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Inconsistencies in horse coat color registration: A case study

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Grullo is a dun dilution on a black coat that is common in the Campolina horse: an autochthonous Brazilian breed. The aims of this case study were to evaluate inconsistencies in grullo coat color registration and to explain their possible causes. A total of 3,270 grullo Campolina horses were evaluated. To confirm the genetic possibility of having grullo animals, the coat color genotypes of parents were inferred by phenotype and compared with those of progeny. A total of 242 horses that were registered as grullos could not have this coat based on their parents' information. Possible explanations for incorrect registration are errors of paternity and in coat color identification. We suggest maintaining obligatory paternity testing and enhancing training in coat color identification.

Key words: *breeder association, Equus caballus, grullo, outline diagram*

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Coat color is a trait of added value in horses [4] and has been the target of artificial selection since domestication of the species [9]. Breed registries are important because they contain information used to explain the mating results of animals with different coat colors. Accurate coat color identification is important for legal purposes and health records. Coat color affects other phenotypes, such as behavior [6], racing performance [2] and genetic diseases [3, 7, 11–13]. In addition, this trait is of paramount importance for breeders who wish to produce foals with certain coats [14].

Among the coat color classifications, the grullo coat is a dun dilution on a black coat, usually with a dark head and primitive marks [14] (Fig. 1). It is also known as blue dun. Primitive marks have a camouflage function, indicating a possible vestige of existence prior to domestication [5]. The black coat in horses is due to the epistatic combination of three loci. The genotypes are B_ (locus B), aa (locus A) and dd (locus D). The grullo coat is defined by the presence of a D allele (genotype: B_ aa D_; see Table 1). The "d" allele, also known as the non-dun allele, is characterized by a 1.6 kb deletion in the noncoding region of the *TBX3* gene [5].

The Campolina breed is an autochthonous horse breed in Brazil. It is a gaited saddle horse [1, 8] of large size, which is the result of phenotypic selection for increased withers height. The grullo coat is found in slightly less than 8% of animals born in the last decade [10]. However, an increase of about 270% compared with the previous decade was reported [10], indicating the occurrence of artificial selection for this coat color.

The grullo coat is found less frequently than other coat colors, but its frequency is increasing. This lower frequency may cause confusion with other dilutions of black coat color, among others. The aims of this study were to evaluate the existence of inconsistencies in grullo coat color registration in Campolina horses and to explain their possible causes.

This case study uses breeder association registration data. The data used were kindly provided by the Brazilian Association of Campolina Horse Breeders (ABCCC; Portuguese acronym). No animals were used in experiments, so no ethical statement is needed. There were 3,871 grullo animals registered with the association between 1970 and 2016. After excluding records for animals with uncertain coat colors for parents, 3,270 grullo animals remained, and these animals were used for the study and for coat color identification of their respective parents. Based on a pedigree analysis (sire, dam and offspring) of the breed registry, the genotypes of the parents for coat color were inferred using their phenotypes and the phenotype of the

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Fig. 1. Grullo Campolina mare named *Inédita* from Luanda ranch (Salvador, Bahia, Brazil) (photo, Paulo Rocha; owner, Paulo Rocha).

Table 1. Genotypes of the most frequent coat colors in horses[a] and their grullo progeny number[b]

Coat color	Locus B	Locus A	Locus D	Black	Chestnut	Bay	Dun	Grullo	Roan	Grey
Black	B_	aa	dd	**27**						
Chestnut[c]	bb	_ _	dd	**69**	**62**					
Bay[d]	B_	A_	dd	**10**	**59**	**15**				
Dun	B_	A_	D_	229	536	282	722			
Grullo	B_	aa	D_	147	196	112	518	121		
Roan[e]	_ _	_ _	_ _	0	3	3	9	0	0	
Grey[f]	B_	_ _	_ _	8	26	21	72	20	3	0

