SHORT COMMUNICATION

A lost Sorraia maternal lineage found in the Lusitano horse breed

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Summary

A common female founder individual of the Portuguese horse breeds Sorraia and Lusitano was found while conducting research on the variation of the Lusitano mitochondrial DNA lineages in relation to studbook information. We obtained 416-bp control region sequences from 16 descendents of a female Sorraia founder (Pomba) still represented in the living population of the Lusitano, according to the most recent edition of this breed's studbook. The same haplotype was found for all analysed samples and belongs to the haplogroup described by several authors as having predominantly Iberian, South American and North African haplotypes bringing new insights on the relationship between the Sorraia and the other Iberian breeds. This work illustrates how weak the boundary of breed establishment can be, especially at the same geographical region. Using the same founders in different breeds is surely one of the explanations to frequently shared haplotypes among recent breeds, resulting in a lack of consistency between mtDNA sequences and breeds and/or geographical regions.

Introduction

The Sorraia horse, one of the three Portuguese native horse breeds, was recovered by Ruy d'Andrade in 1937, from 12 founders: five males and seven females. This breed shares, along with the Przewalski, the extinct Tarpan, the Konik and the horses from the Norwegian fjords, characteristics considered primitive such as: yellow dun or mouse dun (grullo) colour, black dorsal stripe, zebra stripes on legs, and sometimes shoulder stripes and darker rings or stripes on the foreheads (cobwebbing) (Andrade 1926; Lusis 1942; Strachurska 1999). The Sorraia also is believed to be the ancestor of the Lusitano and Andalusian breeds and thus an important ancestor of the entire World's light saddle horses and also of the horses in the New World (Andrade 1926, 1937, 1945; Oom 1992; Oom & Cothran 1994).

Therefore, addressing the phylogenetic relationships of the Sorraia with other horse breeds had an increased interest in the past few years, and has been attempted in several publications concerning horse relationships and domestication (Jansen et al. 2002; Cozzi et al. 2004; Lopes et al. 2005; Royo et al. 2005; Luís et al. 2006).

In a work combining pedigree and mitochondrial DNA (mtDNA) information, Luís et al. (2002) demonstrated the existence of only two maternal lineages still represented in the living Sorraia horses, corresponding to two haplotypes that differ by three nucleotides. Other authors, using a different set of samples, also found only these two maternal haplotypes (Jansen et al. 2002; Lopes et al. 2005) and in many publications it has been emphasized that the Sorraia haplotypes do not cluster with the mtDNA haplotypes more frequently found in Iberian horses

(Jansen *et al.* 2002; Lopes *et al.* 2005; Royo *et al.* 2005; Luís *et al.* 2006).

While conducting research on the variation of the Lusitano horse mtDNA lineages compared with studbook data we found that there was a common female founder (Pomba) to both the Sorraia and the Lusitano breeds. This maternal lineage is still represented in the Lusitano breed, but is no longer represented in the Sorraia. In 1937, the female Pomba was selected by Ruy d'Andrade to be a Sorraia founder, because of its primitive characteristics. However, in 1970, during the third year of the Lusitano horse Studbook's establishment, two mares tracing back to Pomba before she was chosen as a founder of the Sorraia breed, were selected to be included in the Lusitano breed. Over the years these two mares contributed a total of 196 descendents to the Lusitano breed population.

Besides describing a lost Sorraia maternal lineage found to be still represented in the Lusitano pedigree records, the present work aims to provide more information about the mtDNA variation existing in the Sorraia female founders. The contribution of this maternal lineage to a new phylogenetic relationship approach between the Sorraia and other Iberian horse breeds is also addressed.

Materials and methods

According to the most recent edition of the Lusitano studbook there are 20 descendents of Pomba in the living population. We used DNA from 16 of these samples to obtain 416-bp control region sequences, following the procedure described in Luís *et al.* (2002). Using CLUSTAL W software (Thompson *et al.* 1994), this sequence was aligned with the other two remaining Sorraia haplotypes and also with the reference sample (Swedish horse – GenBank X79547) to seek for differences between them.

A median-joining network (Bandelt *et al.* 1999) was generated using NETWORK 4.1.0.8 software (available at http://www.fluxus-engineering.com) to check the positioning of Pomba haplotype in relation to two other Sorraia founders and other 148 Iberian haplotypes, available from GenBank, belonging to the following breeds: Andalusian, Asturcon, Caballo de Corro, Cartujano, Garrano Losino, Lusitano, Marismeño and Potoka.

Results and discussion

In some works previously performed in other breeds, namely the Lipizzan and the Thoroughbred, some disagreement was found between maternal lines and haplotypes (Kavar *et al.* 1999, 2002; Hill *et al.* 2002) revealing the existence of pedigree errors.

