Genetic origin of donkeys in Brazil

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Abstract

Introduction 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.

Material and Methods This study aimed to evaluate the variation and origin of maternal lineages of groups of naturalized donkeys in Brazil (Brazilian, Nordestino, and Pêga).

Results and Discussion We detected five mitochondrial haplotypes with 19 polymorphic sites, two of them exclusively found in the Nordestino donkey; this group is in fact more distant from the others. 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, reported here for the first time.

Conclusion 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.

Keywords Conservation · D-loop · Native donkey · Local breed · Genetic variability

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. It originated in the northeastern region of the Brazil and is characterized by adaptation to the adverse conditions of the local semi-arid climate (e.g., hooves adapted to dry soil) and a small size, feature that results in lower maintenance requirements. The Nordestino donkey is commonly used for transportation, traction and as a saddle animal associated with family farming. It has been suffering intensely from the indiscriminate slaughter to produce a gelatin-based traditional medicine, called eijao, and it is 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, the Brazilian Association of Pêga Donkey Breeders (ABCJPêga, in Portuguese acronym). 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), and it 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 genetic groups, as well as to the definition of conservation strategies. According to the NJ tree (Figure S1), the positions of clades I and II were known. The results showed that clade I was divided into two sub-clades: Ia and Ib. Sub-clade Ia was divided into two sub-clades: Ia1 and Ia2. Sub-clade Ia1 was divided into two sub-clades: Ia1a and Ia1b. Sub-clade Ia2 was divided into two sub-clades: Ia2a and Ia2b. Sub-clade Ib was divided into two sub-clades: Ib1 and Ib2. Sub-clade Ib1 was divided into two sub-clades: Ib1a and Ib1b. Sub-clade Ib2 was divided into two sub-clades: Ib2a and Ib2b. The results showed that clade II was divided into two sub-clades: IIa and IIb. Sub-clade IIa was divided into two sub-clades: IIa1 and IIa2. Sub-clade IIa1 was divided into two sub-clades: IIa1a and IIa1b. Sub-clade IIa2 was divided into two sub-clades: IIa2a and IIa2b. Sub-clade IIb was divided into two sub-clades: IIb1 and IIb2. Sub-clade IIb1 was divided into two sub-clades: IIb1a and IIb1b. Sub-clade IIb2 was divided into two sub-clades: IIb2a and IIb2b.

Materials and methods

Sample collection

Biological samples (hair follicles) were collected from 30 donkeys (Equus asinus) of three native Brazilian breeds: Brazilian donkey (10 samples); Nordestino donkey (10 samples), and Pêga donkey (10 samples) (Figure S1, Table S1). For phylogenetic inferences, the species Equus caballus (NC_001640.1; Xu and Arnason 1994) were selected as an outlier group. It was used ten samples per group since a strong population structure was previously reported (Alves et al. 2021) and the aim was to identify the clade origin.

Molecular data collection and alignment

The DNA samples of the Brazilian donkey were kindly provided by the Brazilian Agricultural Research Corporation (Embrapa) through a Material Transfer Agreement (23,066.043532/2019–91). Total DNA was extracted from the hair follicle samples using the DNA NucleoSpin® Tissue kit (Macherey–Nagel).

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, MgCl₂, and Taq polymerase), and 15.4 μl Milli-Q water.

The primers described by Lopez et al. (2005) were used for amplification and sequencing of fragments of a mitochondrial locus, control region (displacement loop, D-loop) (F: CTGGTCTTGTAACCC and R: ACAGTTATGTGTGAGCATGG). The sequences were amplified between positions 15,351 and 15,949 bp. Donkey sequences available in GenBank were added, as well as sequences of Equus caballus as an outlier group, Equus africanus africanus of clade I, and Equus africanus somaliensis of clade II (Table 1, Table S2).

The amplification conditions were one cycle of initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min, and a final extension at 72 °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).

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), with the donkey reference (X97337.1; Xu et al. 1996). The final alignment length was 524 bp. All sequences generated in this study were deposited in GenBank (NCBI—National Center for Biotechnology Information) under the following accession numbers: Brazilian donkey (OM416453—OM416462); Nordestino donkey (OM416433—OM416442); Pêga donkey (OM416443—OM416452).

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 software were used for these analyses: DnaSP (Librado and Rozas 2009), PopART (Population Analysis with Reticulate Trees) (Leigh et al. 2015), Arlequin v.3.5.2.2 (Excoffier and Lischer 2010), 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 (Tanabe 2011).

For Bayesian inference, two independent runs of 10 million iterations, with four Markov Monte Carlo chains
(MCMC), sampling a tree every 10,000 iterations, were performed. 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, 1000 bootstrap replicates we used.

Results

Specifically in the three genetic groups of donkeys, 19 polymorphic sites were identified in the mitochondrial DNA control region (524 bp) of the 30 individuals. The sites were parsimony informative and one of them carried singletons, generating five haplotypes (H1–H5) with haplotype (Hd = 0.740) and nucleotide (π = 0.01477) diversities. The same parameters were also presented for each group separately (Table 2).

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 2).

The topologies recovered by Bayesian inference and maximum likelihood estimation based on the dataset were similar to other clusters of the species. Bayesian inference (Fig. 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, while 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

With this study was 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 having 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 still from both clades.

Brazil colonization began in the north-eastern 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. It is possible to 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.

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Table 2  Estimation of genetic variation of a D-loop gene fragment (mtDNA) of the Brazilian donkey breeds

| Breed              | n  | S     | SPI | SS | NH  | PH   | SH   | Hd ± s.d | π ± s.d | k   |
|--------------------|----|-------|-----|----|-----|------|------|---------|--------|-----|
| Brazilian donkey   | 10 | 0     | 0   | 0  | 1   | 0    | 1    | 0.00 ± 0.00 | 0.00 ± 0.00 | -   |
| Nordestino donkey  | 10 | 19    | 18  | 1  | 3   | 2    | 1    | 0.689 ± 0.104 | 0.01264 ± 0.00478 | 6.62 |
| Pêga donkey        | 10 | 18    | 17  | 1  | 3   | 1    | 2    | 0.689 ± 0.010 | 0.01192 ± 0.00487 | 6.24 |

Sample size (n), total polymorphic sites (S), parsimony informative (SPI), and singleton site (SS), number of haplotypes (NH), private haplotype (PH), shared haplotype (SH), haplotype diversity (hd), nucleotide diversity (π) with their standard deviations (s.d.), and average number of nucleotide differences (k) within and across the three populations.

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Fig. 1  Topology recovered by Bayesian inference and maximum likelihood estimation.
The formation of the Brazilian donkey occurred more recently, and its history is clear 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 Fig. 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 Nordsetino donkey are because the donkey from Brazil came from the USA. We do not believe this to be the most adequate explanation. The contribution of the two clades to the formation of the Iberian donkey breeds is a more suitable hypothesis.

AMOVA results show significant variation between the donkey breeds (Table S2), 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 their formation 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.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11250-022-03280-x.

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Author 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 Albanó de Araújo Oliveira. Financial support was provided by Pierre Barnabé Escodro, Luis Fernando Batista Pinto, Raphael Bernal Costa, and Gregório Miguel Ferreira de Camargo. All authors discussed the results, revised the manuscript, and approved the final manuscript.

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Data availability GenBank accession numbers from OM416433 to OM416462.

Declarations

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.

Conflict of interest The authors declare no competing interests.

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