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Genetic variation within the Lidia bovine breed

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Summary

The results of an exhaustive data collection from a bovine population with a low level of exchangeability, the Lidia breed, are presented. A total of 1683 individuals from 79 herds were sampled and genetic diversity within and among lineages was assessed using 24 microsatellite loci on 22 different chromosomes. Expected heterozygosity ranged between 0.46 and 0.68 per lineage and there was significant inbreeding in the lineages, which included several farms [mean $F_{IS} = 0.11$, bootstrap 95% confidence interval (0.09, 0.14)], mainly because of the high genetic divergence between herds within those lineages. High genetic differentiation between lineages was also found with a mean F_{ST} of 0.18 [bootstrap 95% confidence interval (0.17, 0.19)], and all pairwise values, which ranged from 0.07 to 0.35, were highly significant. The relationships among lineages showed weak statistical support. Nonetheless, lineages were highly discrete when analysed using correspondence analysis and a great proportion of the individuals were correctly assigned to their own lineage when performing standard assignment procedures.

Keywords bovine, fighting bull, genetic diversity, microsatellite.

Introduction

Most studies usually treat breeds as the management unit within the species, failing to analyse the within-breed structure (Buchanan *et al.* 1994; Blott *et al.* 1998), thus neglecting potential within-breed differentiation. However, the analysis of the genetic structure of certain breeds can also be of interest. Such might be the case of the Lidia bovine breed. The Lidia bovine breed, otherwise known as the fighting bull, is the most successful domestic breed of any Spanish animal, being one of the largest and most inclusive intergrading bovine breeds. It has spread through European countries, such as France, Portugal and Spain, and numerous Central and South American countries (http://www.toroslidia.com/).

This breed has some peculiarities, which make it a population that deserves separate genetic analysis. First, it is the only bovine population selected for behavioural traits with a long history of isolation from the rest of the domestic bovine breeds (see Silva *et al.* 2002, 2006 for the registered behavioural traits). Second, the term Lidia refers to a racial grouping of native bovines of the Iberian Peninsula, which survive almost exclusively in the Mediterranean forest ecosystem traditionally known as La Dehesa (pastureland interspersed with Mediterranean oaks). Their behavioural characteristics, the uniqueness of their management and the hundreds of years of genetic isolation make it difficult to find a Lidia animal immersed in other populations or occupying different ecological niches, thus showing low ecological exchangeability (Crandall *et al.* 2000). In other words, the Lidia breed occupies an adaptive zone different from that of any other breed in its range and evolves separately from the breeds outside its range. Although there is a desirable pattern of aggressiveness present in the behaviour of the whole breed, different types of traditional popular events demand different types of behaviour. These variations in behavioural needs have been one of the main reasons why this racial group has fragmented into small lineages, traditionally called *encastes* (Boletin Oficial del Estado 2001), with different levels of gene flow among them and different behavioural traits favoured in each one. Some of the more isolated lineages, with a low number of animals contributing to the gene pool, can suffer from a loss of genetic variation and inbreeding depression, while others could be the result of a mixture of ancestral *encastes*.

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In summary, the distinct Mediterranean ecosystem inappropriate for most of the specialized bovine breeds, the presence of several lineages or *encastes* and the extreme aggressiveness are characteristics that strongly prevent the exchangeability (Crandall *et al.* 2000) of this breed with others.

Recently, the genetic position of this breed with respect to a collection of European cattle breeds has been addressed (see fig. 2 in Negrini *et al.* 2007) but a more precise knowledge of the structure of such a particular breed should be of interest to classify their lineages and identify the underlying amount and distribution of genetic variability, which can be used in the future to define conservation priorities in the fighting-bull breed.

Materials and methods

Sampling of populations

Detailed figures of the number of herds and animals sampled in each lineage or *encaste* are shown in Table 1. Some of the lineages are composed of a single herd, from which the samples for analysis were taken, e.g. Pablo Romero, Miura, Arauz de Robles or Cuadri. For each of the remaining lineages, a wide range of herds were selected and sampled.

For each lineage, individuals from the same generation were randomly sampled. Fresh blood collected in a buffer formulated to prevent DNA degradation (Dunner & Cañón

Table 1 Description of 29 lineages of the Lidia breed.

