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Quantitative analysis of parent-of-origin effect in reproductive and morphological selection criteria in the Pura Raza Española horse

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Abstract

It is generally assumed that parents make a genetically equal contribution to their offspring, but this assumption might not always hold. This is because the expression of a gene can be blocked by methylation during gametogenesis, and the degree of methylation can depend on the origin of the parental gene (imprinting) or by preferential management associated with genetic merit. The first consequences of this for quantitative genetics is that the mean phenotypes of reciprocal heterozygotes need no longer be the same, as would be expected according to Mendelian heritage. We analysed three mare reproductive traits (reproductive efficiency, age at first foaling and foaling number) and three morphological traits (height at withers, thoracic circumference, and scapula-ischial length) in the Pura Raza Española (PRE) horse population, which possesses a deep and reliable pedigree, making it a perfect breed for analysing the quantitative effect of parent-of-origin. The number of animals analysed ranged from 44,038 to 144,191, all of them with both parents known. The model comparison between a model without parent-of-origin effects and three different models with parent-of-origin effects revealed that both maternal and paternal gametic effects influence all the analysed traits. The maternal gametic effect had a higher influence on most traits, accounting for between 3% and 11% of the total phenotypic variance, while the paternal gametic effect accounted for a higher proportion of variance in one trait, age at first foaling (4%). As expected, the Pearson's correlations between additive breeding values of models that consider parent-of-origin and that do not consider parent-of-origin were very high; however, the percentage of coincident animals slightly decreases when comparing animals with the highest estimated breeding values. Ultimately, this work demonstrates that parent-of-origin effects exist in horse gene transmission from a quantitative point of view. Additionally, including an estimate of the parent-of-origin effect within the PRE horse breeding program could be a great tool for a better parent's selection and that could be of

interest for breeders, as this value will determine whether the animals acquire genetic categories and are much more highly valued.

1 | INTRODUCTION

Early genetic improvement schemes in livestock assumed that the expression of relevant genes is independent of the parent-of-origin. However, some experiments (Graham & Deussen, 1974) suggest that the assumption of equal parental contribution might not always hold. This phenomenon, called the "parent-of-origin effect" or "gametic imprinting", occurs when the expression of a gene varies with the sex of the parent from which it was inherited and is caused by an epigenetic effect but also by herd management. With complete imprinting, the gene inherited from one parent is silent, while with partial imprinting, imprinted genes are expressed at a lower level than the copy from the other parent, and this differential expression can be observed in the individual phenotype. In each generation, the imprint is newly established during gametogenesis, so although a maternal (or paternal) gene may be silenced or limited in an individual, it could be expressed in its progeny if it is its sire (or dam). One of the well-known examples of imprinted genes in livestock is the IGF2 gene in pigs (Jeon et al., 1999; Nezer et al., 1999; Van Laere et al., 2003), which was identified as the main reason for an imprinted quantitative trait loci (QTL) in an F2-line cross experiment, expressed in the paternal line and repressed in the maternal line. These experiments were carried out in F2 families derived from crossing different pig lines, whose family type allows the Qq and qQ genotypes to be separated in the QTL, where the first allele is paternal, and then statistically test for phenotypic differences between them, as expected under imprinted inheritance (De Koning et al., 2000, 2001).

Resemblance between relatives is one of the basic genetic phenomena exhibited by metric traits, and the degree of resemblance is a trait property that can be determined by relatively simple measurements taken in the population (Falconer & Mackay, 1996). The evaluation of the degree of resemblance is based on the partition of the phenotypic variance into different components due to familiar grouping, e.g., between and within groups, and allows one to estimate the magnitude of additive variance, known as heritability. Thus, resemblance between relatives can be conceived as the similarity between individuals of the same group or as the difference between individuals of different groups. This proportion of the total phenotypic variance due to kinship (no related to additive effects) is known as covariance between animal's parent-of-origin effects and is a function of causal components that vary in quantity and proportion according to the type of kinship (Meyer & Tier, 2012). The first results on how much imprinted genes contribute to genetic variation in livestock were presented by De Vries et al. (1994), who found that approximately 4%–5% of the phenotypic variance of pig carcass and growth traits were affected by imprinting. Works such as De Vries et al. (1994) and Engellandt and Tier (2002) described models with a single gametic effect together with the additive breeding value. Later, (Neugebauer, Luther, & Reinsch, 2010; Neugebauer, Räder, et al., 2010) modelled imprinting effects for both paternal and maternal gametes and estimated their variances and the covariance between them; however, their approach included partitioning the maternal and paternal contributions from a single estimate of the imprinting variance. Finally, an alternative interpretation was made by Tier & Meyer (2012), who developed different models to explore the effects of the parent-of-origin effect, and discussing the limits which differentiate between complete and partial imprinting effects. Those authors showed that it is generally not possible to estimate the partial imprinting of both types of parents independently from a quantitative point of view.

The idea behind the estimation of gametic variance assumes that additive genetic effects are associated with gametes and are transmitted to offspring with Mendelian sampling because of meiosis. From a quantitative point of the view, the estimation of variance components is achieved by means of the information provided by the resemblance between relatives (Varona et al., 2015). Various genetic models have been used to evaluate the effects of imprinting on quantitative traits in beef cattle (Blunk et al., 2017a, 2017b; Engellandt & Tier, 2002; Neugebauer, Räder, et al., 2010; Tier & Meyer, 2012), dairy cattle (Essl & Voith, 2002), and pigs (de Vries et al., 1994; Neugebauer, Luther, & Reinsch, 2010). Over the last few decades, imprinted genes have been described in mice (Morison et al., 2005), sheep (Georges et al., 2003), beef (Engellandt & Tier, 2002; Imumorin et al., 2011), pig (de Vries et al., 1994), and humans (Morison et al., 2005), highlighting their relevance, despite often being ignored in routine genetic evaluations. However, imprinted genes in quantitative traits have never been described in horses.

