Genetic diversity, structure, and breed relationships in Iberian cattle[1]

I. Martín-Burriel,[*2] C. Rodellar,[*] J. Cañón,[†] O. Cortés,[†] S. Dunner,[†] V. Landi,[‡]
A. Martínez-Martínez,[‡] L. T. Gama,[§] C. Ginja,[§#] M. C. T. Penedo,[#]
A. Sanz,[*] P. Zaragoza,[*] and J. V. Delgado[‡]

[*]Laboratorio de Genética Bioquímica, Facultad de Veterinaria, Universidad de Zaragoza, 50013 Zaragoza, Spain;
[†]Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040
Madrid, Spain; [‡]Departamento de Genética, Universidad de Córdoba, Campus Rabanales C-5,
14071 Córdoba, Spain; [§]L-INIA, Instituto Nacional dos Recursos Biológicos, 2005-048 Vale de Santarém,
Portugal; and [#]University of California, Veterinary Genetics Laboratory, One Shields Avenue, Davis 95616

ABSTRACT: In Iberia there are 51 officially recognized cattle breeds of which 15 are found in Portugal and 38 in Spain. We present here a comprehensive analysis of the genetic diversity and structure of Iberian cattle. Forty of these breeds were genotyped with 19 highly polymorphic microsatellite markers. Asturiana de los Valles displayed the greatest allelic diversity and Mallorquina the least. Unbiased heterozygosity values ranged from 0.596 to 0.787. The network based on Reynolds distances was star-shaped with few pairs of interrelated breeds and a clear cluster of 4 breeds (Alistana/Arouquesa/Marinhoa/Mirandesa). The analysis of the genetic structure of Iberian cattle indicated that the most probable number of population clusters included in the study would be 36. Distance results were supported by the STRUCTURE software indicating a relatively recent origin or possible crossbreeding or both between pairs or small groups of breeds. Five clusters included 2 different breeds (Betizu/Pirenaica, Morucha/Avileña, Parda de Montaña/Bruna de los Pirineos, Barrosã/Cachena, and Toro de Lidia/Brava de Lide), 3 breeds (Berrenda en Negro, Negra Andaluza, and Mertolenga) were divided in 2 independent clusters each, and 2 breeds were considered admixed (Asturiana de los Valles and Berrenda en Colorado). Individual assignation to breeds was not possible in the 2 admixed breeds and the pair Parda de Montaña/Bruna de los Pirineos. The relationship between Iberian cattle reflects their geographical origin rather than their morphotypes. Exceptions to this geographic clustering are most probably a consequence of crossbreeding with foreign breeds. The relative genetic isolation within their geographical origin, the consequent genetic drift, the adaptation to specific environment and production systems, and the influence of African and European cattle have contributed to the current genetic status of Iberian cattle, which are grouped according to their geographical origin. The greater degree of admixture observed in some breeds should be taken into account before using molecular markers for genetic assignment of individuals to breeds.

Key words: admixture, *Bos taurus*, genetic diversity, Iberia, microsatellite marker, structure and relationship

INTRODUCTION

It is generally assumed that most cattle breeds from the Iberian Peninsula originated in the Near East, from where they followed several migration routes (Troy et al., 2001; Beja-Pereira et al., 2003). Because of its geographical location, the Iberian Peninsula can be considered a contact zone between the African and European continents through the Strait of Gibraltar. The peoples inhabiting the Iberian Peninsula throughout history have contributed to the wide variety of autochthonous cattle breeds extant in this territory.

After domestication, a slow genetic improvement of European cattle occurred until the 19th century. Then

a rapid increase in beef and dairy production was achieved through the use of a limited number of breeds (Medugorac et al., 2009), and during the last century, many cattle breeds became extinct (Köhler-Rollefson, 2000).

Officially recognized autochthonous Iberian cattle include 15 Portuguese and 38 Spanish breeds. Molecular characterization of animal genetic resources may contribute to a rational approach to conservation (European Cattle Diversity Consortium, 2006). During the last few years, partial genetic studies used microsatellite markers to characterize Spanish or Portuguese cattle (Cañón et al., 2001; Beja-Pereira et al., 2003; Cymbron et al., 2005; Martin-Burriel et al., 2007; Ginja et al., 2010b), but a thorough analysis of Iberian cattle breeds has not been carried out thus far.

A consortium of research groups was created in 2007 in the framework of the CONBIAND network (http://www.uco.es/conbiand), and one of its goals was to characterize the genetic diversity of Iberian cattle. In this study, we merged previously obtained genotypic information for Portuguese and Spanish breeds, and also included newly generated genotypic data for 12 breeds from the Northern and Southern Iberian Peninsula and the Canary Islands, assembling a large microsatellite data set on Spanish and Portuguese cattle breeds, which was used to investigate their global genetic variability, structure, and breed relationships.

MATERIALS AND METHODS

The procedure for blood collection from commercial farm animals followed the recommendations of the Joint Working Group on Refinement (1993).

DNA Sampling and Microsatellite Analyses

Forty autochthonous populations from Spain (n = 27) and Portugal (n = 13) were included in this study to represent Spanish and Portuguese cattle: Alistana (**ALIS**), Asturiana de los Valles (**ASTV**), Asturiana de las Montañas (**ASTM**), Avileña (**AVIL**), Berrenda en Colorado (**BCOL**), Berrenda en Negro (**BNEG**), Betizu (**BETI**), Bruna de los Pirineos (**BRUP**), Mallorquina (**MALL**), Marismeña (**MARS**), Menorquina (**MENO**), Monchina (**MONC**), Morucha (**MORU**), Mostrenca (**MOST**), Negra Andaluza (**NAND**), Pajuna (**PAJU**), Parda de Montaña (**PMON**), Pasiega (**PASI**), Pirenaica (**PIRE**), Retinta (**RETI**), Rubia Gallega (**RGAL**), Sayaguesa (**SAYA**), Serrana de Teruel (**STER**), Toro de Lidia (**TLID**), Tudanca (**TUDA**), Vaca Canaria (**VCAN**), Vaca Palmera (**PALM**), Alentejana (**ALEN**), Arouquesa (**AROU**), Barrosã (**BARR**), Brava de Lide (**BRAV**), Cachena (**CACH**), Garvonesa (**GARV**), Marinhoa (**MARI**), Maronesa (**MARO**), Mertolenga (**MERT**), Minhota (**MINH**), Mirandesa (**MIRA**), Preta (**PRET**), and Ramo Grande-Azores (**RAMO**). Twenty to 70 individuals were sampled in each breed with an overall av-erage of approximately 48 animals per breed (Table 1). Efforts were made to ensure that samples were from unrelated animals, registered in herd books, and from a broad geographic area. In the case of the Fighting Bull, samples from Spain and Portugal were collected, whereas for MARS 2 subpopulations were considered (i.e., MARS, which includes animals sampled from several private farms located in the area of Doñana National Park; and MOST, including animals of the same type owned and raised by the Biological Station of Doñana, which have been mostly kept as a closed herd for several years).

The DNA was extracted from blood or hair roots from a total of 1,924 animals, using previously described procedures (Martinez et al., 2000; Martin-Burriel et al., 2007; Ginja et al., 2010b). Twenty-one microsatellite loci distributed across 18 bovine chromosomes and recommended by the Food and Agriculture Organization (**FAO**, 2004) for use in genetic diversity studies were analyzed: *BM1818*, *BM1824*, *BM2113*, *CSRM60*, *CSSM66*, *ETH3*, *ETH10*, *ETH185*, *ETH225*, *HAUT27*, *HEL9*, *ILSTS6*, *INRA32*, *INRA35*, *INRA37*, *INRA63*, *MM12*, *SPS115*, *TGLA53*, *TGLA122*, and *TGLA227*. Microsatellite loci were amplified through PCR using either α[^{32}P]-deoxycytidine triphosphate or fluorescence-labeled primers as described previously (Martin-Burriel et al., 1999; Ginja et al., 2010b). Products of PCR were separated by electrophoresis in 6% denaturing polyacrylamide gels or with capillary genetic analyzers (ABI 377 XL and ABI 3130, Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. Allele sizes were determined with the internal size standards GeneScan-400HD ROX and GeneScan-500 LIZ (Applied Biosystems, Warrington, UK), respectively. Allele nomenclature was standardized following a former European research project (EU RESGEN CT 98-118) on cattle diversity. To ensure compatibility of results from different equipment and laboratories, a total of 30 samples representing the entire allele ranges for this set of markers were genotyped in all participating laboratories. Reference samples were also used in each electrophoresis to standardize results. Data for *INRA35* and *INRA37* were removed from the further analysis of genetic variability, distances, and structure due to the presence of null and 1-bp insertion/deletion alleles, respectively.

Genetic Variability and Neutrality

Allele frequencies for each locus were obtained by direct counting with GENEPOP (Raymond and Rousset, 1995). This program was also used to test for deviations from Hardy-Weinberg equilibrium (**HWE**) and for heterozygote deficit using a Markov chain method to estimate the *P*-values. Fisher's method was applied to calculate the significance of the HWE probabilities across loci.

