Genetic characterization of local Criollo pig breeds from the Americas using microsatellite markers[1]

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ABSTRACT: Little is known about local Criollo pig genetic resources and relationships among the various populations. In this paper, genetic diversity and relationships among 17 Criollo pig populations from 11 American countries were assessed with 24 microsatellite markers. Heterozygosities, *F*-statistics, and genetic distances were estimated, and multivariate, genetic structure and admixture analyses were performed. The overall means for genetic variability parameters based on the 24 microsatellite markers were the following: mean number of alleles per locus of 6.25 ± 2.3; effective number of alleles per locus of 3.33 ± 1.56; allelic richness per locus of 4.61 ± 1.37; expected and observed heterozygosity of 0.62 ± 0.04 and 0.57 ± 0.02, respectively; within-population inbreeding coefficient of 0.089; and proportion of genetic variability accounted for by differences among breeds of 0.11 ± 0.01. Genetic differences were not significantly associated with the geographical location to which breeds were assigned or their country of origin. Still, the NeighborNet dendrogram depicted the clustering by geographic origin of several South American breeds (Criollo Boliviano, Criollo of northeastern Argentina wet, and Criollo of northeastern Argentina dry), but some unexpected results were also observed, such as the grouping of breeds from countries as distant as El Salvador, Mexico, Ecuador, and Cuba. The results of genetic structure and admixture analyses indicated that the most likely number of ancestral populations was 11, and most breeds clustered separately when this was the number of predefined populations, with the exception of some closely related breeds that shared the same cluster and others that were admixed. These results indicate that Criollo pigs represent important reservoirs of pig genetic diversity useful for local development as well as for the pig industry.

Keywords: admixture, biodiversity, Criollo pigs, genetic resources, genetic structure, microsatellite

J. Anim. Sci. 2014.92:4823–4832
doi:10.2527/jas2014-7848

INTRODUCTION

To meet the challenges of feeding the world population while maintaining an appropriate social and environmental balance, local breeds of livestock play a key role, and efforts aimed at their characterization, conservation, and genetic improvement are crucial (FAO, 2007). In several countries, local breeds with untapped potential still exist, which have been maintained mostly by small farmers in marginal produc-

[1]The authors wish to express thanks to the different breeders associations and research groups who kindly provided biological samples used in this study. The authors gratefully thank the members of the CYTED XII-H and CONBIAND networks for valuable cooperation over the years. The authors wish to acknowledge the financial support received by the Project RZ00-15 of the Programa Nacional de Recursos y Tecnologías Agroalimentarias of Ministerio de Ciencia y Tecnología of Spain. This work has received support from contract BIO4-CT98-0188 (PigBioDiv) of the European Union, which is gratefully acknowledged.

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Received March 18, 2014.
Accepted September 1, 2014

tion systems. In the Americas, these breeds are known as Criollo and descend from animals brought by Iberian settlers since the beginning of colonization in the late 15th century and may have also received the influence of exotic and commercial breeds of European and Asiatic origin.

Criollo pig breeds may represent an important reservoir of genes, especially because they have gone through a long process of selection for adaptation to extreme environmental conditions, but very little is known about them (Blackburn et al., 2003; Mariante et al., 2003). Several studies have assessed pig genetic diversity in different parts of the world (Martínez et al., 2000; Li et al., 2004; Ollivier et al., 2005; SanCristobal et al., 2006; Gama et al., 2013), but studies with genetic markers in Iberoamerican breeds have been very scarce (Lemus-Flores et al., 2001; Pérez-Pineda et al., 2006; Sollero et al., 2009; Ramírez et al., 2009; Revidatti et al., 2010; Burgos-Paz et al., 2013), and most of them included a limited number of samples, markers, or breeds, making it difficult to establish comparisons in a wider geographic scenario.

The objectives of our study were to assess within-breed genetic diversity, breed relationships, and population structure in a broad sample of Criollo pig breeds from the Americas. Our hypotheses were 1) Criollo pig populations hold significant amounts of genetic diversity and 2) Criollo pig populations have their own identity, and at least for some of them, there is enough genetic differentiation and structure to justify management at the breed level.

MATERIAL AND METHODS

Molecular Markers

A set of 24 microsatellite markers was selected from the panel recommended for genetic diversity studies by the International Society for Animal Genetics / Food and Agriculture Organization of the United Nations working group (FAO, 2011). The markers chosen were *IGF1*, *S0002*, *S0005*, *S0026*, *S0068*, *S0090*, *S0101*, *S0155*, *S0178*, *S0215*, *S0225*, *S0226*, *S0227*, *S0228*, *S0355*, *S0386*, *SW024*, *SW072*, *SW240*, *SW632*, *SW857*, *SW911*, *SW936*, and *SW951*.