The Pura Raza Española (PRE) horse is an autochthonous Spanish horse with more than 260,000 active animals distributed in 65 countries over the five continents, particularly in Spain, Mexico, the U.S.A., France, and Costa Rica, among others. This breed has a large pedigree, with a stud book created in 1912 with a high completeness of the pedigree (Perdomo-González et al., 2020) and over 40 years of proven parental information by serum biochemical polymorphism and DNA microsatellites (de Andres Cara & Kaminsky, 1985; Kaminski & de Andres Cara, 1986; Negro et al., 2016), which make it a perfect population for analysing the quantitative effect of imprinted loci.

The purpose of this work was to determine whether a parent-of-origin effect on economically important traits exists in PRE horses (reproductive and morphological traits) and, if so, to estimate the paternal and/or maternal contribution to the parent-of-origin variance in those traits. To assess the relative importance of parent-of-origin effect for the genetic variation of economically relevant traits, we applied four different genetic models combining additive, gametic paternal, and gametic maternal effects. Predicting the gametic imprinting effects separately from the direct genetic effects for a breeding animal could be useful for livestock production, because it could be an efficient way of producing superior progeny, while ignoring it could bias the estimated breeding values and the estimates of genetic parameters. For all these reasons, the implementation of genealogical imprinting estimation could be important for the PRE horse breeding program.

2 | METHODS

2.1 | Dataset

Genealogical and morphological data sets were provided by the Real Asociación Nacional de Criadores de Caballos de Pura Raza Española (ANCCE). The complete PRE horse pedigree data base contains a total of 365,117 horses—178,367 males and 186,750 females—born from the early 19th century up to the year 2021. Females with both known parents and at least one offspring were selected to make up the reproductive traits data set, comprising a total of 81,468 mares with information about reproductive efficiency (RE), age at the first foaling (AFF) and foaling number (FN). More specifically, only mares from farms whose main activity was foal production (more than 12 foals per year) were selected. RE was implemented as a relationship between the real number of foalings the mare had had and the optimal number the mare could have had throughout her entire life, as described by Perdomo-González et al. (2021). The morphological data

set consisted of 44,038, 140,980 and 144,191 horses with known parents and information on height at the withers (HatW, distance between the ground and the highest point of the withers), thoracic circumference (TC, measured at its midpoint), and scapula-ischial length (SIL, distance between the greater tubercle of the humerus, caudal part, and ischial tuberosity), respectively. Morphological information was systematically collected in official breed controls using standard measuring sticks and non-elastic measuring tape, as described by Sánchez-Guerrero et al. (2016). All the measurements were taken from the left side of the horse while it was standing on a firm, flat surface, assuming a natural position. A pedigree was generated from each data set containing all the known generations. As a result, the genealogical databases included 96,772 animals for reproductive traits and 94,535, 160,244, and 161,451 horses for HatW, TC, and SIL, respectively.

2.2 | Estimation of imprinting effects

The Mendelian mean genotypic value for a population is given by $\mu = a(p - q) + 2dpq$ (Falconer & Mackay, 1996), where a and d are genotypic values of homozygotic and heterozygotic genes, respectively, and p and q are allele frequencies. However, in the presence of imprinting, the values for d differ according to maternal or paternal allele silencing. Therefore, when $d1$ (genotypic value for A1A2) and $d2$ (genotypic value for A2A1) are different, the mean mendelian genotypic value for the entire population can be given by $\mu = a(p - q) + (d1 + d2)pq$. This implies that the genotypic deviation of a particular genotype can be calculated as the difference between its genotypic value and the population mean. On calculating the breeding values for each genotypic class, defined as twice the difference between the mean genotypic value of that class's offspring and the population mean (Falconer & Mackay, 1996), these deviations are found to be different for males and females because the genotypic classes arising from reciprocal crosses may differ.

In the present work, it was developed a univariate general linear model (results not shown) for each trait to assess the statistical significance of the non-genetic effects which could influence them when included in the respective model. This was followed by a Tukey post hoc test to study non-genetic effects. The statistical analyses were performed with package agricolae for R (de Mendiburu, 2022).

A sequence of 4 genetic models was examined to determine the presence or absence of parent-of-origin effects in important economic traits of the PRE horse, along the same lines as in models developed by different authors (de Vries et al., 1994; Engellandt & Tier, 2002; Neugebauer,

Luther, & Reinsch, 2010; Neugebauer, Räder, et al., 2010; Varona et al., 2015). The models analysed are as follows:

1. Additive $\quad y = Xb + Whs + Z_a a + e$

2. Additive + paternal GE $\quad y = Xb + Whs + Z_a a + Z_p s + e$

3. Additive + maternal GE $\quad y = Xb + Whs + Z_a a + Z_m d + e$

4. Additive + paternal GE + maternal GE $\quad y = Xb + Whs + Z_a a + Z_p s + Z_m d + e,$

where y is the vector of the observed traits, b is the vector of fixed effects, hs is the vector of herd-stallion interaction random effect, a is the vector of additive genetic effect, s and d are vectors of paternal and maternal gametic effects (GE), respectively, e is a vector of random residual effects, and X, W, Z_a, Z_s, and Z_d are incidence matrices of fixed, herd-stallion, additive, paternal gametic, and maternal gametic effects, respectively.