The mean number of alleles per locus (**MNA**) and allelic richness, which corresponds to the corrected mean

Table 1. Breeds studied, corresponding abbreviation, country of origin, geographical location, breed status, sample size, and diversity estimates (SD in parentheses)

Breed name	Abbreviation	Cntry[1]	Loc[2]	St[3]	n[4]	tA[5]	pA[6]	rA[7]	MNA[8]	R_t[9]	H_E[10]	H_O[11]	F_{IS}[12]	LHWEd[13]	LHD[14]
Alistana[15]	ALIS	Spain	N-W	E	50	140	1	12	7.9 (2.6)	5.5 (1.4)	0.690 (0.023)	0.643 (0.014)	0.068***	4***	6
Asturiana de los Valles[15]	ASTV	Spain	North	I	50	164†	1	22†	9.7 (4.0)†	6.5 (2.1)†	0.736 (0.025)	0.715 (0.013)	0.034	3***	3
Asturiana Montañas[15]	ASTM	Spain	North	E	50	136	0	13	7.9 (3.1)	5.7 (1.6)	0.715 (0.024)	0.674 (0.014)	0.069***	3***	8
Avileña[15]	AVIL	Spain	Cent	I	50	140	1	10	8.1 (3.2)	5.8 (1.7)	0.721 (0.024)	0.656 (0.014)	0.060***	7***	5
Berrenda en Colorado	BCOL	Spain	South	E	40	146	0	11	8.6 (3.9)	6.5 (2.1)†	0.750 (0.025)†	0.690 (0.015)	0.063***	3*	4
Berrenda en Negro	BNEG	Spain	South	E	30	99	1	4	5.6 (1.9)	4.6 (1.4)	0.608 (0.027)	0.555 (0.019)	0.064	9***†	4
Betizu[16]	BETI	Spain	North	SF-HE	20	108	0	8	6.2 (2.3)	5.5 (1.7)	0.707 (0.019)	0.634 (0.024)	0.092	2*	1
Bruna de los Pirineos[15]	BRUP	Spain	N-E	E	50	142	1	12	8.2 (3.1)	5.5 (1.6)	0.698 (0.024)	0.677 (0.014)	0.014	1	2
Mallorquina[16]	MALL	Spain	BI	HE	50	83†	1	4	4.8 (2.3)†	3.8 (1.4)†	0.554 (0.041)†	0.518 (0.015)†	0.061	4***	2
Marismeña	MARS	Spain	South	SF-HE	50	147	3†	13	8.3 (3.0)	5.8 (1.5)	0.717 (0.024)	0.698 (0.013)	0.021	1	1
Menorquina[16]	MENO	Spain	CI	HE	50	111	0	3	6.6 (3.4)	4.7 (1.8)	0.632 (0.026)	0.625 (0.014)	−0.013	2	2
Monchina[16]	MONC	Spain	North	SF-E	50	153	0	17	9.0 (3.8)	6.1 (1.8)	0.732 (0.025)	0.699 (0.013)	0.026	2*	2
Morucha[15]	MORU	Spain	Cent	I	50	146	0	14	8.3 (2.4)	6.0 (1.5)	0.729 (0.026)	0.685 (0.013)	0.067***	2*	4
Mostrenca	MOST	Spain	South	SF-HE	50	103	0	4	5.8 (1.8)	4.3 (1.1)	0.603 (0.023)	0.595 (0.014)	0.010	3**	1
Negra Andaluza	NAND	Spain	South	E	21	117	1	7	6.6 (2.6)	5.5 (1.8)	0.696 (0.023)	0.634 (0.022)	0.107***	3**	6
Pajuna	PAJU	Spain	South	HE	38	140	1	7	8.2 (3.9)	5.8 (1.8)	0.715 (0.025)	0.683 (0.015)	0.042	4***	2
Parda de Montaña	PMON	Spain	N-E	Com	50	142	1	13	8.2 (3.0)	5.6 (1.5)	0.701 (0.021)	0.691 (0.013)	0.014	1	1
Pasiega	PASI	Spain	North	HE	50	142	2	13	8.5 (3.6)	5.9 (1.9)	0.705 (0.027)	0.666 (0.014)	0.049	3	2
Pirenaica[15]	PIRE	Spain	N-E	I	50	136	0	11	7.9 (3.2)	5.7 (1.6)	0.699 (0.024)	0.666 (0.014)	0.029	4*	4
Retinta[15]	RETI	Spain	South	I	50	143	0	13	8.1 (3.1)	6.0 (1.6)	0.735 (0.021)	0.678 (0.013)	0.030	1	2
Rubia Gallega	RGAL	Spain	N-W	I	50	142	0	13	8.3 (3.2)	5.7 (1.7)	0.701 (0.030)	0.666 (0.014)	0.024	1***	4
Sayaguesa[15]	SAYA	Spain	N-W	HE	50	139	2	12	7.9 (2.4)	5.9 (1.5)	0.730 (0.020)	0.671 (0.014)	0.078***	6***	5

Continued

Table 1 (Continued). Breeds studied, corresponding abbreviation, country of origin, geographical location, breed status, sample size, and diversity estimates (SD in parentheses)

Breed name	Abbreviation	Cntry[1]	Loc[2]	St[3]	n[4]	tA[5]	pA[6]	rA[7]	MNA[8]	R_t[9]	H_E[10]	H_O[11]	F_{IS}[12]	LHWEd[13]	LHD[14]
Serrana de Teruel[16]	STER	Spain	Cent	HE	50	151	1	15	8.7 (3.3)	6.1 (1.7)	0.737 (0.023)	0.698 (0.013)	0.045	6***	4
Toro de Lidia[15]	TLID	Spain	Cent	SF-I	50	120	0	2	7.0 (3.3)	5.1 (2.0)	0.628 (0.035)	0.554 (0.015)	0.099***	7***	5
Tudanca[16]	TUDA	Spain	North	E	50	136	0	10	7.9 (3.6)	5.6 (2.0)	0.695 (0.026)	0.631 (0.014)	0.054	6***	5
Vaca Canaria	VCAN	Spain	CI	E	50	152	3†	16	8.8 (3.3)	6.1 (1.7)	0.725 (0.028)	0.682 (0.014)	0.052	2	4
Vaca Palmera	PALM	Spain	CI	HE	50	106	0	8	6.3 (3.4)	4.7 (2.2)	0.582 (0.037)	0.591 (0.014)	−0.011	3*	1
Alentejana[17]	ALEN	Port	S-W	E	38	110	0	7	6.4 (2.3)	5.0 (1.3)	0.666 (0.034)	0.623 (0.017)	0.059	2*	3
Arouquesa[17]	AROU	Port	W	E	70	152	0	16	8.9 (3.7)	6.0 (1.8)	0.726 (0.026)	0.695 (0.012)	0.038	4**	5
Barrosã[17]	BARR	Port	N-W	E	69	124	0	8	7.3 (2.9)	5.2 (1.5)	0.678 (0.037)	0.673 (0.012)	−0.001	1	0†
Brava de Lide[17]	BRAV	Port	Cent	SF-E	43	104	0	2	6.2 (2.9)	4.5 (1.7)	0.610 (0.038)	0.543 (0.016)	0.107***	6***	7
Cachena[17]	CACH	Port	N-W	HE	51	146	0	12	8.2 (2.4)	5.7 (1.3)	0.716 (0.034)	0.708 (0.014)	−0.001	1	2
Garvonesa[17]	GARV	Port	S-W	HE	39	117	0	8	6.7 (2.8)	5.0 (1.8)	0.633 (0.040)	0.652 (0.016)	−0.041	1	2
Marinhoa[17]	MARI	Port	W	E	46	116	0	4	6.7 (2.5)	5.1 (1.6)	0.682 (0.028)	0.673 (0.015)	0.005	1	1
Maronesa[17]	MARO	Port	N-W	E	47	127	0	9	7.3 (2.5)	5.2 (1.2)	0.649 (0.038)	0.642 (0.015)	0.012	1	4
Mertolenga[17]	MERT	Port	S-W	E	64	147	0	13	8.6 (3.8)	5.8 (1.9)	0.706 (0.030)	0.619 (0.013)	0.113***,†	7***	9†
Minhota[17]	MINH	Port	N-W	E	50	145	2	10	8.5 (3.2)	5.9 (1.8)	0.717 (0.028)	0.748 (0.013)†	−0.059***,†	0	1
Mirandesa[17]	MIRA	Port	N-W	E	54	106	0	6	6.3 (2.1)	4.4 (1.3)	0.614 (0.023)	0.586 (0.014)	0.012	2	2
Preta[17]	PRET	Port	S-W	E	60	133	1	5	7.7 (3.4)	5.3 (1.7)	0.669 (0.032)	0.642 (0.013)	0.032	0†	2
Ramo Grande-Azores[17]	RAMO	Port	AzI	HE	44	143	1	9	8.3 (3.5)	5.7 (1.8)	0.705 (0.025)	0.667 (0.015)	0.045	1	3

Continued

Table 1 (Continued). Breeds studied, corresponding abbreviation, country of origin, geographical location, breed status, sample size, and diversity estimates (SD in parentheses)

Breed name	Abbreviation	Cntry[1]	Loc[2]	St[3]	n[4]	tA[5]	pA[6]	rA[7]	MNA[8]	R_t[9]	H_E[10]	H_O[11]	F_{IS}[12]	LHWEd[13]	LHD[14]
Overall mean value					48.1	131.3	0.6	9.9	7.6 (1.1)	5.5 (2.3)	0.685 (0.049)	0.651 (0.050)	0.039	2.85	3.3