Lineage	Number of herds	Number of samples	H_E	H_O	Number of alleles	Allelic richness[1]	F_{IS}
Antonio Pérez	1	45	0.560	0.539	4.4	3.8	0.04
Arauz de Robles	1	52	0.536	0.530	4.8	3.9	0.01
Atanasio Fernández	6	97	0.552	0.511	5.0	3.8	0.07*
Baltasar Iban	2	52	0.577	0.534	4.7	4.0	0.07*
Braganza	1	25	0.587	0.575	4.4	–	0.02
Carlos Núñez	6	71	0.646	0.573	5.8	4.7	0.11*
Concha y Sierra	1	49	0.650	0.603	5.1	4.5	0.07*
Conde de la Corte	1	30	0.465	0.467	3.5	3.1	−0.003
Conde Santa Coloma	10	182	0.673	0.550	7.0	5.0	0.18*
Contreras	4	59	0.684	0.589	6.0	5.1	0.14*
Cuadri	1	50	0.464	0.430	4.3	3.4	0.07*
Félix Gómez	1	46	0.612	0.583	4.9	4.2	0.05*
Gamero Cívico	4	57	0.543	0.433	4.7	3.9	0.20*
Hidalgo Barquero	4	57	0.610	0.514	5.2	4.3	0.16*
José Marzal	1	50	0.600	0.590	5.3	4.4	0.02*
Juan Pedro Domecq	9	212	0.578	0.489	6.2	4.2	0.15*
Manuel Arranz	1	32	0.548	0.559	4.0	3.7	−0.02
Maria Montalvo	1	11	0.544	0.548	3.7	–	−0.008
Marqués de Albasarreda	3	46	0.528	0.480	4.2	3.4	0.09*
Marqués de Villamarta	3	60	0.610	0.517	5.3	4.4	0.15*
Miura	1	46	0.588	0.525	4.7	4.0	0.11*
Murube	4	51	0.564	0.463	4.8	4.1	0.18*
Pablo Romero	1	50	0.569	0.539	4.4	3.8	0.05*
Pedrajas	2	48	0.570	0.485	4.8	3.9	0.15*
Saltillo	4	54	0.652	0.507	5.5	4.6	0.22*
Torrestrella	1	50	0.571	0.570	4.8	4.1	0.001
Urcola	1	23	0.643	0.593	4.4	–	0.08*
Vega Villar	3	46	0.614	0.458	5.0	4.3	0.25*
Veragua	1	32	0.638	0.566	5.6	4.1	0.11*
Averaged across lineages	2.7	58.0	0.59	0.530	4.9	4.1	0.12*
All lineages	79	1683	0.715	0.524	9.8	5.7	0.27*
Avileña		50	0.764	0.688	8.0	6.5	0.10*
Morucha		50	0.764	0.725	8.0	6.6	0.05*
Retinta		50	0.780	0.707	8.6	6.8	0.09*
Averaged across breeds		50	0.769	0.707	8.2	6.6	0.08*
All local breeds		150	0.791	0.710	10.3	7.2	0.10*

*Values different from 0 ($P < 0.01$).

[1] Based on a minimum sample size of 15 individuals.

2006) was taken from 1683 individuals, with approximately the same number of males and females.

Additionally, 50 animals from each of three local bovine breeds (Morucha, Retinta and Avileña) of similar geographical origin and with a production system similar to that of the Lidia breed were also included in the analysis as reference for some population genetic parameters.

Genetic loci

The 24 microsatellite loci studied, their chromosomal locations and the numbers of alleles in the population analysed are described in Table S1. Nineteen of these overlap with the panel used in the Resgen project CT98-118 'Towards a strategy for the conservation of the genetic diversity of European cattle', and the remaining five were considered of interest for this study because of the high variability found in previous analyses with this breed.

Statistical analysis

Genetic analyses were performed assuming the existence of traditional lineages or *encastes* to which the herds were assigned (Table 1). These assignments were made following the standards set by the UCTL breeders' association (Unión de Criadores de Toros de Lidia).

For each lineage or *encaste*, the observed heterozygosity, H_O, and unbiased expected heterozygosity, H_E, were estimated according to Nei (1978) and averaged over loci following Nei (1987) (Table 1). Allelic richness based on a minimum sample size of 15 individuals was calculated with the FSTAT v.2.9.3 program (http://www2.unil.ch/popgen/softwares/fstat.htm) and averaged over the markers. The Braganza, María Montalvo and Urcola lineages were excluded from this analysis because of their low sample size.

