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Genetic characterization of Latin-American Creole cattle using microsatellite markers

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Summary

Genetic diversity in and relationships among 26 Creole cattle breeds from 10 American countries were assessed using 19 microsatellites. Heterozygosities, *F*-statistics estimates, genetic distances, multivariate analyses and assignment tests were performed. The levels of within-breed diversity detected in Creole cattle were considerable and higher than those previously reported for European breeds, but similar to those found in other Latin American breeds. Differences among breeds accounted for 8.4% of the total genetic variability. Most breeds clustered separately when the number of pre-defined populations was 21 (the most probable *K* value), with the exception of some closely related breeds that shared the same cluster and others that were admixed. Despite the high genetic diversity detected, significant inbreeding was also observed within some breeds, and heterozygote excess was detected in others. These results indicate that Creoles represent important reservoirs of cattle genetic diversity and that appropriate conservation measures should be implemented for these native breeds in order to minimize inbreeding and uncontrolled crossbreeding.

Keywords admixture, biodiversity, DNA markers, genetic diversity, genetic structure.

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Introduction

Nearly one-third of world cattle are in the American continents. Approximately 65% of these are in South America

(http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#ancor). Creole cattle were for a long time the basis of beef production in many American countries, and still are in a few of these. Creole cattle have undergone a long process of expansion and adaptation to different environments of the Western Hemisphere. Although many of the modern Creole cattle are recognized as breeds and are recorded with breed organizations, these populations are not well characterized genetically. Information about genetic diversity and population structure of Creole cattle can provide a rational basis for the conservation and possible use of native cattle breeds as genetic resources to meet potential future demand for beef.

Microsatellite markers have been widely used for population genetic analyses of livestock species, as they are informative and can successfully elucidate the relationships between individuals and populations. Microsatellites have been commonly used to assess within-breed genetic diversity and inbreeding levels, introgression from other species, genetic differentiation, and admixture among breeds (García *et al.* 2006; Tapio *et al.* 2006; Ginja *et al.* 2009a; Li & Kantanen 2009; Qi *et al.* 2009). Several studies have been conducted in European and Eurasian cattle (*Bos taurus)*, in which microsatellites were used to assess genetic diversity and differentiation (e.g. Cañón *et al.* 2001; European Cattle Genetic Diversity Consortium 2006; Tapio *et al.* 2006; Li & Kantanen 2009). For Creole breeds, very few studies with microsatellite markers have been done (Martínez *et al.* 2005; Armstrong *et al.* 2006; Quiroz-Valiente *et al.* 2006; Aquino *et al.* 2008; Ulloa-Arvizu *et al.* 2008; Martínez-Correal *et al.* 2009). Information about genetic diversity and differentiation of Creole breeds is essential as a first step towards the establishment of appropriate conservation and sustainable management programmes in order to prevent extinction and genetic erosion of these breeds, some of which are endangered, such as Criollo Ecuatoriano, Chino Santandereano or Costeño con Cuernos (FAO 2000).

The objectives of this study were to use microsatellite markers to characterize the within-breed genetic diversity of Creole cattle, to establish breed relationships, and to assess their population structure.

Material and methods

Molecular markers

Six laboratories were involved in this study (University of Córdoba, Complutense University of Madrid and University of Zaragoza in Spain, Instituto Nacional dos Recursos Biológicos in Portugal, University of California Davis in the United States of America, and National University of Colombia in Palmira in Colombia). A common set of 19 microsatellites was selected from a panel of 30 markers recommended by the International Society for Animal Genetics (ISAG)/Food and Agriculture Organization of the United Nations (FAO) working group (FAO, 2004): *BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH3, ETH10, ETH185, ETH225, HAUT27, HEL9, ILSTS006, INRA032, INRA063, MM12, SPS115, TGLA53, TGLA122* and *TGLA227*. Only three of the laboratories were involved in genotyping, because of their extensive experience with cattle microsatellite typing. The other three labs performed DNA extractions.

Sampling strategy

A total of 26 Creole cattle breeds were sampled in 10 countries, representing North, Central, South America and the Caribbean Islands. Overall, 857 individuals were analysed, of which 217 were from six Creole populations from North America (USA and Mexico), 61 were from two breeds in Central America (Panama), 50 were from the Criollo Cubano breed representing the Caribbean Islands and 529 were from 17 breeds located in South America, from Colombia to southern Argentina. The breeds and corresponding sample sizes are shown in Table 1.