[1]Country of origin: Spain or Portugal (Port).
[2]Geographical location: North, Northwest (N-W), Northeast (N-E), South, Southwest (S-W), West (W), Central (Cent), Azores Islands (AzI), Balearic Islands (BI), Canary Islands (CI).
[3]Breed status: commercial (Com), increasing census (I), endangered (E), highly endangered (HE), semiferal (SF).
[4]Sample size (n).
[5]Total number of alleles observed in each breed.
[6]Number of private alleles observed in each breed.
[7]Number of rare alleles observed in each breed.
[8]Mean number of alleles per locus.
[9]Allelic richness values.
[10]Expected heterozygosity values.
[11]Observed heterozygosity values.
[12]Inbreeding coefficient (F_{IS}) and significance (***$P < 0.001$).
[13]Number of loci in Hardy-Weinberg disequilibrium (LHWEd) and the significance of Hardy-Weinberg equilibrium across loci using Fisher's method (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
[14]Number of loci showing a significant heterozygote deficit (LHD).
[15]Data set included in Cañón et al. (2001).
[16]Data set included in Martín-Burriel et al. (2007).
[17]Data set included in Ginja et al. (2010b).
†Indicates the maximum and minimum values.

number of alleles per population based on a minimum sample size (n = 10), were calculated using FSTAT (Goudet, 2002). Unbiased expected ($\mathbf{H_E}$) and observed ($\mathbf{H_O}$) heterozygosities were calculated with the MICROSATELLITE Toolkit software for Excel (Park, 2001). Alleles were classified based on their frequency as described by Medugorac et al. (2009) and according to the following: common alleles, if they appeared across most populations; private alleles ($\mathbf{pA}$), those observed exclusively in 1 breed; and rare alleles ($\mathbf{rA}$), the nonprivate alleles with frequencies less than 0.01 across the entire data set. The inbreeding coefficient ($\mathbf{F_{IS}}$) per population and the corresponding 95% confidence interval after 1,000 bootstraps were calculated using GENETIX software (Belkhir, 2001). Estimators of F_{IT} (inbreeding coefficient of an individual relative to the total population), F_{ST} (the effect of subpopulations compared with the total population), and F_{IS} (inbreeding coefficient of an individual relative to the subpopulation; Weir and Cockerham, 1984) were calculated per locus with FSTAT (Goudet, 2002).

The probability that each locus is under selection was estimated using a Bayesian method (Foll and Gaggiotti, 2008) as implemented in the program BayeScan (http://www-leca.ujf-grenoble.fr/logiciels.htm). We applied a decisive Jeffreys' scale of evidence that corresponds to a posterior probability >0.99.

Genetic Distances

Breed relationships were analyzed with Reynolds genetic distances estimated in PHYLIP (Felsenstein, 1993). A neighbor-joining tree (Saitou and Nei, 1987) was obtained with the CONSENSE application of this software and 1,000 bootstrap replicates. The neighbor-net method (Bryant and Moulton, 2004) as implemented in SPLITSTREE software (Huson and Bryant, 2006) was also used to compute a network based on Reynolds distances to graphically represent breed relationships and admixture.

Genetic Structure

The Bayesian model-based method developed by Pritchard et al. (2000) and implemented in the STRUCTURE software (http://pritch.bsd.uchicago.edu/structure.html) was used to investigate population structure and define clusters of individuals on the basis of multilocus genotypes for 19 microsatellite markers. The number of assumed populations ($\mathbf{K}$) varied between 2 and 40. For each K, 10 independent runs were performed with a burn-in of 10^5 and Markov chain Monte Carlo length of 10^6 iterations under an admixture and correlated allele frequencies model. The average and SD of the logarithmic likelihood [L(K)] of the data were estimated across 10 runs for each K value. The most probable number of population clusters was determined by plotting L(K) and also using the distribution of ΔK (Evanno et al., 2005). To investigate further popula-
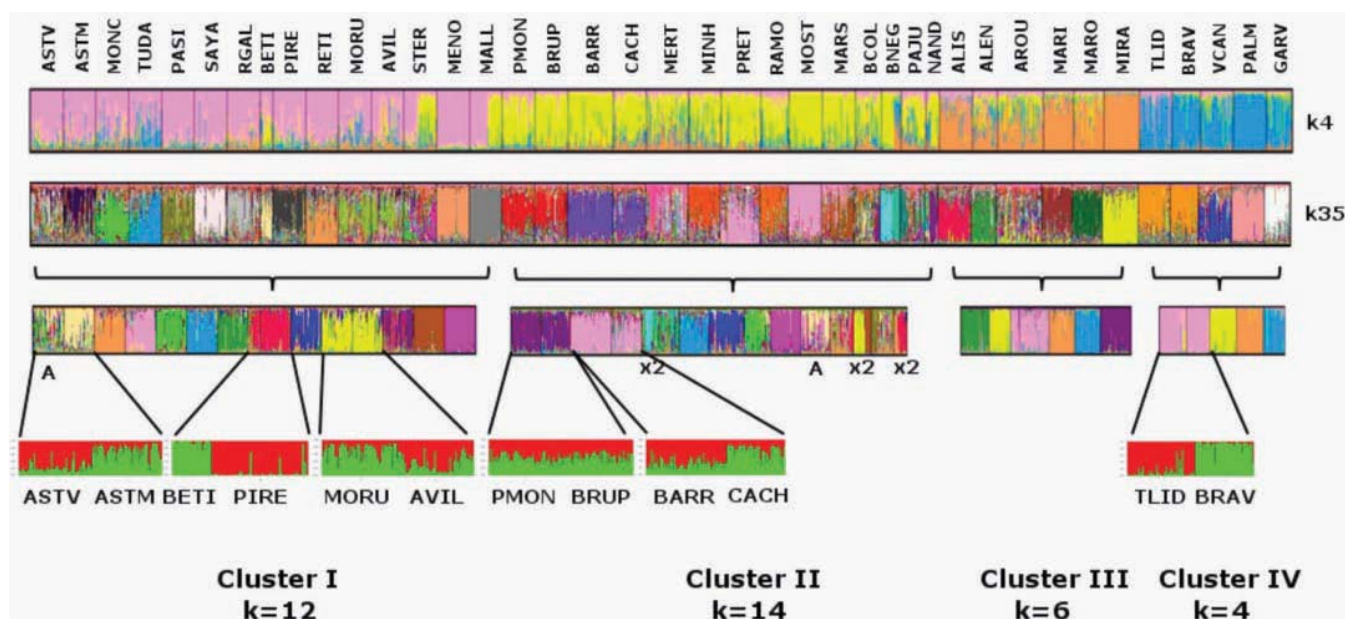
tion subdivisions, the major clusters identified with STRUCTURE were reanalyzed using the same settings and assuming K = 2 to K = n + 3 (n being the number of predefined breeds included in each cluster). After assessing the most likely number of underlying populations, the results were graphically displayed with

DISTRUCT (available at http://rosenberglab.bioinformatics.med.umich.edu/distruct.html; last accessed October 15, 2009).

