	<0.001	1.00	0.98	0.99	<0.001	0.37	0.71
ACS	0.95	0.94	0.75	0.004	0.97	0.97	0.88	<0.001	-3.98	<0.01
GTD	0.87	0.75	0.80	<0.001	0.84	0.71	0.76	<0.001	2.51	0.02
GTDX	0.98	0.97	0.96	<0.001	0.99	0.98	0.97	<0.001	-2.44	0.04
RMS	1.00	0.98	1.00	<0.001	1.00	0.99	1.00	<0.001	4.15	<0.01

CCC: concordance correlation coefficient, r: Pearson correlations, Rho: Spearman correlations. All correlations are significant with $P < 0.05$.

^aTraits: ACS: Acuteness; BA: Brachial angle; BIA: Back inclination angle; BL: Body length; CFA: Coxofemoral angle; CHW: Chest width; CL: Croup length; CRH: Croup height; CW: Croup width; EXT: Extension; FL: Femur length; FPL: Forelimb pastern length; FPP: Forelimb pastern perimeter; FTA: Femorotibial angle; GTD: Gluteal temperature difference; GTDX: Maximum gluteal temperature difference; HA: Hock angle; HL: Head length; HPL: Hindlimb pastern length; HPP: Hindlimb pastern perimeter; HW: Head width; MC: Metacarpal circumference; MCL: Metacarpal length; MDH: Mid-dorsal height; MT: Metatarsal circumference; MTL: Metatarsal length; NL: Neck length; OVR: Overreach; RMS: Vertical acceleration; SH: Sub-pectoral height; SHA: Scapulohumeral angle; TER: Term; TG: Thoracic girth; UL: Upper arm length; WH: Withers height.

^bResults of a comparison of two overlapping correlations based on dependent groups. Comparison between r.jk (EBV, EBV_F) and r.jh (EBV, EBV_ΔF). Difference: r.jk - r.jh. Null hypothesis: r.jk is equal to r.jh Alternative hypothesis: r.jk is not equal to r.jh (two-sided). P-values from Steiger's test were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. Alpha: 0.05.

**Figure 3.**

Concordance correlation coefficients (CCC) of estimated breeding values (EBVs) for the top 20 and bottom 20 animals, comparing baseline models with those adjusted for the inbreeding coefficient (F , blue points) and the individual rate of inbreeding (ΔF , orange points). Traits are ordered by CCC values within each group. Gray lines connect the CCC values of the F -adjusted and ΔF -adjusted models for each trait, illustrating the shift in concordance when accounting for different inbreeding metrics. The red dashed vertical line indicates a reference CCC threshold of 0.95. Dual-sided trait labels are provided to facilitate the identification of specific traits across the top and bottom ranking groups.

Although some traits exhibited mild deviations from normality (Anderson-Darling test), Pearson correlations and Lin's concordance correlation coefficient (CCC) were used to evaluate the association and agreement between baseline and inbreeding-adjusted EBVs. Lin's CCC was specifically included to complement Pearson by directly measuring agreement. To ensure that these inferences were robust to distributional deviations, Spearman's rank correlations were computed as sensitivity checks. These produced similar rankings, thereby confirming the reliability of the main results. Given the small sample sizes in the top- and bottom-20 EBV groups, these specific comparisons were interpreted with caution and supported by rank-based metrics. Finally, detailed Pearson's and Spearman's correlations are provided in Supplementary Material 3, presented separately depending on whether the EBV values followed a normal distribution.

Because the number of phenotyped animals was limited and varied among traits, Bayesian animal model estimates of heritability and EBVs should be interpreted as prelimi-

nary, particularly for traits with small sample sizes, sparse fixed-effect levels, low ESS, or wide posterior uncertainty.