Sampling Strategy

A total of 17 local Criollo pig populations were sampled in 11 countries, representing North, Central, and South America, plus the Caribbean Islands (Fig. S1). Overall, 613 individuals were analyzed, including animals from 5 Criollo populations from North America, specifically Mulefoot (**USA-MF**), Red Wattle hog (**USA-RWH**), and Guinea hog (**USA-GH**) from the United States and Pelón Mexicano (**MEX-PE**) and Baja California (**MEX-BC**) from Mexico; 1 breed from Central America (Criollo Salvadoreño [**SAL**] from El Salvador); 2 breeds representing the Caribbean Islands (Criollo Cubano [**CUB**] from Cuba and Criollo de Guadeloupe [**GUA**] from Guadeloupe Island); and 9 breeds from South America, specifically the Criollo Venezolano (**VEN**) from Venezuela, Zungo (**COL-ZU**), San Pedreño (**COL-SP**), and Criollo del Pacifico (**COL-CP**) from Colombia, Criollo Ecuatoriano (**ECU**) from Ecuador, Criollo Boliviano (**BOL**) from Bolivia, Pampa Rocha (**URU-PR**) from Uruguay, and Criollo from Northeast Argentina Wet (**ARG-NW**) and Dry (**ARG-ND**) regions. Even though the major focus of this study was the assessment of genetic diversity and breed relationships in Criollo pig breeds, the possibility of admixture with commercial breeds of worldwide expansion was also considered. Therefore, 3 commercial breeds representing different trunks, that is, Duroc, Large White, and Meishan, were also sampled and included in complementary analyses. The sample size per breed ranged from 14 to 53 (Table 1). Every attempt was made to ensure that samples were collected from unrelated animals registered in herdbooks in the few cases where these exist and covering a broad geographical area. Blood and hair root samples were collected by qualified veterinarians through their routine practice, in the framework of official programs aimed at the identification, health control, and parentage confirmation of the populations included in the current study. Genotypes of the GUA and Meishan populations belong to the PigBiodiv European Project database (Russell et al., 2003).

Deoxyribonucleic Acid Extraction and PCR Amplification

Genomic DNA was extracted and the 24 microsatellite markers were amplified in multiplex PCR using fluorescence-labeled primers, according to the methods described by Martínez et al. (2000).

Amplicons obtained by PCR were separated by electrophoresis on ABI 377XL instruments (Applied Biosystems, Foster City, CA) according to manufacturer recommendations, and allele sizing was accomplished by using the internal size standard GeneScan-400HD ROX (Applied Biosystems).

Statistical Analyses

Within-breed diversity was ascertained by calculating the mean number of alleles (**MNA**), observed heterozygosity (**Ho**) and unbiased expected heterozygosity (**He**) in each population (Nei, 1973), and their SD.

Table 1. Breed names, country of origin, acronym, number of individuals per population (*N*), mean number of alleles (MNA)/locus, effective number of alleles (Ne)/locus, allelic richness (Ar) per locus corrected for sample size corresponding to a minimum sample size of 10 individuals, mean expected heterozygosity (He) and observed heterozygosity (Ho) and SD, within-population inbreeding coefficient (F_{IS}), and confidence intervals estimated for 24 microsatellites in 17 American pig breeds, and the number of loci with significant deviations from Hardy-Weinberg equilibrium (HWE) after Bonferroni correction ($P < 0.05$)