Assignment tests were performed with STRUCTURE without using prior information of source breeds. The proportion of the genotype of each individual in each
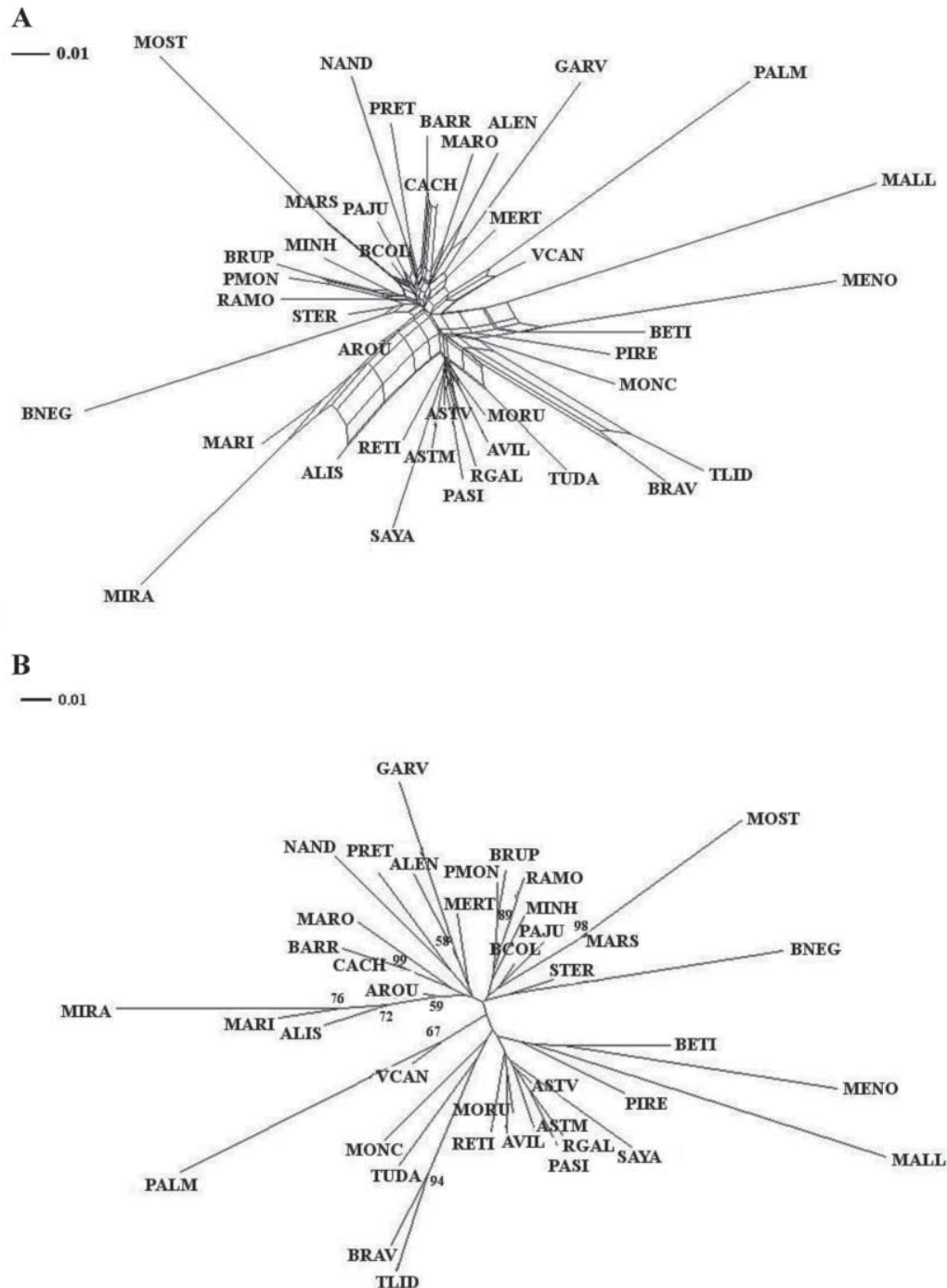
Figure 1. Neighbor-net (A) and neighbor-joining tree (B) summarizing the Reynolds distances among 40 native cattle from Spain and Portugal. Bootstrap values greater than 50% are shown. Abbreviations: Alistana (ALIS), Asturiana de los Valles (ASTV), Asturiana de las Montañas (ASTM), Avileña (AVIL), Berrenda en Colorado (BCOL), Berrenda en Negro (BNEG), Betizu (BETI), Bruna de los Pirineos (BRUP), Mallorquina (MALL), Marismeña (MARS), Menorquina (MENO), Monchina (MONC), Morucha (MORU), Mostrenca (MOST), Negra Andaluza (NAND), Pajuna (PAJU), Parda de Montaña (PMON), Pasiega (PASI), Pirenaica (PIRE), Retinta (RETI), Rubia Gallega (RGAL), Sayaguesa (SAYA), Serrana de Teruel (STER), Toro de Lidia (TLID), Tudanca (TUDA), Vaca Canaria (VCAN), Vaca Palmera (PALM), Alentejana (ALEN), Arouquesa (AROU), Barrosã (BARR), Brava de Lide (BRAV), Cachena (CACH), Garvonesa (GARV), Marinhoa (MARI), Maronesa (MARO), Mertolenga (MERT), Minhota (MINH), Mirandesa (MIRA), Preta (PRET), and Ramo Grande-Azores (RAMO).

Figure 2. Genetic structure of 40 Spanish and Portuguese autochthonous cattle breeds. These populations were grouped in 4 ancestral clusters [number of assumed populations (K) = 4] and reanalyzed to infer further structure. The final clustering identified 36 groups including 3 substructured breeds [Mertolenga (MERT), Berrenda en Negro (BNEG), and Negra Andaluza (NAND)] and 2 admixed breeds [M: Asturiana de los Valles (ASTV) and Berrenda en Colorado (BCOL)]. Individual analyses with K = 2 were run for clusters containing the pairs ASTV/Asturiana de las Montañas (ASTM), Betizu (BETI)/Pirenaica (PIRE), Morucha (MORU)/Avileña (AVIL), Parda de Montaña (PMON)/Bruna de los Pirineos (BRUP), Barrosã (BARR)/Cachena (CACH), and Toro de Lidia (TLID)/Brava de Lide (BRAV). Other abbreviations: Alistana (ALIS), Mallorquina (MALL), Marismeña (MARS), Menorquina (MENO), Monchina (MONC), Mostrenca (MOST), Pajuna (PAJU), Pasiega (PASI), Retinta (RETI), Rubia Gallega (RGAL), Sayaguesa (SAYA), Serrana de Teruel (STER), Tudanca (TUDA), Vaca Canaria (VCAN), Vaca Palmera (PALM), Alentejana (ALEN), Arouquesa (AROU), Garvonesa (GARV), Marinhoa (MARI), Maronesa (MARO), Minhota (MINH), Mirandesa (MIRA), Preta (PRET), and Ramo Grande-Azores (RAMO). Color version available in the online PDF.

cluster or breed (q) obtained with STRUCTURE without using prior information of source breeds were used for assignments. The percentage of individuals correctly assigned to source breeds was calculated for $q > 0.80$ and $q > 0.95$ thresholds. For clusters involving 2 breeds, additional runs were performed for K = 2 to obtain individual q values (Leroy et al., 2009).

RESULTS

Diversity of Portuguese and Spanish Cattle Populations

A total of 248 alleles were detected across 19 microsatellite loci in the 40 Spanish and Portuguese cattle breeds studied. Diversity values per locus are shown in Supplemental Table S1 (available at http://jas.fass.org/content/vol89/issue4/). The MNA per locus was 13, ranging between 9 (*BM1824*, *ETH10*, and *INRA063*) and 22 (*TGLA122*). The genetic diversity observed in the breeds studied is summarized in Table 1. Predominant alleles differed across populations and the greatest number of pA was 3 in VCAN and MARS breeds. The greatest number of rA was observed in ASTV (22). In most cases, pA and rA had decreased frequencies (≤0.05), but the pA observed in SAYA at *INRA32* (170 and 172) had increased frequencies (0.45 and 0.190, respectively), being the predominant alleles in this breed.

Rare allele 128 at *HAUT27* in BETI also had a greater frequency (0.237). Overall, MALL showed the least MNA per locus (4.789) and allelic richness (3.762), whereas ASTV displayed the greatest values for both of these variables (9.737 and 6.499, respectively, Table 1).

Three zebu-diagnostic alleles described in locus *ETH225* (MacHugh, 1996) were detected in Spanish cattle, with allele *154* detected in MOST and MONC, allele *160* in MARS, and allele *158*, which has been described in greater frequencies in African zebu, in VCAN and RGAL. The *TGLA227-79* zebu-diagnostic allele (Lirón et al., 2006) was observed in 19 breeds (TUDA, PASI, RGAL, BETI, MORU, STER, BARR, CACH, PRET, RAMO, MARS, BCOL, BNEG, PAJU, AROU, MARI, TLID, BRAV, and GARV). Finally, West African taurine allele *BM2113-123* (MacHugh, 1996) was detected mainly in southern Iberian breeds (VCAN, GARV, ALEN, NAND, BNEG, BCOL, MARS, MERT, and RETI), but also in 3 northern Spanish cattle (BETI, ASTM, ASTV). On the contrary, the African zebu allele *BM2113-131* (MacHugh, 1996) was mainly present in northern populations (ASTV, ASTM, MONCH, TUDA, PASI, SAYA, RGAL, PIRE, RET, PAJU, AROU, MARO, and MINH).

Overall genetic diversity of Iberian cattle was high, with MNA = 7.61 ± 1.10, $H_E = 0.685 \pm 0.049$, and $H_O = 0.651 \pm 0.050$. Expected heterozygosity by breed

ranged from 0.554 (MALL) to 0.750 (BCOL), and observed heterozygosity varied between 0.518 (MALL) and 0.748 (MINH). A negative F_{IS} value, indicating observed heterozygosity values greater than expected, was significant in MINH (-0.059) for a 95% confidence interval. Ten populations displayed highly significant

F_{IS} values, indicating a deficit of heterozygotes (Table 1), with the greatest deficit observed in MERT (0.113), NAND (0.107), BRAV (0.107), and TLID (0.099). The estimates of global F-statistics and their 95% confidence interval were 0.030 (0.003 to 0.054) for F_{IS}, 0.114 (0.082 to 0.141) for F_{IT}, and 0.086 (0.072 to 0.103) for

Table 2. Proportional contribution of the inferred ancestral clusters (K = 4) in each breed studied and percentages of individuals correctly assigned to their known population for thresholds $q^1 > 0.95$ and $q > 0.8$

