Genetic origin of donkeys in Brazil

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Research Article

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Abstract

The genetic groups of native donkeys in Brazil are characterized by adaptation to the local environment. However, the donkey population in the country is declining, mainly because of agricultural mechanization and transportation that has led to the abandonment and the consequent indiscriminate slaughter of these animals. There are three local genetic groups of distinct geographic and temporal formation. However, analyses of their origin, phylogenetic relationship, and population structure are scarce. Within this context, molecular markers such as the mitochondrial control region (D-loop) are useful for these analyses. This study aimed to evaluate the variation and origin of maternal lineages of groups of naturalized donkeys in Brazil (Brazilian, Nordestino and Pêga). We detected five mitochondrial haplotypes with 18 polymorphic sites, two of them exclusively found in the Nordestino donkey. This group is more distant from the other groups. Phylogenetic analysis indicates maternal contributions of two clades (Nubian and Somali) to the formation of the genetic groups of donkeys, a fact that explains the high diversity, structure and distances of the groups. It was firstly reported here. This analysis contributes production and conservation of native donkey breeds. It also gives clues about the formation of the Iberian breeds from which Brazilian donkeys originated.

Introduction

Donkeys (*Equus asinus*) were first introduced in Brazil around 1534 during the period of Portuguese colonization (Mariante and Cavalcante 2006). Currently, the country harbors three local genetic groups of donkeys: Brazilian donkey, Nordestino donkey, and Pêga donkey (Figure S1). Alves et al. (2021) found wide intra- and inter-breed genetic variability and a strong genetic structure, indicating a high differentiation between the local groups of the country.

The most threatened genetic group (ecotype) is the Nordestino donkey. This group originated in the northeastern region of the Brazil and is characterized by adaptation to the adverse conditions of the local semi-arid climate (for example, hooves adapted to dry soil) and a small size, a feature that results in lower maintenance requirements. The Nordestino donkey is commonly used for transportation and traction and as a saddle animal associated with family farming. It has been suffering intensely from the indiscriminate slaughter for the production of a gelatin-based traditional medicine, called eijao, and has been officially under the protection of nongovernmental organizations (McManus et al. 2010; Carneiro et al. 2018; Bittencourt et al. 2021; Brandão et al. 2021).

The Pêga donkey is the only genetic group identified as a breed with a genealogy record in an association in Brazil. Originating in the state of Minas Gerais, it is used for the production of gaited animals for competitions, in addition to the production of gaited mules (ABCJPêga 2022). The Brazilian donkey has a more recent origin and is located in the state of São Paulo. It originated from crosses between animals brought from Italy (McManus et al. 2010; Carneiro et al. 2018). Brazilian donkey is known for his aptitude for saddle, transportation of goods, traction, and production of mules (McManus et al. 2010).

The donkey population in Brazil has decreased dramatically in recent years. The estimated population was 886,506 animals in 2020, with a decrease of almost 500,000 animals over 24 years (https://www.fao.org/faostat/en/#data/QCL/). Several factors may have contributed to this decline, particularly their reduced use due to agricultural mechanization and abandonment associated with indiscriminate slaughter (Carneiro et al. 2018).

It is known that the origin and domestication of donkeys occurred in Northwest Africa in two distinct clades: clade I whose representative is the Nubian wild ass (*Equus africanus africanus*) and clade II whose representative is the Somali wild ass (*Equus africanus somaliensis*) (Beja-Pereira et al. 2004). Both clades influenced the formation of European breeds and clade II also influenced the formation of Asian breeds (Xia et al. 2019). However, little is known about the origin of the local donkeys in Brazil. Alves et al. (2021) suggested an origin from different clades, given the differentiation and structure detected. Identification of the genetic origin contributes to the understanding of the process of historical formation of genetic groups, as well as to the definition of conservation strategies. Therefore, this study aimed to evaluate the phylogenetic relationship between the local groups of donkeys in Brazil that suffered a population decline, and to identify the clades of origin based on the mitochondrial D-loop region.