DNA extraction and PCR amplification

Genomic DNA was extracted using the procedures described by Martínez *et al.* (2000), Martin-Burriel *et al.* (2007) and Ginja *et al.* (2009a). The 19 microsatellite markers were amplified in multiplex polymerase chain reactions (PCRs) using fluorescence-labelled primers and according to the method used by Ginja *et al.* (2009a).

Amplicons obtained by PCR were separated by electrophoresis on ABI instruments (3730, 3130 and 377XL, Applied Biosystems) according to manufacturer recommendations, and allele sizing was accomplished by using the internal size standards GeneScan™-500 LIZ™ and GeneScan-400HD ROX (Applied Biosystems). Allele nomenclature followed was that used in a previous European research project on cattle diversity (EU RESGEN CT 98-118). To merge the datasets generated in the different laboratories, the set of selected loci was examined by all laboratories. Samples (n = 30) with reference genotypes representing the entire allele range for the marker set were exchanged and genotyped in the three laboratories. Allele sizing was discussed and pooling was decided specifically in the cases with different allele calling. Each laboratory adjusted allele sizing based on these reference samples. Each genotyping laboratory included two reference samples in each assay to control for variation between electrophoresis runs.

Statistical analysis

Allele frequencies for each locus, total number of alleles per locus (NA), and estimated observed (H_o) and unbiased expected (H_e) heterozygosities were calculated. The amount of inbreeding within population (f), and the amount of differentiation among populations (Theta) per locus were

Table 1 Breeds, country of origin, number of individuals per population (*N*), mean of number of alleles/locus (MNA), mean effective number of alleles/locus (NE), mean allelic richness per locus corrected for sample size and breed corresponding to a minimum sample size of six individuals (R_t), mean observed (H_o) and expected heterozygosity (H_e) and their standard deviations (SD), within-population inbreeding coefficient (F_{IS}) and their confidence interval, and number of loci with Hardy–Weinberg equilibrium (HWE) deviations estimated with 19 microsatellites in 26 American Creole breeds ($P < 0.05$).