Breed	Country	Acronym	*N*	MNA (SD)	Ne (SD)	Ar (SD)	He (SD)	Ho (SD)	F_{IS}	HWE
Mulefoot	United States	USA-MF	41	3.38 (1.17)	1.91 (0.60)	2.62 (0.73)	0.427 (0.040)	0.396 (0.017)	0.074 (–0.009/0.125)	2
Red Wattle hog	United States	USA-RWH	35	4.25 (2.13)	2.14 (0.67)	3.28 (0.84)	0.494 (0.034)	0.480 (0.018)	0.028 (–0.051/0.077)	0
Guinea hog	United States	USA-GH	34	5.17 (1.55)	2.65 (1.07)	3.87 (1.08)	0.561 (0.045)	0.483 (0.019)	0.141 (0.051/0.190)*	2
Criollo Baja California	Mexico	MEX-BC	20	5.42 (2.28)	3.31 (1.56)	4.60 (1.77)	0.628 (0.047)	0.554 (0.025)	0.122 (0.022/0.147)*	0
Pelón Mexicano	Mexico	MEX-PE	49	6.50 (2.09)	3.49 (1.74)	4.92 (1.52)	0.629 (0.045)	0.451 (0.015)	0.285 (0.215/0.331)*	1
Criollo Salvadoreño	El Salvador	SAL	21	6.33 (2.55)	3.80 (2.10)	5.06 (1.53)	0.668 (0.039)	0.571 (0.023)	0.149 (0.039/0.212)*	0
Criollo Cubano	Cuba	CUB	50	7.65 (2.23)	3.48 (1.46)	5.18 (1.40)	0.649 (0.043)	0.647 (0.015)	0.003 (–0.051/0.036)	0
Criollo de Guadeloupe	Guadeloupe Island	GUA	35	7.13 (2.88)	3.93 (1.85)	5.00 (1.62)	0.689 (0.038)	0.639 (0.017)	0.074 (0.018/0.092)*	2
Criollo Venezolano	Venezuela	VEN	30	6.33 (2.48)	3.64 (1.98)	4.76 (1.61)	0.645 (0.045)	0.593 (0.019)	0.083 (0.009/0.114)*	1
Zungo	Colombia	COL-ZU	33	5.17 (1.88)	2.89 (1.14)	4.15 (1.17)	0.604 (0.037)	0.577 (0.019)	0.044 (–0.030/0.087)	0
San Pedreño	Colombia	COL-SP	14	5.04 (2.01)	3.28 (1.79)	4.43 (1.53)	0.634 (0.041)	0.644 (0.027)	–0.017 (–0.150/0.016)	0
Criollo del Pacifico	Colombia	COL-CP	42	8.00 (2.30)	3.86 (1.71)	5.45 (1.08)	0.691 (0.033)	0.637 (0.016)	0.079 (0.029/0.101)*	0
Criollo Ecuatoriano	Ecuador	ECU	50	8.71 (3.20)	4.20 (2.21)	5.55 (1.42)	0.696 (0.036)	0.610 (0.014)	0.125 (0.076/0.155)*	1
Criollo Boliviano	Bolivia	BOL	34	7.29 (3.32)	3.85 (2.04)	5.12 (1.71)	0.657 (0.045)	0.630 (0.017)	0.042 (–0.028/0.080)	0
Pampa Rocha	Uruguay	URU-PR	32	5.13 (2.09)	2.83 (1.19)	4.28 (1.44)	0.581 (0.042)	0.570 (0.018)	0.019 (–0.049/0.048)	0
Criollo from Northeast Argentina Wet	Argentina	ARG-NW	53	8.29 (2.14)	3.93 (1.75)	5.47 (1.24)	0.698 (0.031)	0.604 (0.014)	0.135 (0.087/0.154)*	1
Criollo from Northeast Argentina Dry	Argentina	ARG-ND	40	6.83 (3.02)	3.35 (1.62)	4.62 (1.64)	0.626 (0.044)	0.549 (0.016)	0.124 (0.066/0.157)*	1
Means (SD)				6.25 ± 2.30	3.33 ± 1.56	4.61 ± 1.37	0.622 ± 0.040	0.567 ± 0.018	0.089 (0.058/0.125) *	

*$P < 0.05$.

The inbreeding coefficient (**F_{IS}**) was estimated within breeds with a 95% confidence interval, determined from 10,000 bootstrap samples. The effective number of alleles (**Ne**) was calculated as a measure of the number of equally frequent alleles needed to achieve a given level of gene diversity (Yeh and Boyle, 1997). Allelic richness (**Ar**) was estimated as a measure of the number of different alleles independent of sample size. A hierarchical ANOVA was performed to analyze the partition of the total genetic variance into components of interindividual, interbreed, and intergroup differences. Breeds were grouped by geographic region, defined as North America (including USA-MF, USA-RWH, USA-GH, MEX-BC, and MEX-PE); Central America, the Caribbean, and the northern part of South America (including SAL, CUB, GUA, VEN, COL-ZU, COL-SP, and COL-CP); and South America (with ECU, BOL, URU-PR, ARG-NW, and ARG-ND). An analysis of molecular variance (**AMOVA**) was performed, in which variance components were used to compute fixation indices (Excoffier et al., 2007). Divergence among breeds was analyzed with the pairwise F_{ST} distance of Wright (1965) and the D_A genetic distance of Nei et al. (1983) and a Neighbornet was constructed to graphically represent the relationships

between breeds as well as to depict admixture. The genetic relationships among populations were also assessed by a factorial correspondence analysis, which condenses into a few synthetic variables the information contained in the loci analyzed and allows the representation in space of the populations considered, with respect to the defined axes (Cañón et al., 2001).

The genetic structure of the 17 pig populations was investigated with Bayesian simulation procedures with the Structure software (Pritchard et al., 2000) to identify ancestral groups, substructure, and admixture. The grouping of individuals was tested assuming an increasing number of clusters (***K***) and an admixture model with correlated allele frequencies (Falush et al., 2003). Runs of 600,000 iterations after a burn-in period of 200,000 iterations were performed for each *K*. Ten independent simulations for *K* equal to 2 to 17 were performed to identify the most probable *K* through determination of the modal distribution of Δ*K* (Evanno et al., 2005). Average genotype membership fractions (***Q***) of the predefined breeds in each ancestral population were calculated, and a graphical display of individual membership coefficients (***q***) in the ancestral populations was obtained from the run with the highest posterior probability of the data at each *K* value. The software used to carry out each of the statistical analyses is listed in Table S1.