Materials And Methods

Samples of hair follicles from native donkeys (Brazilian donkey: 10 samples; Nordestino donkey: 10 samples; Pêga donkey: 10 samples) were studied (Table S1). The 524-bp mitochondrial DNA D-loop sequences of the present study were deposited in GenBank (accession numbers OM416433 - OM416462).

Sample collection

We collected biological samples (hair follicles) from 30 donkeys (Equus asinus) of three Brazilian breeds: Brazilian donkey (10 samples); Nordestino donkey (10 samples), and Pêga donkey (10 samples) (Figure S1). For phylogenetic inferences, we selected the species Equus caballus (NC_001640.1; Xu and Arnason 1994) as outlier group.

Collection of molecular data

Total DNA was extracted from the hair follicle samples using the DNA NucleoSpin® Tissue kit (Macherey-Nagel). The DNA samples of the Brazilian donkey were kindly provided by the Brazilian Agricultural Research Corporation (Embrapa) through a Material Transfer Agreement (23066.043532/2019-91). The PCR assays were conducted in a Veriti 96 Well Thermal Cycler (Applied Biosystems) in a final volume of 25 µl containing 2.0 µl DNA (9 to 15 ng), 0.5 µl of each primer (10 pmol/µl), 6.6 µl of Taq mix (dNTP, buffer, $MgCl_2$ and Taq polymerase), and 15.4 µl Milli-Q water.

The primers described by Lopes et al. (2005) were used for amplification and sequencing of fragments of a mitochondrial locus, control region (displacement loop, D-loop) (F: CTGGTCTTGTAAACC and R: ACAGTTATGTGTGAGCATGG). The sequences were amplified between positions 15351 and 15949 bp. Donkey sequences available in GenBank were added, as well as sequences of *Equus caballus* as outlier group, *Equus africanus* of clade I, and *Equus africanus somaliensis* of clade II (Table S1).

The amplification conditions were one cycle of initial denaturation at 94^{o}C for 5 min, followed by 35 cycles at 94^{o}C for 1 min, 54^{o}C for 1 min and 72^{o}C for 1 min, and a final extension at 72^{o}C for 5 min. The PCR products were purified using precipitation with 20% polyethylene glycol (Sambrook et al. 1989) and sequenced in an ABI PRISM 3500 automatic sequencer (Applied Biosystems). All sequences generated in this study were deposited in GenBank under the following accession numbers: Brazilian donkey (OM416453 - OM416462); Nordestino donkey (OM416433 - OM416442); Pêga donkey (OM416443 - OM416452).

The BioEdit v.7.0.9.0 software (Hall 1999) was used to edit the sequences. The sequences were aligned using the ClustalW Multiple alignment algorithm implemented in BioEdit (Hall 1999; Thompson et al. 2002) and the CLUSTAL algorithm in MEGA 5 (Tamura et al. 2011). The final alignment length was 525 bp.

Phylogenetic reconstruction, population structure and genetic diversity

After editing, the sequences were used for analysis of the number of haplotypes (H), haplotype diversity (*Hd*), nucleotide diversity (π), molecular analysis of variance (AMOVA), haplotype structure, and phylogenetic reconstruction using Bayesian inference and the maximum likelihood method. The following softwares were used for these analyses: DnaSP (Librado and Rozas 2009), PopART (Population Analysis with Reticulate Trees) (Leigh and Bryant 2015), Arlequin, MrBayes v. 3.2.6 (Ronquist et al. 2012), and RAxML-HPC v.8.2.12 (Stamatakis 2014), respectively. All analyses were performed remotely through the CIPRES Science Gateway 3.3 (http://www.phylo.org/index.php/portal/) (Miller et al. 2011). The evolutionary model HKY + 1 was calculated by Bayesian inference using the Kakusan4 software (Tanab, 2011).

For Bayesian inference, we performed two independent runs of 10 million iterations, with four Markov Monte Carlo chains (MCMC), sampling a tree every 10,000 iterations. Stationarity and convergence of the runs (effective sample size - ESS > 200) were evaluated with Tracer v.1.6 (http://beast.bio.ed.ac.uk/Tracer). The potential scale reduction factor was also used to check convergence of the chain and burn-in (Gelman and Rubin 1992). For maximum likelihood estimation, we used 1000 bootstrap replicates.