Wright's *F*-statistics (F_{IT}, F_{IS} and F_{ST}, Wright 1951) were used to assess the distribution of genetic variability within and among lineages following Weir & Cockerham's (1984) approach. Departures from Hardy–Weinberg proportions in the whole sample and within each lineage were determined with Wright's F_{IT} and F_{IS} respectively. The null distributions of F_{IT} and F_{IS} were approximated by permuting alleles 1000 times within the whole set or within each lineage respectively. Random permutations of genotypes among samples were performed to test whether F_{ST} departed significantly from zero. These computations were performed using FSTAT and sequential Bonferroni (Rice 1989) was applied for multiple test correction.

Genetic relationships between lineages were inferred using two approaches. First, pairwise Reynolds' distances (Reynolds *et al.* 1983) were computed for all pairs of the 29 lineages and used to construct a neighbour-joining (NJ) tree with 1000 bootstrap resamplings to assess their consistency. PHYLIP software (http://evolution.genetics.washington.edu/phylip.html) was used to perform this analysis. These genetic distances were also used to obtain a transformed matrix with ultrametric properties (Weitzman 1992, 1993), and a NJ tree for this new matrix was drawn using MEGA 2.0 software (Kumar *et al.* 2001). Because of the nature of this matrix, the tree is unique in the sense that any grouping algorithm (NJ, UPGMA, etc.) would return the same structure and this tree structure is the one that has the highest probability of explaining the presence of all the taxonomical units today (Weitzman 1992). Examples of the use of this kind of tree can be found in Thaon d'Arnoldi *et al.* (1998), Cañón *et al.* (2001), Barker *et al.* (2001) and Reist-Marti *et al.* (2003).

Second, a multivariate approach was considered. More specifically, a correspondence analysis (Benzécri 1973) was performed using GENETIX 4.04 (Belkhir *et al.* 2001) to plot the representation of lineages in a metric space.

Alternatively, the unsupervised method of Pritchard *et al.* (2000) implemented in the STRUCTURE program (http://pritch.bsd.uchicago.edu/structure.html) was used to assess the support that the molecular information offered to the *a priori* assumptions on the population structure. The whole set of animals was pooled into a single unit to analyse their underlying genetic structure. We examined clustering and whether or not these clusters corresponded to the assumed lineages. For the ancestry model, animals were allowed to have mixed ancestry, and burn-in and run-in lengths of 300 000 and 500 000 respectively were used. After determining the maximum likelihood value of the number of ancestral populations (31), the clustering success rate was assessed following the procedure described in fig. 1 of Rosenberg *et al.* (2001). Animals were then grouped into herds (some herds were pooled into one when they were similarly managed or when they were recently split from an initial herd after its owner's death) and pairwise distances were calculated following Cañón *et al.* (2006) to represent the relationships obtained by the STRUCTURE algorithm, which were then displayed in an NJ tree built with these distances.

Results

Within-lineage diversity and deviation from Hardy–Weinberg equilibrium

In total, 234 alleles were detected across the 24 loci and 1683 individuals sampled from 29 lineages. Average H_E across loci varied between 0.46 (Cuadri) and 0.68 (Contreras) (Table 1). Conde de la Corte, which had the second lowest heterozygosity, also had the lowest average number of alleles and allelic richness. In contrast, the Conde de Santa Coloma lineage possessed the largest number of alleles and Contreras, the largest allelic richness (Table 1). The reference local cattle breeds registered higher values for some of the diversity parameters, such as H_O and allelic richness, which were 33% and 60% higher respectively.

