Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
http://dx.doi.org/10.5424/sjar/2014124-6173

Spanish Journal of Agricultural Research 2014 12(4): 1029-1037
ISSN: 1695-971X
eISSN: 2171-9292

RESEARCH ARTICLE

OPEN ACCESS

Genetic relationships between six eastern Pyrenean sheep breeds assessed using microsatellites

Ainhoa Ferrando[1], Félix Goyache[2], Pere-Miquel Parés[3], Carlos Carrión[1], Jordi Miró[4] and Jordi Jordana[1,*]

[1] Universitat Autònoma de Barcelona. Departament de Ciència Animal i dels Aliments. 08193 Bellaterra (Cerdanyola del Vallès), Barcelona, Spain. [2] SERIDA-Deva. Área de Genética y Reproducción Animal. Camino de Rioseco, 1225. 33394 Gijón, Spain. [3] Universitat de Lleida. Departament de Producció Animal. 25198 Lleida, Spain. [4] Universitat Autònoma de Barcelona. Departament de Medicina i Cirurgia Animals. 08193 Bellaterra (Cerdanyola del Vallès), Barcelona, Spain

Abstract

The knowledge of the genetic composition and relationships among livestock breeds is a necessary step for the implementation of management and conservation plans. This study aims to characterise the genetic diversity and relationships among six sheep breeds of meat aptitude that are spread through the eastern Pyrenees: Tarasconnaise, Castillonnaise and Rouge du Roussillon from France, and Aranesa, Xisqueta and Ripollesa from Spain. All but Tarasconnaise are catalogued as endangered. These breeds do not share the same ancestral origin but commercial trades and gene flow between herds are known to have occurred for centuries. Additionally, two outgroup breeds were included: the Guirra, from a different geographical location, and the Lacaune, a highly selected breed of dairy aptitude. A total of 410 individuals were typed using a panel of 12 microsatellite markers. Statistical, phylogenetic and Bayesian analyses showed that eastern Pyrenean breeds retained high levels of genetic diversity and low, but significant, levels of genetic differentiation (F_{ST} = 4.1%). While outgroups were clearly differentiated from other breeds, Pyrenean breeds tended to form two clusters. The first encompassed Tarasconnaise and Aranesa, which probably descend from a common meta-population. The second tended to group the other four breeds. However, none reached high mean Q-values of membership to a discrete cluster. This is consistent with the recent past gene flow between breeds, despite different ancestral genetic origins. The genetic characterisation carried out of the eastern Pyrenean sheep populations provides useful information to support decision making on their conservation and focusing efforts and resources to more singular breeds.

Additional key words: genetic diversity; genetic resources; endangered breeds; European sheep.

Introduction

Genetic documentation of existing livestock breeds enables the sustainable management and conservation of domestic animal diversity (FAO, 2009). The characterisation of genetic diversity is one of the key points to match future demands of the agri-food markets (Ajmone-Marsan & Globaldiv Consortium, 2010). Molecular techniques (*e.g.* based on microsatellite markers) can be used to assess within-population genetic diversity and between-population differentiation and, therefore, make inferences on the degree of uniqueness of the analysed populations (Groeneveld *et al*., 2010). Together with the overviews of the sheep genetics scenario in the whole continent (Handley *et al*., 2007; Peter *et al*., 2007), during the last decade, such studies have become frequent in European sheep, both considering those sheep breeds spread in a given geographical area (Arranz *et al*., 2001; Baumung *et al*., 2006; Ligda *et al*., 2009; Calvo *et al.,* 2011; Ciani *et al.,*

* Corresponding author: jordi.jordana@uab.cat
Received: 30-04-14. Accepted: 13-11-14.

This work has 2 supplementary tables and 1 supplementary figure that do not appear in the printed article but that accompany the paper online.