Results

Specifically in the three genetic groups of donkeys, 18 polymorphic sites were identified in the mitochondrial DNA control region (524 bp) of the 30 individuals. The 18 sites were parsimony informative

and none of them carried singletons (Table S2), generating five haplotypes (H1-H5) with haplotype (Hd =0.740) and nucleotide (π=0.01477) diversities.

Two of the five haplotypes identified were exclusive to the Nordestino donkey and one to the Pêga donkey. The predominant haplotypes were H1 and H4. The H1 haplotype was found in seven individuals, five belonging to the Pêga donkey and two to the Nordestino donkey. The H4 haplotype was present in 13 individuals, three of the Pêga donkey as well as all the individuals of the Brazilian donkey (Figure S2, Table S3).

The topologies recovered by Bayesian inference and maximum likelihood estimation based on the dataset were similar to other clusters of the species. Bayesian inference (Figure 1) rooted in *Equus caballus* revealed two main branches. Most samples (80%) of the Nordestino donkey were allocated along with the Nubian wild ass (*Equus africanus africanus*), clade I. In contrast, all samples (100%) of the Brazilian donkey and most samples (80%) of the Pêga donkey were assigned to the cluster of the Somali wild ass (*Equus africanus somaliensis*), clade II.

Discussion

We identified, for the first time, that the three donkey genetic groups from Brazil were influenced by both domestication clades of the species. Both ass clades are known to have influenced the formation of European breeds (Xia et al. 2019). The local breeds in Brazil originated during Portuguese/Iberian colonization. Thus, the local groups of the country have representatives of both clades (like the European breeds). Although there are no similar studies available for Portuguese and Spanish donkey breeds, the contribution of Iberian breeds may have been influenced by the Muslim invasion in the Middle Ages (which differs from the rest of Europe). This fact is also reflected in the genetic composition of Peruvian donkeys (Xia et al. 2019) whose genetic groups are also from both clades.

The colonization of Brazil began in the northeastern region (origin of the Nordestino donkey) (clade I) with the exploration of sugar cane, and then moved towards the southeast (origin of the Pêga donkey) (clade II) with the exploration of gold. We therefore believe that the formation of the local breeds from Brazil occurred at different times and has different origins of Portuguese/Iberian donkey breeds since they belong to different clades.

The formation of the Brazilian donkey occurred more recently and its history is better understood since it is known to be the result of mating between breeds of Italian origin. This can be easily observed in Figure S3, in which the Brazilian donkey is close to Italian breeds (clade II). Figure S3 shows a Bayesian phylogenetic tree constructed based not only on the sequences of wild donkeys (as in Figure 1) but also of domestic donkeys from other countries.

Differently from the results, herein, obtained, Xia et al. (2019) reported the Nordestino donkey belonging to clade II, while we observed a greater participation of individuals in clade I. This finding may be due to a sampling effect. The unique genetic group from Brazil studied by Xia et al. (2019) was the Nordsetino

donkey. Moreover, Xia et al. (2019) suggested the great differences between Peruvian donkey breeds and Nordestino donkey are due to the fact that Brazil imported donkeys of European origin from the United States. We do not believe this to be the most adequate explanation for the existing distances but rather the contribution of the two clades to the formation of the Iberian donkey breeds.

The results of AMOVA show significant variation between the donkey breeds (Table S4), as also reported by Alves et al. (2021) using another mitochondrial DNA region (D-loop).

The local donkey breeds in Brazil have different clades of origin, indicating different maternal contributions to the formation of these breeds and, possibly, to the formation of breeds from the Iberian Peninsula. A better understanding of the history of formation of these genetic groups, their high genetic variability and stratification will contribute to the conservation and production of these animals. Moreover, it provides genetic information that can be used as the first criterion to identify ecotypes (Nordestino and Brazilian donkeys) as breeds.

