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Genetic analysis of the Venezuelan Criollo horse

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ABSTRACT. Various horse populations in the Americas have an origin in Spain; they are remnants of the first livestock introduced to the continent early in the colonial period (16th and 17th centuries). We evaluated genetic variability within the Venezuelan Criollo horse and its relationship with other horse breeds. We observed high levels of genetic diversity within the Criollo breed. Significant population differentiation was observed between all South American breeds. The Venezuelan Criollo horse showed high levels of genetic diversity, and from a conservation standpoint, there is no immediate danger of losing variation unless there is a large drop in population size.

Key words: Microsatellites; South America; Horse; Venezuelan Criollo

INTRODUCTION

After the extinction of North and South American *Equus* species about 10,000 years ago, from causes still not completely understood (see Clutton-Brock, 1996), horses only returned to the American continent (the New World) with the second voyage of Christopher Columbus, in 1493. The horse populations increased during the subsequent Spanish and Portuguese colonization period (Bort, 2004; Primo, 2004). Historical records report the presence of around 70 horses on the first colony of La Espanola (Dominican Republic and Haiti) by the year 1503. Subsequently, horses were taken to Panama (1514), Mexico (1524), Brazil (1531), Peru (1532), Argentina (1535), and Florida (1538) (Digard, 1994). By 1553, there were some 10,000 free-roaming horses in the area of Queretaro (Mexico) that spread throughout North and South America (Clutton-Brock, 1992).

The Venezuelan Criollo is considered a local breed and its breeding began as part of the settlement of the city of Coro founded in 1526 by Santo Domingo's ruling Councilor, Juan de Ampíes. In 1528, the Welser governors, of German origin, were licensed by the King of Spain, Carlos V, to import horses and other stock from Hispaniola (Santo Domingo), San Juan (Puerto Rico), and Santiago (Cuba) to Venezuela. Although the majority of horses that went to Venezuela during those years were of Antillean origin, it is known that horses also came directly from Spain, brought by the Welsers or by colonizing settlers. Ambrosio de Alfinger, one of the German governors, departed from San Lucas de Barrarneda (Spain), with more than 80 horses on his way to Venezuela (Lacas, 1953). In 1545, Cristóbal Rodríguez, a colonizing settler of the Venezuelan flatlands (llanos), took ten mares and two Andalusian colts from Jerez de la Frontera, Spain. Therefore, the history of the Venezuelan horse has a deep Spanish influence, at least concerning its origins.

The Venezuelan Criollo breed is extremely well adapted to the local conditions and is dispersed throughout Venezuela, with the majority of the horses being used on large cattle breeding ranches. There is no studbook or census on the number of animals that belong to this breed. Animals from Apure, Aragua and Merida States are phenotypically similar and crossbreeding with other types of horses is rare because of the poor survival of crossbreds and adaptation to harsh conditions in the areas where the Venezuelan Criollo horse is used. The study of phenotypic traits, physiology, behavior, and disease-resistance of the Venezuelan Criollo horse was carried out by De Amas (1946). Recently, awareness of this horse breed has increased and conservation efforts, future breeding strategies and management plans are guided by a group at the Lisandro Alvarado Centro-Occidental University, Barquisimeto, Venezuela.

Genetic characterization of populations can be a useful tool in breed conservation and may have implications for future breeding strategies and management plans. Within the past decade, microsatellites have become the most popular genetic marker and have been successfully applied to parentage and relatedness testing in horses (e.g., Bowling et al., 1997), and to investigations of inter- and intrabreed variability in domestic and feral horse populations (e.g., Canon et al., 2000; Bjornstad et al., 2000; Juras et al., 2003; Aberle et al., 2004; Solis et al., 2005; Luís et. al., 2006; Plante et al., 2007).

In the present study, we estimated the genetic diversity of the Venezuelan Criollo horse and determined the amount of genetic differentiation compared to other South American breeds. Relationships among South American horse breeds and breeds from other parts of the world were analyzed by Luís et al. (2007). In this study, we focused on the comparison of

South American breeds with emphasis on the Venezuelan Criollo. We applied several different approaches to determine the distribution of genetic diversity, and this information can be helpful for an improved management strategy of the Venezuelan Criollo breed.

