

# SNP-Based Heritability of Morphological Traits in the Pura Raza Español Horses

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## Summary

The objective of this study was to estimate heritabilities of conformation traits in PRE with three different approaches. The dataset consisted on 7152 morphological records and 41,888 animals in the pedigree, obtained from the Royal National Association of Spanish Horse Breeders (ANCCE). A total of 2916 animals were genotyped with 60,136 SNPs and included in the analysis. Measurements from 5 zoometric traits were analysed: width of croup (WC), length of croup (LC), angle of shoulder (AS), lateral hock angle (LHA), and direction of hock rear view (DHRV). Firstly, heritabilities were estimated with classical approach with pedigree-based REML and single-step GREML with the HiBlup v1.3.1 software. Then, SNP-based heritabilities were estimated using GREML and accounting for patterns of regional linkage disequilibrium with the LDAK 5.2 software program. Heritabilities were similar for the five traits in the first two approaches using the A matrix (REML) or the H matrix (ssGREML), except for DHRV. Their estimates oscillated between 0.23 for AS and LHA in ssGREML and 0.52 for WC. The SNP-based heritabilities were clearly smoother than those from REML and ssGREML approaches for all traits and they varied between 0.16 for AS and 0.42 for WC. The largest heritability estimates were obtained when we employed REML or ssGREML analysis in this population.

*Keywords: conformation, genomic relationship matrices, linkage-disequilibrium, SNP-based heritability, horse.*

## Introduction

Conformation traits are very important in the Pura Raza Español horse breed because of its relationship with the functionality since PRE horses are mainly used for dressage competitions (Sánchez-Guerrero et al., 2016). To maintain a healthy and profitable equine operation, successful breeding program is crucial. For that reason, obtaining reliable genetic parameters is essential in genetic improvement programs.

Historically, in animal populations, estimated breeding values have been calculated from phenotype and pedigree information and extended literature exists, however, genomic estimates are expected to be more accurate because they do not depend on the quality and completeness of the pedigree. Since the last two decades, with the development of genotyping technologies and the continuous advances in genetic evaluations algorithms, the inclusion of genomic information into the genetic evaluations has become possible. Several methodologies have been developed for this purpose, of which the single-step genomic BLUP (ssGBLUP) approach represents the reference methodology and the largely used for genomic evaluations worldwide.

On the other hand, population-based approaches generally utilize single nucleotide polymorphisms (SNPs) to estimate realized genetic similarity, and are usually called SNP-heritability estimation approaches (Speed et al., 2012). SNP-heritability measures the proportion of phenotypic variance explained by all measured SNPs. Accurate estimation of SNP heritability can help to better understand the degree to which measured genetic variants influence phenotypes (Zhu et al., 2020). In

addition, this methodology allows to correct the bias generated by the fact that there are SNPs located in regions with high or low LD.

Thus, the aim of this study was to estimate heritabilities of conformation traits in PRE with three different approaches. In the first two methods, heritabilities were estimated with classical approach with pedigree-based REML and single-step GREML. Then, LDAK analysis was conducted to estimate SNP-based heritabilities using a linkage-disequilibrium weighted genomic relationship matrix based on unrelated individuals.

## **Materials and methods**

### ***Phenotypic, pedigree data and genotypes***

The datasets used in the present study consisted on 7152 conformation records and 41,888 animals in the pedigree belonging to the Pura Raza Español horse (PRE), provided by the Royal National Association of Spanish Horse Breeders (ANCCE). A total of 2916 individuals were genotyped with medium density GGP Equine Array (NEOGEN), including over 70,000 evenly distributed SNPs. The raw genotype data was filtered using PLINK software v1.9: SNPs with call-rate > 0.95 were retained. The final genomic data included 60,136 SNPs located on autosomal and X chromosomes. Measurements from five conformation zoometric traits were analysed: width of croup (WC), length of croup (LC), angle of shoulder (AS), lateral hock angle (LHA), and direction of hock rear view (DHRV). These traits were systematically collected by trained evaluators using standard measuring sticks and non-elastic measuring tape, as described by (Sánchez-Guerrero et al., 2016).

### ***Estimation of heritabilities***

Heritabilities for conformation traits were firstly estimated using the based-pedigree REML and single step GREML (ssGREML) approaches with the HiBlup v1.3.1 software (Yin et al., 2023). Subsequent estimations were performed using the Linkage-Disequilibrium Adjusted Kinships (LDAK) as implemented in LDAK 5.2 software (Zhang et al., 2021) and the integrated REML analysis (Speed et al., 2012), resulting in estimations of SNP-based heritabilities.