Declarations

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Authors contribution The study conception and supervision were conducted by Gregório Miguel Ferreira de Camargo. Material preparation, data collection, analysis and first draft were performed by Jackeline Santos Alves. Data collection was performed by Chiara Albano de Araújo Oliveira. Financial support was provided by Pierre Barnabé Escodro, Luis Fernando Batista Pinto, Raphael Bermal Costa, and Gregório Miguel Ferreira de Camargo. All authors discussed the results, revised the manuscript and approved the final manuscript

Ethics approval The Ethics Committee on Animal Use of the Universidade Federal da Bahia, Salvador, Bahia, Brazil, approved the study (number 08/2019).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare no potential conflicts of interest.

Data availability GenBank accession numbers OM416433 and OM416462.

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References

1. Alves, J.S., Anjos, M.S., Bastos, M.S., Oliveira, L.S.M., Oliveira, I.P.P., Pinto, L.F.B., Oliveira, C.A.A., Costa, R.B., Camargo, G.M.F. 2021. Variability analyses of the maternal lineage of horses and donkeys. Gene, 769, 145231.
2. Associação Brasileira dos Criadores de Jumento Pêga. 2022. Available at: https://abcjpega.org.br/ (Accessed: 10 January 2022).
3. Beja-Pereira, A., England, P.R., Ferrand, N., Jordan, S., Bakhiet, A.O., Abdalla, M.A., Mashkour, M., Jordana, J., Taberlet, P., Luikart, G. 2004. African origins of the domestic donkey. Science, 304, 1781-1781.
4. Bittencourt, T.C.B.S.C., Silva, E.M., Oliveira, C.A.A. 2021. How much is a donkey worth? Brazilian Journal of Veterinary Research and Animal Science, 58, e174335.
5. Brandão, G.J., Medeiros, J.H., Barros, G.L., Pessoa, A.W.P., Zanella, A.J., Oliveira, C.A.A., Macgregor, E., Nunes, V.P. 2021. How much is a donkey worth? Brazilian Journal of Veterinary Research and Animal Science, 58, e174335.
6. Carneiro G.F., Lucena, J.E.C, Barros, L.O. 2018. The current situation and trend of the donkey industry in South America. Journal of Equine Veterinary Science 65, 106-110.
7. Gelman, A. and Rubim, D.B. 1992. Inference from iterative simulation using multiple sequences. Statistical Science.
8. Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser (Oxf), 41, 95-98.
9. Leigh, J., Bryant, D., Steel, M. 2015. PopART (Population Analysis with Reticulate Trees).
10. Librado, P. and Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25, 1451-1452.
11. Lopez, C.L., Alonso, R., Aluja, A.S.D. 2005. Study of the genetic origin of the Mexican creole donkey (*Equus asinus*) by means of the analysis of the D-loop region of mitochondrial DNA. Tropical Animal Health & Production, 37, 173–88.
12. Mariante A.S. and Cavalcante N. 2006. Animais do Descobrimento: raças domésticas da história do Brasil. Brasília: Embrapa Sede / Embrapa Recursos Genéticos e Biotecnologia, 274p.
13. McManus, C., Paiva, S., Louvandini, H., Melo, C., Seixas, L. 2010. Jumentos no Brasil. In INCT Informaçãos Genético-Sanitária da Pecuária, 10 pp.
14. Miller, M.A., Pfeiffer, W., Schwartz, T. 2011. The CIPRES science gateway: a community resource for phylogenetic analyses. In Proceedings of the TeraGrid Conference: extreme digital discovery.
15. Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu L., Suchard, M.A., Huelsenbeck, J.P. 2012. Mrbayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539-542.
16. Sambrook, J., Fritschi, E.F., Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor: Cold Spring Harbor Press.

17. Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30, 1312-1313.
18. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. Program MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. Molecular Biology & Evolution, 28, 2731-2739.
19. Tanabe, A.S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. Molecular Ecology Resources, 11, 914-921.
20. Thompson, J.D., Gibson, T.J., Higgins DG. Multiple Sequence Alignment Using ClustalW and ClustalX. Current Protocols Bioinf. 2002;2.3.1-2.3.22.
21. Xia, X., Yu, J., Zhao, X., Yao, Y., Zeng, L., Ahmed, Z., Shen, S., Dang, R., Lei, C. 2019. Genetic diversity and maternal origin of Northeast African and South American donkey populations. Animal Genetics, 50, 266-270.
22. Xu, X., Árnason, U. 1994. The complete mitochondrial DNA sequence of the horse, *Equus caballus*: Extensive heteroplasmy of the control region. Gene, 148, 357-362.