DISCUSSION

Pedigree structure

The pedigree presented high connectivity: 95% of the sires and dams in the total population were known, and 100% in the phenotyped subset, which is a critical factor for the unbiased estimation of genetic parameters (Leroy, 2014). Pedigree depth, with average maximum generations of 11.58 for the total population and 16.00 for the phenotyped subset, was consistent with the findings in domestic species, in which greater depth improves the accuracy of genetic diversity assessments (Goyache et al., 2003). Inbreeding levels (6.74% in the total population and 9.02% in the phenotyped group) indicated moderate inbreeding, which fell within the range observed in species under intense selection (Curik et al., 2014). The higher homoge-

neity in the phenotyped group suggests selective mating practices or demographic bottlenecks. The high average relatedness (8.37% and 11.98% for the total and phenotyped populations, respectively) reflects strong genetic interconnectivity, which, according to Sørensen *et al.* (2005), increases the risk of inbreeding depression without proper management of genetic diversity. The generation interval (8.90 years in the total population and 9.04 years in the phenotyped group) was large compared to other species, likely linked to the extended reproductive lifespan and management practices. Although this slows the accumulation of inbreeding, it also limits the population's capacity to adapt to environmental or economic changes (McParland *et al.*, 2007).

Analysis of inbreeding, temporal trends, and genetic impact

Since 1970, inbreeding (F and ΔF) has markedly increased, following minimal levels observed between 1945 and 1950, when the genetic base was broad, and artificial selection was less intense (Goyache *et al.*, 2003). This rise is associated with intensive breeding practices and the repeated use of a limited number of breeding animals, which reduces genetic diversity (Leroy, 2014). The current annual ΔF , which ranges between 1% and 1.5%, exceeds the recommended threshold of 1% (FAO, 2011), thereby compromising long-term maintenance of genetic viability. The EG has increased from 1.19 (1945) to 8.42 (2023), reflecting improved pedigree recording and greater accuracy in inbreeding calculations. However, the concurrent increase in both F and ΔF indicates a genuine loss of genetic diversity, posing a risk to the future health and performance of the breed (Boichard *et al.*, 1997).

Wright's fixation indices (FIS, FST, FIT) assess genetic structure. The FIS was 0.025 (total population) and 0.0265 (phenotyped subset), indicating a slight excess of homozygosity, a pattern commonly observed in horse breeds under selection (Leroy, 2014; Curik *et al.*, 2014). In the PPH, this may be attributed to the selection of specific traits (morphological or functional), which reduces genetic variability within subpopulations (Li *et al.*, 2023; Frigo *et al.*, 2009). The FST was very low (0.0016 in the total population; 0.000082 in the phenotyped group), indicating minimal genetic differentiation, which is typical of horse populations with consistent gene flow (Goyache *et al.*, 2003). Similar results have been reported for Croatian horse breeds, with an FST of 0.0260 (Ivanković *et al.*, 2009), and Pantaneiro horses, in which the FST ranged from 0.008 to 0.056 (Nogueira *et al.*, 2021). In the context of inbreeding depression, this low genetic differentiation suggests that inbreeding-related risks affect the entire population, underscoring the need to implement comprehensive population-wide strategies to manage genetic diversity.

The observed FIT was consistent with previous studies in horses (McParland *et al.*, 2007), in which moderate in-

breeding allows for effective management of inbreeding without compromising population viability. The FIS, FST, and FIT values aligned with those reported for breeds such as Andalusians (Valera *et al.*, 2005) and Arabians (Cervantes *et al.*, 2008). Although genetic variability is still present, the current inbreeding levels (6.74% in the total population; 9.02% in the phenotyped subset) necessitate proactive strategies, such as breeding animal rotation or the introduction of new lineages, to preserve genetic diversity.

Heritability estimates for morphological traits in our study (h^2 ranging from 0.10 to 0.96) align with recent Bayesian MCMC estimates in the same breed, which reported moderate to high heritabilities for height at withers ($h^2 = 0.56$), height at croup ($h^2 = 0.61$), and thorax perimeter ($h^2 = 0.58$) in PPH (Karlaui *et al.*, 2025). The similarity of some estimates with previous studies supports their biological plausibility, although the precision of the present estimates remains constrained by the limited phenotypic sample size.