The models were run using a Bayesian approach with a Gibbs sampler (Gelfand & Smith, 1990). The prior distributions for systematic effects and variance components were assumed to be bounded uniform, and the prior distributions for the genetic effects were multivariate Gaussian distributions with mean zero and variance defined as follows:

$$\text{var}\begin{pmatrix} a \\ e \end{pmatrix} = \begin{pmatrix} A\sigma_a^2 & 0 \\ 0 & I\sigma_e^2 \end{pmatrix} \text{ and } \text{var}\begin{pmatrix} s \\ d \end{pmatrix} = G_1 \otimes G,$$

where $G_1 = \begin{pmatrix} \sigma_s^2 & 0 \\ 0 & \sigma_d^2 \end{pmatrix},$

σ_a^2 is the additive variance, σ_s^2 and σ_d^2 are the variances of s and d, respectively, and σ_e^2 is the residual term variance. G is the matrix of gametic relationships (Schaeffer et al., 1989); and $\otimes$ indicates the Kronecker product. A is the pedigree-based numerator relationship matrix, and I is an identity matrix.

More specifically, b included the coat colour (4 levels: grey, bay, black, and chestnut) and the geographic zone (3 levels: Spain, the rest of the Europe and the rest of world) for both reproductive and morphological traits. Stud size (4 levels: two or less foals per year, between 3 and 6 foals per year, between 7 and 12 foals per year, over 12 foals per year) and birth decade (12 levels: from the early twentieth century to 2017 grouped by decades) were included for reproductive traits, while sex (2 levels: male and female) and evaluation age (3 levels: less or equal to 3, between 4 and 7 and 8 or higher) were included for morphological traits. The coat colour was included as fixed effects due to its crucial role in the reproductive management of the Pura Raza Española horse. As certain coat colours are more desirable due to their

potential impact on the future foal's price, breeders often specifically select mates based on coat colour. Also, in genetic parameters estimation models related to conformation and functional traits in PRE horses, the coat colour factor has been included as an important effect most likely because the different coat colour is related to different original trunks to the PRE breed (Perdomo-González et al., 2021; Poyato-Bonilla et al., 2018; Sánchez et al., 2017). Heritability was expressed as the ratios of the total additive genetic (σ_a^2) variances to the total phenotypic variance (σ_p^2). The herd-stallion ratio (hs²) was expressed as $\sigma_{hs}^2 / \sigma_p^2$, and the paternal and maternal gametic ratios (gs² and gd², respectively), were expressed as σ_s^2 / σ_p^2 and σ_d^2 / σ_p^2, respectively.

For each trait, we applied the models with parent-of-origin effects (2, 3, and 4) and the equivalent model without parent-of-origin effects (model 1). The gametic relationships matrix was calculated using ad hoc software written in FORTRAN90. Variance component analyses were run for each dataset using GIBBSF90+ and POSTGIBSF90 programs (Misztal et al., 2018) as a single chain of 250,000 cycles, with the first 50,000 iterations being discarded. All the samples were stored to calculate summary statistics. Comparisons between the models were made using the Deviance Information Criterion (DIC), where the model with the lowest DIC is the model that best compromise adjustment and model complexity. Estimated breeding values (EBV) for additive, paternal gametic and maternal gametic effects were also obtained from each model. For each paternal and maternal gametic effects two paternal gametic breeding values and two maternal gametic breeding values can be obtained, as a form of modelling animal transmission both as male and female, although biologically it can only act as one of them. As a result, the offspring will receive from his father it 50% additive breeding value and the paternal gametic breeding values mean value from his father and the 50% of the additive breeding value and the maternal gametic breeding value mean value from his mother.

3 | RESULTS

Table 1 shows the descriptive statistics of the traits analysed for the Pura Raza Española horse. Average mean values for reproductive traits are 38.95 (RE), 65.05 (AFF), and 4.32 (FN), and average mean values for morphology traits are 160.99 (HatW), 190.10 (TC), and 159.50 (SIL). Reproductive traits show higher coefficient of variation than morphology traits, which range from 35.38 to 76.89 and 3.10 to 4.57, respectively.

The comparison for reproductive (Table 2) and morphological (Table 3) traits using the DIC highlight model 4 (Additive + paternal GE + maternal GE) is the model that best fits the data for all traits. After that, the next best fitted model

TABLE 1 Descriptive statistics of analysed traits in the Pura Raza Española horse.

Traits	n	Mean $\pm$ SD	CV	Min	Max
Reproductive traits					
RE	81,468	38.95 ± 17.49	44.90	4.09	133.33
AFF	81,468	65.05 ± 23.02	35.38	36.00	288.00
FN	81,468	4.32 ± 3.32	76.89	1.00	24.00
Morphological traits (cm)					
HatW	44,038	160.99 ± 4.99	3.10	140.00	182.00
TC	140,980	190.10 ± 8.69	4.57	155.00	225.00
SIL	144,191	159.50 ± 5.38	3.37	130.00	190.00

Abbreviations: AFF, age at first foaling in months; FN, foaling number; HatW, height at withers; RE, Reproductive efficiency in percentage; SD, standard deviation; SIL, scapula-ischial length; TC, thoracic circumference.

for RE, FN, HatW, TC, and SIL is model 3 (Additive + maternal GE), while for AFF it was model 2 (Additive + paternal GE). For all traits, model 1 (additive) had the worst fit.