Breed	Country	*N*	MNA (SD)	NE	R_t	H_e (SD)	H_o (SD)	F_{IS}	HWE
Guabalá	Panama	25	5.79 (1.96)	3.53	4.10	0.660 (0.045)	0.629 (0.022)	0.048 (−0.047/0.093)	7
Guaymí	Panama	36	7.79 (1.65)	4.26	4.93	0.756 (0.017)	0.735 (0.017)	0.028 (−0.031/0.053)	2
Texas Longhorn	USA	80	8.05 (2.46)	4.39	4.78	0.740 (0.025)	0.707 (0.012)	0.045 (0.010/0.067)*	2
Criollo Poblano	Mexico	43	8.37 (2.01)	4.67	5.08	0.774 (0.017)	0.693 (0.016)	0.105 (0.052/0.133)*	7
Criollo de Baja California	Mexico	21	7.05 (1.58)	4.18	4.98	0.760 (0.019)	0.742 (0.024)	0.025 (−0.071/0.048)	4
Criollo de Chihuahua	Mexico	19	6.68 (1.49)	4.33	5.14	0.777 (0.018)	0.719 (0.026)	0.077 (−0.027/0.105)	6
Criollo de Nayarit	Mexico	24	7.74 (1.94)	4.72	5.25	0.788 (0.018)	0.749 (0.021)	0.050 (−0.019/0.066)	2
Criollo de Chiapas	Mexico	30	7.84 (1.57)	4.77	5.23	0.782 (0.021)	0.741 (0.019)	0.053 (−0.013/0.082)	6
Blanco Orejinegro	Colombia	25	5.74 (1.76)	3.39	4.10	0.697 (0.023)	0.737 (0.020)	−0.059 (−0.138/−0.027)*	0
Caqueteño	Colombia	25	7.58 (1.57)	4.78	5.22	0.787 (0.017)	0.780 (0.019)	0.009 (−0.070/0.040)	2
Sanmartinero	Colombia	25	6.37 (1.16)	3.60	4.39	0.721 (0.018)	0.692 (0.022)	0.042 (−0.046/0.076)	4
Romosinuano	Colombia	25	5.11 (1.59)	3.21	3.94	0.669 (0.030)	0.651 (0.023)	0.028 (−0.066/0.071)	4
Costeño con Cuernos	Colombia	25	5.26 (1.37)	3.29	3.94	0.671 (0.031)	0.692 (0.022)	−0.032 (−0.120/0.007)	2
Chino Santandereano	Colombia	25	7.32 (1.73)	4.38	5.03	0.776 (0.013)	0.726 (0.021)	0.066 (−0.004/0.089)	4
Velasquez	Colombia	25	6.79 (1.44)	4.31	4.92	0.769 (0.016)	0.730 (0.021)	0.053 (−0.033/0.086)	3
Lucerna	Colombia	24	6.63 (2.06)	3.82	4.69	0.717 (0.025)	0.673 (0.024)	0.063 (−0.027/0.092)	2
Hartón del Valle	Colombia	22	7.74 (1.73)	4.60	5.24	0.783 (0.016)	0.783 (0.021)	−0.000 (−0.087/0346)	2
Criollo Casanareño	Colombia	35	8.00 (1.65)	3.52	4.13	0.766 (0.020)	0.739 (0.019)	0.035 (−0.022/0.060)	2
Criollo Ecuatorinao	Ecuador	12	6.63 (2.11)	4.30	5.23	0.771 (0.023)	0.732 (0.029)	0.054 (−0.074/0.076)	1
Criollo Uruguayo	Uruguay	43	5.63 (1.67)	3.22	3.97	0.674 (0.019)	0.668 (0.017)	0.009 (−0.046/0.035)	2
Pampa Chaqueño	Paraguay	50	8.11 (1.79)	4.62	5.05	0.771 (0.017)	0.750 (0.014)	0.028 (−0.015/0.051)	2
Criollo Pilcomayo	Paraguay	36	7.53 (1.74)	4.68	5.07	0.768 (0.022)	0.764 (0.016)	0.006 (−0.043/0.023)	2
Criollo Argentino	Argentina	50	6.26 (1.66)	3.33	4.00	0.678 (0.023)	0.673 (0.015)	0.007 (−0.049/0.041)	1
Criollo Patagónico	Argentina	35	5.32 (1.57)	3.22	3.84	0.670 (0.025)	0.629 (0.019)	0.062 (−0.012/0.104)	3
Caracú	Brazil	47	6.74 (1.73)	3.70	4.32	0.711 (0.022)	0.733 (0.015)	−0.030 (−0.080/−0.006)*	2
Criollo Cubano	Cuba	50	7.58 (2.36)	4.61	4.92	0.761 (0.018)	0.793 (0.013)	−0.043 (−0.084/−0.024)*	8
		Means	*6.92 ± 0.99*	*4.06 ± 0.58*	*4.67 ± 0.51*	*0.738 ± 0.045*	*0.718 ± 0.045*	*0.028 ± 0.039*	*3.15 ± 2.07*

In italic means and standard error * $P < 0.05$

estimated according to Weir & Cockerham (1984), with corresponding *P*-values obtained based on 1000 randomizations.