Effect of inbreeding on traits

Inbreeding may be associated with a gradual reduction in genetic variability, negatively affecting morphological traits, such as WH in the PPH (Larrea Izurieta *et al.*, 2022), consistent with findings in other species (Leroy, 2014; Curik *et al.*, 2014; Bussiman *et al.*, 2018). However, WH was not affected in Mangalarga Marchador horses (Gonçalves *et al.*, 2012), indicating that the impact of inbreeding varies among breeds or populations. The SHA trait also showed a significant association with both F and ΔF . After FDR correction, only SHA remained suggestive ($q = 0.07$) for both F and ΔF . All other traits had $q \geq 0.21$, indicating that uncorrected $P < 0.05$ results should be interpreted as exploratory rather than confirmatory. Studies on morphological traits and their response to inbreeding depression suggest that SHA may be similarly susceptible to inbreeding-related changes (Gómez *et al.*, 2009; Poyato-Bonilla *et al.*, 2020) because of their dependence on complex genetic interactions (Goyache *et al.*, 2003). The BA and MCL traits were also associated with F and ΔF , indicating that inbreeding could affect not only linear measurements but also angles, thereby altering key proportions relevant to performance (Cervantes *et al.*, 2008). Although there are no direct studies on MCL in Spanish Purebred horses, cannon circumference appears to be tentatively affected by inbreeding (Gómez *et al.*, 2009), suggesting a potentially similar impact on MCL.

The functional/physiological traits of ACS and GTD may exhibit a potential association with ΔF ; notably, ACS was also associated with F . Interestingly, F had a positive effect on ACS, contrary to expectations. While this statistical association could theoretically reflect historical selection for specific gait characteristics that incidentally co-occurred with increased homozygosity, pedigree-based analyses alone cannot confirm the fixation of favorable alleles or

distinguish between directional selection and random genetic drift (Poyato-Bonilla *et al.*, 2020). However, this finding contrasts with other studies that have demonstrated that inbreeding can impair functional traits related to physical performance in horses (Leroy *et al.*, 2013). Therefore, such positive associations should be interpreted with caution, as they may also arise from trait-specific genetic architectures, environmental covariation, or statistical variability inherent to moderate sample sizes. The authors emphasize that a greater elevation angle (ACS) does not necessarily imply improved functionality or health, as an excessive increase in ACS could raise energy expenditure or biomechanical stress, potentially compromising the horse's overall functionality in contexts other than PPH competitions (Bartolomé *et al.*, 2021).

Similarly, the negative associations between inbreeding metrics and gluteal temperature traits (GTD and GTDX) suggest a negative regression coefficient with both F and ΔF , indicating that higher inbreeding reduces thermoregulatory capacity, possibly due to the genetic load affecting metabolism or key physiological functions involved in thermoregulation (Oh, 2012; Jensen *et al.*, 2014). However, these analyses were based on a limited subset of animals ($n = 84$) with high phenotypic variability ($CV > 125\%$), which substantially reduced the reliability of the physiological inferences. Similar results have been observed in cattle under heat stress (Sørensen *et al.*, 2005). Although some traits were significantly associated with both F and ΔF , the magnitudes of their regression coefficients differed markedly. This can be explained by the fact that F reflects accumulated homozygosity (exerting a gradual effect on heritable traits), whereas ΔF captures recent inbreeding, which can have a more immediate and severe impact due to the rapid accumulation of deleterious alleles (McParland *et al.*, 2007). Therefore, ΔF may be a critical parameter for assessing inbreeding depression in horses and other species. In addition, while FDR correction reduced the number of statistically robust associations, the consistent directional trends across morphometric, functional, and physiological traits support the biological plausibility of the effects of inbreeding in PPH. These exploratory findings provide a foundation for targeted validation in larger cohorts.