Average posterior means of estimates can also be seen in Table 2 and Table 3. As expected, additive variance values become less from model 1 to 4 for each trait. For reproductive traits (Table 2), additive genetic variance values decreased from 25.88 to 18.65, from 39.33 to 27.58, and from 0.45 to 0.12 for RE, AFF, and FN, respectively. For morphology traits (Table 3), additive genetic variance values decreased from 15.08 to 13.34, from 20.73 to 13.35, and from 13.21 to 11.63 for HatW, TC, and SIL, respectively. Residual variance values varied slightly from model 1 to model 4, varying from 235.44 to 232.20, 394.08 to 388.02, and 6.34 to 6.14 for RE, AFF, and FN, respectively, and from 4.59 to 4.16, 40.14 to 37.38, and 11.68 to 11.15 for HatW, TC, and SIL, respectively. As a result of this, the heritability values decreased slightly from 0.09 to 0.07, 0.08 to 0.05, and 0.06 to 0.02 for RE, AFF, and FN, respectively, and from 0.72 to 0.64, 0.29 to 0.18, and 0.48 to 0.42 for HatW, TC, and SIL, respectively.

Average posterior means of estimates for paternal and maternal gametic variance values were higher when evaluated together (model 4) than when evaluated separately (models 2 and 3) for AFF, FN, and TC. The average means of paternal gametic variances (σ_s^2) values for model 4 were 1.73, 21.10, and 0.09 for RE, AFF, and FN, respectively, while for morphology traits, they were 0.05, 4.48, and 0.24 for HatW, TC, and SIL, respectively. The average means of maternal gametic variances (σ_d^2) values for model 4 were 9.89, 4.30, and 0.52 for RE, AFF, and FN, respectively, while for morphology traits, they were 2.19, 7.77, and 2.12 for HatW, TC, and SIL, respectively. Paternal and maternal gametic ratios had very similar magnitudes between models. In model 4, reproductive traits obtained paternal gametic ratios of 0.01, 0.04, and 0.01 for RE, AFF, and FN, respectively, while maternal gametic ratios were 0.03, 0.01, and 0.07, respectively. Morphological traits obtained paternal gametic ratios of

TABLE 2 Model comparison and (co)variance components of reproductive traits.

Trait	Model	DIC	σ_{hs}^2 (PSD)	σ_a^2 (PSD)	σ_s^2 (PSD)	σ_d^2 (PSD)	σ_e^2 (PSD)	σ_{total}^2 (PSD)	hs^2 (PSD)	h^2 (PSD)	gs^2 (PSD)	gd^2 (PSD)
RE (%)	1	687040.85	22.90 (1.060)	25.88 (1.527)			235.44 (1.719)	284.22 (1.469)	0.08 (0.005)	0.09 (0.004)		
	2	686967.85	22.16 (1.049)	24.72 (1.521)	3.37 (0.645)		234.45 (1.718)	284.70 (1.473)	0.08 (0.004)	0.09 (0.005)	0.01 (0.002)	
	3	686818.59	22.38 (1.058)	19.89 (1.577)		9.92 (1.596)	232.34 (1.775)	284.50 (1.472)	0.08 (0.004)	0.07 (0.006)		0.03 (0.006)
	4	686810.85	22.19 (1.067)	18.65 (1.827)	1.73 (0.463)	9.89 (2.052)	232.20 (1.791)	284.67 (1.470)	0.08 (0.004)	0.07 (0.006)	0.01 (0.002)	0.03 (0.007)
AFF	1	731658.71	76.87 (2.836)	39.33 (3.120)			394.08 (3.220)	510.28 (2.751)	0.15 (0.005)	0.08 (0.006)		
	2	731462.70	73.64 (2.838)	32.64 (3.301)	16.46 (3.496)		390.14 (3.403)	512.88 (2.854)	0.14 (0.005)	0.06 (0.006)	0.03 (0.007)	
	3	731629.89	76.69 (2.770)	38.18 (3.142)		2.12 (0.635)	393.37 (3.226)	510.36 (2.756)	0.15 (0.005)	0.07 (0.006)		0.00 (0.001)
	4	731413.67	72.82 (2.849)	27.58 (3.982)	21.10 (3.719)	4.30 (1.699)	388.02 (3.364)	513.82 (2.850)	0.14 (0.005)	0.05 (0.008)	0.04 (0.007)	0.01 (0.003)
FN	1	391603.63	0.75 (0.028)	0.45 (0.030)			6.34 (0.042)	7.53 (0.038)	0.10 (0.004)	0.06 (0.004)		
	2	391556.91	0.73 (0.028)	0.44 (0.030)	0.05 (0.010)		6.32 (0.042)	7.54 (0.039)	0.10 (0.004)	0.06 (0.004)	0.01 (0.001)	
	3	391086.15	0.72 (0.028)	0.21 (0.026)		0.47 (0.041)	6.16 (0.045)	7.55 (0.039)	0.10 (0.004)	0.03 (0.003)		0.06 (0.005)
	4	391012.26	0.71 (0.028)	0.12 (0.026)	0.09 (0.020)	0.52 (0.045)	6.14 (0.045)	7.58 (0.039)	0.09 (0.004)	0.02 (0.003)	0.01 (0.003)	0.07 (0.006)

Note: Model comparison and average of the marginal posterior distribution of (co)variance components of reproductive traits.

Abbreviations: σ_{hs}^2, herd-stallion interaction variance; σ_a^2, additive genetic variance; σ_e^2, residual variance; σ_s^2, paternal gametic variance; σ_d^2, maternal gametic variance; AFF, age at first foaling in months; FN, foaling number; gd^2, maternal gametic ratio; gs^2, paternal gametic ratio; h^2, heritability; hs^2, herd-stallion ratio; PSD, Posterior standard deviations; RE, reproductive efficiency.