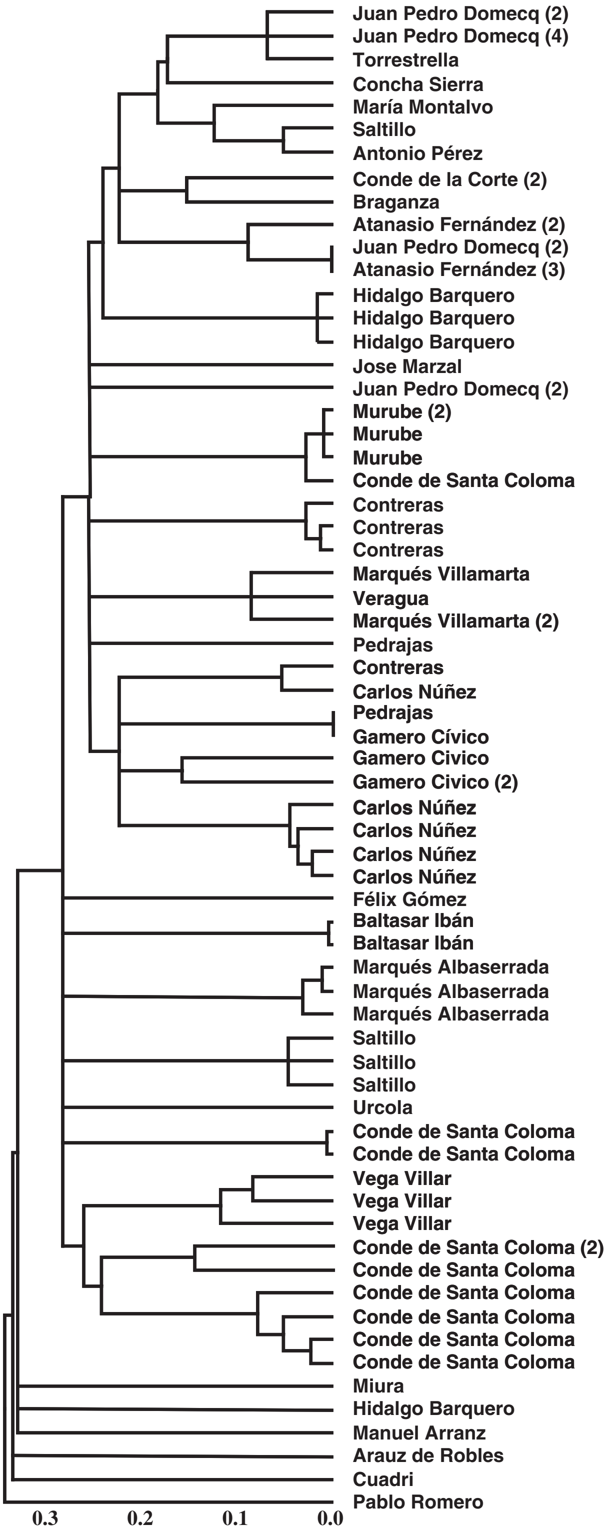

Figure 1 Neighbour-joining tree based on distances obtained from STRUCTURE output. The number of herds in each lineage is in parentheses.

Pearson correlation coefficients between heterozygosity and number of alleles and between number of alleles and allelic richness were 0.73 and 0.86 respectively.

The average F_{IT} value for all loci across populations (0.27) was highly significant [bootstrap 95% confidence interval (0.25, 0.30)] indicating a strong deficit of heterozygotes. This was caused both by significant departures from Hardy–Weinberg expectation within lineages [14 lineages exhibited significant across loci F_{IS} values, average $F_{IS} = 0.11$, bootstrap 95% confidence interval (0.09, 0.14)] and by differentiation between lineages [average $F_{ST} = 0.18$, bootstrap 95% confidence interval (0.17, 0.19)].

Differentiation between lineages

Genetic differentiation was high and significant with an average F_{ST} of 0.18. All pairwise F_{ST} values were highly significant, ranging from 0.05 (Torrestrella vs. Juan Pedro Domecq) to 0.35 (Marqués de Albaserrada vs. Conde de la Corte) indicating strong differentiation and reproductive isolation among the lineages. For each population, the average of Reynolds' distance with the rest of the lineages was calculated as an indicator of the divergence of the populations, and results arranged in descending order appear in Table S2. On average, the Cuadri lineage appeared as the most genetically isolated from the rest of the lineages, and Contreras, the least. The first two axes of the correspondence analysis, which contribute 12.9% and 9.4% of the total inertia respectively, are shown in Fig. S1. The local cattle breeds are separated from the Lidia lineages on axis 1.

Despite the high levels of genetic differentiation observed between the lineages, there was low statistical support for the neighbour-joining cluster analysis (data not shown) and only four branching events exhibited bootstrap support greater than 70%. This lack of a strong clustering pattern indicated that the exact relationship between lineages could not be reliably established with these 24 markers. The dendrogram in Fig. S2, obtained with the Reynolds' distances transformed into ultrametric distances, showed that the lineages grouped into two main clusters. The length of the branches corresponded to the level of inbreeding; therefore, the Cuadri, Arauz de Robles, Marqués de Albaserrada, Pablo Romero and Miura lineages appear to have undergone higher levels of inbreeding than the rest.

