


Morphological and genetic diversity of Pura Raza Español horse with regard to the coat colour

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Abstract

Gene mutations influencing melanocytes also impact on physiological and behavioural functions. In this study, we investigated their association with four different coat colours in the Pura Raza Español (PRE) horse using morphological traits and molecular datasets. Four different subpopulations were identified according to individual coat colour: grey, bay, chestnut and black. Coat colour significantly associated with morphological measurements. Observed and expected heterozygosity values were low in grey compared with the other three subpopulations, suggesting the presence of unique ancestral alleles probably arisen by genetic drift and selection mechanism effects. Nei's distance demonstrated a clear division among subpopulations, the grey being the most divergent group. Gene flow estimates were similar, showing the lowest values in grey. Divergence times among subpopulations assessed with the average square distance suggested that grey was the original PRE population which diverged from bay, chestnut and black. Our results also demonstrated a clear morphological differentiation according to coat colour. The close genetic structure of bay and chestnut PRE subpopulations and the clear differences in most morphological traits of grey and chestnut PRE mares would suggest the pleiotropic effect of genomic regions determining coat colour in horses. However, further analysis including genomic information would be necessary to elucidate the mechanisms involved.

KEYWORDS

coat colour, genetic distance, horse, microsatellite DNA, morphological traits

1 | INTRODUCTION

Horse production is one of the main agricultural activities in Spain, responsible for 0.5% of the gross national product. Within this, the Pura Raza Español (PRE) horse is the most important breed in terms of census and economic impact on international trade (Sánchez-Guerrero, Molina, Gómez, Peña, & Valera, 2016). Currently, this breed has 231,003 live horses around the world and a long-time established genetic selection programme which

includes some registration restrictions regarding the coat colour of individuals.

Coat colour is one of the most noticeable animal features which has interested and intrigued mankind for centuries (Pruvost et al., 2011). Accordingly, the genetic control of pigmentation was one of the first systems to be determined, including more than 130 loci and 1,000 mutations (Steingrímsson, Copeland, & Jenkins, 2006). Based on this knowledge, several unique coat colour phenotypic variations could be produced in horses, increasing the value of the animal (Bellone, 2010; Thiruvankadan, Kandasamy, & Panneerselvam, 2008). Some of them could even involve selective advantages essential for

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survival under certain environmental conditions (Dikmen, Dahl, Cole, Null, & Hansen, 2017; Reissmann & Ludwig, 2013). However, it is believed that most of the phenotypes currently observed in the modern horse are the result of domestication and selective breeding (Horváth et al., 2010; Ludwig et al., 2009). For example, some breeds as Menorca Purebred (Solé, Santos, Gómez, Galisteo, & Valera, 2013), American Paint horse and Appaloosa horse are mostly defined by the colour and patterns of their coats as a result of the breeders' selection criteria, based probably on their beauty among other traits. Similarly, the existence of pleiotropic effects associated with specific coat colours is also well known. This is the case of grey horses, whose mutations are involved in the development of melanocytes (e.g. melanocortin receptor or MCR genes) and the appearance of genetic diseases (Bellone, 2010). Moreover, in several species coat colour could also be associated with behavioural (Ducrest, Keller, & Roulin, 2008), morphological (Bize, Gasparini, Klopfenstein, Altwegg, & Roulin, 2006) and physiological traits (Roulin, 2004).

Conformational traits have been widely studied in horses due to their relationship with sporting performance, longevity or predisposition to lameness (Koenen, van Veldhuizen, & Brascamp, 1995; Saastamoinen & Barrey, 2000; Sánchez-Guerrero, Cervantes, Molin, Gutiérrez, & Valera, 2016; Weller, Pfau, Verheyen, May, & Wilson, 2006). For instance, some relationships between conformational traits and body dimensions were described as advantageous for dressage discipline (Sánchez-Guerrero, Cervantes, et al., 2016; Sánchez-Guerrero, Molina, et al., 2016). Despite the fact that the genetic basis of horse conformational and locomotive traits is poorly known, several studies have shown high heritabilities on those traits, thus determining the existence of a specific genetic control system (Ducro, Bovenhuis, & Back, 2009; Koenen et al., 1995; Sánchez-Guerrero, Cervantes, et al., 2016).

Although some studies have reported the possible pleiotropic effect of coat colour on horses (Finn et al., 2016; Horváth et al., 2010; Ludwig et al., 2009; Thiruvankadan et al., 2008), they mainly focused on their association with genetic diseases. However, to our knowledge, a systematic large-scale study assessing the influence of coat colour in the morphology of the individuals has not yet been performed.

Therefore, the aim of this study was to determine the effect of coat colour on the morphological and genetic structure of PRE. For that purpose, two large-scale databases including morphological measurements (MM) of more than 90,000 individuals were analysed and more than 174,000 individuals genotyped. Additionally, morphological and genetic divergences among PRE populations based on molecular data were studied.

2 | MATERIALS AND METHODS

2.1 | DNA records

In total, 174,590 individual molecular records belonging to the breeding programme of the Asociación Nacional de Criadores de

Caballos de Pura Raza Española (ANCCE) were used. Blood samples were obtained from the jugular vein by the official veterinary services of the ANCCE. DNA was extracted using the QIAamp 96 DNA Blood Kit (Qiagen, Hilden, Germany) and genotyped using the standard STR panel for horses of the International Society for Animal Genetics (Table S1, Demyda-Peyrás et al., 2013).

2.2 | Morphological records

The phenotypic database included morphological traits of 90,941 individuals from 36 countries (15,272 stallions and 75,669 mares). All records were collected by the ANCCE official veterinary services between 2012 and 2016. The horse population was also clustered in four subpopulations: grey (9,026 stallions and 45,546 mares), bay (4,490 stallions and 22,290 mares), black (1,270 stallions and 6,380 mares) and chestnut (486 stallions and 1,453 mares). For each individual, 23 MM were systematically collected in official controls of the breed (Sánchez-Guerrero, Molina, et al., 2016). All measurements were taken from the left side of the horse while it was standing on a hard surface and flat ground assuming a natural position, using standard measuring sticks, nonelastic measuring tapes and zoometric compasses, according to the variable to determine. Horses were positioned with the front legs and hind feet parallel and as near as possible to the perpendicular with the toes lined up. The use of sedatives during measurements was strictly forbidden.