TABLE 3 Model comparison and (co)variance components of morphological traits.

Trait	Model	DIC	σ_{hs}^2 (PSD)	σ_a^2 (PSD)	σ_s^2 (PSD)	σ_d^2 (PSD)	σ_e^2 (PSD)	σ_{total}^2 (PSD)	hs^2 (PSD)	h^2 (PSD)	gs^2 (PSD)	gd^2 (PSD)
HatW	1	222855.45	1.27 (0.102)	15.08 (0.322)			4.59 (0.199)	20.94 (0.176)	0.06 (0.005)	0.72 (0.011)		
	2	221910.42	1.21 (0.102)	14.81 (0.329)	0.56 (0.136)		4.46 (0.206)	21.04 (0.177)	0.06 (0.005)	0.70 (0.120)	0.03 (0.006)	
	3	219796.98	1.18 (0.102)	13.31 (0.378)		2.22 (0.274)	4.19 (0.207)	20.89 (0.173)	0.06 (0.005)	0.64 (0.015)		0.11 (0.013)
	4	219658.48	1.17 (0.103)	13.34 (0.372)	0.05 (0.016)	2.19 (0.282)	4.16 (0.206)	20.91 (0.173)	0.06 (0.005)	0.64 (0.015)	0.01 (0.001)	0.10 (0.013)
TC	1	965533.15	11.38 (0.263)	20.73 (0.460)			40.14 (0.327)	72.25 (0.336)	0.16 (0.003)	0.29 (0.006)		
	2	963901.85	10.75 (0.265)	19.90 (0.485)	3.54 (0.487)		38.89 (0.367)	73.08 (0.361)	0.15 (0.004)	0.27 (0.006)	0.05 (0.007)	
	3	963636.23	10.79 (0.259)	16.40 (0.582)		6.25 (0.576)	38.77 (0.344)	72.21 (0.332)	0.15 (0.003)	0.23 (0.008)		0.09 (0.008)
	4	961706.70	10.17 (0.272)	13.35 (0.874)	4.48 (0.836)	7.77 (0.728)	37.38 (0.440)	73.15 (0.393)	0.14 (0.004)	0.18 (0.012)	0.06 (0.011)	0.11 (0.010)
SIL	1	825961.63	2.53 (0.077)	13.21 (0.192)			11.68 (0.117)	27.42 (0.132)	0.09 (0.003)	0.48 (0.005)		
	2	824514.94	2.44 (0.076)	13.04 (0.193)	0.69 (0.115)		11.43 (0.122)	27.59 (0.136)	0.09 (0.003)	0.47 (0.006)	0.03 (0.004)	
	3	823169.33	2.38 (0.076)	11.75 (0.231)		2.09 (0.200)	11.22 (0.123)	27.44 (0.132)	0.09 (0.003)	0.43 (0.007)		0.08 (0.007)
	4	822760.73	2.35 (0.076)	11.63 (0.244)	0.24 (0.074)	2.12 (0.208)	11.15 (0.125)	27.49 (0.133)	0.09 (0.003)	0.42 (0.008)	0.01 (0.003)	0.08 (0.008)

Note: Model comparison and average of the marginal posterior distribution of (co)variance components of morphological traits.

Abbreviations: σ_{hs}^2, herd-stallion interaction variance; σ_a^2, additive genetic variance; σ_s^2, paternal gametic variance; σ_d^2, maternal gametic variance; σ_e^2, residual variance; σ_{total}^2, total variance; gd^2, maternal gametic ratio; gs^2, paternal gametic ratio; h^2, heritability; hs^2, herd-stallion ratio; PSD, Posterior standard deviations; SIL, scapula-ischial length; TC, thoracic circumference. HatW, height at withers;

TABLE 4 Additive estimated breeding values comparison between models with and without parent-of-origin effects.

	Reproductive traits			Morphological traits		
	ER	AFF	FN	HatW	TC	SIL
r_{p1-2}	0.99*	0.98*	0.99*	0.99*	0.99*	0.99*
r_{p1-3}	0.98*	0.99*	0.95*	0.99*	0.98*	0.99*
r_{p1-4}	0.99*	0.99*	0.97*	0.99*	0.99*	0.99*
5% upper†	88.1	92.5	74.4	92.4	90.92	93.4
20% upper†	91.8	93.2	83.4	95.1	93.80	95.6
20% lower†	93.0	91.5	85.5	92.9	92.90	93.9

Note: Pearson's correlations between additive EBV (r_p), percentage of coincident animals within additive EBV between models 1 and 4 (†).

Abbreviations: AFF, age at first foaling in months; FN, foaling number; HatW, height at withers; RE, Reproductive efficiency; SIL, scapula-ischial length; TC, thoracic circumference.

*$p < 0.001$.

0.01, 0.06, and 0.01 for HatW, TC, and SIL, respectively, while for model 4, morphological traits obtained maternal gametic ratios of 0.10, 0.11, and 0.08, respectively.

The upper part of Table 4 shows Pearson's correlations between additive genetic values, comparing the additive model (1) with the imprinting models (2–4). For all traits, the correlations were significant, positive, and of a high value, very close to 1. The correlation value between models 1 and 3 for FN showed the lowest value, 0.95. The percentages of co-incident animals within the upper 5%, upper 20%, and lower 20% EBV between models 1 and 4 can be seen in the lower part of Table 4. Within the upper 5% of EBV, the percentage of coincident animals varied between 74.4% (FN) and 93.4% (SIL); nevertheless, the percentage of coincident animals increased when the upper 20% of EBV is compared, ranging from 83.4% (FN) to 95.6% (SIL). A high percentage of coincident animals was also found when comparing the lower 20% of EBV, ranging from 85.5% (FN) to 93.9% (SIL).