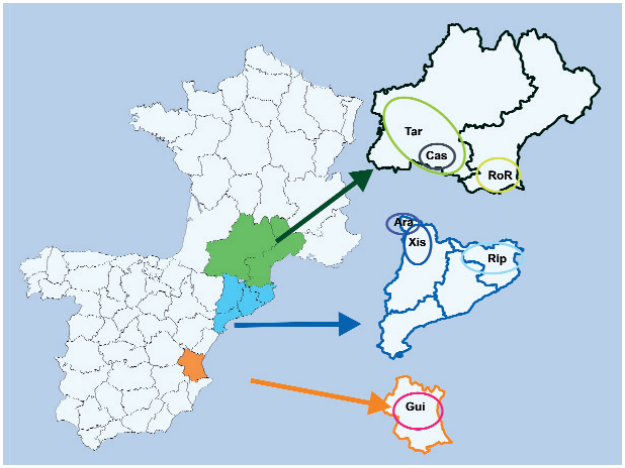

Figure 1. Map illustrating the main areas of location of the analysed eastern Pyrenean sheep breeds. Censuses range from roughly 120,000 individuals belonging to the Tarasconnaise breed, to roughly 2,000 belonging to the Castillonnaise and Aranesa breeds. Abbreviations are as follows: Aranesa (Ara), Tarasconnaise (Tar), Xisqueta (Xis), Ripollesa (Rip), Rouge du Roussillon (RoR) and Castillonnaise (Cas). The location of the Guirra (Gui) sheep breed is also given.

2013), or types of sheep (Santos-Silva *et al*., 2008; Kusza *et al*., 2009). Regardless of the approach used, most of the studies carried out are limited to a single country (Álvarez *et al*., 2004; Glowatzki-Mullis *et al*., 2009), while historical relationships among neighbouring sheep populations suggest the need of cross-border analyses (Tapio *et al*., 2005, 2010; Cinkulov *et al*., 2008; Kusza *et al.,* 2008; Salamon *et al.,* 2014).

Six different local sheep breeds are spread in the eastern Pyrenees area (Fig. 1): Tarasconnaise, Castillonnaise and Rouge du Roussillon in France, and Aranesa, Xisqueta and Ripollesa in Spain (Sánchez-Belda & Sánchez-Trujillano, 1986; Babo, 2000; Esteban, 2003; Avellanet, 2006; Parés, 2008). Generally, they show large differences in origin and morphology. The Spanish Aranesa (census estimated < 2,000 individuals) is closely related to the French Tarasconnaise, and probably shares more ancestral origin with the Merino sheep breed. The Ripollesa breed (< 16,000 individuals) is known to have been formed by the intercross between local Pyrenean sheep and transhumant Merinos. The Xisqueta breed (< 15,000 individuals), meanwhile, belongs to the Iberian Trunk, which derived from the primitive sheep that arrived from Central Asia. The Rouge du Roussillon (< 2,500 individuals) is a red-coated breed originated in Northern Africa, which is known in Spain as "Berberina" (Berber). The Castillonnaise breed has a small population in the Central Pyrenees (< 2,000 individuals), and is known locally as the «red head», because of its skin pigmentation. All of them are classified as endangered breeds, with the exception of Tarasconnaise breed (> 120,000 individuals).

The aim of this study is to undertake the characterisation of the European sheep genetic resources via the assessment of the genetic relationships between the eastern Pyrenean sheep breeds.

Material and methods

Sampling and genotyping

Blood samples were obtained from 364 individuals chosen at random among different herds and locations, regardless of gender and age. Individuals belonged to the following eastern Pyrenean sheep breeds: Aranesa (94 individuals; from 24 farms, sampled average 4), Tarasconnaise (44 individuals; from 3 farms, sampled average 15), Xisqueta (97 individuals; from 14 farms, sampled average 7), Ripollesa (53 individuals; from 4 farms, sampled average 13), Rouge du Roussillon (52 individuals; from 10 farms, sampled average 5) and Castillonnaise (24 individuals; from 2 farms, sampled average 12). Additionally, the Spanish Guirra (or Levantina Red), a red-coated breed that originated in Northern Africa, and the French Lacaune, a highly selected breed of dairy aptitude with a high census and widely distributed, were used as outgroup populations of reference (33 and 13 individuals, respectively). In this study, the Lacaune breed acted as a "production" outgroup, while the Guirra breed acted as a geographical outgroup. Altogether, the number of individual sheep analysed was 410.

Total DNA was isolated from blood samples following Ausubel *et al*. (1989). A panel of 12 microsatellite markers (see Table 1) was analysed on all the individuals (Avellanet, 2006). Genotyping was performed on an automatic sequencer, ABI PRISM 3730 (Life Technologies, Carlsbad, CA, USA) using GeneMapper v3.7 software (Life Technologies).