2.3 | Sample clustering and assessment

For the first analysis, all the available individuals were clustered according to their coat colour in four subpopulations (grey, bay, black and chestnut), without considering their pedigree (coat colour subpopulations, $n = 174,590$). For the second analysis, only individuals with at least four full generations of ancestors bearing the same coat colour (grey horses) or not bearing the grey coat (bay, black or chestnut) were clustered following the same criteria (pure coat colour subpopulations, $n = 57,633$).

2.4 | Genetic diversity

Genetic diversity across subpopulations was determined in both analyses using the molecular records available. To this end, the mean number of alleles per locus (MNA), observed heterozygosity (H_o), unbiased expected heterozygosity (H_e , corrected for sample size) and Wright's F_{IS} were computed using the microsatellite analyser 4.05 program (Dieringer & Schlötterer, 2003). Genetic differentiation among populations was determined using Wright's F_{ST} and Nei genetic distances (Nei, 1972; corrected for sample size), and plotted in a dendrogram using the Phylip 3.5 package (Felsenstein, 1989). The analysis included the horse subpopulations clustered according to their basic coat colour as well as groups with the remaining PRE individuals with different coat colours (called "other"; Figure 1). A pairwise matrix of the genetic distances was then used to obtain a

neighbor-joining tree (Saitou & Nei, 1987) with a bootstraps of 1,000 replicates in order to test the robustness of tree topology using the DISPAN software (Ota, 1993).

2.5 | Gene flow and population divergence time

Gene flow was determined by the effective number of migrants per generation (N_m ; Wright, 1990) using F_{ST} estimates (Crow & Kimura, 1970) as follows:

$$N_m = (1 - F_{ST})/4F_{ST}.$$

Divergence time between populations was estimated using the genetic distance $(\delta\mu)^2$ (Goldstein, Ruiz Linares, Cavalli-Sforza, & Feldman, 1995):

$$\tau_{gen} = [(\delta\mu)^2/2\beta]g;$$

where β is the mutation rate of microsatellite loci and g is the generation interval. In this study, mutation rate (β) (Bowling et al., 1997) and generation interval (Krüger, Gaillard, Stranzinger, & Rieder, 2005) were set at 4×10^{-4} and 7 years, respectively.

2.6 | Morphological diversity

Morphological differences among subpopulations clustered according to their own coat colour (grey, bay, black and chestnut) were determined using a general linear model (Statistica v. 8.0, StatSoft, 2007). Mares and stallions were analysed separately due to the sexual dimorphism of the PRE breed (Sánchez-Guerrero, Molina, et al., 2016). For each trait, significant differences among the four subpopulations were determined using a Duncan posthoc multitest ($p > 0.05$).

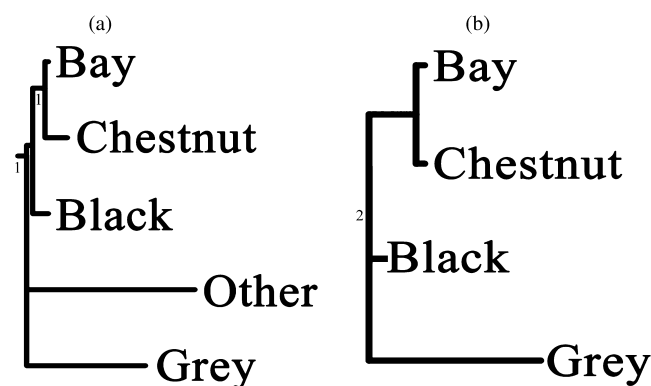


FIGURE 1 Neighbor-joining tree obtained from the Nei genetic distances among different horse subpopulations (Phylip) (1,000 bootstrap): (a) according to their basic coat colour (grey, bay, chestnut, black and others [the rest of different coat colour Pura Raza Español horses]) whose relatives are four generations with the same coat colour; (b) according to their basic coat colour (grey, bay, chestnut, black) whose relatives are four generations without grey coat colour. All the bootstrap values not indicated at the respective nodes are 100%. ¹98.5%. ²95.1%

3 | RESULTS

3.1 | Genetic diversity among coats

The genetic diversity observed among subpopulations is summarized in Table 1. Grey individuals showed the highest MNA in both analyses, being slightly lower in the pure coat colour subpopulation. Oppositely, chestnuts showed the lowest MNA and the lowest number of individuals, with bays and blacks located at closely similar intermediate values. Interestingly, grey horses showed the lowest H_o and H_e compared with the other three subpopulations. These results were even higher among pure individuals and agreed with the F_{IS} values observed, showing an elevated degree of relatedness (8.32% inbreeding).

3.2 | Population structure

The molecular analysis showed a clear division between grey and the other three subpopulations, being bay and chestnut horses the most closely related (Figure 1). For instance, genetic distances varied from 0.003 between bays and chestnuts to 0.04 among greys, bays and chestnuts. Similarly, pairwise F_{ST} values between grey and the rest of the basic coat colour subpopulations were higher, ranging from 0.027 to 0.043.

3.3 | Gene flow and divergence time between populations

Gene flow between subpopulations was estimated through the number of migrants per generation (N_m ; Table 2, upper diagonal). Grey horses were the most divergent subpopulation, being their N_m values eight times lower than those observed among the other three subpopulations. Divergence times among subpopulations, based on the average square distance $(\delta\mu)^2$, ranged from 112 years (0.0256) in the

TABLE 1 Genetic diversity of the two subpopulations regarding to coat colour of the Pura Raza Español horse (174,590 and 57,633 individuals, respectively) based on 17 microsatellite loci

	Coat colour	N	MNA	H_o	H_e	F_{IS}^*
Coat colour subpopulations	Grey	85,888	14.82	0.66	0.67	0.018
	Bay	62,431	14.12	0.69	0.70	0.017
	Chestnut	4,998	10.88	0.69	0.70	0.016
	Black	21,273	13.29	0.68	0.70	0.017
	Total	174,590				
Pure coat colour subpopulations	Grey	30,046	13.65	0.64	0.66	0.026
	Bay	18,079	12.88	0.69	0.71	0.019
	Chestnut	1,209	8.82	0.69	0.71	0.018
	Black	8,299	12.06	0.69	0.70	0.017
	Total	57,633				

Notes. N: sample size; MNA: mean number of alleles per locus; H_o : observed heterozygosity; H_e : expected heterozygosity (corrected for sample size); F_{IS} : heterozygote deficiency coefficient. * $p < 0.05$.