When performing cluster analysis with STRUCTURE, the different herds mostly grouped together as expected from *a priori* assignments of herds to lineages. The maximum likelihood number of clusters turned out to be 31, so the 29 lineages would actually come from 31 genetically differentiated ancestral groups. Pairwise distances were built following Cañón *et al.* (2006) to represent in a hierarchical tree the relationships of the different herds. The associated tree, showing how herds would group according to the STRUCTURE output, is depicted in Fig. 1. Table S2 shows the percentage of successful assignments (individuals assigned to their own lineage) obtained with the procedure of Rosenberg *et al.* (2001). The success rate was generally quite high.

Discussion

Variability and heterozygote deficits within lineages

Genetic diversity within lineages of the Lidia breed is, in general, lower than in European bovine breeds, but this does not extend to the results for the Lidia breed considered as a whole. The number of alleles per lineage ranged between 3.5 and 7, and the average number of alleles per locus observed in the Lidia breed (9.8) was very close to that observed in the three local cattle breeds included in the study (Table 1). Allelic richness (number of alleles standardized for variation in sample size) for the whole breed (5.7) was similar to the average obtained for a set of 69 breeds analysed by the European Cattle Genetic Diversity Consortium (2006) when the parameter is computed based on a minimum of 11 animals. However, it is low when compared to the average of the set of reference local breeds (5.7 vs. 7.2) (Table 1), although in this case the analysis was based on a minimum sample size of 15 animals. Observed heterozygosities within lineages ranged between 0.43 and 0.60, and the value for the whole breed was 0.52. These values are lower than the 0.69 average (range 0.52–0.73) of the 69 breeds considered by the European Cattle Genetic Diversity Consortium (2006).

Gene diversity of the Lidia breed is quite similar to that found in the three local breeds (Table 1), and similar expected heterozygosities were also found in the 69 European breeds analysed by the European Cattle Genetic Diversity Consortium (2006). Nonetheless, the average value within lineages was, as expected, lower than that of the European cattle breeds (0.59 vs. 0.71).

Divergence between gene diversity and H_O shows up as a strong heterozygote deficit (high F_{IS} values) in 14 of the 29 lineages. The likely explanation for these heterozygote deficits is the sampling process within many of the lineages. Most of the lineages with high F_{IS} values included several herds (Table 1), which, in practice, can be genetically differentiated groups, and this stratification was thus present in the analysed sample. After reanalysing the data by taking the herd structure into consideration, the result was that only six of the 146 pairwise F_{ST} values were non-significant. The F_{ST} values within lineages varied between 0.05 (Baltasar Ibán) and 0.27 (Vega Villar) (Table S3), with an unweighted average of 0.15. Most of those F_{ST} values were greater than the average within-lineage F_{IS} value of 0.11, which means that genetic differences between herds were responsible for most, but not all, of the heterozygote deficit. The average F_{ST} value of 0.15 is greater than the average pairwise F_{ST} values obtained between bovine breeds in some recent publications (Cañón *et al.* 2001; European Cattle Genetic Diversity Consortium 2006), which were around 0.07. Although the use of assisted reproductive technologies is rare in the Lidia breed, breeding practices such as inbreeding could lead to small effective population sizes. Actually, the effective number of founders, i.e. the number of equally contributing founders that would be expected to produce the same genetic diversity as that observed in the population under study (Lacy 1989), is below five for most lineages, and effective population size is, on average, around 30 (data not shown). Therefore, the positive F_{IS} values observed within the lineages and even in some herds are explained by the existence of genetic isolation among herds within the lineages and by some degree of inbreeding within herds.

Analysis of within-lineage genetic variability classified the lineages into three categories that almost perfectly matched their breeding practices. The first category comprised those lineages with one single herd, in which inbreeding practices are used to fix desired patterns of behavioural traits. Veragua, Urcola, Miura, Concha y Sierra and Pablo Romero are examples of single-herd lineages with relatively high inbreeding values ($F_{IS} > 0.07$). The second category included lineages with several herds and restricted migration among them. Most of the lineages in Table S3 belong to this category, and all of them showed high heterozygosity deficit levels ($F_{IS} > 0.15$) because of subdivision in genetically different herds. Finally, a third category grouped together lineages that contained either recent crossbreeding with animals from different lineages or recent pooling of different strains or families within the lineages. Antonio Pérez, Braganza, José Marzal, María Montalvo and Torrestrella are examples of the second category, while Arauz de Robles is an example of the third. All of them are characterized by a F_{IS} value that is not different from 0.

Differentiation between lineages and individuals

The level of differentiation between lineages was very large, as demonstrated by an average F_{ST} of 0.18. This value was much higher than values among European cattle breeds, which ranged from 0.07 (Cañón *et al.* 2001) to 0.10 (MacHugh *et al.* 1998). In fact, in the present study, all average F_{ST} values between each lineage and the rest of the lines were greater than 0.12 (Table S2).