Statistical analyses

Usefulness of the typed microsatellite set was tested using the FSTAT v. 2.9.3.2 program (Goudet, 2001) by computing the following parameters: number of obser-

Table 1. Description of the microsatellite markers used in the current study. Additionally, number of observed alleles (n), effective number of alleles (k_e), observed (H_o) and expected (H_e) heterozygosity are given for each locus type, and as overall mean and standard deviation (s.d.)

Locus	Chromosome	Primers (forwards and reverse)	GenBank accession no.	Reference	n	k_e	H_o	H_e
HSC	Oar20	F: CTGCCAATGCAGAGACACAAGA R: GTCTGTCTCCTGTCTTGTCATC	M90759	Scott *et al.* (1991)	17	9.94	0.85	0.90
INRA49	BTA1	F: GTTTGTATTAGTTTGTGTTCTTTGGC R:TTG GCT TCCACAATCACACA	X71588	Vainman *et al.* (1994)	9	2.72	0.57	0.63
MAF65	Oar15	F:AAAGGCCAGAGTATGCAATTAGGAG R: CCACTCCTCCTGAGAATATAACATG	M67437	Buchanan *et al.* (1992)	11	3.98	0.72	0.74
MAF214	Oar16	F:AATGCAGGAGATCTGAGGCAGGGACG R:GGGTGATCTTAGGGAGGTTTTGGAGG	L38982	Buchanan & Crawford (1993)	15	2.38	0.50	0.58
MCM42	Oar9	F:CATCTTTCAAAAGAACTCCGAAAGTG R: CTTGGAATCCTTCCTAACTITCGG	L34281	Hulme *et al.* (1994)	11	3.60	0.65	0.72
MCM527	Oar5	F: GTCCATTGCCTCAAATCAATTC R: AAACCACTTGACTACTCCCCAA	L34277	Hulme *et al.* (1994)	10	4.52	0.72	0.77
MCM218	Oar4	F: CACTAAAAGCTTATGAAAGTTCCAGC R:GATCCTAGCATCAGTCTCCAGATG	L39828	Hulme *et al.* (1996)	13	3.50	0.67	0.71
OARCP49	Oar17	F:CAGACACGGCTTAGCAACTAAACGC R: GTGGGGATGAATATTCCTTCATAAGG	U15702	Ede *et al.* (1995)	24	10.04	0.82	0.90
OARCP20	Oar21	F: GATCCCCTGGAGGAGGAAACGG R: GGCATTTCATGGCTTTAGCAGG	U15695	Ede *et al*. (1995)	12	4.40	0.75	0.77
OARCP34	Oar3	F:GCTGAACAATGTGATATGTTCAGG R:GGGACAATACTGTCTTAGATGCTGC	U15699	Ede *et al.* (1995)	10	4.69	0.75	0.78
OARFCB11	Oar2	F: GCAAGCAGGTTCTTTACCACTAGCACC R:GGCCTGAACTCACAAGTTGATATATCTATCAC	L01531	Buchanan & Crawford (1993)	10	4.73	0.78	0.78
TGLA53	Oar16	F: CAGCAGACAGCTGCAAGAGTTAGC R: CTTTCAGAAATAGTTTGCATTCATGCAG	AC_000173 GPC_000000185	Zimin *et al.* (1999)	11	7.84	0.80	0.87
Mean					12.75	5.19	0.72	0.76
s.d.					4.22	2.61	0.10	0.09

ved alleles (k), effective number of alleles (k_e), average number of alleles per locus adjusted to 13 individuals (A_{13}; from El Mousadik & Petit, 1996), within-population observed (H_o) and expected heterozygosity (H_e), and heterozygote deficiency within population (f) computed following Weir & Cockerham (1984). These parameters were computed at the population level, and also at the marker level to assess the usefulness of typed microsatellite set to achieve the goals of this study. Also, using the FSTAT program (Goudet, 2001) the between-populations F_{ST} (θ) matrix was computed following Weir & Cockerham (1984). In all cases, confidence in the computed values was ascertained by jackknifing over loci using 1920 replicates. The significance level of $p < 0.05$ was adjusted using the Bonferroni correction for multiple independent tests.