RESULTS

Within-Breed Genetic Diversity

Estimates of within-breed genetic diversity are shown in Table 1. The USA-MF and USA-USA-RWH populations showed the lowest expected diversity (He of 0.43 and 0.49, respectively), while the highest levels were found in ECU, ARG-NW, COL-CP, and GUA (He of about 0.70). The CUB had the highest Ho (0.65) and USA-MF the lowest (Ho of about 0.40). The MNA per locus over all populations was 6.25 ± 2.30, with a minimum value of 3.38 in USA-MF and a maximum of 8.71 in ECU. The global mean Ne was 3.33 ± 1.56, varying between 1.91 (USA-MF) and 4.20 (ECU), while Ar had a mean of 4.61 ± 1.37 and ranged between 2.62 (USA-MF) and about 5.50 (ECU, ARG-NW, and COL-CP).

Departures from Hardy-Weinberg equilibrium (HWE) were significant ($P < 0.05$) for 11 of the 408 population–locus combinations (Table 1). In the populations analyzed, the ARG-ND, ARG-NW, GUA, COL-CP, VEN, SAL, MEX-PE, USA-GH, MEX-BC, and ECU showed a significant ($P < 0.05$) deficit in heterozygosity, with an overall F_{IS} of 0.089 ($P < 0.05$) and the highest value of 0.285 observed in the MEX-PE population.

Genetic Relationships between Breeds

The mean *F*-statistics observed across loci (results not shown) suggested that the levels of breed differentiation were considerable, with multilocus F_{ST} values indicating that approximately 11% of the total genetic variation corresponded to differences between breeds, while the remaining 89% were attributed to differences among individuals within a breed.

Partitioning of genetic variability among the different sources of variation, with breeds grouped by region and by country, is shown in Table S2. Neither the country of origin nor the regions of the Americas from which the different breeds were sampled contributes significantly to genetic differentiation, as the *F*-test was nonsignificant and the corresponding variance component was negative in both cases. Therefore, there was no contribution to the total genetic variation that could be explained by these breed groupings.

The D_A ranged from 0.08 for the CUB/ECU pair to 0.44 for USA-MF/USA-RWH (Table S3). Indeed, the USA-MF showed the highest distance values relative to most Criollo breeds, including those located in the same country (USA-RWH and USA-GH).

The NeighborNet dendrogram presented in Fig. 1 shows that the 2 breeds of North America (USA-MF and USA-GH) formed a cluster with the Uruguayan URU-PR and the Mexican MEX-BC. Another cluster is formed by BOL with the ARG-NW and ARG-ND from Argentina, while Colombian breeds (COL-CP, COL-SP, and COL-ZU) clustered with the other North American breed (USA-RWH). The MEX-PE, SAL, CUB, and ECU clustered together in an intermediate position between these 2 clusters. Finally, the GUA from Guadeloupe Island grouped with VEN.

In the factorial correspondence analysis, the first, second, and third axis accounted for 13.0, 11.1, and 9.81% of the total inertia, respectively. The result of this analysis (Fig. 2) indicated that the first axis separates USA-MF; the second axis separates USA-GH, BOL, and USA-RWH; and the third axis separates USA-RWH, MEX-BC, USA-GH, and BOL breeds. The majority of the breeds clustered closely together in the center of the graphic.

Genetic Structure and Admixture Analysis

Bayesian clustering methods allow for the assignment of individuals to groups based on their genetic similarity and provide information on the number of ancestral populations underlying the observed genetic diversity. The results of these analyses indicate that the most likely number of ancestral populations is $K = 11$, as concluded from the modal distribution of Δ*K* (Fig. S2). This result supports the relatively high level of breed differentiation already detected by *F*-statistics, but a few breeds show signs of sharing a common ancestry and/or admixture.