Figures

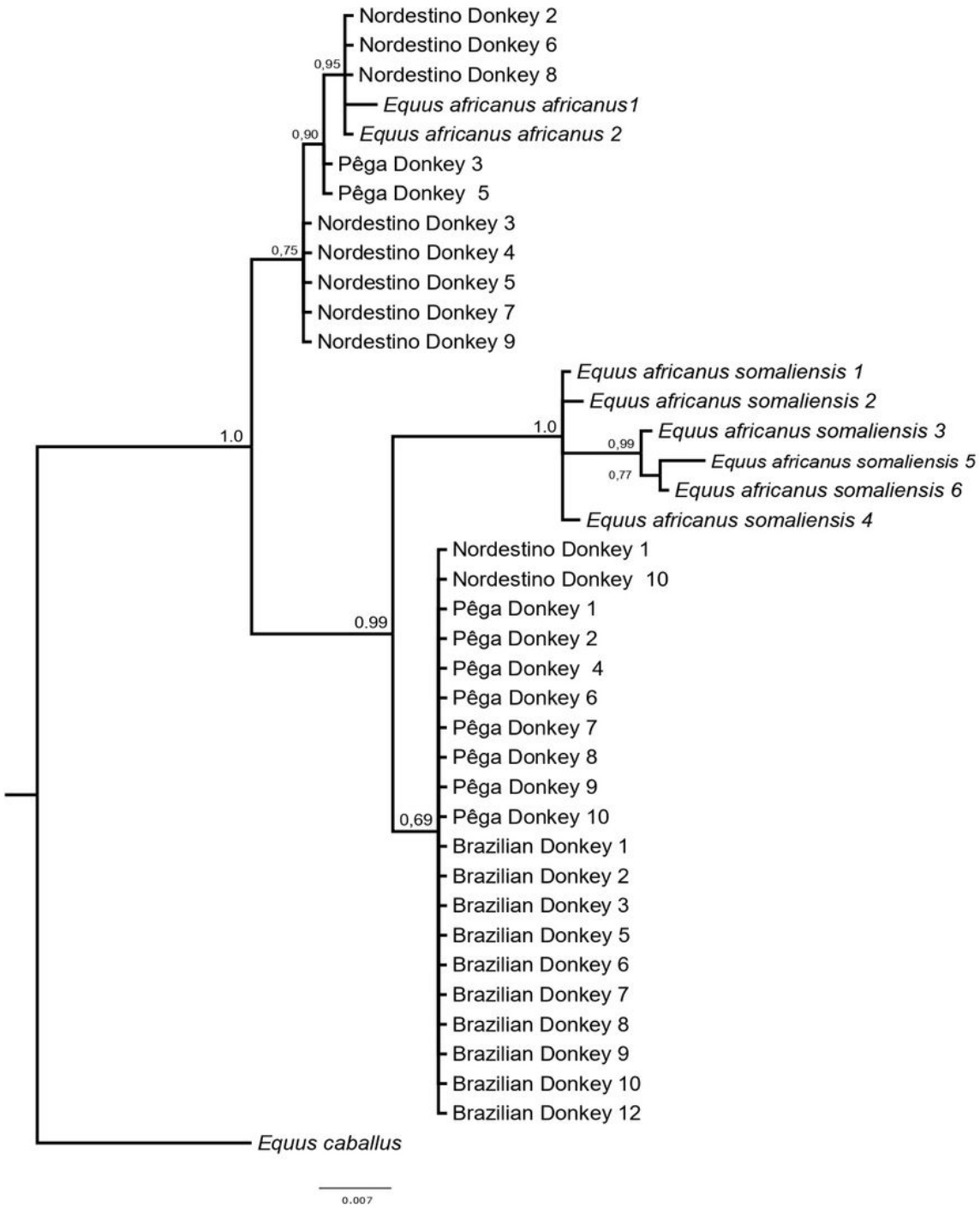

Figure 1

Topology recovered by Bayesian inference and maximum likelihood estimation

Supplementary Files

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