Livestock Science 197 (2017) 1–7

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Contributions to diversity rather than basic measures of genetic diversity characterise the spreading of donkey throughout the American continent

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ARTICLE INFO

Keywords:
Contributions to diversity
Geographic patterns
Gene diversity
Allelic richness
Microsatellites

ABSTRACT

Donkey was introduced into the Americas soon after its discovery in the 15th century. However, there is no historical consensus on how they spread across the continent. In a previous study, two distinct genetic pools (Clusters A -Southern part - and B - Northern part of South America and Central America) were identified, with likely confluence in Colombia. The aim of this study was to evaluate whether the main genetic diversity parameters, such as gene diversity (GD) and allelic richness (k), or the relative contributions of various breeds to these parameters are useful indicators to give genetic support to historical information on putative routes of the spreading of donkeys across the American continent. In full agreement with historical sources suggesting that Greater Antilles were the first breeding nucleus, both total contributions to gene diversity (gGD_T) and to allelic richness ($C_T^{(k)}$) showed a higher ability to identify the "abundant centre" of the species on the Continent. Even though there are historical reports suggesting various entry points of the donkey into the continent (e.g. in Brazil), these parameters suggested that, in our dataset, the Cuban donkey population was the more likely representative of the first breeding nucleus of the species. Central and South American donkey populations in the surroundings of the Caribbean Gulf would more likely be early derivatives of Antillean donkey. The strong North-South genetic structure was confirmed for the American donkey metapopulation. Current analyses suggest that populations classified into Cluster A (South) are essentially a sample of the genetic background of Cluster B (North). The Andean route had the highest importance in the formation of the South American populations. The extinction of either population belonging to Cluster B could lead to a decrease in overall genetic diversity both at the gene diversity level (negative gGD_T values) and the allelic richness level (positive $C_T^{(k)}$ contributions). The opposite pattern is found for populations belonging to Cluster A. The extinction of the

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http://dx.doi.org/10.1016/j.livsci.2016.12.014
Received 8 June 2016; Received in revised form 20 December 2016; Accepted 30 December 2016

populations belonging to Cluster B would decrease the overall American donkey gene diversity in roughly 8% and would dramatically affect the number of alleles in the metapopulation (19.1%). However, the extinction of the donkey populations classified into Cluster A would increase overall gene diversity by 2.2%. Although, the genetic scenario of each individual population varies substantially, the joint conservation of the donkey populations classified into both Clusters A and B is highly advised.

1. Introduction

The introduction of domestic donkeys into America is well documented. After the initial establishment of founding populations in Greater Antilles the species was introduced into the mainland Continent through Mexico, to be spread northwards, and Panama to connect with commercial routes to Colombia, Venezuela and northern Brazil and southerly to Northern Andean countries such as Ecuador and Peru. From here, the species is expected to have been involved in the active trade routes between the Peruvian plateau and Pampas region (Brookshier, 1974; Laguna, 1991; Santos et al., 1992; Sponenberg, 1992; Rodero et al., 1992; Yanes, 2005; Delgado et al., 2010).

Recently, Jordana et al. (2016) analysed the American donkey population, together with Iberian and other Mediterranean donkey breeds, to ascertain their genetic structure and to identify the most likely ancestral donor populations. The authors reported the presence of two distinct genetic clusters (named A and B) in American donkey. Cluster A, formed by Southernmost American donkey populations, showed a very low genetic diversity probably subsequent to an older founder event and no significant influence of recent gene flow from Europe. Cluster B, mainly formed of donkey populations surrounding the Caribbean Gulf, showed higher polymorphism though it was not possible to reject the existence of modern gene flow from Iberian donkeys.

The ascertainment of those two genetic clusters was consistent with the historical information suggesting that the species moved from the initial Greater Antilles stock into the geographical areas surrounding the Caribbean Sea. Later on, a breeding nucleus was created in the Peruvian Plateau, from which Southernmost American donkey populations could have been formed (Laguna, 1991; Yanes, 2005). However, the indication of multiple different local genetic events due to different recent histories in the analysed populations prevented the ascertainment of the most likely routes for the spreading of the species throughout the continent (Jordana et al., 2016).