Although several traits exhibited statistically significant associations with inbreeding ($P < 0.05$), it is essential to distinguish between statistical significance and biological or practical relevance in a breeding context. The unstandardized regression coefficients for F ranged from -0.308 to $+0.369$, indicating the expected change in each trait per one-unit increase in the inbreeding coefficient (0–100 % scale). Because practical inbreeding levels in equine populations typically range between 0.01 and 0.16, the phenotypic impact per 1% increase in F is proportionally smaller (Perdomo-González *et al.*, 2022; Dos Santos *et al.*, 2021). For instance, a coefficient of $b = -0.193$ for WH implies a reduction of approximately 0.193 cm per 1% increase in F , which, while statistically detectable, may not translate

into immediate breeding program adjustments unless accumulated across multiple generations or combined with other selection pressures. Regression coefficients (b) further clarify that, after accounting for measurement scale, traits such as WH and SHA exhibit comparable sensitivity to inbreeding when expressed in standard deviation units. Conversely, several traits with $P < 0.05$ but very small “ b ” values likely reflect statistical sensitivity rather than biologically actionable inbreeding depression. Therefore, genetic management decisions should prioritize traits that demonstrate both statistical support and effect sizes exceeding biologically meaningful thresholds, particularly those directly linked to structural conformation, gait functionality, and thermoregulatory capacity.

Effect of inbreeding on EBVs

Although the correlations between EBVs estimated with and without inbreeding adjustment were generally high ($r \geq 0.90$, $\rho \geq 0.90$, $CCC \geq 0.85$ for most traits), indicating strong overall ranking stability, these results must be interpreted with appropriate caution. High correlations and CCC values indicate that inbreeding adjustment produced limited changes in EBV estimates for most traits. However, these comparisons should be interpreted as model-sensitivity analyses rather than direct evidence of bias or predictive superiority. Trait-specific exceptions, EXT, SHA, WH, BA, and CFA, exhibited lower concordance ($CCC < 0.80$ in certain merit strata) and significant Steiger's z -tests, suggesting that inbreeding adjustment could influence ranking for these characteristics. Among the functional traits evaluated, EXT exhibited the most pronounced indication of an inbreeding-related effect, where both F and ΔF adjustments produced low concordance ($CCC = 0.62$ – 0.72) and potential bias among the top 20 animals, suggesting that failure to account for inbreeding may lead to mis-ranking of elite sires for this key gait-related trait. Similar effects were observed for SHA and WH, where CCC values fell below 0.80, indicating poor to moderate agreement between the adjusted and unadjusted EBVs (Lin, 1989). These findings are similar to those of studies on other livestock species, showing that inbreeding depression can significantly bias genetic evaluations when ignored, especially for fitness- and conformation-related traits (Silió *et al.*, 2013; Bjelland *et al.*, 2013).

Notably, ΔF consistently outperformed static F in preserving EBV ranking stability, as evidenced by higher correlations, improved CCC values, and significant Steiger's z -tests ($P < 0.01$) for EXT, SH, NL, and MDH. These observations could tentatively support the view that recent inbreeding (captured by ΔF) is more deleterious than ancient inbreeding due to the greater likelihood of homozygosity for partially recessive deleterious alleles (Bjelland *et al.*, 2013; Pekkala *et al.*, 2014). Our results tentatively indicate that models including ΔF produced EBVs that were generally more similar to baseline EBVs than models including F for some traits. Whether ΔF provides a more biologically

informative correction should be evaluated using larger datasets with predictive validation. The greater sensitivity observed among top-ranked animals for some traits may reflect model sensitivity within this EBV stratum; however, further analyses are needed to determine whether this pattern is associated with recent inbreeding load.

In contrast, most traits, including BL, TG, MC, and several angular measurements, showed excellent concordance ($CCC > 0.95$) and negligible ranking changes, despite statistically significant P -values (Figure 3). This suggests that while inbreeding effects are statistically detectable, they could be practically irrelevant for selection decisions in these traits, as previously noted in cattle and pigs (Makanjuola *et al.*, 2020). Therefore, rather than implying a broad impact on selection decisions, our preliminary findings indicate that an inbreeding correction could be prioritized for traits showing reduced concordance or known functional sensitivity, whereas standard models remain robust for most other traits. This nuanced interpretation aligns with the observed correlation structure and prevents overstatements of practical breeding implications.