A first approach to within-breed diversity was ascertained by calculating the mean number of alleles, observed and unbiased expected estimates of heterozygosity per population (Nei 1973), and their standard deviations. The within-breed inbreeding coefficient (F_{IS}) was estimated with a 95% confidence interval, determined by 1000 permutations and 10 000 bootstraps. 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 genetic diversity. This estimate allows comparison of populations in which the number and distributions of alleles differ drastically. Allelic richness (R_t) was estimated as a measure of the number of alleles independent of sample size, hence allowing comparisons among different sample sizes. Deviations from Hardy–Weinberg equilibrium (HWE) were also assessed. After defining groups of breeds by geographic region (North America, Central America, South America and Caribbean Islands) and by country of origin, a hierarchical analysis of variance was carried out, which allowed the partitioning of the total genetic variance into components due to inter-individual and inter-breed differences. Variance components were used to compute fixation indices. The interpretation of population structure by *F*-statistics was tested using a non-parametric permutation approach as described by Excoffier *et al.* (1992). Computations were carried out using an AMOVA (Analysis of Molecular Variance) procedure (Excoffier *et al.* 2005). Genetic divergence among breeds was estimated through two commonly used genetic distance measures: D_A (Nei *et al.* 1983) and Reynolds genetic distances (Reynolds *et al.* 1983). A NeighborNet was constructed from Reynolds genetic distances to graphically represent the relationships between breeds, as well as to depict evidence of admixture. The genetic structure of the 26 cattle populations was investigated to identify population substructure and admixture. This is accomplished by decomposing the HWE and linkage disequilibrium so that they could be maximally explained by origin and admixture. The grouping of individuals was tested assuming an increasing number of clusters (*K*) and using an admixture model with correlated allele frequencies (Falush *et al.* 2003). Runs of 500 000 iterations after a burn-in period of 200 000 iterations were performed for each *K*. Five independent simulations for *K* equal to 2–26 were performed to identify the most probable *K* through determining the modal distribution of Δ*K* (Evanno *et al.* 2005). The proportion of each individual's genotype (*q*) in the predefined populations in each cluster was also estimated. A graphical display of individual membership coefficients in each ancestral population was obtained from the run with the highest posterior probability of the data at each *K* value. The particular software used to carry out all the calculations is listed in Table S1.

Results

Molecular markers

Diversity estimates for each locus and *F*-statistics are summarized in Table S2. A total of 259 alleles across all breeds were detected in the 19 markers, with a range of 7 (*BM1824*) to 21 (*TGLA53*) alleles per locus, and an overall mean number of alleles of 13.63 ± 3.52.

Most markers (89.5%) showed high levels of heterozygosity, with H_e and H_o above 0.65, while *HAUT27* and *INRA063* showed lower levels of Ho. The estimated amount of inbreeding within populations (*f*) had an overall mean of 0.027 ± 0.009 ($P < 0.01$), while the amount of differentiation among populations (*theta)* per locus was also significant ($P < 0.01$), with an overall mean of 0.084 ± 0.003. The *BM1818* locus deviated from HWE in only one population ($P < 0.05$), while *ILSTS006* deviated from equilibrium in nine populations.

Within-breed genetic diversity

Estimates of within-breed genetic diversity are shown in Table 1. The Guabalá and Criollo Patagónico breeds showed the lowest diversity (H_e of 0.660 and 0.670, respectively), while the highest levels of H_e were found in some Creole populations from México and Colombia. The Criollo Cubano had the highest H_o value (0.793). The mean number of alleles per locus over all populations was 6.92 ± 0.99, with a minimum value of 5.11 in the Colombian Romosinuano and a maximum of 8.11 in the Paraguayan Pampa Chaqueño. NE (mean value of 4.06 ± 0.58) and R_t (mean value of 4.67 ± 0.51) were similar in all populations, varying within a short range between 3.22 (Criollo Patagónico) and 4.78 (Colombian Caqueteño) for NE, and from 3.84 (Criollo Patagónico) to 5.25 (Mexican Criollo de Nayarit) for R_t, assuming a minimum sample size of six individuals.

Departures from HWE were significant ($P < 0.05$) for 81 loci among the 494 population-locus combinations. The number of markers not in HWE ranged from 0 in the Blanco Orejinegro from Colombia to 8 in Criollo Cubano (Table 1). Only five populations showed significant deviations from 0 ($P < 0.05$) for the F_{IS} value obtained, such that the Texas Longhorn and Mexican Criollo Poblano, which showed heterozygote deficit, while Colombian Blanco Orejinegro, Brazilian Caracú and Criollo Cubano showed an excess of heterozygotes.

Genetic relationship between breeds

The mean *F*-statistics (Table S2) suggested that the apparent levels of breed differentiation were considerable, with multilocus *theta* values indicating that approximately 8.4% of the total genetic variation corresponded to differences

between breeds, while the remaining 91.6% corresponded to differences among individuals.