Breed[2]	Cluster I	Cluster II	Cluster III	Cluster IV	$q > 0.95$	$q > 0.80$
ASTV	0.787†	0.089	0.054	0.070	0*	62*
ASTM	0.751†	0.063	0.096	0.090	0*	44*
MONC	0.670†	0.085	0.097	0.148	4	56
TUDA	0.566†	0.060	0.088	0.286	14	62
PASI	0.836†	0.046	0.039	0.079	4	52
SAYA	0.852†	0.044	0.038	0.066	10	70
RGAL	0.737†	0.069	0.144	0.050	0	46
BETI	0.493†	0.304	0.075	0.128	75*	95*
PIRE	0.748†	0.115	0.032	0.105	76*	92*
RETI	0.794†	0.060	0.090	0.056	2	54
MORU	0.650†	0.074	0.125	0.150	0*	54*
AVIL	0.612†	0.136	0.152	0.100	0*	40*
STER	0.394†	0.386	0.102	0.118	12	40
MENO	0.923†	0.033	0.021	0.023	32	88
MALL	0.561†	0.369	0.023	0.047	76	94
PMON	0.191	0.669†	0.055	0.085	0*	0*
BRUP	0.095	0.778†	0.070	0.056	0*	0*
BARR	0.030	0.777†	0.125	0.067	0*	22*
CACH	0.092	0.618†	0.172	0.118	0*	39*
MERT	0.103	0.428†	0.279	0.190	0/1	17/15
MINH	0.087	0.566†	0.175	0.172	0	44
PRET	0.067	0.725†	0.135	0.073	5	33
RAMO	0.163	0.571†	0.073	0.193	0	45
MOST	0.022	0.877†	0.029	0.073	8	76
MARS	0.061	0.756†	0.062	0.122	0	14
BCOL	0.138	0.498†	0.171	0.193	0	0
BNEG	0.039	0.610†	0.197	0.154	20/10	57/33
PAJU	0.170	0.464†	0.213	0.153	8	21
NAND	0.121	0.559†	0.142	0.178	0/0	28/33
ALIS	0.166	0.035	0.716†	0.083	24	90
ALEN	0.174	0.216	0.447†	0.163	53	87
AROU	0.091	0.245	0.512†	0.152	13	61
MARI	0.043	0.060	0.846†	0.051	30	83
MARO	0.067	0.169	0.708†	0.056	38	83
MIRA	0.019	0.016	0.948†	0.017	48	81
TLID	0.245	0.035	0.046	0.675†	48*	84*
BRAV	0.029	0.071	0.059	0.840†	60*	93*
VCAN	0.093	0.163	0.062	0.682†	66	88
PALM	0.019	0.029	0.029	0.923†	84	94
GARV	0.043	0.169	0.094	0.694†	64	85
Overall	0.318†	0.300†	0.188†	0.191†	22.2†	58.2†

[1]Proportion of the genotype of each individual in each cluster or breed. Individual q values were obtained with the second STRUCTURE analyses of ancestral clusters. Breeds with substructure display 2 values corresponding to the assignation to each cluster.

[2]Alistana (ALIS), Asturiana de los Valles (ASTV), Asturiana de las Montañas (ASTM), Avileña (AVIL), Berrenda en Colorado (BCOL), Berrenda en Negro (BNEG), Betizu (BETI), Bruna de los Pirineos (BRUP), Mallorquina (MALL), Marismeña (MARS), Menorquina (MENO), Monchina (MONC), Morucha (MORU), Mostrenca (MOST), Negra Andaluza (NAND), Pajuna (PAJU), Parda de Montaña (PMON), Pasiega (PASI), Pirenaica (PIRE), Retinta (RETI), Rubia Gallega (RGAL), Sayaguesa (SAYA), Serrana de Teruel (STER), Toro de Lidia (TLID), Tudanca (TUDA), Vaca Canaria (VCAN), Vaca Palmera (PALM), Alentejana (ALEN), Arouquesa (AROU), Barrosã (BARR), Brava de Lide (BRAV), Cachena (CACH), Garvonesa (GARV), Marinhoa (MARI), Maronesa (MARO), Mertolenga (MERT), Minhota (MINH), Mirandesa (MIRA), Preta (PRET), and Ramo Grande-Azores (RAMO).

*Percentages obtained with K = 2.

†Proportion of the predominant cluster.

F_{ST} (Supplemental Table S1, available at http://jas.fass.org/content/vol89/issue4/).

HWE and Neutrality

All populations except MINH and PRET showed deviations from HWE at 1 or more loci (Table 1). Deviations from HWE across loci were significant in 24 populations for a $P < 0.05$ obtained with Fisher's method, in most cases due to heterozygote deficit. The breed that showed the greatest proportion of loci in disequilibrium was BNEG (9 loci) with a significant deficit of heterozygosity for 4 loci, whereas MERT presented 7 loci with HWE deviations and the greatest number of loci with heterozygote deficit (9 loci).

Using a stringent criterion, 4 loci (*SPS115*, *HEL9*, *MM12*, and *BM1818*) showed decisive evidence for directional selection with a posterior probability >99%. Three of these loci coincided in known QTL regions according to Cattle QTLdb (2010). The microsatellite *SPS115* is a flanking marker for a QTL affecting KPH percentage (Kim et al., 2003), *HEL9* for clinical mastitis and somatic cell count (Klungland et al., 2001), and *BM1818* is a flanking marker for 12 different QTL regions (Plante et al., 2001; Ashwell et al., 2004; Cruickshank et al., 2004; Kneeland et al., 2004; Li et al., 2004; Schnabel et al., 2005; Seidenspinner et al., 2009; McClure et al., 2010).

Genetic Relationships Among Breeds

The neighbor-net based on Reynolds distances (Figure 1) had a star-like shape consistent with a close genetic relationship among Portuguese and Spanish cattle breeds. Some clusters and pairs of interrelated breeds were in agreement with the greatest bootstrap values of the neighbor-joining tree. For instance, the Portuguese BARR and CACH grouped together (99% bootstrap), as did Spanish and Portuguese fighting cattle TLID and BRAV (94%), the Doñana populations MOST and MARS (98%), the mountain cattle BRUP and PMON (89%), the 2 Canary breeds (VCA and PALM, 67%), and the cluster containing the Portuguese MIRA, MARI, and AROU and the Spanish ALIS (bootstrap values ranging from 59 to 76%). The most divergent populations were BRAV, BNEG, GARV, MALL, MENO, MIRA, MOST, PALM, and TLID. Reynolds distances were also calculated using the 15 neutral markers, and a neighbor-joining tree was constructed displaying very similar results (data not shown).

Genetic Structure

The results obtained in the analysis with STRUCTURE showed an increase in the likelihood of the data with the number of clusters considered, reaching a plateau at K = 26 to 35 (Supplemental Figure S1, available at http://jas.fass.org/content/vol89/issue4/). The greatest ΔK was obtained at K = 4 (24.4) followed by K = 35 (20.2). The genetic structure detected at low K values might indicate more a ancestral subdivision of cattle populations that could precede breed formation. For K = 4, the following breed groups were identified (Figure 2): cluster I included most of the North and Central Spanish breeds, together with the 2 Balearic cattle; cluster II grouped the 2 Brown Alpine-derived breeds (PMON and BRUP), most of the Andalusian cattle, as well as the Northern (MINH, BARR, CACH) and Southern (PRET and MERT) Portuguese breeds with cattle from Azores (RAMO); cluster III included the Northern Portuguese breeds MARI, ARO, MARO, and MIRA and the Spanish ALS, which inhabits a neighboring geographic area from that of MIRA, and the Southern Portuguese breed ALEN; and cluster IV included the 2 fighting cattle (TLID and BRAV) populations, the 2 Canarian breeds (VCAN and PALM), and the Portuguese GARV. The contribution of each of these clusters to each breed is shown in Table 2. On average, this contribution was greater than 70% in 19 breeds, whereas the remaining breeds displayed a greater degree of admixture. Breed average membership proportions (***Q***) in each ancestral cluster ranged from 0.394 in STER to 0.948 in MIRA. Apart from cluster IV, and the STER and ALEN breeds, the clusters obtained were generally consistent with the neighbor-net.

For each ancestral cluster, further substructure was detected as inferred through L(K) and ΔK (Supplemental Figure S1 and Table S2, available at http://jas.fass.org/content/vol89/issue4/). Cluster I could be split into K = 12 groups with 10 breeds forming independent clusters (ASTM, MONC, TUDA, PASI, SAYA, RGAL, RETI, STER, MENO, and MALL), MORU and AVIL clustering together as well as BETI and PIRE, whereas ASTV was admixed. In 7 breeds the proportional contribution of the inferred clusters was greater than 70%, and 5 other breeds displayed proportions greater than 60%. The breeds that displayed a greater degree of admixture were ASTV, BETI, and STER (Supplemental Table S2, available at http://jas.fass.org/content/vol89/issue4/). The partition of cluster II was consistent with breed histories, with 2 clusters including each 2 closely related breeds (PMON and BRUP; BARR and CACH), whereas 3 other breeds showed substructure (NAND, BNEG, and MERT). Average *Q* values for each of the breeds included in this cluster were somewhat less than those found in other clusters, such that only 3 breeds showed values >70%, whereas the remaining breeds showed some level of admixture. No specific cluster was found for BCOL, with approximately 50% of the animals grouping together with the NAND breed and other 15% of the animals clustering with MARS breed. For cluster III, using the Ln[L(K)] method, it was possible to identify 6 clusters that were consistent with the predefined breeds (ALIS, ALEN, AROU, MARI, MARO, and MIRA). Although ΔK displayed the most probable K = 7, which identified substructure in AROU (data not shown), we used the smallest K as a general precautionary rule as suggested

by Pritchard et al. (2000). In this cluster, average Q values were >75% in all breeds. Finally, and for cluster IV, 3 breeds appeared as independent clusters (VCAN, PALM, GARV), whereas TLID and BRAV clustered together. The 2 Fighting Bull populations did not split in 2 independent clusters until reaching K = 7. Average Q values in each breed within this cluster were high (>88%). In summary, second-order STRUCTURE analyses revealed 36 as the most probable number of clusters. Clusters III and IV included breeds that were more differentiated, whereas the breeds within the other 2 clusters showed greater levels of admixture (Figure 2 and Supplemental Table S2, available at http://jas.fass.org/content/vol89/issue4/).