MATERIAL AND METHODS

Hair samples were collected from a total of 214 Venezuelan Criollo horses. Samples were collected from the Apure, Merida and Aragua States in Venezuela, accounting for 161, 42 and 11 individuals, respectively. All samples from South American and the two Iberian horse breeds, the Andalusian and the Sorraia (Table 1) were previously typed in our laboratory with the same 15 microsatellite panel. Relationships between South American horse breeds and breeds from other parts of the world were well documented by Luís et al. (2007). South American horse breeds belong to the same cluster (group h; Luís et al., 2007), and therefore, we focused only on relationships within this clade.

A total of 15 microsatellite loci (AHT4, AHT5, ASB2, ASB17, ASB23, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX33, and VHL20) were typed using the methods described by Juras et al. (2003).

Allelic frequencies, polymorphic information content (*PIC*) and average exclusion probability (*PE*) were calculated using the Cervus 2.0 software (Marshall et al., 1998). Genetic variability was measured by estimating observed (H_O), expected (H_E) and unbiased expected (UH_E) heterozygosities, effective number of alleles (N_E), and the total number of variants found in each population (N_A). The genetic differentiation between populations (Φ_{PT} and F_{ST}) and the variance components of microsatellite diversity within and between populations for all pairs of populations were analyzed using analysis of molecular variance (AMOVA) with permutations set to 999 in the GENALEX 6 software (Paekall and Smouse, 2006). Departure from Hardy-Weinberg equilibrium was tested using GENEPOP 4.0 (Raymond and Rousset, 1995). To account for multiple simultaneous tests, the sequential Bonferoni procedure was applied (Rice, 1989). F-statistics and gene differentiation coefficient (G_{ST}) were calculated using GENETIX 4.02 (Belkhir et al., 2004). The chord distance (D_C) (Cavalli-Sforza and Edwards, 1967) and Nei's D_A distance (Nei et al., 1983) were calculated with the POPULATIONS 1.2.28. program (written by Langella, 1999). Genetic distance based on the proportion of shared alleles (POSA) (Bowcock et al., 1994) was calculated using Microsatellite Analyser (Dieringer and Schlötterer, 2003). Trees were visualized in either Treeview (Page, 1996) or SplitsTree4 (Huson and Bryant, 2006), depending on the distance method used.

The genetic structure of populations was assessed using Bayesian clustering methods implemented in STRUCTURE 2.2 (Pritchard et al., 2000) and BAPS 5.3 softwares (Corander et al., 2008). The model with admixture and correlated allele frequencies and 10 independent replicates were carried out in each run (*K* between 2 and 13) using a burn-in period of 20,000 followed by 100,000 MCMC repetitions in the STRUCTURE software. Pairwise similarities between runs were computed by CLUMPP (Jakobsson and Rosenberg, 2007). We used the DISTRUCT 1.1 software (Rosenberg, 2004) to graphically display the results produced by STRUCTURE. Monte Carlo resampling approach implemented in the GENECLASS 2.0 software (Piry et al., 2004) was used to perform population assignment and exclusion tests and to calculate the probability of origin for each individual and each sample, applying 10,000 simulated individuals and a type I error of 0.01.

RESULTS

A total of 126 alleles were detected across 15 microsatellite loci, with a mean number of alleles per locus of 8.4 and a range from 4 to 16 in the Venezuelan Criollo horses. The least polymorphic microsatellite was HTG6 (0.626), and the highest diversity was found in the AHT4 (0.836) locus. The highest number of alleles (16) was observed in the ASB17 locus. There were no significant deviations from Hardy-Weinberg expectations observed in the Venezuelan Criollo horses. Several rare alleles were found to be in the whole dataset, although at low frequency. All loci were highly informative for the Venezuelan Criollo breed, with *PIC* values higher than 0.5. The probability of *PE* was 99.96% using this set of microsatellites in the Venezuelan Criollo horse breed. Summary statistics for the breeds used in microsatellite marker analysis of population structure are given in Table 1. Allele frequency data for Venezuelan Criollo horses are available from the authors upon request.

Table 1. Summary statistics for the breeds analyzed using microsatellite markers.