Figure 1 shows the evolution of EBV for additive, paternal gametic and maternal gametic effects for reproductive and morphological traits estimated with the most complete model, model 4 (Additive + paternal GE + maternal GE). The EBV have been standardized with the average EBV of those horses born in 2003, when the PRE breeding program started. The EBV for reproductive traits varied between −1 and 1. While for FN, all the EBV seem to be close to zero until 2008, from which point there was a slight increase, especially for additive, paternal 2 and maternal 1 breeding values. RE and AFF showed different results, with RE having constant EBV values close to zero except for additive and paternal 2 EBV, which slightly increased after the creation of the breeding program. On the other hand, AFF shows regular additive, and both paternal and maternal gametic effects values close to zero throughout the period, except in the period 2015–2017,

FIGURE 1 Evolution of estimated breeding values for additive, paternal and maternal gametic effects over the generations.
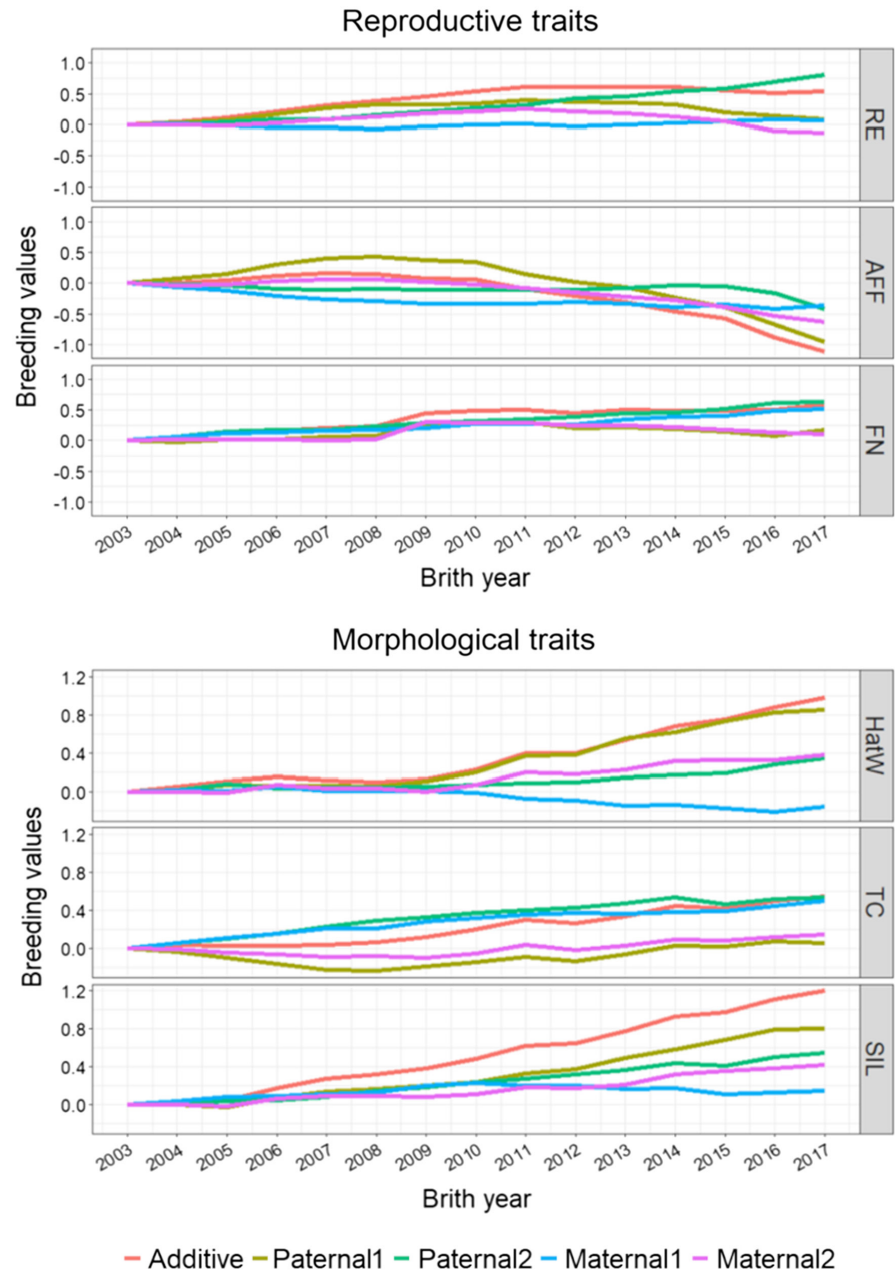
when additive, paternal 1 and maternal 2 have decreased to almost −1. For morphological traits, the EBV ranged between −0.3 and 1.2. All the morphological traits show similar behaviour, with constant or increasing values, except for maternal 1, which has seen a fall in EBV values in recent years for HatW and SIL.

4 | DISCUSSION

In recent decades, a series of works have revealed that imprinted genes are involved in many aspects of development in mammals and plants (Dini et al., 2021; Montgomery & Berger, 2021). Under genomic imprinting, the paternal and maternal copies at a locus are expressed differentially.