TABLE 2 The effective number of migrants per generation (N_m , upper diagonal), and the divergence times (lower diagonal) among the basic coat colour horse subpopulations of the Pura Raza Español horse analysed

	Coat colour	Grey	Bay	Chestnut	Black
Pure coat colour subpopulations	Grey		5.610	5.973	9.030
	Bay	0.1996		78.864	54.695
	Chestnut	0.1781	0.0156		35.011
	Black	0.1225	0.0284	0.0256	

Note. μ (mutation rate per population); the number of effective migrants per generation ($N_m = (1 - F_{ST})/4F_{ST}$); $(\delta\mu)^2 =$ average square distance allows to calculate divergence times with the formula: $[(\delta\mu)^2/2\beta]g$.

chestnut–black pair to 1247 years (0.1996) in the grey–bay pair, whereas black–grey and chestnut–grey pairs diverged 536 (0.1225) and 779 (0.1781) years ago respectively.

3.4 | Morphological diversity among coats

Differences among coat colour subpopulations were significant in 22 and 15 MM in mares and stallions respectively (Table 3). In general, chestnut horses were the smallest and bays the biggest. On the contrary, grey and black subpopulations presented the most homogeneous individuals and only 13.04% and 21.74% of the morphological traits were significantly different among mares and stallions, respectively. In stallions, height at withers (HW) was similar in grey, black and bay subpopulations, but lower in chestnuts. In mares, grey and chestnut were the most divergent groups (78.26% of the traits were statistically different), followed by bay and chestnut mares (73.91%). As an example, bay mares were significantly taller, grey and black subpopulations presented similar intermediate values and chestnuts were the shortest.

4 | DISCUSSION

In this study, we analysed for the first time the relationship between coat colour and morphological variability using a large PRE horse dataset that included 90,000 phenotypes and 175,000 genotypes. To our knowledge, such possible relationship has not been previously analysed in any breed in this species. Furthermore, the comprehensive dataset used in this study (collected during more than 20 years) provides a higher reliability to our results.

Genetic variation in the horse basic coat colour is determined by two loci associated with the *MC1R* and *ASIP* genes (bay, black and chestnut) (Marklund, Moller, Sandberg, & Andersson, 1996; Rieder, Taourit, Maria, Langlois, & Guérin, 2001; Sponenberg, 2009), jointly with the dominant locus *STX17*, which is responsible of the grey coat (Rosengren Pielberg et al., 2008). Those genes, among others associated with melanocyte controls, were previously linked to pleiotropic effects on mammals (Ducrest et al., 2008; Reissmann & Ludwig, 2013). In horses, they were mostly associated with diseases such as the lethal white foal syndrome (McCabe et al., 1990), deafness (Magdesian, Williams, Aleman, Lecouteur, & Madigan, 2009) or the lavender foal syndrome (Brooks, Gabreski, et al., 2010), as reviewed by Bellone (2010), but not with morphological traits.

Coat colour is a major attribute which determines breeding practices in the PRE production system. Nowadays, only individuals showing large white spots in head and limbs, or of any size in the rest of the body (piebald, pinto or appaloosa), are forbidden in the PRE studbook (ANCCE, 2017). Chestnut coat was also forbidden in the PRE population from 1979 to 2002 and therefore, selection intensity in this subpopulation was probably lower compared with the rest of coats. Furthermore, most PRE breeders still raise individuals with limited coat colour variability in their herds, and some of them are specialized in the production of horses with a unique coat colour (García, Valera, Molina, & Rodero, 1998). This could explain the lower percentage of grey ancestors observed in the rest of the subpopulations studied (6.79%, 0.34% and 2.52% in bay, chestnut and black horses, respectively), despite the fact that grey conforms half of the whole PRE population.

Coat colour affects several aspects of the horse behaviour through unclear genetic mechanisms (Finn et al., 2016; Jacobs, Staiger, Albright, & Brooks, 2016). The small differences in performance among individuals bearing several coat colours reported by Stachurska, Pieta, Łojek, and Szulowska (2007) were absent in grey or nongrey horses. Since the grey pattern is originated by the presence of a completely dominant allele (Henner et al., 2002), probably the pleiotropic effects produced by the rest of the genes involved in the coat colour control mechanism were masked. This hypothesis could partially explain our results, considering that grey horses presented intermediate morphological values compared with the other phenotypes assessed. Moreover, none of the genes recently associated with PRE morphometric traits are involved in coat colour patterns (i.e., *LCORL/NCAPG*, *HMG2A*, *USP31* and *MECR*; Sevane, Dunner, Boado, & Cañon, 2017). Therefore, since the coat colour mechanism is masked in grey horses, the genetic effect of coat colour on morphological differences could not be inferred in this case.

A large genetic variability was observed in all the subpopulations of pure and nonpure individuals of the breed, as shown by the high MNA values. Additionally, H_o and H_e values were also high in all the subpopulations except for grey horses, suggesting that their genetic background was wider but more concentrated in some particular lineages, thus leading to an increased genetic drift. These results are among the highest currently reported in this species in studies performed using similar markers, ranging between 0.40 and 0.79 (Achmann et al., 2004; Berber et al., 2014; Leroy et al., 2009; Plante et al., 2007; Prystupa, Juras, Cothran, Buchanan, & Plante, 2012);

TABLE 3 Effects of coat colour within gender in stallions and mares from the PRE worldwide population of Pura Raza Español