Under a pure genetic drift model, assuming no admixture and no mutation, the Reynolds' distance between populations *i* and *j* and their effective sizes were used to estimate time divergence between the two populations using the formula $t \sim D_R/(1/4N_{ei} + 1/4N_{ej})$. On average, time since divergence in generations among lineages was around 13, equivalent to 95 years, for an average generation interval of 7.5 years. They ranged between 4.4 generations (~33 years), for the divergence between the Juan Pedro Domecq and Torrestrella lineages, and 17.5 generations (~137 years), for the divergence between the Conde de la Corte and Marqués de Albaserrada lineages. However, information recovered in the UCTL breeders' association from historical data indicated that at least for some pairs of lineages, longer divergence times were expected. For instance, genetic isolation between the Conde de la Corte and Marqués de Albaserrada lineages has been documented since 1820.

Regarding the correspondence analysis, the most notable results are the very clear separation between the three local bovine breeds and all the lineages of the Lidia breed, and the recognition of most Lidia lineages using only the first two axes, a clear sign of the high degree of genetic differentiation.

The order of the lineages appearing in the tree based on Weitzman's diversity function (Fig. S1) depended on the random drift that caused the observed diversity. On the other hand, despite the lack of statistical support for the tree topology, the accuracy of the assignment of individuals to their own lineage was high (95% on average) and in 13 of the 29 lineages, 100% of the individuals were correctly assigned (Table S2).

Herd clustering into the 31 groups obtained from the STRUCTURE analysis fitted the *a priori* assumptions. There were some mismatches, mostly arising out of three situations: (i) some lineages, initially considered as different, were now clustered together, like Antonio Pérez and María Montalvo, Braganza and Conde de la Corte, Torrestrella and some herds from Juan Pedro Domecq or Veragua and Marqués de Villamarta; (ii) herds belonging to the same lineage now appeared clearly separated, such as those from Conde de Santa Coloma, divided now into three different groups, or those from Juan Pedro Domecq and Hidalgo Barquero, split into two groups each; and (iii) herds from different lineages were joined together into a new lineage, as happens with one herd from Pedrajas and one from Gamero Cívico, or one from Carlos Núñez and one from Contreras. Lineages have often been generated from a low number of animals that shared a particular phenotype (usually behavioural traits). Breeders have also frequently tried to maintain the desired characteristics by mating related animals (i.e. inbreeding). Lineages are traditionally kept within families, which hinder gene flow and rapidly establish significant genetic differentiation between lineages. This fragmentation of the fighting-bull population, and consequently the low population sizes of its lineages, seriously compromises the short-term survival of lineages and the loss of their specific genes. On the other hand, population subdivision can be a powerful way of maintaining genetic variability, because different alleles at a locus can be fixed in different lineages and be permanently kept as a source of variation, as long as the lineage persists. In fact, assuming no mutation, migration or selection, the amount of genetic variance preserved among n lineages approaches $(1 - 1/n)V_g(0)$ after few times N_e/n generations, with $V_g(0)$ being the genetic variance at coalescence and N_e the effective population size of the breed (Lande 1995).

In situations of subdivision and lack of genetic connections between lineages, the use of molecular information to estimate between-population kinship has shown an acceptable efficiency in terms of predicting low error (Eding & Meuwissen 2001). On the other hand, there is very detailed pedigree information within each lineage. Thus, methods that combine between- and within-lineage variation could be used to establish conservation of genetic diversity in the fighting-bull breed (Eding *et al.* 2002; García *et al.* 2005; Ollivier & Foulley 2005), where the between-lineage variability is estimated using the molecular information (Weitzman 1992, 1993; Eding & Meuwissen 2001), and within-lineage variation is based on available pedigree data.

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Supplementary material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2008.01738.x

Figure S1 Projection of 29 lineages of the Lidia breed plus three local breeds (Morucha, Retinta and Avileña) on axes 1 and 2 using the coordinates obtained by a correspondence analysis.

Figure S2 Neighbour-joining tree based on Reynolds' genetic distances transformed to distances with ultrametric properties.

Table S1 Microsatellite names, numbers of alleles and chromosomal locations.

Table S2 Average Reynolds' genetic distances from each lineage to the rest classified in descent order and clustering success rate.

Table S3 Wright's *F*-statistics within each lineage.