Breed	N	H_O	H_E	UH_E	F_{IS}	N_A	N_E
Argentine Criollo (AC)	25	0.741	0.712	0.726	-0.040	6.000	3.655
Puerto Rican Paso Fino (RP)	50	0.688	0.668	0.675	-0.037	6.200	3.178
Andalusian (AN)	30	0.687	0.717	0.729	0.034	5.933	3.846
Peruvian Paso (PP)	30	0.758	0.736	0.748	-0.030	7.133	4.245
Colombian Paso Fino (CF)	50	0.752	0.752	0.759	0.002	7.733	4.305
Campolina (CP)	30	0.710	0.725	0.737	0.026	6.867	4.074
Chilean Criollo (CC)	30	0.691	0.707	0.719	0.026	5.933	3.637
Pantaneiro (PN)	25	0.773	0.738	0.754	-0.043	6.667	4.178
Mangalarga Marchador (MM)	50	0.708	0.712	0.729	0.002	6.800	3.750
Brazilian Criollo (BZ)	50	0.765	0.737	0.745	-0.037	7.200	4.130
Sorraia (SO)	30	0.553	0.510	0.518	-0.083	3.133	2.403
Chilote (CI)	30	0.702	0.737	0.750	0.050	7.333	4.221
Venezuelan Criollo (VC)	214	0.758	0.776	0.778	0.022	8.400	4.782

Sample size (N), observed (H_O), expected (H_E) and unbiased expected (UH_E) heterozygosities, heterozygote deficiency (F_{IS}), the total number of variants found in each population (N_A), and effective number of alleles (N_E).

AMOVA results showed that 16% of the variation originated among the horse breeds and 84% was from within the populations (Φ_{PT} = 0.160, P = 0.010). The F_{IS}, F_{IT} and F_{ST} values were 0.014, 0.090, and 0.077, respectively. The coefficient of gene differentiation (G_{ST}) had an average value of 0.097. Within only South American horse breeds the F_{IS}, F_{IT} and F_{ST} values were 0.014, 0.073, and 0.059, respectively. The values for gene differentiation (F_{ST}) between pairs of South American breeds were from 3.3% between the Venezuelan Criollo and Chilote pair (3.6% for the Venezuelan Criollo and Colombian Paso Fino pair) to 13.3% for the Argentine Criollo and Puerto Rican Paso Fino pair. The F_{ST} value over all loci between the 10 South American horse breeds was 0.059, which indicates that 5.9% of the variability could be attributed to differences between breeds. We also looked to see if there was any evidence of genetic differentiation among the three Venezuelan Criollo horse populations that were sampled (Apure, Aragua and Merida). The mean F_{ST} among these three populations was 2.2% compared to a mean F_{ST} among South American breeds of 5.9%. The sample sizes for the Merida and Aragua States were small; however, it is unlikely that a larger sample would change the results significantly.

The probabilities of individual assignment, based on Bayesian methods with a Monte Carlo resampling approach, revealed that only 74.7% (481 individuals of 644) could be

correctly assigned to their population of origin. For the Venezuelan Criollo, 182 of 214 individuals were correctly assigned (85%). STRUCTURE analysis showed that the independent runs from K = 2 to K = 13 produced consistent results. A plot with the clustering of individuals is presented in Supplementary Figure 1. K = 3 indicated the presence of two clear clusters, one belonging to the Sorraia and the other to Puerto Rican Paso Fino. From K = 4 onwards, assignment reflects the presence of population structure associated with progressive genetic differentiation. At K = 7, all Criollo horses showed a high degree of similarity excluding the Venezuelan Criollo, a similar pattern was observed for Paso Fino breeds, where the Puerto Rican Paso is more isolated. The optimal number of clusters was eight and proportion of membership of each pre-defined population in each of the eight clusters is presented in Table 2. Bayesian analysis of genetic population structure using the BAPS software revealed that the optimal number of partitions is eight of the 13 populations and the neighbor-joining tree based on this observation is presented in Figure 1.

Table 2. Proportion of assignment of each pre-defined population to each of the eight clusters.