Morphological measurements	LSMeans ¹ Stallions				Significance CC	LSMeans ¹ Mares				Significance CC
	Grey	Black	Bay	Chestnut		Grey	Black	Bay	Chestnut	
HW	162.06 ^b	162.12 ^b	162.38 ^b	160.84 ^a	***	159.33 ^b	159.15 ^b	159.71 ^c	158.60 ^a	***
HCr	160.88 ^{bc}	160.71 ^b	161.16 ^c	159.64 ^a	***	158.69 ^{bc}	158.44 ^b	158.95 ^c	158.00 ^a	***
LB	160.72 ^b	160.63 ^b	160.88 ^b	159.91 ^a	*	160.27 ^{bc}	160.07 ^b	160.53 ^c	159.59 ^a	***
LH	62.30 ^b	62.26 ^b	62.31 ^b	61.91 ^a	ns	61.57 ^a	61.54 ^a	61.81 ^b	61.59 ^{ab}	***
WH	23.50	23.63	23.57	23.51	ns	23.14 ^b	23.17 ^b	23.20 ^b	23.00 ^a	**
CL	9.16 ^{ab}	9.26 ^b	9.18 ^b	9.07 ^a	*	9.02 ^{ab}	9.09 ^b	9.09 ^b	8.99 ^a	**
LN	76.09 ^c	75.36 ^{ab}	75.64 ^b	75.16 ^a	***	74.27 ^c	73.62 ^b	73.93 ^b	72.87 ^a	***
LS	65.70 ^b	65.72 ^{ab}	65.70 ^{ab}	65.35 ^a	ns	64.68 ^{ab}	64.58 ^a	65.03 ^c	64.89 ^{bc}	***
WC	41.96 ^b	41.71 ^{ab}	41.84 ^b	41.44 ^a	*	40.54 ^b	40.17 ^a	40.46 ^b	39.95 ^a	***
LFA	47.42	47.31	47.41	47.29	ns	46.66 ^b	46.50 ^b	46.74 ^b	46.04 ^a	***
LBa	30.12	30.24	30.32	30.26	ns	30.04 ^a	30.36 ^{ab}	30.42 ^b	30.14 ^{ab}	***
LL	30.20 ^b	30.04 ^{ab}	30.01 ^{ab}	29.73 ^a	ns	31.41 ^b	31.18 ^b	31.15 ^b	29.92 ^a	***
LCr	52.77 ^b	52.81 ^b	52.73 ^b	52.25 ^a	*	52.69 ^b	52.66 ^b	52.70 ^b	52.23 ^a	**
WCr	52.69 ^b	52.42 ^b	52.55 ^b	51.95 ^a	***	53.86 ^b	53.73 ^b	53.82 ^b	53.12 ^a	***
LF	52.68 ^b	52.39 ^b	52.57 ^b	51.84 ^a	***	51.54 ^b	51.17 ^c	51.54 ^b	50.75 ^a	***
LG	45.21	45.43	45.46	45.19	ns	44.85 ^a	44.68 ^a	45.10 ^{ab}	45.36 ^b	***
DCr	49.09 ^{ab}	49.31 ^b	49.33 ^b	48.73 ^a	ns	49.63	49.65	49.69	49.59	ns
LBU	51.52 ^b	51.50 ^b	51.47 ^b	51.03 ^a	*	50.57 ^b	50.52 ^b	50.61 ^b	50.10 ^a	***
BD	42.67 ^b	42.02 ^a	42.46 ^{ab}	41.56 ^b	***	42.67 ^c	42.02 ^{ab}	42.46 ^{bc}	41.56 ^a	***
DSD	73.68 ^{bc}	73.44 ^{ab}	73.88 ^c	73.27 ^{ab}	**	73.63 ^a	73.57 ^a	73.99 ^b	73.92 ^b	***
PT	188.18 ^c	187.30 ^b	187.51 ^{bc}	185.66 ^a	***	190.94 ^c	189.75 ^b	190.77 ^c	188.17 ^a	***
PK	33.99 ^b	33.91 ^b	34.08 ^b	33.61 ^a	***	31.54 ^b	31.48 ^b	31.59 ^b	31.22 ^a	***
PCB	20.92 ^b	21.00 ^b	21.03 ^b	20.68 ^a	***	20.00 ^{bc}	19.95 ^b	20.04 ^c	19.80 ^a	***

Notes. HW: height at withers; HCr: height at croup; LH: length of head; WH: width of head; CL: commissure of lips; LN: length of neck; LS: length of shoulder; WC: width of chest; LFA: length of forearm; LB: length of body; LBA: length of back; LL: length of loin; LCr: length of croup; WCr: width of croup; LF: length of femur; LG: length of gaskin; DCr: depth of croup; LBU: length of buttock; BD: bicostal diameter; DSD: dorso-sternum diameter; PT: perimeter of thorax; PK: perimeter of knee; PCB: perimeter of cannon bone; CC: coat colour; ns: no significant. ¹Any standard errors are higher than 1.03. Mean measurements are expressed in cm. ^{a,b,c}Values within a row with different superscripts differ significantly at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

furthermore, they validate our preliminary study using an 80 times smaller dataset (2,808 individuals; Negro et al., 2016).

Interestingly, the lowest heterozygosity values were obtained in grey horses, even though this subpopulation has the highest census (near 85,000 horses). This could be explained by the increased selection pressure applied in this particular coat, mainly for functional and morphological traits, due to the existence of widely renowned lineages which are heavily used as breeders (Valera, Molina, Gutiérrez, Gómez, & Goyache, 2005). Thus, the number of stallions employed among breeders is limited considering their census, narrowing its genetic base (Sánchez-Guerrero, Cervantes, Valera, & Gutiérrez, 2014). In this sense, F_{IS} values were also low and positive in most subpopulations, suggesting a slight excess of homozygosity and some kind of directional mating among them. Those differential traits were probably produced by the different primary selection objectives derived from the breeders' aims as well as by restrictions derived from fluctuations of the breeding studbook policies. In the case of pure grey individuals, the

hypothesis of increased selection intensity was particularly supported by F_{IS} values substantially higher (0.026) in comparison with the rest of the subpopulations (0.016–0.019).

F_{ST} values showed that bay and chestnut horses were the most closely related subpopulations, even clustering together in the phylogenetic analysis. On the other hand, grey horses were clearly segregated as an individual genetic cluster, with black horses located in the middle. This could be due to the fact that since grey coat colour is a dominant allele (Locke, Penedo, Bricker, Millon, & Murray, 2002), a horse needs only one copy to be a grey PRE. Such genetic peculiarity is well known by breeders, who take advantage of it to obtain grey horses the majority of times. On the contrary, the genetic control of the remaining three coats is narrower; therefore, their genetic basis would be expected to appear closely clustered.