The genetic distance of Reynolds among the different breeds studied (Table S3) ranged from 0.024 for the Mexican Criollo de Chihuahua/Criollo de Baja California pair to 0.210 for Romosinuano/Criollo Patagónico. The D_A distances ranged from 0.082 for Criollo Poblano/Texas Lonhorn pair to 0.357 for Romosinuano/Criollo Cubano. The Panamanian Guabalá showed the highest distance values relative to most Creole breeds, while distance estimates among Mexican Creoles and among some Colombian breeds were the lowest. Scarce 0 null influence of *Bos indicus* and non-Latin American breeds in most Creole breeds has been found using microsatellites in other studies, such as those of Egito *et al.* (2007), Villalobos Cortés *et al.* (2010) and Liron *et al.* (2006a,b).

Partitioning of genetic variability among the different sources of variation, with breeds grouped by region and by country, is shown in Table S4. Less than 3% of the total genetic difference was explained by the geographical location to which breeds were assigned (1.81%) or by their country of origin (2.98%).

The NeighborNet dendrogram is presented in Fig. 1. The general trend indicates a close genetic relationship between populations from nearby geographical regions. Mexican Criollo Poblano, Criollo de Chihuahua, Criollo de Baja California and Criollo de Nayarit formed a cluster with Texas Longhorn from the United States. The Mexican Criollo de Chiapas appeared in another cluster with breeds originating from distant regions, i.e., Criollo Pilcomayo from Paraguay, Criollo Ecuatoriano from Ecuador, Criollo Cubano from Cuba, and Velasquez and Caqueteño from Colombia. Both Panamanian Guabalá and Guaymí breeds grouped in a different cluster, together with the Colombian breeds Lucerna, Blanco Orejinegro and Hartón del Valle. The other Colombian breeds (Romosinuano, Costeño con Cuernos, Sanmartinero and Criollo Casanareño) clustered together, although Costeño con Cuernos and Romosinuano were genetically closer, while Chino Santandereano split from the centre as an independent branch. The southern breeds Caracú, Criollo Patagónico, Criollo Argentino, Criollo Uruguayo and Pampa Chaqueño formed an independent and rather distant cluster.

Genetic structure and admixture analysis

Bayesian clustering methods allow for the assignment of individuals to groups based on their genetic similarity and provide information about the number of ancestral populations underlying the observed genetic diversity. The results of this analysis indicate that, for the 26 breeds analysed, the most likely number of ancestral populations is 21 (Fig. S1), suggesting that the most significant subdivision was by breeds or by groups of closely related breeds. The proportional membership of individual genotypes in the different clusters (Fig. 2) indicates that, for $K = 2$, one