Breed	N	Inferred clusters							
		I	II	III	IV	V	VI	VII	VIII
Argentine Criollo (AC)	25	0.023	0.108	0.016	0.017	0.042	0.762	0.024	0.008
Puerto Rican Paso Fino (RP)	50	0.028	0.028	0.825	0.023	0.031	0.036	0.017	0.012
Andalusian (AN)	30	0.040	0.074	0.014	0.174	0.028	0.054	0.600	0.016
Peruvian Paso (PP)	30	0.024	0.134	0.041	0.676	0.033	0.050	0.020	0.022
Colombian Paso Fino (CF)	50	0.038	0.179	0.020	0.501	0.174	0.036	0.038	0.014
Campolina (CP)	30	0.018	0.246	0.030	0.358	0.068	0.047	0.213	0.020
Chilean Criollo (CC)	30	0.066	0.030	0.009	0.087	0.061	0.678	0.049	0.019
Pantaneiro (PN)	25	0.038	0.172	0.051	0.415	0.179	0.060	0.069	0.015
Mangalarga Marchador (MM)	50	0.023	0.042	0.020	0.051	0.039	0.081	0.718	0.026
Brazilian Criollo (BZ)	50	0.032	0.093	0.012	0.042	0.055	0.706	0.039	0.021
Sorraia (SO)	30	0.003	0.003	0.004	0.003	0.003	0.003	0.004	0.977
Chilote (CI)	30	0.036	0.618	0.041	0.076	0.069	0.084	0.058	0.018
Venezuelan Criollo (VC)	214	0.358	0.142	0.031	0.030	0.357	0.043	0.022	0.016

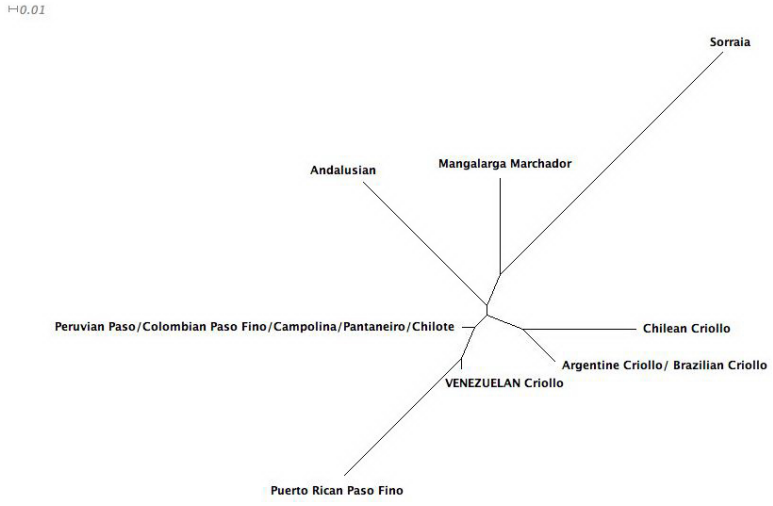

Figure 1. Neighbor-joining tree based on Nei's genetic distance from genetic mixture analysis at the sample population level in BAPS.

Phylogenetic trees based on D_C, the natural logarithm of the POSA and F_{ST} produced trees with topologies similar to the tree based on Nei's D_A distance (Figure 2). Comparison of the Venezuelan Criollo horse with other South American breeds indicates closest relationship to the Puerto Rican Paso Fino breed (Figure 2). When we split the Venezuelan Criollo into the Apure, Merida and Aragua States, they all clustered together, with the same result as observed using Bayesian analysis implemented in BAPS, which reveals one cluster as optimal partition (data not shown), thus reaffirming the lack of population substructure.