The possible presence of genetic bottlenecks in our data was tested performing a two-tails Wilcoxon test, as implemented in the program BOTTLENECK (Luikart *et al.*, 1998; Piry *et al*., 1999), under the conservative stepwise mutation model. The two-tailed test ensures that statistical significance obtained using one-tailed tests for heterozygote deficiency or heterozygote excess is not due to chance (Type I error).

Additionally, for short-term evolution and divergence due to genetic drift only (Takezaki & Nei, 1996), both the Nei *et al*. (1983) D_A and the Reynolds *et al*. (1983) between-populations genetic distance matrices were computed using the POPULATIONS program v.1.2.31 (Langella, 1999). For descriptive purposes, Principal Component Analysis (PCA) was performed on each computed between-populations distance ma-

Table 2. Heterozygote deficiency within population (*f*) computed at the marker level following Weir & Cockerham (1984)

Locus	Aranesa	Tarasconnaise	Xisqueta	Ripollesa	Rouge du Roussillon	Castillonnaise	Guirra	Lacaune
MCM42	0.148*	–0.099	0.004	0.092	0.022	0.338*	0.183	–0.121
INRA49	0.093	0.038	0.128*	0.021	–0.025	0.069	–0.086	–0.333
MCM527	0.046	–0.058	0.124*	–0.038	–0.016	0.053	0.077	–0.286
TGLA53	0.036	0.114	0.063	–0.051	–0.018	–0.031	–0.026	–0.077
MAF65	0.052	–0.074	–0.110	0.001	–0.082	0.059	0.072	–0.244
HSC	0.075*	0.057	0.001	0.105	–0.029	–0.021	0.094	–0.339
OARCP20	0.014	–0.045	0.024	–0.037	–0.005	–0.184	0.224*	–0.213
OARCP34	0.085	0.040	0.049	0.001	0.021	–0.004	–0.092	–0.492
OARCP49	0.106**	0.055	0.081**	0.054	–0.043	–0.009	0.081	–0.425
OARFCB11	–0.119	0.024	0.045	–0.040	–0.167	0.017	0.103	0.209
MCM218	0.030	–0.076	–0.019	0.112	0.141*	0.039	0.079	–0.062
MAF214	0.055	0.065	0.267***	0.102	0.214*	0.121	–0.072	–0.508
Total	0.051**	0.004	0.051**	0.025	–0.005	0.033	0.050*	–0.251

*, **, ***: $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

trix, using the SAS/STAT™ program (SAS Inst. Inc., Cary, NC, USA), to summarise the contents of the information. Only factors with an eigenvalue >1 were retained, and their scores were used to construct a multidimensional plot using Microsoft Excel™.

The Structure program (Pritchard *et al*., 2000) was used to ascertain cryptic genetic structure in the analysed dataset. The program gives Bayesian estimates of the natural logarithm of the probability that a given genotype *X* is part of a given population *K* (ln Pr(*X*/*K*)). As the implemented algorithm uncovers 'hidden structure' without using *a priori* knowledge about the number of clusters (populations or breeds) present in a dataset, the most likely *K*-value in the data set was identified according to Evanno *et al.* (2005) using the Structure Harvester v.0.6.8 website (Earl & vonHoldt, 2012). *K* was set to vary between 1 and 6, and 20 simulations with different starting points for each *K*-value. All runs used burn-in and data collection periods of 100,000 iterations. The program was run under the admixture model, considering correlated allele frequencies.

Finally, population structuring was further assessed by computing an Analysis of Molecular Variance (AMOVA), using the program Arlequin 1.1 (Schneider *et al*., 1997). The statistical significance of the values was estimated by permutation analysis using 1,000 permutations.

Results

The microsatellite set typed allowed a total of 153 alleles to be identified in the whole of the analysed population. Altogether, the markers set used was highly polymorphic ($H_e = 0.76 \pm 0.09$; Table 1). Furthermore, statistically significant deviations of Hardy-Weinberg proportions per locus and breed were few and not consistently found across populations (Table 2). Therefore, these deviations could be due to chance (Type I error). Overall, the marker set typed is sufficient enough to obtain the goals of this research.