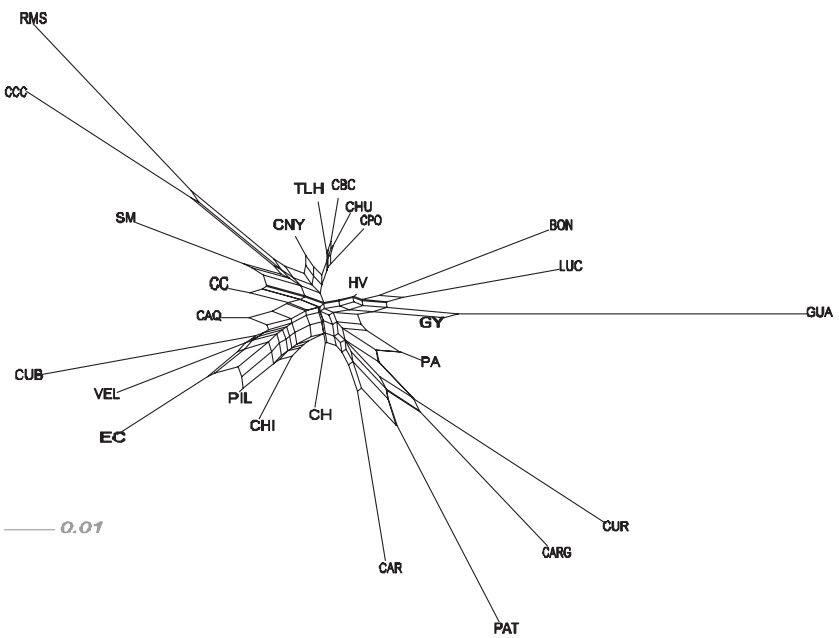

Figure 1 Neighbor Net dendrogram constructed from Reynolds genetic distances among 26 Creole populations. Breed abbreviations: GUA, Guabalá; GY, Guaymí; TLH, Texas Longhorn; CPO, Criollo Poblano; CBC, Criollo de Baja California; CHU, Criollo de Chihuahua; CNY, Criollo de Nayarit; CHI, Criollo de Chiapas; BON, Blanco Orejinegro; CAQ, Caqueteño; SM, Sanmartinero; RMS, Romosinuano; CCC, Costeño con Cuernos; CH, Chino Santandereano; VEL, Velásquez; LUC, Lucerna; HV, Hartón del Valle; CC, Criollo Casanareño; EC, Criollo Ecuatoriano; CUR, Criollo Uruguayo; PA, Pampa Chaqueño; PIL, Criollo Pilcomayo; CARG, Criollo Argentino; PAT, Criollo Patagónico; CAR, Caracú; CUB, Criollo Cubano.

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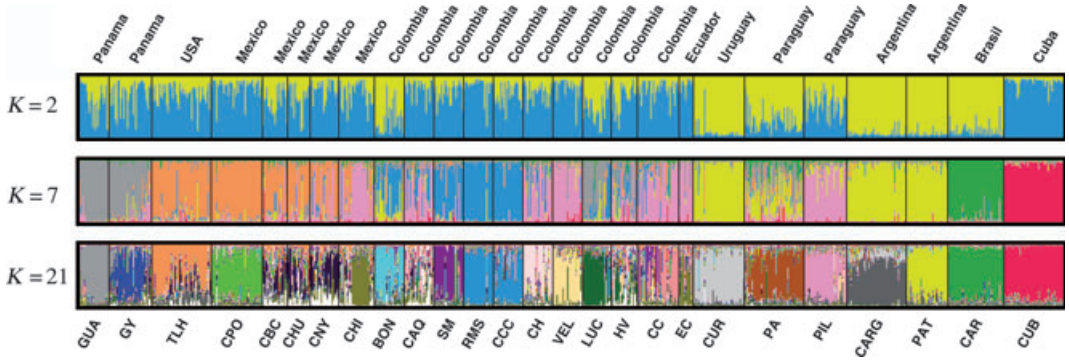

Figure 2 Population structure of 26 cattle populations using the model-based STRUCTURE software. Each animal is represented by a single vertical line divided into *K* colours, where *K* is the number of clusters assumed and the colours show the estimated individual proportions of cluster membership. Results are shown for (a) *K* = 2, (b) *K* = 7 and (c) *K* = 21. Breed abbreviations: GUA, Guabalá; GY, Guaymí; TLH, Texas Longhorn; CPO, Criollo Poblano; CBC, Criollo de Baja California; CHU, Criollo de Chihuahua; CNY, Criollo de Nayarit; CHI, Criollo de Chiapas; BON, Blanco Orejinegro; CAQ, Caqueteño; SM, Sanmartinero; RMS, Romosinuano; CCC, Costeño con Cuernos; CH, Chino Santandereano; VEL, Velásquez; LUC, Lucerna; HV, Hartón del Valle; CC, Criollo Casanareño; EC, Criollo Ecuatoriano; CUR, Criollo Uruguayo; PA, Pampa Chaqueño; PIL, Criollo Pilcomayo; CARG, Criollo Argentino; PAT, Criollo Patagónico; CAR, Caracú; CUB, Criollo Cubano.

cluster includes Brazilian Caracú, Criollo Argentino, Criollo Patagónico and Criollo Uruguayo, with $q > 0.870$, and a second cluster includes the Criollo Cubano; all the remaining populations showed some level of admixture, as was the case for the Colombian Lucerna ($q = 0.501$), Hartón del Valle ($q = 0.668$) and the Paraguayan Criollo Pilcomayo ($q = 0.552$). For $K = 3$, Criollo Cubano grouped in a separate cluster, and for $K = 4$, the Brazilian Caracú formed a different cluster (results not shown).

For $K = 7$, the Panamanian populations were clearly differentiated and formed a separate cluster with the Colombian Lucerna. The North American breeds grouped together, except for the Mexican Criollo de Chiapas, which was in the same cluster as Caqueteño, Chino Santandereano, Velasquez, Criollo Casanareño, Criollo Ecuatoriano and Criollo Pilcomayo. The Colombian breeds Blanco Orejinegro, Sanmartinero, Romosinuano and Costeño con Cuernos formed another cluster, while the Criollo Argentino, Criollo Patagónico and Criollo Uruguayo formed the seventh cluster. This type of grouping was consistent with that obtained in the NeighborNet dendrogram shown in Fig. 1.