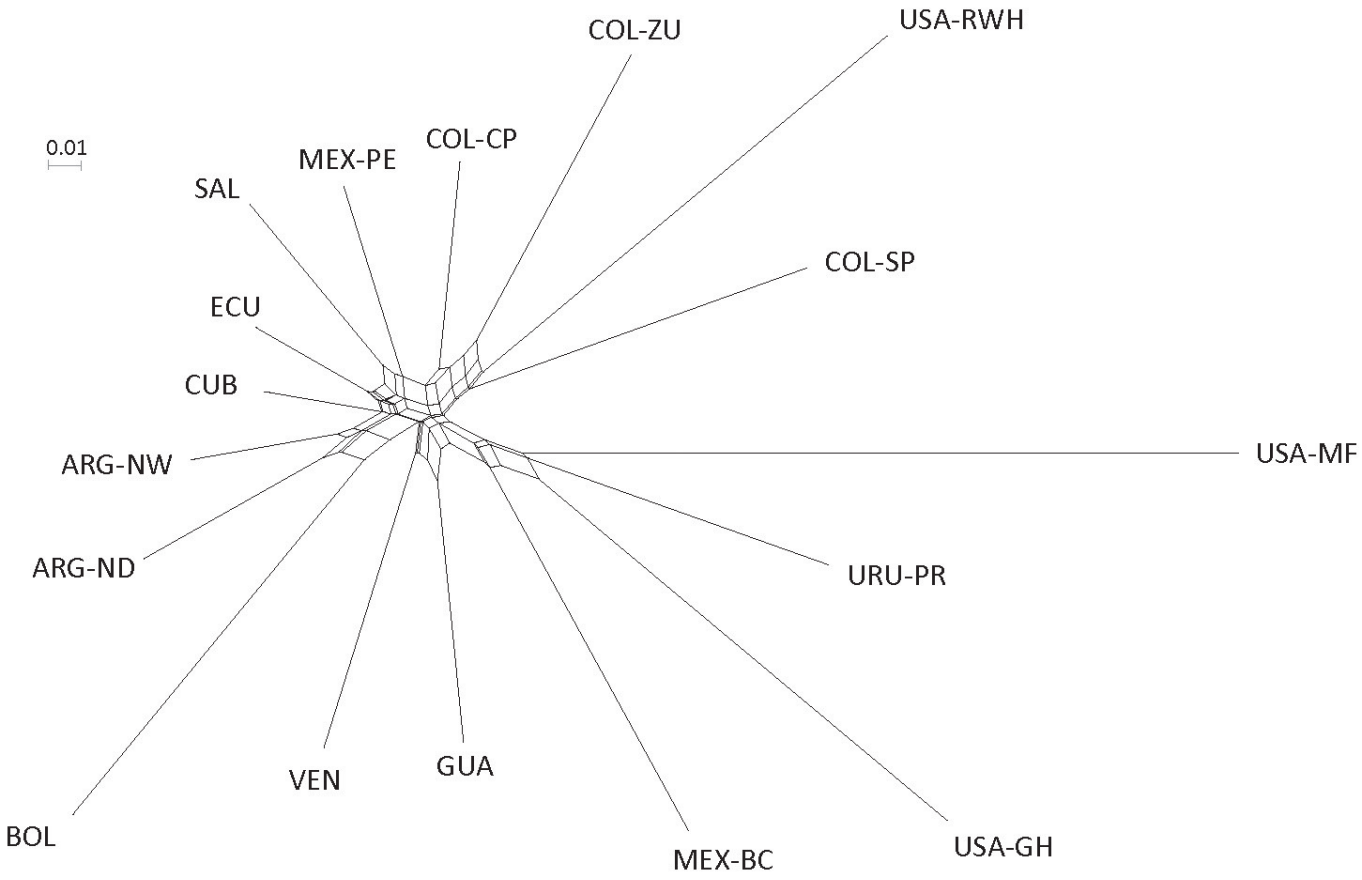

Figure 1. NeighborNet dendrogram constructed from genetic distances among 17 Criollo pig breeds. Breed abbreviations: USA-MF = Mulefoot; USA-RWH = Red Wattle hog; USA-GH = Guinea hog; MEX-BC = Criollo Baja California; MEX-PE = Pelón Mexicano; SAL = Criollo Salvadoreño; CUB = Criollo Cubano; GUA = Criollo de Guadeloupe; VEN = Criollo Venezolano; COL-ZU = Zungo; COL-SP = San Pedreño; COL-CP = Criollo del Pacifico; ECU = Criollo Ecuatoriano; BOL = Criollo Boliviano; URU-PR = Pampa Rocha; ARG-NW = Criollo from Northeast Argentina Wet; ARG-ND = Criollo from Northeast Argentina Dry.

The results of the Bayesian cluster analyses are summarized in Fig. 3, for values of K ranging from 2 to 11. When only 2 ancestral populations are assumed, the clusters are represented by the United States breeds and those from all other countries grouped separately. As K increases, the situation differs among breeds, such that when the estimated probable $K = 11$ is considered (Fig. 3), the USA-MF (the first to split as an independent breed cluster at $K = 3$), USA-RWH, USA-GH, COL-ZU, BOL, and URU-PR show a strong degree of between-breed differentiation and within-breed homogeneity. Two populations show evidence of substructure (MEX-PE and ARG-ND), while 8 other show signs of admixture (SAL, CUB, GUA, VEN, COL-SP, COL-CP, ECU, and ARG-NW). These results are confirmed by the high average genotype memberships ($Q > 0.80$) found for well-differentiated breeds, that is, in 7 out of the 11 predefined clusters estimated with Structure (Table S4).

When 3 international breeds were incorporated in the analyses (Fig. S3), a very minor influence, detectable only in the United States Criollo populations, could be identified. This analysis confirmed a shared ancestry between the USA-MF and USA-GH with Large White ($K = 3$) as well as between USA-RWH and Duroc ($K = 5$). Interestingly, the influence of Meishan pigs in the Large White population was also detected here ($K = 2$), confirming the historical records of admixture between Asian and European pigs (White, 2011; Bosse et al., 2014; Li et al., 2014). Nevertheless, none of the other Criollo breeds showed signs of admixture with the commercial breeds analyzed.