Gene flow (N_m), which is strongly correlated with population divergence (Slatkin, 1995), was low between grey and the other groups of black, bay and chestnut horses. On the contrary, N_m was higher among the nongrey subpopulations, suggesting lower genetic

divergences among them. Those differences could be explained by the indistinct use of black, chestnut and bay stallions among nongrey breeders, in which the true colour of the individual was expressed due to the absence of the grey dominant allele. Additionally, these results are in accordance with the main hypothesis of PRE breeding history, in which the dominant grey coat was considered to have the highest prevalence among the founders of the breed, and from which nongrey coats derived progressively. In this study, the average-square distance ($\delta\mu$)² analysis between diverged populations was also in agreement with the idea of the existence of a single ancestral population—grey horses—which split at some time in the past (around 500 years) to produce the other coats represented in PRE, being nongrey individuals bred in high levels of isolation ever since.

Morphology is critically important in nearly all horse breeds which are subjected to a systematic process of selection over time (Brooks, Makvandi-Nejad, et al., 2010). In our study, 23 different MM were assessed using a comprehensive and systematic dataset to identify the selection patterns of skeletal size and shape that could have existed in the PRE population and their possible association with coat colour. Our results showed that PRE MM values differed depending on coat colour; they were higher in the grey PRE group compared with the other grey breeds with common origins such as Lipizzaner (Zechner et al., 2001), but lower than in another grey breed such as Old Kladruby (Petlachová et al., 2012). On the contrary, MM values in chestnut and black subpopulations were higher than in other same coated breeds such as Arabian Purebred (Cervantes et al., 2009) and Menorca Purebred (Solé, Valera, Gómez, Cervantes, & Fernández, 2013). Besides, bay PRE MM results were lower than in other bay populations such as Dutch Warmblood, Hanoverian, Oldenburg and Trakehner (Brooks, Makvandi-Nejad, et al., 2010). This may indicate some kind of diversifying selection for skeletal size and shape among coated horse breeds which could explain the different levels of genetic diversity observed in the PRE subpopulations and the pattern of positive directional selection (i.e. for HW). However, since populations are dynamic units which can easily adapt their physiology and morphology to their environments and breeding schemes (Agaviezor et al., 2012), it is expectable that this particular population continues evolving to a more “PRE-like” phenotype characterized by its complexity. Interestingly, average heights, widths, depths and perimeters recorded and analysed in all the available PRE databases were similar to those reported in previous studies in which colour coat was not used as a clustering parameter among individuals (Gómez, Goyache, Molina, & Valera, 2009; Molina, Valera, Dos Santos, & Rodero, 1999; Sánchez-Guerrero, Molina, et al., 2016). Furthermore, all of these morphological differentiations were lower among stallions. Despite gene regulatory networks could be differentially controlled between males and females, it is also true that males are usually more selected than females, and therefore tend to be more homogenous. However, gender-related differences are highly variable between and within breeds, and even at diverse ages in the same population (Pinto et al., 2005; Sánchez-Guerrero, Molina, et al., 2016). Thus, the existence of a pleiotropic effect expressed differentially between sexes cannot be disregarded.

On the contrary, our results pointed out the existence of morphological differentiation in mares associated to a particular coat colour. As an example, 78.26% of MM were significantly different between grey and chestnut mares (being chestnut generally smaller). This clear differentiation in the chestnut group could be explained by the fact that they were banned from the PRE studbook in 1979 because they were considered as a crossbreed based on Arabian horses. However, after the description of the coat colour genetic control (*ASIP* and *MC1R* genes) and the demonstration that chestnut horses could be derived from black or bay parents, chestnut were reallocated in the PRE studbook. On the other side, morphological differences between grey and black subpopulations were lower, in agreement with the smaller genetic distance observed using the molecular data and breeding policy history in relation to the coat colour characteristics of the breed. The most genetically related populations (chestnuts and bays) showed one of the highest morphological differences, that is, bay horses were the tallest and biggest and chestnut horses the shortest and smallest, suggesting the possible pleiotropic effect of coat colour. In spite of such common genetic background, chestnut horses showed the highest levels of genetic diversity, in agreement with a lower selection pressure given its prohibition because of PRE studbook rules during 15 years. Thus, the possibility of a genetic effect of chestnut colour showing small phenotypes still remains unclear.

Nowadays, the use of sequence-derived genotype data allows to understand the genetic mechanisms underlying complex traits by increasing the power to detect novel variants. For instance, Frischknecht, Signer-Hasler, Leeb, Rieder, and Neuditschko (2016) recently confirmed the existence of a genetic association for HW in horses and identified a novel QTL on ECA9 associated with other conformation traits using imputation from sequence data. However, most previous association studies in this species using coat colour as clustering parameter were performed in phenotypes associated with health disorders (Reissmann & Ludwig, 2013). This could be explained by the existence of a highly pleiotropic gene network involving the melanocortin system and colour regulation (San-Jose, Ducret, Ducrest, Simon, & Roulin, 2017). This system is also involved in energy metabolism in domestic animals (Andersson, 2003) and animal growth (*MC4R* variant in pig) (Van Den Broeke et al., 2015). Therefore, we suggest that the observed morphological differentiation and the hypothesis of a possible pleiotropic effect need a more detailed study through high-throughput genomic methodologies.

In conclusion, we determined for the first time the existence of a significant morphological variability among horses with different coat colours by using a reliable and systematized methodology which combines molecular markers and a professional and standardized phenotypic characterization in more than 170,000 horses. These results suggest the possible existence of a pleiotropic effect of the coat colour regulatory network associated with the morphology of horses. However, the presence of directional mating among coated populations and the fluctuations of breeding studbook policies do not allow their genetic origin (pleiotropic or genetic selection) to be determined clearly. Besides, our findings could be used as a starting

point to determine which effects are involved in the morphological differences described according to coat colour and to take this effect into consideration for a further analysis including genomic information.