F-statistics computed for the whole population were significantly different from 0 ($p < 0.01$). An average heterozygote deficiency (F_{IS}) of 2.7% was observed at the studied loci. For F_{ST} values, there was a 4.1% genetic differentiation among the breeds studied, and the overall average heterozygote deficiency (F_{IT}) was 6.7%

Table 3 shows the parameters for the genetic variability of each analysed sheep breed. In the case of the Lacaune breed, values are affected by a very low sample size. The Xisqueta breed had the higher raw, (11.25 ± 3.44) and adjusted for sample size (7.12 ± 2.10), average number of alleles per locus. The observed number of alleles for this breed was about twice that observed for the Castillonnaise and Guirra breeds. Regarding expected heterozygosity, this pattern can still be observed: despite most breeds having H_e values of 0.73 or higher, the Xisqueta breed had an expected heterozygosity of 0.76 ± 0.10, while the Castillonnaise and Guirra breeds had values of 0.70 ± 0.10 and 0.68 ± 0.11, respectively. Only the Aranesa, Xisqueta and Guirra breeds showed statistically significant heterozygote deficiency, characterised by positive *f* values (roughly 0.05).

All the computed between-breeds F_{ST} values were statistically significant at the significance level of

Table 3. Number of individuals analysed (N), number of observed alleles (k), effective number of alleles (k_e), average number of alleles per locus adjusted to 13 individuals (A_{13}), within-population observed (H_o) and expected heterozygosity (H_e), and heterozygote deficiency within population (f) computed following Weir & Cockerham (1984). Values are given as a mean ± standard deviation. Estimates (f) were obtained using jackknifing over the loci

Breed	N	k	k_e	A_{13}	H_o	H_e	f
Aranesa	94	9.66±3.25	4.70±2.41	6.45±2.01	0.70±0.11	0.73±0.11	0.051**
Tarasconnaise	44	8.08±2.64	4.56±1.94	6.46±1.98	0.74±0.12	0.73±0.12	0.004
Xisqueta	97	11.25±3.44	5.16±2.58	7.12±2.10	0.72±0.13	0.76±0.10	0.051**
Ripollesa	53	8.91±2.90	4.50±1.61	6.30±1.98	0.73±0.12	0.74±0.10	0.025
Rouge du Roussillon	52	8.91±2.46	4.31±2.08	6.36±1.78	0.74±0.13	0.73±0.08	–0.005
Castillonnaise	24	6.41±2.10	3.69±1.13	5.51±1.53	0.69±0.14	0.70±0.10	0.033
Guirra	33	6.25±1.42	3.51±1.16	5.29±1.19	0.66±0.14	0.68±0.11	0.050*
Lacaune	13	4.25±1.05	2.58±0.65	4.25±1.05	0.75±0.20	0.58±0.11	–0.251

*, **, ***: $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 4. Between-breeds F_{ST} (θ) distance values. All the values are significant at $p < 0.05$

Breeds-F_{ST}	2	3	4	5	6	7	8
1. Aranesa	0.013	0.013	0.021	0.037	0.037	0.090	0.097
2. Tarasconnaise		0.011	0.016	0.040	0.041	0.095	0.103
3. Xisqueta			0.011	0.022	0.026	0.084	0.079
4. Ripollesa				0.037	0.033	0.090	0.108
5. Rouge du Roussillon					0.053	0.087	0.114
6. Castillonnaise						0.109	0.112
7. Guirra							0.146
8. Lacaune							—

$p = 0.05$ (Table 4). The six breeds located in the eastern Pyrenees showed lowerpaired F_{ST} values, but significant with each other, ranging from 0.011 to 0.053. The Guirra and Lacaune breeds, used as reference populations, showed the highest values among them ($F_{ST} = 0.146$), and with respect to Pyrenean breeds (ranging between 0.084 and 0.114). In any case, the pairs formed by any combinations of the Aranesa, Tarasconnaise, Xisqueta and Ripollesa breeds showed very low differentiation values.

Under the conservative stepwise mutation model, the two-tailed Wilcoxon signed-rank test gave statistical support to the presence of a genetic bottleneck due heterozygote deficiency in the Guirra ($p = 0.013$), Ripollesa ($p = 0.006$) and Xisqueta ($p = 0.008$) sheep breeds.