All breeds clustered independently when 21 groups were considered (the most probable *K* value), with the exception of Mexican Criollo de Baja California, Criollo de Chihuahua and Criollo de Nayarit, the Criollo Ecuatoriano, and the Colombian breeds Caqueteño, Hartón del Valle and Criollo Casanareño, which demonstrated considerable common ancestry in two to three clusters and were clearly admixed. For $K = 26$ (number of breeds studied), the results were similar to those obtained for $K = 21$, with increased subdivision of some populations (results not shown).

The results of the Bayesian cluster analysis carried out with STRUCTURE are summarized in Table S5, where the average *q* values in each of the 21 clusters are shown for the different breeds. The most important ancestry membership (or membership fraction) among the breeds ranged between 0.161 in Caqueteño and 0.854 in Criollo Cubano.

Discussion

Published studies reporting the genetic diversity of Latin American Creole cattle using microsatellites are still scarce and are limited to a small numbers of breeds, often from a single country (Ulloa-Arvizu *et al.* 2008; Martínez-Correal *et al.* 2009; Villalobos Cortés *et al.* 2009; Villalobos Cortés *et al.* 2010); there are a few studies examining the genetic diversity in Creole cattle breeds at the regional level (Liron *et al.* 2006b; Martínez *et al.* 2007). Although mtDNA and Y-chromosome markers were recently used to investigate the origins of some Creole cattle (Giovambattista *et al.* 2000; Magee *et al.* 2002; Carvajal-Carmona *et al.* 2003; Liron *et al.* 2006a; Ginja *et al.* 2009b), to our knowledge our study is the most comprehensive genetic diversity analysis of Creole cattle from the Americas based on autosomal markers.

The levels of within-breed diversity detected in Creole cattle were considerable ($H_o = 0.740$, $H_e = 0.711$ and mean of number of alleles/locus (MNA) = 6.92), and were higher than those previously reported for European breeds (Cañón *et al.* 2001; Mateus *et al.* 2004; Martin-Burriel *et al.* 2007; Ginja *et al.* 2009a), but similar to those found in other Latin American breeds (Liron *et al.* 2006b; Egito *et al.* 2007).

The high genetic variability found in Creole cattle, even in endangered populations such as the Criollo Patagónico, Criollo Cubano, Panamanian Creoles and most of the

Colombian breeds, may reflect contributions from animals of distinct origins, as confirmed by the analysis of mtDNA sequences and Y haplotypes, which have noted the heterogeneous genetic composition of Creole cattle through the detection of both European and African influences (Carvajal-Carmona *et al.* 2003; Miretti *et al.* 2004; Ginja *et al.* 2009b).

The degree of genetic differentiation among the Creole breeds studied, which is supported by significant between-population *theta* estimates, indicated relatively low levels of gene flow and some level of reproductive isolation among most Creole breeds. As in other studies using microsatellites, most of the genetic variation corresponded to differences among individuals, and only 8.4% of the total variation was due to breed differences, which is in the range reported for other cattle breeds (Kantanen *et al.* 2000; Cañón *et al.* 2001; Mateus *et al.* 2004; Liron *et al.* 2006b; Ginja *et al.* 2009a). Furthermore, results in the present study indicate that most of the populations included herein show levels of genetic differentiation that are sufficient to consider them as independent breeds according to international conventions (FAO 2007); thus efforts should be made to recognize, protect and promote them.

AMOVA analyses showed that higher levels of genetic diversity were observed among breeds from different regions than among breeds from different countries (F_{SC} estimates of about 6.0% and 4.5%, respectively). This pattern could reflect the regional routes of dispersion of Creole cattle across the Americas. The higher genetic differentiation among the regional groups could be a consequence of the genetic drift that has occurred due to the low number of animals brought to the Western Hemisphere in the 15th and 16th centuries, which has been estimated at less than 1000 individuals (Rouse 1977; Primo 1992). Moreover, regional divergence may also reflect differences between the regions in which exotic germplasm has been introduced over time, especially Zebu (*B. indicus*) introgression during the 20th century, which occurred mainly in regions with a tropical climate (Rouse 1977). Average D_A genetic distances between North and South American breeds (0.191; SD ± 0.038) are bigger than those between Iberian and French breeds (0,126; SD ± 0.18) that were found by Beja-Pereira *et al.* (2003).