We did not find any disagreement within the data set analysed as all samples sequenced here revealed the same haplotype. Therefore, we are confident that this haplotype represents the maternal lineage of Pomba (nucleotide sequence deposited in GenBank with accession number DQ339575). Moreover, we are sure about the paternity assignment of the individuals as parentage testing is compulsory in the Lusitano and Sorraia breeds since 1987. The results from the alignment are shown in Table 1 with the indication of the nucleotide substitutions relative to the reference sample. The Pomba sequence has seven nucleotide differences from the reference sample and differs from eight to nine nucleotides from the Sorraia lineages Cigana and Azambuja respectively.

The Pomba haplotype was found in another Lusitano maternal lineage not directly related to her, with a frequency of only 0.014 among all the lineages analysed by Lopes *et al.* (2005). Despite the low frequency it belongs to the mtDNA haplogroup with a CCG sequence motif (nucleotide positions 15494–495–496), designated haplogroup D by Jansen *et al.* (2002) (Figure 1), where high frequency of Iberian samples have been found in several works: 42% (Royo *et al.* 2005); 55% (Lopes *et al.* 2005); and 44% (Luís *et al.* 2006). Thus, this lost haplotype in the Sorraia that was found in the Lusitano provides the

Table 1 Sorraia horse haplotype sequences and nucleotide substitutions among the control region sequences, when compared with the reference sample GenBank X79547

	Position of nucleotide substitutions relative to GenBank no.X79547 (15411–15770)												
Haplotype	15494	15495	15496	15534	15542	15585	15597	15602	15635	15649	15666	15703	15720
X79547	T	T	A	C	C	G	A	C	C	A	G	T	G
Azambuja AF447764	.	C	.	.	T	.	G	T	T	.	A	C	A
Cigana AF447765	.	C	.	.	T	A	G	T	.	.	A	.	A
Pomba DQ339575	C	C	G	T	.	.	.	T	.	G	.	.	A

The dots indicate concordance with the reference sample. Azambuja, Cigana and Pomba are the three Sorraia maternal lines analysed.
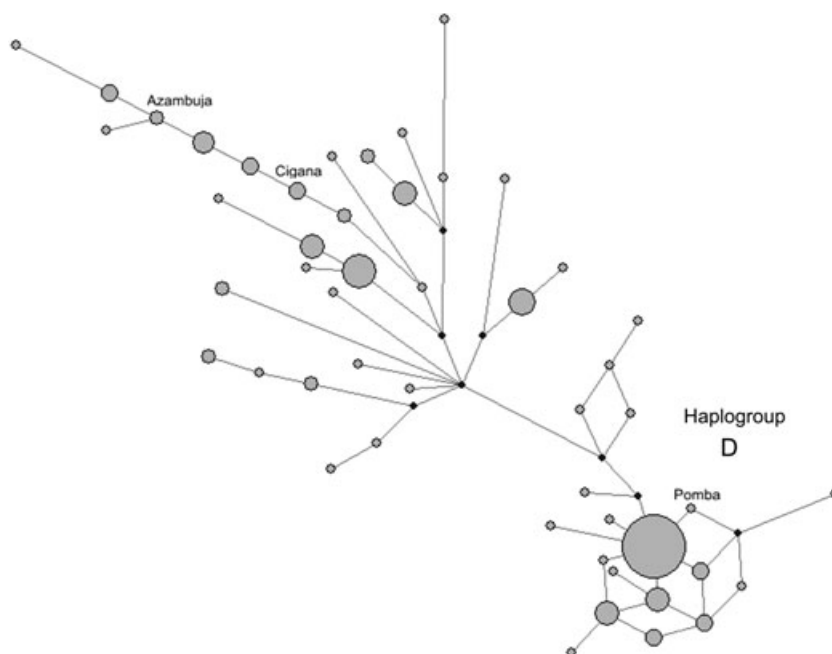
Figure 1 Median-joining network relating the mitochondrial DNA D-loop sequences of Azambuja, Cigana and Pomba with sequences from additional 148 Iberian sequences. Haplogroup D is indicated.

link to the relationship between the Sorraia and the other Iberian breeds that was not seen from the other two haplotypes. This new discovery provides more information about the mtDNA diversity that was present in the Sorraia female founders, information that can be useful when trying to infer the importance of this peculiar breed in the Iberian Peninsula and to worldwide mtDNA diversity.