Fig. 2 summarises the between-breeds genetic relationships assessed using F_{ST} (Fig. 2A), D_A (Fig. 2B) and Reynolds' (Fig. 2C) distance matrices (see also Tables S1 and S2 [pdf online]). The information provided is consistently the same: the eastern Pyrenean sheep breeds and the two outgroups (the Guirra and the Lacaune breeds) were differentiated on the *X*-axis, while differentiation between outgroups could be found on the *Y*-axis. However, while the information provided by using F_{ST} and Reynolds' distance matrices was fully consistent, D_A allowed the Castillonnaise and the Rouge du Roussillon breeds to be differentiated from the rest of the eastern Pyrenean sheep.

Structuring Bayesian analysis (STRUCTURE) enabled three genetic clusters (K = 3) to be identified in our dataset (Table 5; Fig. S1 [pdf online]). Cluster 1 includes the Guirra (outgroup) individuals; Cluster 2 includes most of the Aranesa and Tarasconnaise genotypes, and roughly a third of the Xisqueta and the Ripollesa and Castillonnaise individuals; and, finally, Cluster 3 includes two thirds or more of the Xisqueta, Ripollesa and Rouge du Roussillon individuals. The Castillonnaise breed individuals are equally assigned to Clusters 2 and 3.

Finally, AMOVA analysis showed that most of the genetic variance is explained by between-individuals variation (95.85%), while the remaining 4.15% is explained by between-breeds differences. These estimates were statistically significant for $p < 0.0001$.