The presumed influence of Brown Swiss in 4 Spanish populations (ASTV, BRUP, PMON, and STER) was tested in STRUCTURE without using prior breed information, as well as in 2 Portuguese breeds (MINH and RAMO) that seemed to be closely related to PMON and BRUP in the genetic distance analysis (Figure 1). Assuming K = 2 to K = 7, the settings included a burn-in of 10^5 and an Markov chain Monte Carlo length of 10^6 iterations. Brown Swiss genotypes have been described previously (Martin-Burriel et al., 2007). The analysis revealed K = 6 as the most probable number of clusters, being PMON and BRUP included in a single cluster but clearly differentiated from the BRSW. The genetic proportion (Q) that Spanish breeds shared with Brown Swiss ranged from 4.2% in STER to 7.5% in ASTV (Supplemental Table S3, available at http://jas.fass.org/content/vol89/issue4/). The BRSW contribution in Portuguese breeds was very small (1.6 and 1.2% for MINH and RAMO, respectively). The main contribution to STER resulted from the PMON-BRUP and ASTV clusters (11.1 and 19.8%, respectively), with the proportion shared with BRSW being very small (4.2%).

Breed Assignment

Individual q values obtained in the 4 ancestral clusters were used for breed assignment. Independent analyses were done for K = 2 to assign individuals to the breed pairs ASTV/ASTM, BETI/PIRE, MORU/AVIL, PMON/BRUP, BARR/CACH, and TLID/BRAV. The percentage of individuals assigned to each cluster are shown in Table 2 for $q > 95\%$ and $q > 80\%$ without using prior information on breeds. Approximately 60% of the individuals were classified within their source ancestral population with $q > 0.8$. The Spanish breeds ALIS, MALL, and PALM and the Portuguese BRAV displayed proportions of individuals correctly assigned >90% ($q > 0.8$). By contrast, 12 breeds displayed <50% of the individuals correctly assigned ($q < 0.8$). At the given threshold, individuals of the admixed BCOL and the pair PMON, BRUP could not be reassigned to their given breed label nor to any of the breed labels under study.

DISCUSSION

Using 19 microsatellite markers we studied 40 autochthonous breeds from Portugal and Spain, which included endangered populations, as well as commercial cattle breeds with larger census. To our knowledge, this is the most comprehensive genotypic data set and study on the genetic diversity of native cattle from these 2 countries. The 19 microsatellites included in this data set have been recommended by the FAO for diversity analyses in cattle. These markers were among the most informative of the FAO panel, which in part could explain the greater genetic diversity detected in our study, which was slightly greater when compared with that previously reported for Iberian and other European cattle (MacHugh et al., 1997; Martin-Burriel et al., 1999; Cañón et al., 2001; Mateus et al., 2004; European Cattle Diversity Consortium, 2006; Martin-Burriel et al., 2007; Medugorac et al., 2009; Ginja et al., 2010b). However, the greater genetic diversity found in Iberian cattle is consistent with multiple influences over time, mainly through the Mediterranean route but also from North Africa (Miretti et al., 2004; Cymbron et al., 2005; Cortés et al., 2008), and more recently crossbreeding with other European cattle.

Several publications have reported the presence of zebu and African-type alleles in Portuguese cattle breeds through the analysis of both mitochondrial DNA (**mtDNA**) and short tandem repeat (**STR**) markers (Cymbron et al., 1999; Beja-Pereira et al., 2003; Ginja et al., 2009, 2010b). Zebu-type alleles have also been previously detected in Iberia (Beja-Pereira et al., 2003; Ginja et al., 2010b), as we found in our study. In the early decades of the 20th century, southern Iberian cattle populations were crossed with zebu (Miranda do Vale, 1949) and later on with Santa Gertrudis and Nellore (Ralo and Guerreiro, 1981). Our study confirms the presence of zebu-diagnostic alleles (MacHugh, 1996; Lirón et al., 2006) in Southern breeds (MOST, BCOL, BNEG, PAJU, MARS, AROU, MARI, GARV, and PRET), but this influence is extended to Northern (MINH, BARR, CACH, RGAL, MONC, ASTV, ASTM, TUDA, PASI, BETI, PMON, and BRUP), central (AVIL, MORU, STER, TLID, and BRAV), and island (RAMO) cattle. Moreover, the highly frequent African zebu allele *ETH225-157* appeared in VCAN and RGAL cattle. The presence of such a large number of breeds containing African zebu alleles, even populations raised in Northern Iberia, could question the specificity of these diagnostic zebu-alleles. Nevertheless, the African influence in Iberian cattle is now well characterized at the molecular level (STR, mtDNA, and Y chromosome) and is supported by historical information (Cymbron et al., 1999, 2005; Ginja et al., 2009, 2010b). Accordingly, the presence of West African taurine alleles in 12 breeds dispersed throughout the Iberian Peninsula supported the African cattle influence in the Iberian cattle.

Alleles detected exclusively in a particular breed were found in several loci, generally at very small frequen-

cies. However, SAYA cattle presented a breed-specific allele at the INRA32 locus, with a frequency greater than 0.4, which has been reported as the predominant allele for this marker in this breed by Cañón et al. (2001). Similarly, BETI population displayed a rA at the HAUT27 locus at increased frequency (0.237). Genetic drift could have contributed to the increased frequency found for these alleles because the SAYA and BETI populations are at greater risk of extinction.

Some breeds showed decreased genetic diversity, including fighting cattle (TLID and BRAV), island populations (MALL, MENO, and PALM), and the highly threatened GARV, MOST, NAND, and MIRA breeds. In these breeds, departures from HWE were observed, generally due to heterozygosity deficit. For fighting cattle (TLID and BRAV) heterozygote deficit was detected and is consistent with previous analyses (Martin-Burriel et al., 1999, 2007; Cañón et al., 2008; Ginja et al., 2010b), and is interpreted as a consequence of both inbreeding and the presence of independent lineages (Wahlund effect). Deviations from HWE found in MERT, PAJU, BNEG, and NAND can also be explained by a Wahlund effect due to breed substructure. On the other hand, the recent history of NAND and MIRA indicates that they have suffered genetic erosion in the last decades due to a bottleneck and consequent genetic drift and inbreeding (DAD-IS, 2010). For NAND, subsequent dilution occurred through crossbreeding with AVIL cattle, which is a breed with a large population size. Small population sizes, genetic drift, and selection could have contributed to the heterozygote deficit observed in the other breeds. On the other hand, the excess of heterozygotes observed in MINH population is almost certainly a consequence of crossbreeding (Machado, 2000; Ginja et al., 2010b). The heterogeneity observed in GARV has been explained as the result of genetic erosion of this highly threatened native breed (Ginja et al., 2010b). To summarize, 2 scenarios have been found in these threatened populations: 1) loss of diversity and accumulated inbreeding in Fighting Bull, island, and highly threatened populations, and 2) genetic drift with few animals representing distinct alleles and genetic erosion due to crossbreeding as observed in specific breeds from Portugal and Spain.

The genetic distances of Reynolds were used to analyze breed relationships between Portuguese and Spanish cattle because they reflect mainly the effect of genetic drift. Phenograms displayed low bootstrap values as expected when a large number of populations is analyzed (Felsenstein, 2004), and as previously observed in the analysis of closely related domestic breeds with different genetic distances (Beja-Pereira et al., 2003; Cymbron et al., 2005; Lirón et al., 2006). Although small bootstrap values can be related to the use of a relatively small number of markers, similar results were obtained for subsets of these breeds even when a larger number of polymorphic markers was used (Mateus et al., 2004; Ginja et al., 2010b).