Previous analyses of large sets of horse mtDNA sequence revealed great heterogeneity in horse maternal lines within breeds and extensive sharing of haplotypes among breeds (Vilà *et al.* 2001; Hill *et al.* 2002; Jansen *et al.* 2002; Bruford *et al.* 2003), supporting the statement made by Lister *et al.* (1998) that haplotypes and breeds are not congruent. The use of the same female founder for the Sorraia and Lusitano illustrates how weak the boundary of breed establishment can be, especially within the same geographical region. The inclusion of different animals belonging to the same maternal lineage in the Stud Books of more than one breed is one of the processes that have contributed to the extensive sharing of haplotypes among breeds. This work reinforces an idea defended by Arthur Mourant that '… the actual breeds are no more than temporary integrations in the constant process of admixture that marks the history of all living species' (Sykes 2003). Therefore, to better understand the modern horse evolution and its relationships, we should be cautious when examining relationships with the breed as the operational unit, as no direct inference can be made about the origin of an individual or breed from the sharing of mtDNA sequences. Historical records and other well-defined breed's characters (morphological, physiological, genetic and cultural) can be of great relevance in the analysis of horse breed phylogeny and evolution.

Acknowledgements

References

Andrade R.d' (1926) *Apontamentos Para um Estudo Sobre a Origem e Domesticação do Cavalo na Península Ibérica*. Aproximações, Centro Tipográfico Colonial, Lisboa, Portugal.

Andrade R.d' (1937) Les chevaux du Sorraia. In: *Comptes Rendues XII Congrès International de Zoologie*. Lisbonne pp. 2368–2370.

Andrade R.d' (1945) O Cavalo do Sorraia. *Bol. Pecuário*, **XIII**, 1–13.

Bandelt H.-J., Forster P., Röhl A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, **16**, 37–48.

Bruford M.W., Bradley D.G., Luikart G. (2003) Genetic analysis reveals complexity of livestock domestication. *Nat. Rev. Genet.*, **4**, 900–910.

Cozzi M.C., Strillacci M.G., Valiati P., Bighignoli B., Cancedda M., Zanotti M. (2004) Mitochondrial D-loop sequence variation among Italian horse breeds. *Genet. Sel. Evol.*, **26**, 663–672.

Hill E.W., Bradley D.G., Al-Barody M., Ertugrul O., Splan R.K., Zakharov I., Cunningham E.P. (2002) History and integrity of thoroughbred dam lines revealed in equine mtDNA variation. *Anim. Genet.*, **33**, 287–294.

Jansen T., Forster P., Levine M.A., Oelke H., Hurles M., Renfrew C., Weber J., Olek K. (2002) Mitochondrial DNA and the origins of the domestic horse. *Proc. Natl Acad. Sci. USA*, **99**, 10905–10910.

Kavar T., Habe F., Brem G., Dovc P. (1999) Mitochondrial D-loop sequence variation among the 16 maternal lines of the Lipizzan horse breed. *Anim. Genet.*, **30**, 423–430.

Kavar T., Brem G., Habe F., Sölkner J., Dovc P. (2002) History of Lipizzan horse maternal lines as revealed by mtDNA analysis. *Genet. Sel. Evol.*, **34**, 635–648.

Lister A.M., Kadwell M., Kaagan L.M., Jordan W.C., Richards M.B., Stanley H.F. (1998) Ancient and modern DNA in a study of horse domestication. *Anc. Biomol.*, **2**, 267–280.

Lopes M.S., Mendonça D., Cymbron T., Valera M., Costa-Ferreira J., da Câmara Machado A. (2005) The Lusitano horse maternal lineage based on mitochondrial D-loop sequence variation. *Anim. Genet.*, **36**, 196–202.

Luís C., Bastos-Silveira C., Cothran E.G., Oom M.M. (2002) Variation in the mitochondrial control region sequence between the two maternal lines of the Sorraia horse breed. *Genet. Mol. Biol.*, **25**, 309–311.

Luís C., Bastos-Silveira C., Cothran E.G., Oom M.M. (2006) Iberian origins of New World horses. *J. Hered.*, **97**, 107–113.

Lusis J.A. (1942) Striping patterns in domestic horses. *Genética*, **23**, 31–60.

Oom M.M. (1992) O cavalo Lusitano. Uma raça em recuperação. PhD thesis. University of Lisbon, Lisbon, Portugal.

Oom M.M., Cothran E.G. (1994) The genetic variation of an endangered breed: the Sorraia horse. *Anim. Genet.*, **27**, 35.

Royo L.J., Álvarez I., Beja-Pereira A., Molina A., Fernández I., Jordana J., Gómez E., Gutiérrez J.P., Goyache F. (2005) The origins of the Iberian horses assessed via mitochondrial DNA. *J. Hered.*, **96**, 663–669.

Strachurska A.M. (1999) Inheritance of primitive markings in horses. *J. Anim. Breed. Genet.*, **116**, 29–38.

Sykes B. (2003) *Sete Filhas de Eva*. Editorial Notícias, Lisboa, Portugal.

Thompson J.D., Higgins D.G., Gibson T.J. (1994) CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.

Vilà C., Leonard J.A., Götherstöm A., Marklund S., Sandberg K., Lindén K., Wayne R.K., Ellegren H. (2001) Widespread origin of domestic horse lineages. *Science*, **291**, 474–477.