This means that the average phenotypes of reciprocal heterozygotes need no longer be the same, as expected by the Mendelian heritage. This loss of symmetry undermines the clarity of standard single-locus models of quantitative genetics and their conventional estimate of genetic variance and resemblances among relatives. Although a large number of studies have attempted to measure imprinted genes in different species (Georges et al., 2003; Imumorin et al., 2011; Jeon et al., 1999; Morison et al., 2005), very few works have evaluated imprinting from a quantitative point of view (Neugebauer, Luther, & Reinsch, 2010; Neugebauer, Räder, et al., 2010; Spencer, 2002; Tier & Meyer, 2012; Varona et al., 2015). In this work, we have applied a series of models to differentiate gametic from additive variance. To do this, it is important to have a

long-standing, reliable studbook to be able to estimate resemblances among relatives accurately. As has been described, the Pura Raza Española (PRE) horse breed has a reliable pedigree that makes it the perfect population for analysing the quantitative effect of imprinted loci, with a pedigree completeness level of PRE horses born between 2009 and 2018 (115,005 individuals) of 99.6%, remaining over 99% until the third generation and over 90% until the seventh generation (Perdomo-González et al., 2022).

The three traits of reproductive efficiency analysed in the present work were described by Perdomo-González et al. (2021), who analysed the phenotype of a selected population of breeding mares, while in this work, we have analysed all the mares with the phenotype and both parents known (Table 1). Despite the number of evaluated mares being 3.5 times higher here, the descriptive statistics are very close between the two works. Although the average values of RE and FN are slightly higher, 45.49 and 5.30, respectively, the average value of AFF (57.57) was lower than that found in this work. However, in line with our findings, Goméz et al. (2020) reported a comparable average mean value of 64.6 months for AFF in PRE mares. On the other hand, similar results can also be observed in morphological traits between the descriptive statistics shown in this work and those of the PRE population studied by Sánchez-Guerrero et al. (2017) and Poyato-Bonilla et al. (2020). Sánchez-Guerrero et al. (2017) studied the relationship between morphology and performance in PRE, and found both the TC and the SIL average values for stallions (187.6 and 160.9, respectively) and mares (188.3 and 159.9, respectively) similar to our results, the average values for HatW are more in concordance with the average value for stallions than for mares (160.2 and 157.3, for stallions and mares, respectively). Nevertheless, Poyato-Bonilla et al. (2020) showed average mean SIL values that are consistent with our results (159.38 and 159.01, for stallions and mares, respectively) although they found a more similar HatW mean value for mares than for stallions (162.27 and 159.53 for stallions and mares, respectively), which was higher for those authors.

In this work, we researched for the first time the relative importance of parent-of-origin effect for the genetic variation of economically relevant traits for horse production by applying different models with additive, paternal and/or maternal origin effect. There have been no previous studies on quantitative imprinting effects on reproductive or morphological traits of horses in the literature, which makes comparison difficult. Model comparisons by DIC (Tables 2 and 3) revealed the importance of including both paternal and maternal gametic effects in the models used for the genetic evaluations of all the traits studied. For all traits, except AFF, the model with the second-best fit was additive + maternal GE, which indicates crucial

maternal gametic effects in the analysed traits in the productive context. Tier and Meyer (2012) analysed the quantitative parent-of-origin effect in ultrasonic measurements of body composition traits in Australian beef cattle. They found that the inclusion of parent-of-origin effects in the models reduces the additive variance, and the estimate of variances due to gametic effects is consistent between models with only effect (models 2 and 3) or the model with both (model 4), which agree with the results obtained here. Regarding reproductive traits (Table 2), our additive models obtained similar heritability values to those described by Perdomo-González et al. (2021), 0.10, and 0.08 for RE and FN, respectively, while the AFF value was lower (0.16), with the estimations using a model to determine inbreeding depression load variance components. Nevertheless, the most complete imprinting model (model 4) showed lower heritability values for all the reproductive traits. In general, heritability values for reproductive traits were lower than those obtained from other horse breeds. Gómez et al. (2020) described heritability values for AFF in PRE, Arab, Anglo Arab, and Spanish Sport Horse mares (0.15, 0.20, 0.16, and 0.32, respectively) and (Taveira & Dias, 2007) reported an AFF heritability value of 0.38 in Thoroughbred mares. Similar heritability values for FN have been described in Standardbred (0.01) and Finn horses (0.03) (Sairanen et al., 2009) but lower to those reported by Wolc et al. (2009) in Warmblood horses (0.12). For morphological traits (Table 3), additive models obtained values similar to those in the literature for estimating the genetic parameters for the same breed for HatW, with higher values for SIL (0.39) and TC (0.67) (Sánchez-Guerrero et al., 2017). In addition, heritability values were lower than those obtained in models with estimation of the inbreeding depression load variance for HatW (0.80) and higher for SIL (0.34) (Poyato-Bonilla et al., 2020). Similar heritability values was described for HatW (0.8) but lower for TC (0.67) in a previous work with PRE horse (Sánchez-Guerrero et al., 2016), while lower value was reported for HatW (0.58) and higher for TC (0.45) and SIL (0.51) in a meta-analysis study in horse conformation traits (Hossein-Zadeh, 2021).