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REFERENCES

- Achmann, R., Curik, I., Dovc, P., Kavar, T., Bodo, I., Habe, F., ... Brem, G. (2004). Microsatellite diversity, population subdivision and gene flow in the Lipizzan horse. *Animal Genetics*, 35, 285–292. <https://doi.org/10.1111/j.1365-2052.2004.01157.x>
- Agaviézor, B. O., Peters, S. O., Adefenwa, M. A., Yakubu, A., Adebambo, O. A., Ozoje, M. O., ... Imumorin, I. G. (2012). Morphological and microsatellite DNA diversity of Nigerian indigenous sheep. *Journal of Animal Science and Biotechnology*, 3, 38. <https://doi.org/10.1186/2049-1891-3-38>
- ANCCE. (2017). *Programa de mejora del Pura Raza Español*. Retrieved from http://www.lgancce.com/web2/sites/default/files/pages_relatod_documents/anexo_ii_programa_de_mejora_del_pre.pdf
- Andersson, L. (2003). Melanocortin receptor variants with phenotypic effects in horse, pig, and chicken. *Annals of the New York Academy of Sciences*, 994, 313–318. <https://doi.org/10.1111/j.1749-6632.2003.tb03195.x>
- Bellone, R. R. (2010). Pleiotropic effects of pigmentation genes in horses. *Animal Genetics*, 41(Suppl. 2), 100–110. <https://doi.org/10.1111/j.1365-2052.2010.02116.x>
- Berber, N., Gaouar, S., Leroy, G., Kdidi, S., Tabet Aouel, N., & Säidi Meh-tar, N. (2014). Molecular characterization and differentiation of five horse breeds raised in Algeria using polymorphic microsatellite markers. *Journal of Animal Breeding and Genetics*, 131, 387–394. <https://doi.org/10.1111/jbg.12092>
- Bize, P., Gasparini, J., Klopfenstein, A., Altwegg, R., & Roulin, A. (2006). Melanin-based coloration is a nondirectionally selected sex-specific signal of offspring development in the alpine swift. *Evolution*, 60, 2370–2380. <https://doi.org/10.1554/06-155.1>
- Bowling, A. T., Eggleston-Stott, M. L., Byrns, G., Clark, R. S., Dileanis, S., & Wictim, E. (1997). Validation of microsatellite markers for routine horse parentage testing. *Animal Genetics*, 28, 247–252. <https://doi.org/10.1111/j.1365-2052.1997.00123.x>
- Brooks, S. A., Gabreski, N., Miller, D., Brisbin, A., Brown, H. E., Streeter, C., ... Antczak, D. F. (2010). Whole-genome SNP association in the horse: Identification of a deletion in myosin Va responsible for Lavender Foal Syndrome. *PLoS Genetics*, 6, <https://doi.org/10.1371/journal.pgen.1000909>
- Brooks, S. A., Makvandi-Nejad, S., Chu, E., Allen, J. J., Streeter, C., Gu, E., ... Sutter, N. B. (2010). Morphological variation in the horse: Defining complex traits of body size and shape. *Animal Genetics*, 41, 159–165. <https://doi.org/10.1111/j.1365-2052.2010.02127.x>
- Cervantes, I., Baumung, R., Molina, A., Druml, T., Gutiérrez, J. P., Sölkner, J., & Valera, M. (2009). Size and shape analysis of morphofunctional traits in the Spanish Arab horse. *Livestock Science*, 125, 43–49. <https://doi.org/10.1016/j.livsci.2009.03.006>
- Crow, J. F., & Kimura, M. (1970). *An introduction to population genetics theory*. New York, NY: Harper and Row.
- Demyda-Peyrás, S., Membrillo, A., Bugno-Poniewierska, M., Pawlina, K., Anaya, G., & Moreno-Millán, M. (2013). The use of molecular and cytogenetic methods as a valuable tool in the detection of chromosomal abnormalities in horses: A case of sex chromosome chimerism in a Spanish purebred colt. *Cytogenetic and Genome Research*, 141, 277–283. <https://doi.org/10.1159/000351225>
- Dieringer, D., & Schlötterer, C. (2003). Microsatellite analyser (MSA): A platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, 3, 167–169. <https://doi.org/10.1046/j.1471-8286.2003.00351.x>
- Dikmen, S., Dahl, G. E., Cole, J. B., Null, D. J., & Hansen, P. J. (2017). The larson blue coat color phenotype in holsteins: Characteristics and effects on body temperature regulation and production in lactating cows in a hot climate. *Journal of Animal Science*, 95, 1164–1169.
- Ducrest, A. L., Keller, L., & Roulin, A. (2008). Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology and Evolution*, 23, 502–510. <https://doi.org/10.1016/j.tree.2008.06.001>
- Ducro, B. J., Bovenhuis, H., & Back, W. (2009). Heritability of foot conformation and its relationship to sports performance in a Dutch Warmblood horse population. *Equine Veterinary Journal*, 41, 139–143. <https://doi.org/10.2746/042516409X366130>
- Felsenstein, J. (1989). PHYLIP—phylogeny inference package (Version 3.2). *Cladistics*, 5, 164–166.
- Finn, J. L., Haase, B., Willet, C. E., van Rooy, D., Chew, T., Wade, C. M., ... Velie, B. D. (2016). The relationship between coat colour phenotype and equine behaviour: A pilot study. *Applied Animal Behaviour Science*, 174, 66–69. <https://doi.org/10.1016/j.applanim.2015.11.004>
- Frischknecht, M., Signer-Hasler, H., Leeb, T., Rieder, S., & Neuditschko, M. (2016). Genome-wide association studies based on sequence-derived genotypes reveal new QTL associated with conformation and performance traits in the Franches-Montagnes horse breed. *Animal Genetics*, 47, 227–229. <https://doi.org/10.1111/age.12406>
- García, A., Valera, M., Molina, A., & Rodero, A. (1998). Genetic study of the colour coat in the characterisation of the equine breeds. *Arch Zootec*, 47, 247–253.
- Goldstein, D. B., Ruiz Linares, A., Cavalli-Sforza, L. L., & Feldman, M. W. (1995). Genetic absolute dating based on microsatellites and the origin of modern humans. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 6723–6727. <https://doi.org/10.1073/pnas.92.15.6723>
- Gómez, M. D., Goyache, F., Molina, A., & Valera, M. (2009). Sire × stud interaction for body measurement traits in Spanish Purebred horses. *Journal of Animal Science*, 87, 2502–2509. <https://doi.org/10.2527/jas.2008-0841>
- Henner, J., Poncet, P. A., Guérin, G., Hagger, C., Stranzinger, G., & Rieder, S. (2002). Genetic mapping of the (G)-locus, responsible for the coat color phenotype “progressive greying with age” in horses (*Equus caballus*). *Mammalian Genome*, 13, 535–537. <https://doi.org/10.1007/s00335-002-2174-7>
- Horváth, G., Blahó, M., Kriska, G., Hegedüs, R., Gerics, B., Farkas, R., & Ákesson, S. (2010). An unexpected advantage of whiteness in horses: The most horsefly-proof horse has a depolarizing white coat. *Proceedings of the Royal Society B: Biological Sciences*, 277, 1643–1650. <https://doi.org/10.1098/rspb.2009.2202>
- Jacobs, L. N., Staiger, E. A., Albright, J. D., & Brooks, S. A. (2016). The MC1R and ASIP coat color loci may impact behavior in the horse.