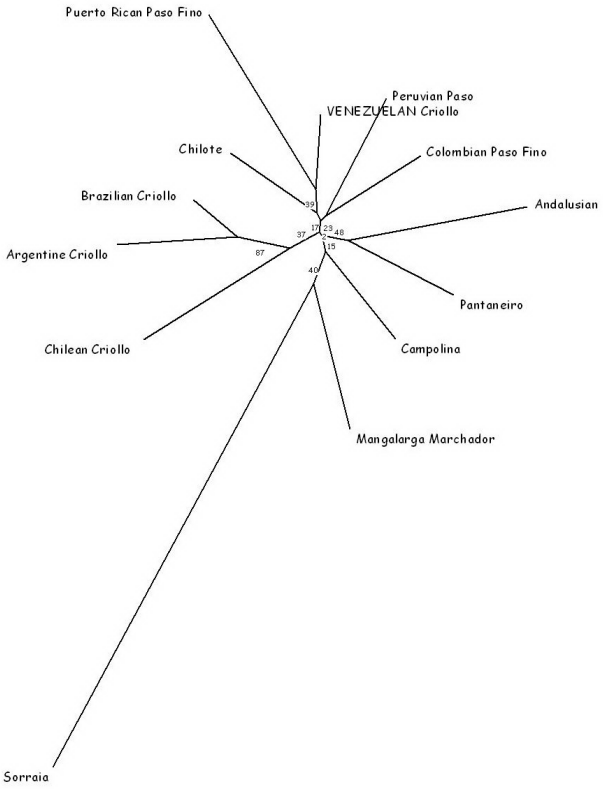

Figure 2. Neighbor-joining tree based on Nei's D_A distance.

DISCUSSION

The Venezuelan Criollo horse showed high levels of genetic diversity similar to other domestic horse populations analyzed (e.g., Bjornstad et al., 2000; Aberle et al., 2004; Leroy et al., 2009), and from a conservation standpoint, there is no immediate danger of losing variation unless there is a large drop in population size.

The overall proportion of genetic variation attributed to the differences between the South American horse breeds was 5.9% of the total variation, and the remaining 94.1% due to the differences between individuals. This is somewhat lower than the 12% observed in Norwegian breeds (Bjornstad et al., 2000), 10% found in French horses (Leroy et al., 2009), 8%

found in Spanish horse breeds (Canon et al., 2000), and only higher than the 1.5% reported in south European native horse breeds (Solis et al., 2005). Lower levels of genetic differentiation within South American horse breeds could be attributed to the limited genetic material that was brought to New World.

Using a wide array of breeds and different clustering methods, the Venezuelan Criollo horse consistently clusters with South American breeds. South American breeds belong to a group that also clusters with the Iberian Andalusian, Lusitano and Sorraia breeds (see previously published data, Luís et al., 2007). The introduction of horses to the New World by the Spanish and Portuguese explorers and colonists is well documented (Bort, 2004). During the course of history, the South American breeds likely had introductions of blood from breeds other than Iberian breeds. However, genetic evidence based on the use of mtDNA (Mirol et al., 2002; Luís et al., 2006), microsatellites and protein markers (Luís et al, 2007) agrees with a greater influence of Spanish and Portuguese horses than of other types of breeds on the New World breeds.

Within South American breeds, the Venezuelan Criollo horses cluster with the Paso Fino horses rather than with other Criollo horses (Figure 2). This is not a very surprising result as historical records indicate that the first horses to arrive in Venezuela, Colombia and Peru were from the Caribbean Islands: Dominican Republic, Cuba and Puerto Rico (Del Rio Moreno, 1992). Similar results were observed in a study of Uruguayan Criollo horses, where it was found to have closer relationship with Peruvian Paso, Barb and Paso Fino horses rather than with the Spanish Pure Bred (Kelly et al., 2002). Although there are no historical data confirming the introduction of Barb horses in America, the authors proposed that Spanish Pure Breds were repeatedly crossbred in Spain with different breeds during the XVI-XVIII centuries, and the present-day Barb (as they stayed relatively free of crossbreeding) is more similar to the old-type Spanish Pure Breds (Kelly et al., 2002). Similarly, genetic analysis of the Pantaneiro horse showed clear genetic differentiation between the Pantaneiro and the Uruguayan Criollo horse despite close geographical location (Sereno et al., 2008). When Venezuelan Criollo horses are separated into three subpopulations from the States of Apure, Merida and Aragua, they cluster within the same branch and show no differentiation. The Venezuelan Criollo horses appear to be both phenotypically and genotypically quite uniform, despite the high genetic diversity.