DISCUSSION

Published studies reporting the genetic diversity of Criollo pigs using microsatellites are still scarce and limited to a small number of breeds, often from a single country (Lemus-Flores et al., 2001; Kelly et al., 2004; Canul et al., 2005; Sierra et al., 2005; Pérez-Pineda et al., 2006; Castro et al., 2007; Sollero et al., 2009), or to a few studies examining the genetic diversity in some Criollo pig breeds at the regional level (Revidatti et al., 2010). Monoparental genetic markers such as mitochondrial DNA and Y-chromosome markers have been used to investigate the origins of a few Criollo pig breeds (Ojeda et al., 2008; Ramírez et al., 2009; Souza et al., 2009), and recently, genetic diversity was examined in

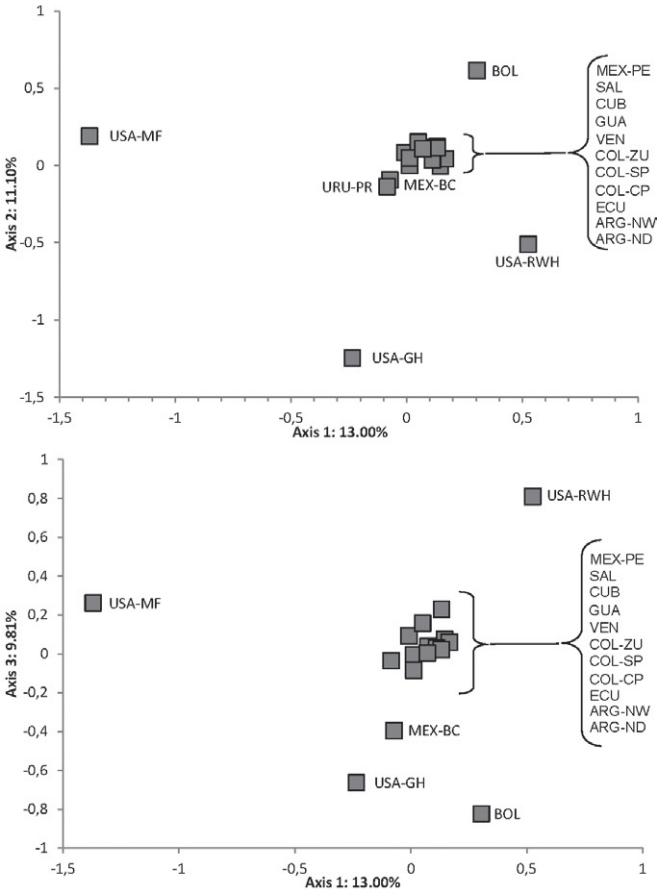

Figure 2. Factorial correspondence analysis obtained from 24 microsatellite loci for 17 Criollo pig breeds. Breed abbreviations: USA-MF = Mulefoot; USA-RWH = Red Wattle hog; USA-GH = Guinea hog; MEX-BC = Criollo Baja California; MEX-PE = Pelón Mexicano; SAL = Criollo Salvadoreño; CUB = Criollo Cubano; GUA = Criollo de Guadeloupe; VEN = Criollo Venezolano; COL-ZU = Zungo; COL-SP = San Pedreño; COL-CP = Criollo del Pacifico; ECU = Criollo Ecuatoriano; BOL = Criollo Boliviano; URU-PR = Pampa Rocha; ARG-NW = Criollo from Northeast Argentina Wet; ARG-ND = Criollo from Northeast Argentina Dry.

some Criollo breeds of pigs using a high-density panel of SNP (Burgos-Paz et al., 2013). In our study, we used a panel of autosomal markers, which provides an in-depth insight on the genetic structure, diversity, and relationships among a comprehensive sample of Criollo breeds of pigs and allows for comparisons with similar studies that have been performed with other breeds of pigs throughout the world.

The levels of within-breed diversity detected in Criollo from the Americas or local pigs were considerable (means of Ho = 0.57, He = 0.62, MNA = 6.25, and Ar = 4.61), such that the MNA and Ar was higher than those obtained for obtained for Duroc, Large White and Meishan pigs (data not shown) and those previously reported for European breeds (Laval et al., 2000; Martínez et al., 2000, 2003, 2007; Vicente et al., 2008; Gama et al., 2013; Boitard et al., 2010) but lower than the results reported for Chinese breeds (Yang et al., 2003; Zhang et al., 2003; Li et al., 2004). Furthermore, the Ne of 3.33 was similar to that reported for Portuguese (Vicente et al., 2008) and Australian (Li et al., 2000) pig breeds; lower than the estimates reported for other Iberian, Chinese, Thai, Indian, and Vietnamese breeds (Li et al., 2000, 2004; Behl et al., 2002; Fan et al., 2002, 2003; Yang et al., 2003, 2012; Zhang et al., 2003; Fabuel et al., 2004; Huang et al., 2005; Thuy et al., 2006; Fang et al., 2009); but higher than that observed in other European and Brazilian pig populations (Laval et al., 2000; Sollero et al., 2009; Gama et al., 2013). In general, the He detected in our study was higher than that reported for some endangered Spanish pig breeds (Martínez et al., 2003, 2007; Vega-Pla et al., 2004) but similar to the relatively high levels of genetic diversity obtained for the Iberian pig (Martínez et al., 2000) and some Brazilian breeds (Sollero et al., 2009). However, the genetic diversity reported for Asian pig breeds tends to be even higher than that estimated here (Li et al., 2000; Fan et al., 2002; Yang et al., 2003; Zhang et al., 2003). Some Criollo breeds deviated from HWE, probably due to admixture (SAL, GUA, VEN, and ARG-NW) or substructure (MEX-PE) as was demonstrated by the results of the Bayesian clustering analysis.