Iberian cattle have been classified into 3 distinct morphologic groups: Red Convex (Turdetano), Brown Concave (Blond-brown Cantábrico), and Black Orthoid (Iberian; Sánchez-Belda, 1984). This grouping, however, is not universally agreed upon, and discrepancies exist in the classification of Iberian cattle (Sánchez-Belda, 1984; Fellius, 1995). In agreement with other studies (Mateus et al., 2004; Ginja et al., 2010b), our results indicate that Spanish and Portuguese breeds can be grouped according to their geographical location rather than by their morphotypes. Although this assertion is valid for most breeds, some exceptions were found, with the greatest genetic distances being observed between geographical neighbor pairs (i.e., MINH with RGAL, and ALEN with RETI). Admixture with imported commercial breeds could have contributed to this divergence; for example, historical evidence supports crossbreeding in MINH mainly with German Yellow (Machado, 2000; Lenstra and Econogene, 2008), and in RETI with Limousine (Pérez et al., 2005) before the establishment of herd books. Using both sets of 19 and 15 neutral microsatellite markers, increased bootstrap values were observed only for pairs of breeds that have separated somehow recently and that are genetically entangled, such as BRUP and PMON (which are descendants from the Brown Alpine), BARR and CACH [whose close genetic relationship has been recently reported (Ginja et al., 2010b)], and MARI with the MIRA breed from which it presumably derives (Gama et al., 2004). The last 2 breeds formed a clear cluster with ALIS and AROU, which was also supported by STRUCTURE. As for fighting cattle, the clustering of Portuguese and Spanish populations (BRAV and TLID) reflects a common origin or a more recent crossbreeding between these populations or both as is also shown by the STRUCTURE analysis. Despite the greater divergence of PALM, the 2 Canarian populations (PALM and VCAN) clustered together with a relatively large bootstrap value. The presence of cattle in the Canary Islands is relatively recent because cattle did not populate them at the moment of their colonization in the 15th century. Phenotypically, both Canary breeds show greater similarity with northwestern Iberian breeds, but the influence of southern breeds cannot be discarded because the archipelago was an intermediate port during the colonization of America (Rodero et al., 1992). On the other hand, these islands are located less than 100 km from the African coast, and the influence of African cattle is very likely. Recent mtDNA and Y chromosome haplotype analyses indicated that VCAN was influenced by African cattle either directly or through the influence of southern Iberian breeds (Ginja et al., 2010a). Accordingly, the African zebu allele *ETH225.158* allele (MacHugh, 1996) was detected in VCAN. Studying Y-chromosome markers, Ginja et al. (2010a) found an increased frequency of the H4Y1 haplotype in VCAN, which is common in Northern European commercial dairy cattle, such as Friesian, and

suggests further admixture in that breed. On the contrary, PAL showed no African influence and was almost fixed for a highly distinct Y1 patriline (Ginja et al., 2010a). Although insularity certainly contributed to the isolation of PAL cattle, their genetic distinctiveness could lend support to the application of conservation measures. The possible relationship between MINH and RAMO with Brown Swiss due to their clustering with PMON and BRUP breeds was not confirmed by the STRUCTURE analysis. The influence of other breeds in MINH has been discussed above, and the contribution of other European breeds not included in this analysis could probably explain the clustering of these breeds. For example, crossbreeding between RAMO and Friesian has been reported (Ginja et al., 2009, 2010b).

The results of the analysis with STRUCTURE were mostly in agreement with the genetic distance analysis. Although the first level of population structure was detected at K = 4, consistent with major clusters of the neighbor-joining distance phenogram, subsequent analyses allowed differentiation of most of the breeds included in this study as independent clusters, with the exception of PMON and BRUP, which are the less differentiated populations. The set of 19 microsatellite markers used in this work was useful to differentiate recently derived breeds such as PMON/BRUP from BRSW, MOST from MARS, and MARI from MIRA. Although increasing the number of polymorphic markers used could help to obtain greater resolution, unpublished results from our group indicate that some of the highly related breeds (e.g., PMON and BRUP) might be hard if not impossible to differentiate. Substructure was also detected in a few breeds (MERT, BNEG, and NAND), which is most probably a result of their breeding system. The presence of independent lineages corresponding to distinct morphologic types has been reported in MERT (Ginja et al., 2010b), and substructure in BNEG and NAND could also be a consequence of reproductive isolation of breed subpopulations. The fact that some breeds are substructured can have consequences to their future management, which could aim at facilitating admixtures between subpopulations, although some degree of heterogeneity should be accepted as part of breed histories, rather than considered a depreciative feature (European Cattle Diversity Consortium, 2006).

Admixture was detected in 3 of the ancestral clusters (I to III), whereas the breeds within cluster IV were more differentiated, such that the average Q for cluster IV was 0.763, whereas the other clusters have averages less than 0.7. In cluster I, genetic admixture was observed for ASTV, with the influence of 3 clusters corresponding to neighboring populations (ASTM, PASI, and RGAL). In addition, the genetic information shared between Brown Swiss and ASTV was confirmed in an independent STRUCTURE analysis. Similarly, PIRE and MONC clusters contributed to BETI, and both MONC and BETI share a similar feral behavior, morphology, and geographical origin (Martin-Burriel et al., 2007). In this cluster, 2 representatives of the Iberian trunk, MORU and AVIL, grouped together. In addition to their common morphotype, their location in neighbor regions could have facilitated more recent admixtures. Although STER was included in cluster I, this population did not show admixture with the other breeds included in the cluster. Moreover, STER clustered with Brown Swiss-derived breeds in the neighbor net. We have previously reported the influence of mountain cattle breeds in this population, including Brown Swiss (Martin-Burriel et al., 2007). The present work confirmed indirect influence of Brown Swiss in STER through PMON/BRUP and showed a common genetic background between STER and ASTV.

In general, high admixture levels were observed in Andalusian breeds (BCOL, BNEG, MARS, MOST, NAND, PAJU, and RETI), reflecting crossbreeding between southern breeds. Two reasons can explain this admixture: 1) the traditional extensive management system of southern breeds, and 2) the late arrival of modern genetic management, such as parentage testing, for breed registration. In the past, these animals were moved in seasonal migrations in search for pastures, and animals from different regions were grouped in specific areas, justifying their admixture. Until the mid 20th century, breeds were officially recognized according to geographical criteria and animals were registered based on phenotypic standards. For instance, the breeds Cardena, RETI, and NAND were considered to be the gray, red, and black varieties, respectively, of the Andalusian breed. Only in the 1970s, Andalusian breeds were managed independently, but their past genetic admixture could still be detected in the genetic analyses.

Our results showed that Portuguese and Spanish breeds were grouped mainly according to their geographic origin, with a weak effect of country delimitations on their genetic differentiation. Historically, there is a strong relationship among the Portuguese and Spanish cattle breeds that are raised in neighboring regions of these 2 countries. In the past, the border was an artificial line in terms of animal breeding. Animal migrations were common for centuries and until herd books were established in the 1970s of the past century. Despite their recent histories and common origins, Portuguese and Spanish cattle are highly structured and most breeds are well differentiated, and in some cases, high genetic distances are found between neighboring breeds. In the near future as a consequence of recent technical advances and development of high-throughput systems, it will be possible to analyze a large number of markers and samples in short time and at considerably less cost. A combined analysis of neutral as well as selected markers should provide more accurate information on breed relationships and population genetic structure.

In conclusion, we report here a comprehensive characterization of the genetic diversity and breed relationships of native cattle from Spain and Portugal. Repro-

ductive isolation, inbreeding, and crossbreeding have contributed to the extant genetic composition of these breeds. Commercial European breeds as well as African cattle have contributed to the genetic composition of Iberian cattle; therefore, further work in a wider context of a meta-analysis of European/World cattle is necessary to better understand breed relationships and admixture. Relationships among Iberian cattle reflect their geographical distribution rather than phenotypic breed groups. Management and breeding programs must be carefully considered to minimize inbreeding, maintain overall genetic and allelic diversities, and account for within-breed genetic structure.

LITERATURE CITED

Ashwell, M. S., D. W. Heyen, T. S. Sonstegard, C. P. Van Tassell, Y. Da, P. M. VanRaden, M. Ron, J. I. Weller, and H. A. Lewin. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. J. Dairy Sci. 87:468–475.

Beja-Pereira, A., P. Alexandrino, I. Bessa, Y. Carretero, S. Dunner, N. Ferrand, J. Jordana, D. Laloe, K. Moazami-Goudarzi, A. Sanchez, and J. Cañón. 2003. Genetic characterization of southwestern European bovine breeds: A historical and biogeographical reassessment with a set of 16 microsatellites. J. Hered. 94:243–250.

Belkhir, K. 2001. GENETIX, logiciel sous WindowsTM pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France.

Bryant, D., and V. Moulton. 2004. Neighbor-net: An agglomerative method for the construction of phylogenetic networks. Mol. Biol. Evol. 21:255–265.

Cañón, J., P. Alexandrino, I. Bessa, C. Carleos, Y. Carretero, S. Dunner, N. Ferran, D. Garcia, J. Jordana, D. Laloe, A. Pereira, A. Sanchez, and K. Moazami-Goudarzi. 2001. Genetic diversity measures of local European beef cattle breeds for conservation purposes. Genet. Sel. Evol. 33:311–332.

Cañón, J., I. Tupac-Yupanqui, M. A. Garcia-Atance, O. Cortés, D. Garcia, J. Fernandez, and S. Dunner. 2008. Genetic variation within the Lidia bovine breed. Anim. Genet. 39:439–445.

Cattle QTLdb. 2010. Cattle Quantitative Trait Locus (QTL) database. Accessed Dec. 7, 2010. http://www.genome.iastate.edu/cgi-bin/QTLdb/BT/index.

Cortés, O., I. Tupac-Yupanqui, S. Dunner, M. A. Garcia-Atance, D. Garcia, J. Fernandez, and J. Cañón. 2008. Ancestral matrilineages and mitochondrial DNA diversity of the Lidia cattle breed. Anim. Genet. 39:649–654.

Cruickshank, J., M. R. Dentine, P. J. Berger, and B. W. Kirkpatrick. 2004. Evidence for quantitative trait loci affecting twinning rate in North American Holstein cattle. Anim. Genet. 35:206–212.

Cymbron, T., A. R. Freeman, M. Isabel Malheiro, J. D. Vigne, and D. G. Bradley. 2005. Microsatellite diversity suggests different histories for Mediterranean and Northern European cattle populations. Proc. Biol. Sci. 272:1837–1843.