The parent-of-origin effects can be identified as originating from, first, loci which are completely suppressed by one or another parent and are modelled with independent gametic effects, and second, loci which are modified by one or other parent, with genes modelled as pairs of gametes. Estimates of gametic variance components fluctuate between the traits. Maternal imprinting effects accounted for between 1% (AFF) and 7% (FN) of the total phenotypic variance for reproductive traits (Table 2), while represented between 8% (SIL) and 11% (TC) of the total phenotypic variance for morphological traits (Table 3). Amir Rooudbar et al. (2017, 2018) found maternal imprinting effects to

be significant sources of phenotypic variance for weaning weight and different monthly weight in Iran-Black sheep and Lori-Bakhtiari sheep, finding maternal imprinting ratios between 13% and 23%, higher than the values described here. Meanwhile, (Mokhtari et al., 2022) found slightly lower values for maternal gametic ratios in growth traits and yearling greasy fleece weight in Raeini Cashmere goats.

Estimates of paternal imprinting variance had a similar or slightly lower magnitude than maternal imprinting variances for all the traits analysed (Tables 2 and 3), except for AFF. The greatest difference can be seen for AFF (Table 2), where the paternal imprinting effect is almost five times higher than the maternal imprinting effect (21.10 and 4.30, respectively). The reason for the higher paternal variance component on AFF could be due to a breeder effect, where a valuable stallion's female offspring are also the mares who become pregnant earlier than the others. In other words, there is an association that generates a greater resemblance between relatives when certain genes are inherited from the sire than when they are generated from the dam, and this is due to management because it is the breeders who choose the stallions to fix those traits in the population. It is worth noting, too, that FN maternal imprinting variance is over four times higher than the additive variance that may be due to an intrinsic herd management effect, which selects daughters of mares with a large number of offspring to act as breeders more frequently. On the other hand, morphology traits (Table 3), which are less influenced by herd reproductive management, show typical additive behaviour, with additive values higher than both the paternal and the maternal gametic values. The results in this work show higher morphological traits than those reported by Mokhtori et al. (2022) in the Raeini Cashmere goat, where the paternal imprinting ratios were close to zero for body weight traits at different ages and were considered as not significant for those traits. The same occurs in (Amiri Roudbar et al., 2017, 2018), where the authors found no influence of paternal imprinting effects on body weight in the Iran-Black sheep breed and on reproductive traits in Lori-Bakhtiari sheep, respectively. In general, maternal gametic ratios tend to be higher than paternal gametic ratios, which suggests that offspring phenotype is more influenced by the mother. This may be attributed to the fact that PRE mothers typically exhibit greater genetic diversity than males, who experience stronger selection pressures and are relatively more homogeneous.

As expected, the Pearson's correlation between the genetic additive breeding values for models with parent-of-origin effects and models without parent-of-origin effects is high, which implies that the additive estimated breeding values between models are coherent. However, it is also important to compare the coincident animals in the upper and lower parts of the EBV, because the correlation can be diluted at the extremes. From this, we can verify that animals with high additive genetic value under a normal animal model also have a high probability of having high additive genetic value under an imprinting model. In addition to the very high Pearson correlations between EBV from models 1 and 4, the proportion of coincident animals remains high when comparing animals in the higher percentiles. For this reason, this type of model is advisable be used routinely in the PRE breeding program, since the value will determine whether the animals acquire a good genetic category and are much more highly valued.

For genetic evaluations, parent-of-origin effect can be accounted for by adding two additive gametic effects per animal to the additive genetic effect, the paternal and the maternal expression patterns of imprinted genes. These two new additive effects are composed of paternal and maternal genes, respectively. At the same time, from a quantitative point of view, the two alleles an individual receives from its parents' alleles produce two paternal gametic breeding values and two maternal gametic values. Eventually, a stallion transmits to its descendants 50% of its additive breeding value plus the mean paternal gametic values, while a mare transmits to its descendants 50% of its additive breeding value plus the mean maternal gametic values. Finally, Figure 1 shows model 4 EBV standardized with the average EBV of horses born in 2003, when the PRE breeding program started, with a clearly visible increase in most breeding values until to the present day. In general, EBV for RE and FN show constant or increasingly values, whereas AFF shows decreasing values, which also indicates an improvement or reduction in the age at the first foaling, especially over the last few years. Regarding the morphological traits, a general upwards progression can be seen, especially in the additive and paternal 1 breeding values for HatW and SIL, while maternal 1 appears to be constant or slightly decreasing. For all these reasons, under parent-of-origin effects, the variance of EBVs for imprinting at the genetic level differs from their variance at the phenotypic level, and gametes have full potential to be expressed in gran-progeny and subsequent descendants, while expression in their progeny can be limited when inherited from parents of the opposite gender (Tier & Meyer, 2012).

5 | CONCLUSIONS

Understanding the imprinting phenomenon gives us an insight into this fascinating mechanism of controlling gene expression. In this work, we present evidence that parts of the additive genetic variance in PRE horse traits are influenced by parent-of-origin effects. The model comparisons revealed the importance of including both paternal

and maternal gametic effects in the models considered for genetic evaluations of all the traits studied. Most traits analysed are highly influenced by the maternal imprinting effect, even when the paternal imprinting effect has had a major relevance in one of them (AFF). The estimation of variance components for parent-of-origin could be included in the genetic evaluations of the Pura Raza Española horse by estimating four parental breeding values per animal. Ultimately, traits are measured in individuals, although breeders' decisions are also very important, especially in horses, where higher paternal influence can be observed, which, in fact, is really the breeder's influence, from the moment the stallion is chosen.

AUTHOR CONTRIBUTIONS

AM and MV conceived and designed the analyses. DPG and LV performed and executed the genetic models. DPG and NL wrote the first draft of the manuscript. All authors contributed to the interpretations of the results, the discussion and prepared the final manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The dataset supporting the results of this study was supplied by the National Association of Pura Raza Española Horse Breeders (ANCCE). The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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