The degree of genetic differentiation among the Criollo local breeds studied was supported by the significant overall F_{ST} estimate, which indicates relatively low levels of gene flow and some level of reproductive isolation for most of the breeds analyzed. These results could be a consequence of the diversity of origins, as pigs were gradually introduced in the Americas, with a first wave arriving during the primary conquest, essentially brought by soldiers who carried pigs on board their ships during the long journey from the Iberian Peninsula, as a source of fresh meat to fight scurvy. A second wave probably arrived with the colonizers and settlers who came afterward (Rodero et al., 1992). Over the centuries, these pig populations spread throughout the American continents (Primo, 2004) and went through a process of selective adaptation to a vast diversity of environmental conditions (Blackburn et al., 2003, Mariante et al., 2003). Throughout this period, selection, genetic drift, and possible admixture events with international pig breeds originated different populations currently known as Criollo, which nevertheless show distinct features and are well differentiated from a morphological standpoint.

In our study with microsatellite markers, most of the observed genetic variability corresponded to differences among individuals and about 11% of the total variation was due to breed differences, which is in line with the results obtained in other studies where microsatellites were used to characterize genetic diversity in European pig breeds (Laval et al., 2000; Martínez et al., 2000; SanCristobal et al., 2006; Vicente et al., 2008; Gama et al., 2013). For Asian breeds, however, the amount of

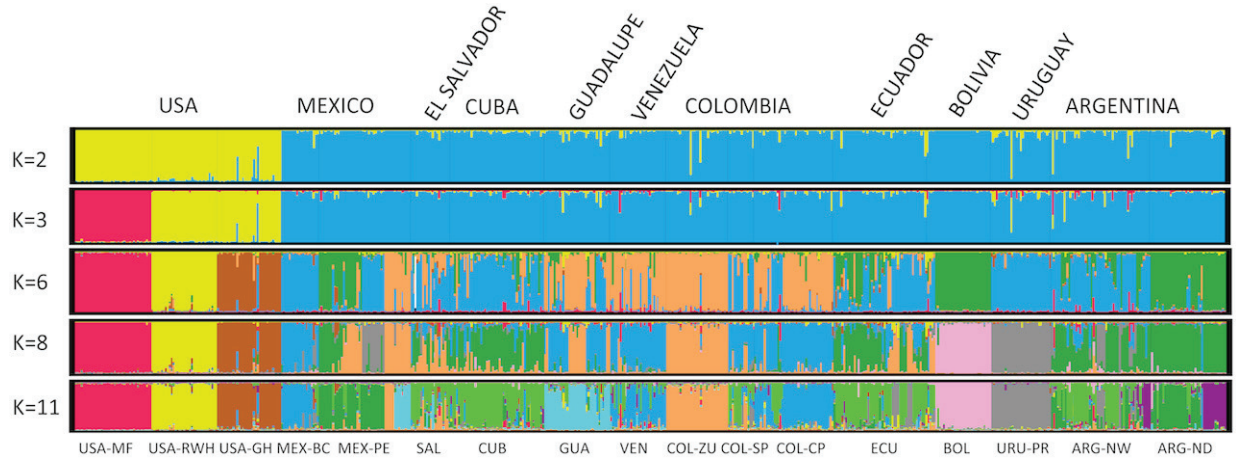

Figure 3. Population structure of 17 Criollo pig populations, as inferred from Bayesian analysis. For each animal, a single vertical line divided into *K* colors is drawn, where *K* is the number of assumed ancestral clusters that ranged from 2 to 11. The individual's estimated genotype membership proportion in a given cluster is represented by the corresponding color. Breed abbreviations: USA-MF = Mulefoot; USA-RWH = Red Wattle hog; USA-GH = Guinea hog; MEX-BC = Criollo Baja California; MEX-PE = Pelón Mexicano; SAL = Criollo Salvadoreño; CUB = Criollo Cubano; GUA = Criollo de Guadeloupe; VEN = Criollo Venezolano; COL-ZU = Zungo; COL-SP = San Pedreño; COL-CP = Criollo del Pacifico; ECU = Criollo Ecuatoriano; BOL = Criollo Boliviano; URU-PR = Pampa Rocha; ARG-NW = Criollo from Northeast Argentina Wet; ARG-ND = Criollo from Northeast Argentina Dry.

genetic variability accounted for by differences among breeds tends to be higher, in the range of about 20% (Fan et al., 2002; Li et al., 2004; Kim et al., 2005).