Cymbron, T., R. T. Loftus, M. I. Malheiro, and D. G. Bradley. 1999. Mitochondrial sequence variation suggests an African influence in Portuguese cattle. Proc. Biol. Sci. 266:597–603.

DAD-IS. 2010. Domestic Animal Diversity Information System. Accessed Dec. 7, 2010. http://dad.fao.org.

European Cattle Diversity Consortium. 2006. Marker-assisted conservation of European cattle breeds: An evaluation. Anim. Genet. 37:475–481.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14:2611–2620.

FAO. 2004. FAO Standing Committee. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsatellite Markers. Accessed May 10, 2010. http://dad.fao.org/cgi-bin/getblob.cgi?sid=ca5 3b91a6f7c80be8e7066f4a5066dc1,50006220.

Fellius, M. 1995. Cattle Breeds—An Encyclopedia. Misset. Doetinchem, the Netherlands.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Felsenstein, J. 2004. Inferring Phylogenies. Sinauer Associates Inc., Sunderland, MA.

Foll, M., and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. Genetics 180:977–993.

Gama, L. T., N. Carolino, M. S. Costa, and C. P. Matos. 2004. Recursos genéticos animais em Portugal. Relatório Nacional. Vale de Santarém (Portugal). Food and Agriculture Organization of the United Nations: 68.

Ginja, C., M. C. Penedo, L. Melucci, J. Quiroz, O. R. Martinez Lopez, M. A. Revidatti, A. Martinez-Martinez, J. V. Delgado, and L. T. Gama. 2010a. Origins and genetic diversity of New World Creole cattle: inferences from mitochondrial and Y chromosome polymorphisms. Anim. Genet. 41:128–141.

Ginja, C., L. Telo da Gama, and M. C. Penedo. 2009. Y chromosome haplotype analysis in Portuguese cattle breeds using SNPs and STRs. J. Hered. 100:148–157.

Ginja, C., L. Telo Da Gama, and M. C. Penedo. 2010b. Analysis of STR markers reveals high genetic structure in Portuguese native cattle. J. Hered. 101:201–210.

Goudet, J. 2002. Fstat version 2.9.3.2. (Feb. 2002). Institute of Ecology. Biology Building, UNIL, CH-1015, Lausanne, Switzerland.

Huson, D. H., and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23:254–267.

Joint Working Group on Refinement. 1993. Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Lab. Anim. 27:1–22.

Kim, J. J., F. Farnir, J. Savell, and J. F. Taylor. 2003. Detection of quantitative trait loci for growth and beef carcass fatness traits in a cross between *Bos taurus* (Angus) and *Bos indicus* (Brahman) cattle. J. Anim. Sci. 81:1933–1942.

Klungland, H., A. Sabry, B. Heringstad, H. G. Olsen, L. Gomez-Raya, D. I. Vage, I. Olsaker, J. Odegard, G. Klemetsdal, N. Schulman, J. Vilkki, J. Ruane, M. Aasland, K. Ronningen, and S. Lien. 2001. Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. Mamm. Genome 12:837–842.

Kneeland, J., C. Li, J. Basarab, W. M. Snelling, B. Benkel, B. Murdoch, C. Hansen, and S. S. Moore. 2004. Identification and fine mapping of quantitative trait loci for growth traits on bovine chromosomes 2, 6, 14, 19, 21, and 23 within one commercial line of *Bos taurus*. J. Anim. Sci. 82:3405–3414.

Köhler-Rollefson, I. 2000. Implementing the Convention on Biodiversity with respect to domestic animal diversity. International Workshop on Animal and Plant Genetic Resources in Agriculture at the Biosphere Reserve, Schorfheide-Chorin, Germany. Accessed Oct. 21, 2009. http://lifeinitiative.net/html/implementing_cbd.htm.

Lenstra, J. A., and Econogene. 2008. What's in a breed? Criteria for conservation ESF Workshop: Diversity, selection and adaptation in wildlife and livestock—Molecular approaches. The European Science Foundation (ESF), Salzburg, Austria.

Leroy, G., E. Verrier, J. C. Meriaux, and X. Rognon. 2009. Genetic diversity of dog breeds: Within-breed diversity comparing genealogical and molecular data. Anim. Genet. 40:323–332.

Li, C., J. Basarab, W. M. Snelling, B. Benkel, J. Kneeland, B. Murdoch, C. Hansen, and S. S. Moore. 2004. Identification and fine mapping of quantitative trait loci for backfat on bovine

chromosomes 2, 5, 6, 19, 21, and 23 in a commercial line of *Bos taurus*. J. Anim. Sci. 82:967–972.

Lirón, J. P., P. Peral-Garcia, and G. Giovambattista. 2006. Genetic characterization of Argentine and Bolivian Creole cattle breeds assessed through microsatellites. J. Hered. 97:331–339.

Machado, M. A. 2000. Galega/Minhota. Pages 117–129 in Catalogo de Raças Autóctones de Castela e Leao (Espanha)-Regiao Norte de Portugal: Espécies Bovina e Equina. E. Garcia, ed. Fundaçao Rei Alfonso Henriques, Salamanca, Spain.

MacHugh, D. E. 1996. Molecular biogeography and genetic structure of domesticated cattle. PhD Thesis. University of Dublin, Dublin, Ireland.

MacHugh, D. E., M. D. Shriver, R. T. Loftus, P. Cunningham, and D. G. Bradley. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146:1071–1086.

Martin-Burriel, I., E. Garcia-Muro, and P. Zaragoza. 1999. Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. Anim. Genet. 30:177–182.

Martin-Burriel, I., C. Rodellar, J. A. Lenstra, A. Sanz, C. Cons, R. Osta, M. Reta, S. De Arguello, and P. Zaragoza. 2007. Genetic diversity and relationships of endangered Spanish cattle breeds. J. Hered. 98:687–691.

Martinez, A. M., J. V. Delgado, A. Rodero, and J. L. Vega-Pla. 2000. Genetic structure of the Iberian pig breed using microsatellites. Anim. Genet. 31:295–301.

Mateus, J. C., M. C. Penedo, V. C. Alves, M. Ramos, and T. Rangel-Figueiredo. 2004. Genetic diversity and differentiation in Portuguese cattle breeds using microsatellites. Anim. Genet. 35:106–113.

McClure, M. C., N. S. Morsci, R. D. Schnabel, J. W. Kim, P. Yao, M. M. Rolf, S. D. McKay, S. J. Gregg, R. H. Chapple, S. L. Northcutt, and J. F. Taylor. 2010. A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. Anim. Genet. 41:597–607.

Medugorac, I., A. Medugorac, I. Russ, C. E. Veit-Kensch, P. Taberlet, B. Luntz, H. M. Mix, and M. Forster. 2009. Genetic diversity of European cattle breeds highlights the conservation value of traditional unselected breeds with high effective population size. Mol. Ecol. 18:3394–3410.

Miranda do Vale, J. 1949. Gado Bissulco. Livraria Sá da Costa, Lisboa, Portugal.

Miretti, M. M., S. Dunner, M. Naves, E. P. Contel, and J. A. Ferro. 2004. Predominant African-derived mtDNA in Caribbean and

Brazilian Creole cattle is also found in Spanish cattle (*Bos taurus*). J. Hered. 95:450–453.

Park, S. D. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. PhD Thesis. University of Dublin, Dublin, Ireland.

Pérez, J. A., F. Álvarez, M. Valera, P. J. Azor, A. Membrillo, and A. Molina. 2005. Selection program of Retinta beef native cattle breed: Preliminary results of the control nucleus. Arch. Zootec. 206:401–408.

Plante, Y., J. P. Gibson, J. Nadesalingam, H. Mehrabani-Yeganeh, S. Lefebvre, G. Vandervoort, and G. B. Jansen. 2001. Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. J. Dairy Sci. 84:1516–1524.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Ralo, A., and A. Guerreiro. 1981. Raças exóticas e cruzamentos. In Bovinos em Portugal. Lisboa: Direcção Geral dos Serviços Veterinários, Lisboa, Portugal.

Raymond, M., and F. Rousset. 1995. GENEPOP (ver. 1.2): A population genetics software for exact test and ecumenicism. J. Hered. 86:248–249.

Rodero, A., J. V. Delgado, and E. Rodero. 1992. Primitive Andalusian livestock and their implications in the discovery of America. Arch. Zootec. 41:383–400.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Sánchez-Belda, A. 1984. Razas bovinas españolas. Publicaciones de Extensión Agraria. MAPA, Madrid, Spain.

Schnabel, R. D., T. S. Sonstegard, J. F. Taylor, and M. S. Ashwell. 2005. Whole-genome scan to detect QTL for milk production, conformation, fertility and functional traits in two US Holstein families. Anim. Genet. 36:408–416.

Seidenspinner, T., J. Bennewitz, F. Reinhardt, and G. Thaller. 2009. Need for sharp phenotypes in QTL detection for calving traits in dairy cattle. J. Anim. Breed. Genet. 126:455–462.

Troy, C. S., D. E. MacHugh, J. F. Bailey, D. A. Magee, R. T. Loftus, P. Cunningham, A. T. Chamberlain, B. C. Sykes, and D. G. Bradley. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature 410:1088–1091.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.