For the three approaches, the fixed effects of sex, age, coat colour and geographical area were considered, and included in a univariate animal models equal to those described in (Sánchez et al., 2013). The additive genetic effect was modeled using different kinds of genetic covariance structures: for the REML evaluation, a matrix A that denotes the pedigree-based additive genetic relationship was used, and, for ssGREML, the A matrix was replaced by H, the pedigree-genomic relationship matrix obtained by blending the matrix A with the genomic matrix G. For the LDAK model, the genomic relationship matrix was adjusted for the linkage disequilibrium (Speed et al., 2012).

## **Results and discussion**

This study has established, for the first time, an estimation of SNP-heritability weighting the SNP markers to adjust for linkage disequilibrium for measures of horse morphology and in a Spanish breed.

The descriptive statistics for the conformation traits in the PRE are presented in Table 1. The coefficients of variation were low in this study, ranging from 5.91% for LC to 21.53% for DHRV, which could indicate a low genetic variation for these traits in this population.

**Table 1.** Descriptive statistics of conformation traits in the Pura Raza Español horses.

Trait	N	Mean±SD	Minimum	Maximum	CV(%)
WC	7087	53.47±3.31	32	68	6.19
LC	7149	52.82±3.12	35	68	5.91
AS	6942	55.39±6.68	2	82	12.05
LHA	7151	5.14±0.95	1	9	18.39
DHRV	7151	4.22±0.91	1	7	21.53

SD: standard deviation; CV: coefficient of variation; WC: width of croup; LC: length of croup; AS: angle of shoulder; LHA: lateral hock angle; DHRV: direction of hock rear view.

The results of our heritability estimates for conformation traits in the PRE breed are given in Table 2. Heritabilities were similar for the five traits in the first two approaches using the A matrix (REML) or the H matrix (ssGREML), except for DHRV. Their estimates oscillated between 0.23 for AS and LHA in ssGREML and 0.52 for WC. The SNP-based heritabilities were clearly smoother than those from REML and ssGREML approaches for all traits and they varied between 0.16 for AS and 0.42 for WC. The LDAK approach assumes that heritability varies according to local levels of LD, whereas REML and ssGREML assume that heritability is independent of LD.

**Table 2.** Heritability estimates (SE between parenthesis) of conformation traits given by REML, ssGREML and LDAK in the Pura Raza Español horses.

Trait	Approach		
	REML	ssGREML	LDAK
WC	0.52 (0.043)	0.50 (0.040)	0.42 (0.054)
LC	0.41 (0.036)	0.40 (0.034)	0.36 (0.045)
AS	0.27 (0.033)	0.23 (0.030)	0.16 (0.043)
LHA	0.25 (0.032)	0.23 (0.029)	0.17 (0.041)
DHRV	0.48 (0.040)	0.40 (0.036)	0.27 (0.047)

WC: width of croup; LC: length of croup; AS: angle of shoulder; LHA: lateral hock angle; DHRV: direction of hock rear view.

In the literature, high heritability estimates were reported for conformation traits in horse breeds (Solé et al., 2014 in Menorca horse population; Gómez et al., 2021 in PRE horse breed; Vosgerau et al., 2022 in German Warmblood horses). These estimates were performed with classical evaluation (pedigree based relationship matrix). Genomic analysis such as genome-wide association studies (GWASs) of the horse morphology was performed in other breeds with heritabilities ranging between 0.14 and 0.58 (Gmel et al., 2019 in Franches-Montagnes and Lipizzan breeds; Ricard et al., 2023 in French jumping horses). Nevertheless, until present, SNP-based heritability has been reported only for the osteochondrosis trait in horse (Zimmermann and Distl, 2023). In this study, it is notable that the use of a LD-weighted relatedness matrix had a substantial impact on estimates of heritability. The higher estimates were obtained when using REML and ssGREML than LDAK.

In this study, it is noticeable that the use of a LD-weighted relatedness matrix had a substantial impact on estimates of heritability of traits, being the higher estimates obtained when using REML and ssGREML than LDAK. LDAK suggests modification of the relationship matrix according to local LD contribution of the SNPs to the genetic similarity between a pair of individuals weighted according to the LD with their neighboring SNPs. Estimating heritability using genetic similarity adjusted for local LD reduces the potential bias and increases the precision of the heritability estimate (Speed et al., 2012).

## Conclusions

The results obtained from the present study allow us to assume that analyses based on pedigree data and ssGREML result in higher heritability estimates compared to estimates obtained with LD-weighted genomic relationship matrix in the Pura Raza Español Horse population.

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