- Journal of Heredity*, 107, 214–219. <https://doi.org/10.1093/jhered/esw007>
- Koenen, E. P. C., van Veldhuizen, A. E., & Brascamp, E. W. (1995). Genetic parameters of linear scored conformation traits and their relation to dressage and show-jumping performance in the Dutch Warmblood Riding Horse population. *Livestock Production Science*, 43, 85–94. [https://doi.org/10.1016/0301-6226\(95\)00010-1](https://doi.org/10.1016/0301-6226(95)00010-1)
- Krüger, K., Gaillard, C., Stranzinger, G., & Rieder, S. (2005). Phylogenetic analysis and species allocation of individual equids using microsatellite data. *Journal of Animal Breeding and Genetics*, 122, 78–86. <https://doi.org/10.1111/j.1439-0388.2005.00505.x>
- Leroy, G., Callède, L., Verrier, E., Mériaux, J. C., Ricard, A., Danchin-Burge, C., & Rognon, X. (2009). Genetic diversity of a large set of horse breeds raised in France assessed by microsatellite polymorphism. *Genetics Selection Evolution*, 41, 31. <https://doi.org/10.1186/1297-9686-41-31>
- Locke, M. M., Penedo, M. C. T., Bricker, S. J., Millon, L. V., & Murray, J. D. (2002). Linkage of the grey coat colour locus to microsatellites on horse chromosome 25. *Animal Genetics*, 33, 329–337. <https://doi.org/10.1046/j.1365-2052.2002.00885.x>
- Ludwig, A., Pruvost, M., Reissmann, M., Benecke, N., Brockmann, G. A., Castañes, P., ... Hofreiter, M. (2009). Coat color variation at the beginning of horse domestication. *Science*, 324, 485. <https://doi.org/10.1126/science.1172750>
- Magdesian, K. G., Williams, D. C., Aleman, M., Lecouteur, R. A., & Madigan, J. E. (2009). Evaluation of deafness in American Paint Horses by phenotype, brainstem auditory-evoked responses, and endothelin receptor B genotype. *Journal of the American Veterinary Medical Association*, 235, 1204–1211. <https://doi.org/10.2460/javma.235.10.1204>
- Marklund, L., Moller, M. J., Sandberg, K., & Andersson, L. (1996). A missense mutation in the gene for melanocyte-stimulating hormone receptor (MC1R) is associated with the chestnut coat color in horses. *Mammalian Genome*, 7, 895–899. <https://doi.org/10.1007/s003359900264>
- McCabe, L., Griffin, L. D., Kinzer, A., Chandler, M., Beckwith, J. B., & McCabe, E. R. (1990). Overo lethal white foal syndrome: Equine model of aganglionic megacolon (Hirschsprung disease). *American Journal of Medical Genetics*, 36, 336–340. <https://doi.org/10.1002/ajmg.1320360319>
- Molina, A., Valera, M., Dos Santos, R., & Rodero, A. (1999). Genetic parameters of morphofunctional traits in Andalusian horse. *Livestock Production Science*, 60, 295–303. [https://doi.org/10.1016/S0301-6226\(99\)00101-3](https://doi.org/10.1016/S0301-6226(99)00101-3)
- Negro, S., Solé, M., Pelayo, R., Gómez, M. D., Azor, P. J., & Valera, M. (2016). Molecular diversity between two cohorts of six Spanish riding-horse breeds: Impact of selection in Crossbred vs Purebred populations. *Livestock Science*, 193, 88–91. <https://doi.org/10.1016/j.livsci.2016.09.013>
- Nei, M. (1972). Genetic distance between populations. *The American Naturalist*, 106, 283–292. <https://doi.org/10.1086/282771>
- Ota, T. (1993). *DISPAN: Genetic distance and phylogenetic analysis software*. University Park, PA: Institute of Molecular Evolutionary Genetics, Pennsylvania State University.
- Petlachová, T., Sobotková, E., Jiskrová, I., Pířová, M., Bihuncová, I., Černohorská, H., & Kostuková, M. (2012). Evaluation of the conformation of stallions of selected horse breeds. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 60, 375–382. Retrieved from <http://www.scopus.com/inward/record.url?eid=2-s2.0-84874095975&partnerID=tZotx3y1>
- Pinto, L. F. B., De Almeida, F. Q., Quirino, C. R., Cabral, G. C., De Azevedo, P. C. N., & Santos, E. M. (2005). Multivariate analysis of body measures in Mangalarga Marchador foals: Discriminant analysis [Análise multivariada das medidas morfométricas de potros da raça Mangalarga Marchador: Análise discriminante]. *Revista Brasileira de Zootecnia*, 34, 600–612. <https://doi.org/10.1590/S1516-35982005000200030>
- Plante, Y., Vega-Pla, J. L., Lucas, Z., Colling, D., De March, B., & Buchanan, F. (2007). Genetic diversity in a feral horse population from Sable Island, Canada. *Journal of Heredity*, 98, 594–602. <https://doi.org/10.1093/jhered/esm064>
- Pruvost, M., Bellone, R., Benecke, N., Sandoval-Castellanos, E., Cieslak, M., Kuznetsova, T., ... Ludwig, A. (2011). Genotypes of predomestic horses match phenotypes painted in Paleolithic works of cave art. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 18626–18630. <https://doi.org/10.1073/pnas.1108982108>
- Prystupa, J. M., Juras, R., Cothran, E. G., Buchanan, F. C., & Plante, Y. (2012). Genetic diversity and admixture among Canadian, Mountain and Moorland and Nordic pony populations. *Animal*, 6, 19–30. <https://doi.org/10.1017/S1751731111001212>
- Reissmann, M., & Ludwig, A. (2013). Pleiotropic effects of coat colour-associated mutations in humans, mice and other mammals. *Seminars in Cell and Developmental Biology*, 24, 576–586. <https://doi.org/10.1016/j.semcdb.2013.03.014>
- Rieder, S., Taourit, S., Maria, D., Langlois, B., & Guérin, G. (2001). Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (*Equus caballus*). *Mammalian Genome*, 12, 450–455. <https://doi.org/10.1007/s003350020017>
- Rosengren Pielberg, G., Golovko, A., Sundström, E., Curik, I., Lennartsson, J., Seltenhammer, M. H., ... Andersson, L. (2008). A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nature Genetics*, 40, 1004–1009. <https://doi.org/10.1038/ng.185>
- Roulin, A. (2004). The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews of the Cambridge Philosophical Society*, 79, 815–848. <https://doi.org/10.1017/S1464793104006487>
- Saastamoinen, M. A., & Barrey, E. (2000). Genetics of conformation, locomotion and physiological traits. In A. T. Bowling & A. Ruvinsky (Eds), *Genet horse* (pp. 439–472). London, UK: CABI. <https://doi.org/10.1079/9780851994291.0000>
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology Evolution Journal*, 4, 406–425.
- Sánchez-Guerrero, M. J., Cervantes, I., Molin, A., Gutiérrez, J. P., & Valera, M. (2016). Designing an early selection morphological linear traits index for dressage in the Pura Raza Español horse. *Animal*, 11, 948–957. <https://doi.org/10.1017/S1751731116002214>
- Sánchez-Guerrero, M. J., Cervantes, I., Valera, M., & Gutiérrez, J. P. (2014). Modelling genetic evaluation for dressage in Pura Raza Español horses with focus on the rider effect. *Journal of Animal Breeding and Genetics*, 131, 395–402. <https://doi.org/10.1111/jbg.12088>
- Sánchez-Guerrero, M. J., Molina, A., Gómez, M. D., Peña, F., & Valera, M. (2016). Relationship between morphology and performance: Signature of mass-selection in Pura Raza Español horse. *Livestock Science*, 185, 148–155. <https://doi.org/10.1016/j.livsci.2016.01.003>
- San-Jose, L. M., Ducret, V., Ducrest, A.-L., Simon, C., & Roulin, A. (2017). Beyond mean allelic effects: A locus at the major color gene MC1R associates also with differing levels of phenotypic and genetic (co)-variance for coloration in barn owls. *Evolution*, 71, 2469–2483. <https://doi.org/10.1111/evo.13343>
- Sevane, N., Dunner, S., Boado, A., & Cañon, J. (2017). Polymorphisms in ten candidate genes are associated with conformational and locomotive traits in Spanish Purebred horses. *Journal of Applied Genetics*, 58, 355–361. <https://doi.org/10.1007/s13353-016-0385-y>
- Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139, 457–462.
- Solé, M., Santos, R., Gómez, M. D., Galisteo, A. M., & Valera, M. (2013). Evaluation of conformation against traits associated with dressage ability in unriden Iberian horses at the trot. *Research in Veterinary Science*, 95, 660–666. <https://doi.org/10.1016/j.rvsc.2013.06.017>