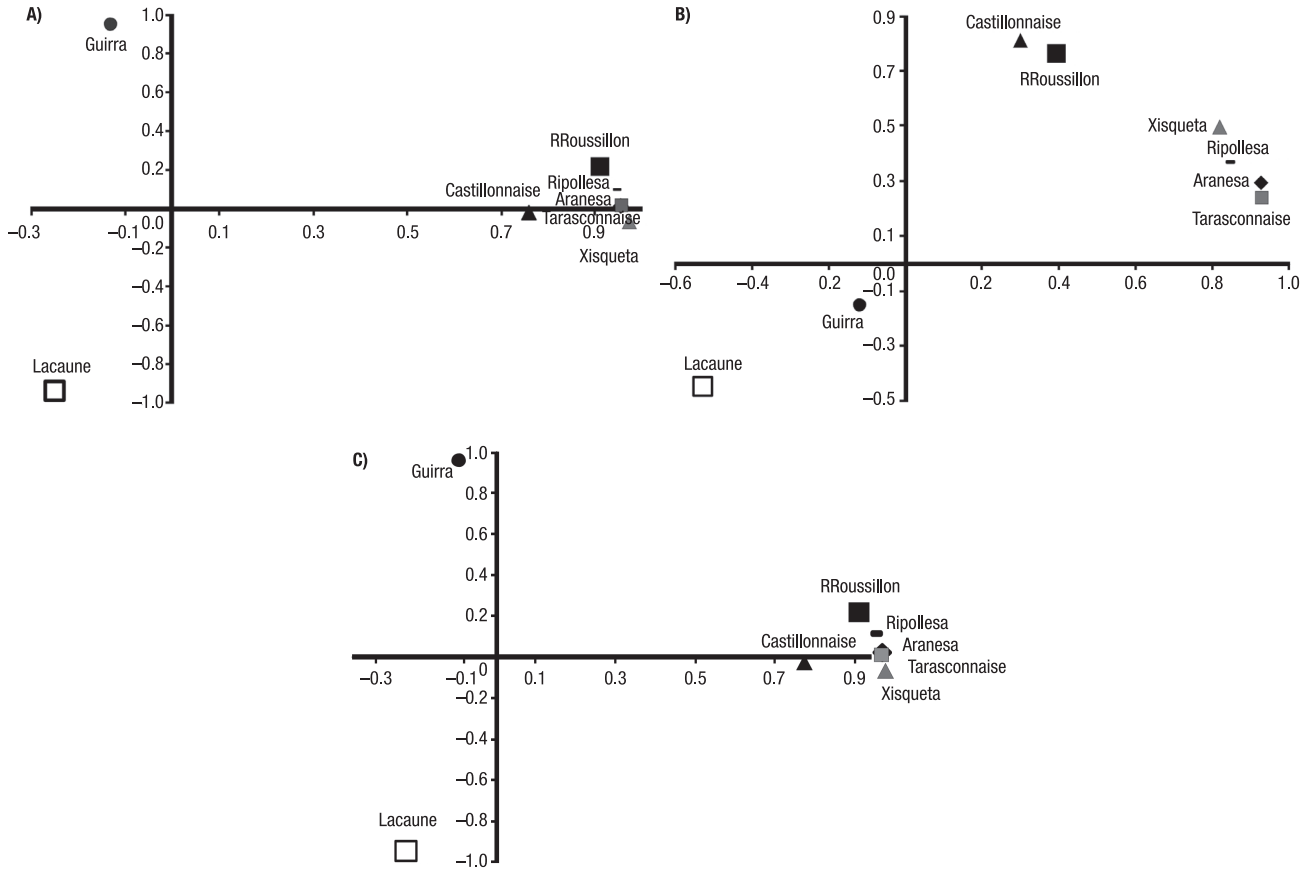

Figure 2. Distribution of the analysed sheep populations according to the results of the Principal Component Analysis performed on the between-populations F_{ST} (Plot A), D_A (Plot B) and Reynolds' (Plot C) distance matrices (see Tables S1 and S2 [pdf online] for further details). Factor 1 is on the *X*-axis while Factor 2 is on the *Y*-axis.

Discussion

Even though the analysed sheep breeds have limited census and are classified as "at risk" in their respective countries (Babo, 2000; Esteban, 2003), the current populations illustrate that they still retain a noticeable genetic variability. Although they are not directly comparable with the current research due to the different microsatellite set tested, the expected heterozygosity assessed in eastern Pyrenean sheep is comparable with that previously reported in different Iberian sheep sets, ranging from 0.66 (Alvarez *et al*., 2004) to 0.77 (Arranz *et al*., 2001). Handley *et al*. (2007) argue that, when compared with Northern European populations, Southern European sheep breeds keep higher genetic diversity due to their usually higher census sizes and the strong trading movement that Southern sheep populations have experienced during centuries.

In any case, similar scenarios like that of eastern Pyrenean sheep have been previously reported in limited geographical areas gathering sheep populations under low selective pressure and high between-populations gene flow (Tolone *et al*., 2012). The eastern Pyrenean sheep breeds are found in valleys, historically sharing summer pastures, livestock markets, and having similar external influences due to transhumance. In this respect, it is necessary to highlight the well-documented influence of the Merino breed in the formation of the Aranesa and Ripollesa breeds, and the fact that the Aranesa is considered the Spanish representative of the Tarasconnaise breed, and that the Xisqueta and the Aranesa breeds have actually interchanged individuals for centuries (Babo, 2000; Esteban, 2003; Parés, 2008; Parés *et al*., 2011).

Even though both the Guirra and the Xisqueta breeds have recently experienced a strong population bottleneck that has given a traceable signal using molecular markers, the genetic scenario of the Ripollesa breed is less clear. The Ripollesa breed is undergoing a selection programme probably leading to the existen-

Table 5. Proportion of membership of each sampled population in each of the three clusters inferred in the most likely run using the STRUCTURE program. Proportions of membership higher than 0.5 are in bold

Breed	Inferred clusters		
	1	2	3
1. Aranesa	0.03	**0.76**	0.19
2. Tarasconnaise	0.01	**0.78**	0.20
3. Xisqueta	0.03	0.30	**0.65**
4. Ripollesa	0.05	0.31	**0.63**
5. Rouge du Roussillon	0.04	0.08	**0.87**
6. Castillonnaise	0.05	0.41	**0.53**
7. Guirra	**0.90**	0.03	0.06

ce of multiple local bottlenecks, at a farm level, that have not been solved via between-farms gene flow (*i.e.* sharing artificial insemination rams). In any case, since between-breeds differentiation follows, as expected, a geographical pattern (see Fig. 2), the genetic bottlenecks identified are not likely to affect our results.