The AMOVA showed that levels of genetic diversity observed between breeds from different geographical areas or from different countries were negligible, indicating that there is not a defined pattern that would reflect a dispersion route of Criollo pigs in the Americas, as reported for other species such as cattle (Delgado et al., 2012). Introduction models of pigs in the Americas are probably different from other species, because pigs were most often used as fresh meat in the transatlantic shipping stages of the conquest. Occasionally, surviving individual pigs were released forming feral populations such as those found in Argentina or the Pantanal region of Brazil, among others. After colonization, frequent interactions between feral and domestic pools were likely established, which could affect the geo-evolutive map found today.

Relationships among breeds, assessed by their genetic distances, indicate that USA-MF was the breed most distant from all others, whereas the closest relationships were found between ECU and CUB. Moreover, in the Bayesian analysis, these 2 breeds showed strong signs of admixture from various ancestral populations, which is a possible indicator of the influence that the same original stock may have had in their development in the past.

The dendrogram depicting distances among the Criollo pig breeds analyzed indicates that the existence of several clusters can be observed, for example, the cluster comprising the North American breeds USA-MF, USA-GH, and MEX-BC plus the Uruguayan URU-PR, suggesting the existence of a common origin for these breeds. The separation of USA-MF, USA-GH, and USA-RWH from each other and from the remaining breeds was also supported by the factorial correspondence analysis, confirming the stronger identity and differentiation of these breeds.

While most North American breeds belong to the same cluster, they are very distinct from each other, such that the mean D_A among breeds in the North American group was higher (0.337 ± 0.056) than for Central America and the Caribbean (0.191 ± 0.035) and South American breeds (0.194 ± 0.064). This reflects the more pronounced isolation among North American breeds and perhaps different influences from international germplasm and a higher proximity and some degree of admixture among breeds in Central and South America. Lemus-Flores et al. (2001) found genetic distances among 7 varieties of Mexican hairless pig breeds with Landrace and Hampshire of 0.19 and 0.49, respectively, while Martínez et al. (2005) reported genetic distances between Cuban Criollo and Iberian breeds varying between 0.24 and 0.38.

The occurrence of clustering by geographic origin was also observed when Argentinean pigs (ARG-ND and ARG-NW) clustered with the nearby BOL and when Colombian breeds clustered together, even though in this case they were also joined by the North American USA-RWH. However, some unexpected results were also observed, such as the grouping of breeds from countries as distant as El Salvador, Mexico, Ecuador, and Cuba, but these results could have been affected by influence of commercial breeds, which nevertheless was not detected in the Bayesian analysis. Another important fact to be considered is that soon after the discovery of the Americas, Cuba became an important distribution center of zoogenetic resources, because this island was a main base from which colonization of the rest of the Americas disseminated (Rodero et al., 1992). Thereby, Cuban genetic resources could have influenced the successive

bottlenecks that gave origin to the different continental Criollo breeds.

The Bayesian analysis suggests that commercial breeds have had a minor influence in most Criollo pig populations (Fig. S3), and this influence is restricted to North American pig breeds. These results are in agreement with those reported by Burgos-Paz et al. (2013) using a high density SNP panel of 60K, who concluded that some of the Criollo populations studied here are equidistant between Landrace, Large White, and Iberian breeds and more distant from Duroc. Further studies are warranted to clarify the origins of Criollo pigs, using a broader sample of breeds contributing to their genetic pool, namely their Iberian counterparts.

Some of the breeds included in our study represent a well-differentiated population, that is, COL-ZU, USA-MF, BOL, USA-RWH, VEN, USA-GH, URU-PR, and ARG-ND, with high genotype membership coefficients (Q) to each of their clusters. Other breeds had lower membership coefficients, corresponding to some degree of substructure (MEX-PE and COL-CP) and/or admixture (the remaining populations). For example, it was not possible to differentiate MEX-BC, SAL, CUB, COL-SP, and ECU, because these breeds grouped in the same cluster, as well as COL-CP with GUA, while ARG-NW and ARG-ND also showed a considerable level of genetic admixture, at least in some of the animals. A similar pattern was found in similar studies performed in Criollo breeds of other species such as cattle (Delgado et al., 2012) and goats (Martínez et al., 2012) and possibly reflects some degree of admixture when animals migrated through the Caribbean Islands and the American continent (Primo, 2004).

Despite the high genetic diversity detected within Criollo pigs, significant inbreeding or substructuring was also observed in some breeds, especially in MEX-PE, which tends to be raised in several closed and independent herds. With SNP, Burgos-Paz et al. (2013) could not find this substructure, possibly because of sampling effects and the more reduced sample size, because with this type of genetic marker it is common to have far more markers per individual but fewer individuals per marker.

Our results show a clear differentiation among Criollo pigs with respect to international pig breeds analyzed. Given the adaptation of Criollo pigs to harsh conditions, conservation plans that also foster their use for local meat consumption, maybe under a label of quality, are warranted.

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