In the NeighborNet representation of the Reynolds genetic distances, five different clusters can be recognized, and each may correspond to a different path of cattle dispersion into the Americas or, in some cases, perhaps to a more recent introgression of germplasm from other breeds. The Mexican breeds and the Texas Longhorn, which is near the centre of the radial tree, and which could correspond to one of the first paths of cattle dispersion into Central and North America, form the first group. A second cluster is established by the Panamanian and some Colombian breeds (Blanco Orejinegro, Lucerna and Hartón del Valle); this could correspond to the first route of cattle introduction into South America from the Caribbean Islands, as well as the important commercial exchange between the Panamanian and Colombian ports in the 16th century (Primo 2004). The cluster formed by the Brazilian Caracú, Criollo Argentino, Criollo Patagónico and Criollo Uruguayo, with the Pampa Chaqueño located nearby, is likely reflective of the Rio de la Plata and Brazilian routes of colonization, and cattle flow into South America. A fourth group made up of four Colombian breeds, raised either close to the Atlantic coast (Romosinuano and Costeño con Cuernos) or in the Eastern Llanos (Sanmartinero and Criollo Casanareño), may share a common ancestry, as it is known that, for example, the Costeño con Cuernos was used in the foundation of the Romosinuano (Primo 1992). The last cluster includes breeds with a widely dispersed geographical distribution, such as the Creoles from Cuba and Ecuador, Velasquez and Caqueteño from Colombia, Criollo de Chiapas from Mexico and Criollo Pilcomayo from Paraguay. The introduction of *B. indicus* in many tropical areas could be the major factor causing the genetic proximity among these breeds. The influence of *B. indicus* has been demonstrated, for example, in Criollo Cubano (Uffo *et al.* 2006) and Criollo de Chiapas (Ginja *et al.* 2009b), and it is known that *B. indicus* was used in the development of the Velasquez breed (Martínez-Correal *et al.* 2009).

Analysis with STRUCTURE confirmed the general features observed in the NeighborNet dendrogram and revealed a high level of genetic admixture in some populations, thus explaining the differences between these graphic representations. For example, it was not possible to differentiate the Mexican Creoles from Baja California, Chihuahua and Nayarit, because these breeds grouped in the same cluster. Similarly, it was not possible to differentiate between the Colombian Hartón del Valle, Criollo Casanareño Creole and Caqueteño, which also showed a considerable level of genetic admixture.

Despite the high genetic diversity detected within Creole cattle, significant inbreeding was also observed within some breeds, especially in Criollo Poblano, which tend to be raised in several closed and independent herds. Heterozygote excess was detected in Colombian Blanco Orejinegro, Caracú and Criollo Cubano, which have suffered recent bottlenecks. Some crossing among different herds, possibly even with other breeds, may also have taken place. These results indicate that appropriate management programs must be implemented to ensure that the genetic pool represented by these breeds is not lost due to further genetic erosion or uncontrolled crossbreeding.

In conclusion, Creole cattle populations retain high levels of genetic diversity, especially when compared with breeds that are under more intensive breeding programs. In spite of the general name of Creole that is used to classify most of the populations analysed, there are important breed differences among them, even though some show signs of genetic admixture. Inbreeding was

detected in some breeds, suggesting the need for appropriate measures to be taken to avoid its negative effects. The results presented herein can be used to support breed recognition and promotion, and to assist all stakeholders in the implementation of conservation measures and breeding programs adjusted to the specific characteristics of each country and cattle population.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Description of ΔK values computed by STRUCTURE software.

Table S1 List of software used for statistical analyses.

Table S2 Microsatellite loci analysed.

Table S3 Pairwise population genetic distance values among 26 Creole breeds.

Table S4 Partitioning of genetic variability among the different sources of variation, with breeds grouped by a) region and b) country.

Table S5 Estimated membership fractions in each cluster.

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