Before presenting the conclusions, the authors wish to acknowledge certain limitations of this study that deserve mention. Although the modest phenotyped sample size presents a common constraint in equine genetic studies regarding statistical power and variance estimation, this study implemented advanced Bayesian frameworks. Bayesian animal models use the available pedigree relationship matrix to estimate genetic parameters; however, the limited number of phenotyped animals constrains the precision of EBVs and variance component estimates. The reliability of these estimates was further secured through strict convergence criteria ($ESS > 200$) and parsimonious model selection via the Deviance Information Criterion (DIC), which optimized the inclusion of fixed effects such as sex, age, breeder, and gait velocity. Moreover, by combining a stratified analysis of extreme EBV groups with Steiger's z -tests, we established a highly sensitive approach to quantify inbreeding biases, which might be ignored in limited datasets using traditional methodology. However, while the extensive pedigree structure and Bayesian modeling framework helped stabilize parameter estimation, they could not compensate for the scarcity of direct phenotypic records. Consequently, the EBVs and inbreeding effect estimates reported herein should be interpreted as preliminary indicators of trait sensitivity, rather than definitive selection criteria. These findings underscore the necessity for future studies to incorporate substantially larger, longitudinally collected phenotypic datasets, ideally combined with genomic information, to validate these patterns and produce robust, selection-ready genetic evaluations.

Although our findings are constrained by the limited phenotypic sample size and the associated uncertainty in EBV estimation for functional traits, they nonetheless highlight the critical importance of monitoring and managing inbreeding in PPH populations. The observed

upward trend in inbreeding coefficients underscores the need for proactive management strategies to preserve genetic diversity and support the long-term viability of this emblematic breed. Consistent with Wright's fixation indices, these results suggest that breeding programs should strive to balance the selection of desirable conformation and gait traits with the active conservation of genetic variability. Given the exploratory nature of the present EBV comparisons, definitive selection recommendations require validation in larger, more deeply connected cohorts. Therefore, future studies should integrate molecular genomic data with expanded phenotypic records to refine these preliminary patterns and enable more precise and robust assessments of inbreeding depression and population structure.

Although the extensive pedigree structure and Bayesian modeling framework provide analytical stability, they cannot compensate for the scarcity of direct phenotypic records, and the resulting EBVs inherently carry substantial uncertainty. Nevertheless, the consistent patterns observed across morphometric, angular, functional, and physiological traits provide preliminary evidence that inbreeding exerts measurable, trait-specific effects in the PPH. The comparison between the baseline and inbreeding-adjusted models suggests that ΔF may influence EBV estimates for some traits. However, larger datasets and predictive validation are needed before the routine incorporation of ΔF into genetic evaluation can be recommended. Therefore, these results should be interpreted as exploratory indicators of bias direction and potential magnitude rather than finalized selection criteria. Establishing robust, selection-ready EBV rankings will require substantially larger, longitudinally collected phenotypic datasets, ideally integrated with genomic information, to distinguish recent from historical inbreeding effects. In the interim, this study established a methodological baseline and reinforced the practical necessity of incorporating ΔF into routine genetic assessments to support the long-term genetic health and performance sustainability of the breed.

Finally, our findings support trait- and stratum-specific modelling of inbreeding in PPH genetic evaluations. While most traits showed high concordance between adjusted and unadjusted EBVs, specific functional and conformation traits (e.g., EXT, SHA, SH, WH, MDH, and NL) exhibited greater sensitivity to inbreeding adjustment, particularly among lower-merit animals. These preliminary results suggest that future studies should evaluate whether ΔF -based corrections can improve genetic evaluations of key functional traits. Definitive implementation in breeding programs will require validation in larger, multi-environmental cohorts.

Competing Interests Statement

The authors state that records of eight animals owned by José Dextre were used. It is also stated that José Dextre was Chairman of the Board of the Universidad Científica del Sur during the development of the methodological phase of this research.

Ethics Statement

The study was approved by the Institutional Committee of Ethics in Research with Animals and Biodiversity of the Universidad Científica del Sur (N° 075-CIEI-AB-CIENTÍFICA-2022)

Data Availability Statement

The data sets presented in this article are not readily available because the data is not published due to privacy restrictions.

Author contributions

JLVV, contributed to the conduct and design of the study. JLVV, PGMQD, MLVM, executed the experiment. JLVV organized, edited, processed and analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

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