- Solé, M., Valera, M., Gómez, M. D., Cervantes, I., & Fernández, J. (2013). Implementation of optimum contributions selection in endangered local breeds: The case of the Menorca Horse population. *Journal of Animal Breeding and Genetics*, 130, 218–226. <https://doi.org/10.1111/jbg.12023>
- Sponenberg, P. (2009). Colors built from the basic colors. *Equine color genet* (pp. 39–72). John Wiley & Sons.
- Stachurska, A., Pieta, M., Łojek, J., & Szulowska, J. (2007). Performance in racehorses of various colours. *Livestock Science*, 106, 282–286. <https://doi.org/10.1016/j.livsci.2006.07.017>
- StatSoft. (2007). *STATISTICA (data analysis software system)*. Retrieved from www.statsoft.com
- Steingrímsson, E., Copeland, N. G., & Jenkins, N. A. (2006). Mouse coat color mutations: From fancy mice to functional genomics. *Developmental Dynamics*, 235, 2401–2411. <https://doi.org/10.1002/dvdy.20840>
- Thiruvankadan, A. K., Kandasamy, N., & Panneerselvam, S. (2008). Coat colour inheritance in horses. *Livestock Science*, 117, 109–129. <https://doi.org/10.1016/j.livsci.2008.05.008>
- Valera, M., Molina, A., Gutiérrez, J. P., Gómez, J., & Goyache, F. (2005). Pedigree analysis in the Andalusian horse: Population structure, genetic variability and influence of the Carthusian strain. *Livestock Production Science*, 95, 57–66. <https://doi.org/10.1016/j.livprodsci.2004.12.004>
- Van Den Broeke, A., Aluwé, M., Tuytens, F. A. M., Ampe, B., Vanhaecke, L., Wauters, J., ... Millet, S. (2015). An intervention study demonstrates effects of MC4R genotype on boar taint and performances of growing–finishing pigs. *Journal of Animal Science*, 93, 934–943. <https://doi.org/10.2527/jas.2014-8184>
- Weller, R., Pfau, T., Verheyen, K., May, S. A., & Wilson, A. M. (2006). The effect of conformation on orthopaedic health and performance in a cohort of National Hunt racehorses: Preliminary results. *Equine Veterinary Journal*, 38, 622–627. <https://doi.org/10.2746/042516406X159034>
- Wright, S. (1990). Evolution in mendelian populations. *Bulletin of Mathematical Biology*, 52, 241–295. <https://doi.org/10.1007/BF02459575>
- Zechner, P., Zohman, F., Sölkner, J., Bodo, I., Habe, F., Marti, E., & Brem, G. (2001). Morphological description of the Lipizzan horse population. *Livestock Production Science*, 69, 163–177. [https://doi.org/10.1016/S0301-6226\(00\)00254-2](https://doi.org/10.1016/S0301-6226(00)00254-2)

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