[a]Light and dark variations caused by other dilution genes were not considered in coat color definitions. The tobiano coat pattern was disregarded, as it is caused by an epistatic gene and does not affect the background color. [b]Inconsitencies are indicated by bold numbers. [c]Red chestnut and grey-chestnut were also grouped here. [d]Seal-brown was also grouped here. [e]Roan is a white hair pattern that may occur in any background coat (genotype: R_). [f]Grey is a white hair pattern that may occur in any background coat (genotype: G_). Herein, the genotype B_ was considered to be grey, as grey-chestnut was differentiated in a studbook annotation and grouped with "chestnut".

grullo offspring. The genotypes of the most frequent coat colors are shown in Table 1. The predicted genotypes of the parents were compared with the predicted genotype of the grullo descendant. The parental genotypes should contain alleles that are compatible with the genotype alleles of the grullo offspring. Comparisons were made, and inconsistencies were recorded.

Of the 3,270 grullo animals evaluated, it was not possible for 242 of the animals registered as grullo (7.4%) to actually be grullo according to the coat colors of their parents. This inconsistency was due to the genetic incompatibility of the alleles that composed the coat color genotypes of the sires, dams and offspring. So, it was considered that the coat colors of the parents or offspring were recorded incorrectly.

Incorrect parentage is a likely explanation for the observed discrepancies. The distribution of the 242 inconsistent records was analyzed and compared between before and after 2007, since the breeder association started to require paternity testing for registration in that year. One hundred sixty-nine animals (69.9%) were born before 2007,

and 73 (30.1%) were born after 2007. The mean number of inconsistent records was 4.6 per year before 2007, and it was 8.1 per year after 2007.

The inconsistency was due to the difference in alleles of the grullo genotype (offspring) from those of the parent(s) genotype(s). The numbers of inconsistencies, according to parental coat color, are shown in Table 1. There were some grullo animals whose parents both had chestnut coat colors. This is impossible, as grullo animals are B_ at locus B and chestnut animals are bb (Table 1). Parents with black, bay, or chestnut coat colors in any combination are other examples of inconsistencies. For all these coat colors, the genotype at locus D is dd. A grullo animal must be D_. Hence, one of the parents of a grullo animal must carry the D allele (Table 1). Mendes *et al*. observed similar inconsistencies for other coat colors too [10].

Analysis of the inconsistent records before and after 2007 showed a lower incidence (~30%) after the implementation of obligatory paternity testing for registration. However, the mean number of inconsistent records per year was higher after 2007. These findings suggest that errors in pedigree data resulting from the lack of paternity testing may have caused inconsistencies before 2007 but that they are not the only cause of erroneous records, as errors continued to exist (with higher annual mean numbers).

Another possible reason for inconsistencies is errors in the identification of the grullo coat color. Technicians may have misidentified the coat color during completion of the outline diagram for registration. The grullo coat color is difficult to identify and may be easily confused with other coat color dilutions.

About 23.8% (n=722) of the grullo horses had both parents registered as duns, which is the most frequent mating (Table 1). Since dun is the prevalent coat color in Campolina horses [10], this agrees with practical knowledge indicating that grullo animals are frequently born from dun animals.

The coat color of a horse is an important factor for breeders, since it adds value to the animal and is intimately associated with predilections. Erroneous records of this phenotype are still observed. The present case study analyzed the grullo coat color of Campolina horses and demonstrated inconsistencies. Maintenance of obligatory paternity testing for registration with the breed association is essential to reduce the number of animals mistakenly registered as grullo. In addition, enhanced training of technicians in the identification of grullo coat color is recommended. Inconsistencies in coat registration could be remedied completely if genetic tests for coat color were carried out; however, the costs of such tests make this difficult to implement in practice.