In the wild, in scenarios where gene sources and expansion patterns are known, gene diversity (expected heterozygosity; H_e) and allelic richness adjusted for sample size (rarefacted number of alleles per locus; $k_{(n)}$) have shown to be superior to other diversity measures to deal with the task of defining conservation priorities (Comps et al., 2001; Petit et al., 2003). Actually, those parameters have different sensitivity to stochastic processes (Eckert et al., 2008) and are able to differentiate between geographical areas acting as genetic sources (abundant centres), colonised zones and, furthermore, contact zones in which some variability parameters can show “artificially” increased values (Comps et al., 2001). From a practical point of view, both gene diversity and number of alleles per locus have the advantage of straightforward interpretation, because gene diversity illustrates the existence of balanced allelic frequencies in a population and allelic richness can characterise the degree of genetic uniqueness or distinctiveness of a population (Petit et al., 1998; Caballero and Toro, 2002). In a metapopulation both parameters can be decomposed into within- and between-population fractions (Petit et al., 1998; Caballero and Toro, 2002), therefore allowing accounting for recent local events affecting genetic signals in the populations studied. The aim of this research was to assess the usefulness of two methods for the estimation of genetic contributions to diversity, to ascertain if historical information on the routes of spreading of donkey across the American continent has genetic support. Furthermore, the potential genetic consequences of losing the populations assessed will be discussed.

2. Material and methods

2.1. Data available

The 350 American donkey DNA samples, obtained in 13 different countries (Mexico, Guatemala, Cuba, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay, Chile, Argentina, Uruguay and Brazil; Table 1), analysed in Jordana et al. (2016) using 14 microsatellites (VHL20, AHT4, HMS7, AHT5, HMS6, HTG10, HTG7, HMS2, HTG4, HTG6, HMS3, HTG15, HMS5 and ASB23) were available. The geographical location of sampled animals is shown in Fig. S1. Sampling strategies are described in detail in Jordana et al. (2016). DNA extraction and genotype scoring were carried out following Jordana et al. (2016) and Aranguren-Méndez et al. (2001). Following Jordana et al. (2016), for descriptive purposes samples were grouped when necessary into Cluster A (formed of Ecuador, Peru, Bolivia, Paraguay, Chile, Argentina and Uruguay samples) and Cluster B (formed of Mexico, Guatemala, Cuba, Venezuela, Colombia and Brazil samples).

2.2. Genetic diversity analyses

Statistical analyses were carried out using the software MolKin (current version v3.1; Gutiérrez et al., 2005). Parameters characterising genetic diversity, such as expected heterozygosity (H_e; Nei, 1987), heterozygote deficiency due to population inbreeding or subdivision (F_{IS}; Nei, 1987), raw and rarefacted ($k_{(n)}$; Hurlbert, 1971) average number of alleles per locus, and the between-population Nei's minimum distance (Nei, 1987) and molecular coancestry (Caballero and Toro, 2002) were computed. To avoid bias due to low and unequal sample sizes, parameters listed above, except for rarefacted allelic richness, were adjusted for sampling size following Cervantes et al. (2011) using as sample size the harmonic mean of the national donkey populations available (23). For the same purposes, statistical significance of the computed parameters was assessed by bootstrapping using 1000 samples and fitting sample size to 23 individuals per population. In turn, the rarefacted average number of alleles per locus was adjusted to 24 copies ($k_{(24)}$), which is twice the minimum number of individuals within a population with genotype known for all the microsatellites to allow a direct between-populations comparison of the results presented. See the MolKin User's Guide (freely available at http://www.ucm.es/info/prodanim/html/JP_Web.htm) for a detailed description of the methodologies used).

Using also MolKin, contributions to diversity were assessed following Caballero and Toro (2002) and Petit et al. (1998). Caballero and Toro (2002) proposed setting priorities for conservation using the maintenance of the maximum overall Nei's (1987) gene diversity (GD) in the preserved set of breeds as the criterion. Notice that this is equivalent to minimising the overall molecular coancestry ($\overline{f}$) because $GD = 1 - \overline{f}$. Therefore, the average GD of a given population depends on the within-subpopulation coancestry and its average distance relative to other subpopulations. This allowing the contributions to the total GD to be separated due to the within-breed diversity (f_{ii}) and the between-breed genetic distance. In this scenario, $GD_T = GD_W + GD_B$, where GD_T is the total contribution to GD, GD_W is the contribution to the within-breeds diversity and GD_B the contribution to the