The individual clustering obtained using the Bayesian approach indicated that the Sorraia, which is highly inbred, formed a single-, well-defined cluster. It was difficult to assign the Venezuelan Criollo horses to a single cluster, as was observed for the Appaloosa and Hanoverian horses (Plante et al., 2007), indicating that these horses are highly variable. A similar result was also obtained in studies of Franches-Montagnes (Glowatzki-Mullis et al., 2006), Sable Island horses (Plante et al., 2007), the Pantaneiro breed (Sereno et al., 2008), and a large set of breeds raised in France (Leroy et al., 2009). In recent studies of human populations, the most difficult regions to detect population structure were the ones that had the smallest between-population variance, while the isolated and relatively homogeneous groups could be efficiently detected, even if the time since the populations diverged was short (Rosenberg et al., 2002; Wang et al., 2007). A recent study of goat diversity revealed moderate genetic differentiation levels (7%) and a low individual assignment to the breeds of origin, i.e., 74.9% (Canon et al., 2006). The proportion of correct assignments of individuals was correlated with the F_{ST} value and was negatively correlated with heterozygosity (Canon et al., 2006). A similar result was observed in our study of the Venezuelan Criollo horse population.

The Venezuelan Criollo is a unique breed, well adapted to local conditions and harsh

environment, as is typical of other Criollo type breeds from South America. Population numbers currently appear large enough to sustain the relatively high genetic diversity, now found in the breed, but future breeding strategies, studbook, management, and conservation plans should be established for this breed to ensure that variation can be maintained if the circumstances for the breed change.

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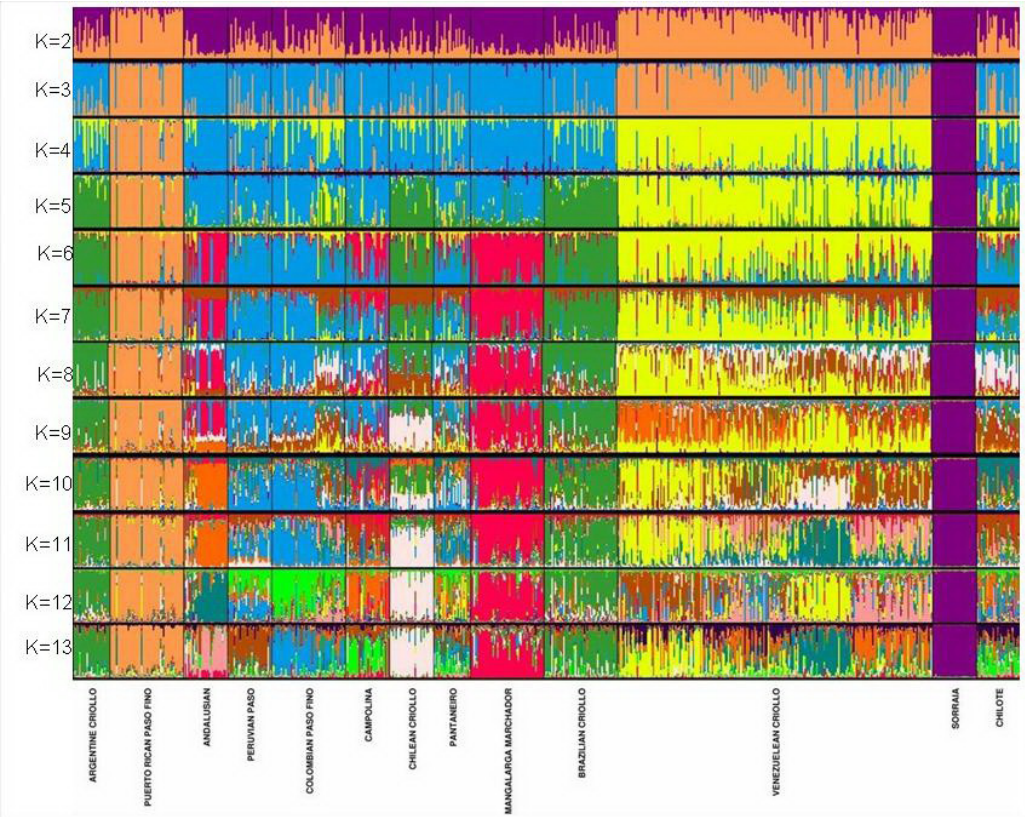
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Graphical presentation of the population structure analysis of 644 horses. Individual horses are represented by a single vertical line, broken into K color segments, with lengths proportional to the estimated membership of the inferred cluster.