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References

1. de Sousa, A.S., de Jesus, I.I.C., Araújo Oliveira, C.A., Costa, R.B., and de Godoi, F.N. 2018. How is the morphometry of stallions and mares show-winning and non-winning campolina brazilian breed with batida and picada gaits? *J. Equine Vet. Sci.* **64**: 34–40. [Medline] [CrossRef]
2. Jäderkvist Fegraeus, K., Velie, B.D., Axelsson, J., Ang, R., Hamilton, N.A., Andersson, L., Meadows, J.R.S., and Lindgren, G. 2018. A potential regulatory region near the EDN3 gene may control both harness racing performance and coat color variation in horses. *Physiol. Rep.* **6**: e13700. [Medline] [CrossRef]
3. Henkel, J., Lafayette, C., Brooks, S.A., Martin, K., Patterson-Rosa, L., Cook, D., Jagannathan, V., and Leeb, T. 2019. Whole-genome sequencing reveals a large deletion in the *MITF* gene in horses with white spotted coat colour and increased risk of deafness. *Anim. Genet.* **50**: 172–174. [Medline] [CrossRef]
4. Hennessy, K.D., Quinn, K.M., and Murphy, J. 2008. Producer or purchaser: different expectations may lead to equine wastage and welfare concerns. *J. Appl. Anim. Welf. Sci.* **11**: 232–235. [Medline] [CrossRef]
5. Imsland, F., McGowan, K., Rubin, C.J., Henegar, C., Sundström, E., Berglund, J., Schwochow, D., Gustafson, U., Imsland, P., Lindblad-Toh, K., Lindgren, G., Mikko, S., Millon, L., Wade, C., Schubert, M., Orlando, L., Penedo, M.C., Barsh, G.S., and Andersson, L. 2016. Regulatory mutations in *TBX3* disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses. *Nat. Genet.* **48**: 152–158. [Medline] [CrossRef]
6. Jacobs, L.N., Staiger, E.A., Albright, J.D., and Brooks, S.A. 2016. The *MC1R* and ASIP coat color loci may impact behavior in the horse. *J. Hered.* **107**: 214–219. [Medline] [CrossRef]
7. Johansson, M.K., Jäderkvist Fegraeus, K., Lindgren, G., and Ekesten, B. 2017. The refractive state of the eye in Icelandic horses with the Silver mutation. *BMC Vet. Res.* **13**: 153. [Medline] [CrossRef]
8. Lucena, J.E.C., Vianna, S.A.B., Berbari-Neto, F., Sales-Filho, R.L.M., and Diniz, W.J.S. 2016. Caracterização morfométrica de fêmeas, garanhões e castrados da raça Campolina baseada em índices. *Arq. Bras. Med. Vet. Zootec.* **68**: 431–438. [CrossRef]
9. Ludwig, A., Pruvost, M., Reissmann, M., Benecke, N.,

Brockmann, G.A., Castaños, P., Cieslak, M., Lippold, S., Llorente, L., Malaspinas, A.S., Slatkin, M., and Hofreiter, M. 2009. Coat color variation at the beginning of horse domestication. *Science* **324**: 485–485. [Medline] [CrossRef]

10. Mendes, L.J., Pereira, L.F.L., Wenceslau, R.R., Costa, M.D., Jayme, D.G., Maia, H.G.O., Teixeira, G.L., and Oliveira, N.J.F. 2019. [Characterization of coat colors horses from the Campolina breed]. *Arq. Bras. Med. Vet. Zootec.* **71**: 1364–1374 (in Portuguese). [CrossRef]
11. Metallinos, D.L., Bowling, A.T., and Rine, J. 1998. A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: an equine version of Hirschsprung disease. *Mamm. Genome* **9**: 426–431. [Medline] [CrossRef]
12. Rosengren Pielberg, G., Golovko, A., Sundström, E., Curik, I., Lennartsson, J., Seltenhammer, M.H., Druml, T., Binns, M., Fitzsimmons, C., Lindgren, G., Sandberg, K., Baumung, R., Vetterlein, M., Strömberg, S., Grabherr, M., Wade, C., Lindblad-Toh, K., Pontén, F., Heldin, C.H., Sölkner, J., and Andersson, L. 2008. A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nat. Genet.* **40**: 1004–1009. [Medline] [CrossRef]
13. Sánchez, M.J., Azor, P.J., Molina, A., Parkin, T., Rivero, J.L.L., and Valera, M. 2017. Prevalence, risk factors and genetic parameters of cresty neck in Pura Raza Español horses. *Equine Vet. J.* **49**: 196–200. [Medline] [CrossRef]
14. Sponenberg, D.P., and Bellone, R. 2017. Equine Color Genetics, 4th ed., John Wiley & Sons, Hoboken.