This scenario of poor genetic differentiation is clearly illustrated by the between-breeds genetic distances computed (Table 4; Table S1 [pdf online]; Fig. 2). While all the eastern Pyrenean sheep breeds are certainly separated from the outgroups, the group formed by the Aranesa, Tarasconnaise, Xisqueta and Ripollesa breeds clearly shows a noticeable genetic identity. While one could expect that the Rouge du Roussillon breed would show the highest differentiation within the eastern Pyrenean group, this was the situation detected for Castillonnaise breed. Even though the Castillonnaise breed could be classified as belonging to the Pyrenean "Entrefino" sheep group by its fleece characteristics (Parés, 2008) and, therefore, it would be expected to be genetically closer to the Aranesa, Tarasconnaise, Xisqueta and Ripollesa breeds, probably the recent history of the breed, in which its inter-crossing with British 'Southdown' individuals is documented (Babo, 2000), has probably lead to some differentiation. Interestingly enough, in spite of their common geographical North-African origin, the Rouge du Roussillon sheep has a genetic differentiation with the Guirra breed similar to those sheep assessed for the other eastern Pyrenean group. This suggests that the genetic background of the North-African red-coat sheep that reached the Mediterranean coasts of Southern Europe was diluted through inter-crossing with local sheep in each area. Similar results, with respect to Rouge du Roussillon breed, were obtained by Parés *et al*. (2011) in a study of fleece characteristics in Mediterranean red-coat sheep breeds.

This overall scenario was confirmed via STRUCTURE analysis. Even though the Aranesa and Tarasconnaise individuals were grouped together into Cluster 2, a significant proportion of the other eastern Pyrenean sheep individuals were in this cluster, and a non-negligible proportion of Aranesa and Tarasconnaise individuals were assigned to Cluster 3.

In conclusion, the genetic characterisation of the eastern Pyrenean sheep populations carried out provides useful information to support decision making on their conservation. While the partition of the eastern Pyrenean sheep into different breeds explains a significant part of their genetic variation, as suggested by the AMOVA analyses, the analysed populations form quite uniform clusters that could be considered, as a whole, for further trans-boundary initiatives for livestock diversity conservation (Hoffmann, 2011). Our results also confirm the historical information suggesting that both the Tarasconnaise and the Aranesa breeds belong to a common meta-population. However, the current analysis also shows that there has been a significant gene flow among neighbouring sheep breeds and, therefore, a comprehensive understanding of the eastern Pyrenean sheep is necessary before the implementation of conservation strategies in such populations.

Acknowledgments

This paper was partially funded by the Ministry of Agriculture, Livestock, Fisheries, Food and the Natural Environment of the Government of Catalonia. We also thank the farmers and the various breeders' associations for their help and collaboration throughout the sampling period.

References

Ajmone-Marsan P, Globaldiv Consortium, 2010. A global view of livestock biodiversity and conservation. Anim Genet 41: 1-5.

Alvarez I, Royo LJ, Fernández I, Gutiérrez JP, Gómez E, Goyache F, 2004. Genetic relationships and admixture among sheep breeds from Northern Spain assessed using microsatellites. J Anim Sci 82: 2246-2252.

Arranz JJ, Bayón Y, San Primitivo F, 2001. Genetic differentiation among Spanish sheep breeds using microsatellites. Genet Sel Evol 33: 529-542.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, 1989. Currents Protocols in Molecular Biology. Vol. 2. John Wiley and Sons Inc., New York. 1600 pp.

Avellanet R, 2006. Conservación de recursos genéticos ovinos en la raza Xisqueta: caracterización estructural, racial y gestión de la diversidad en programas "in situ". Doctoral thesis. Universidad Autónoma de Barcelona, Barcelona.

Babo D, 2000. Races ovines et caprines françaises, Edits. France Agricole, Paris. 204 pp.

Baumung R, Cubric-Curik V, Schwend K, Achmann R, Söelkner J, 2006. Genetic characterisation and breed assignment in Austrian sheep breeds using microsatellite marker information. J Anim Breed Genet 123: 265-271.

Buchanan FC, Crawford AM, 1993. Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. Anim Genet 24: 145.

Buchanan FC, Swarbrick PA, Crawford AM, 1992. Ovine dinucleotide repeat polymorphism at the MAF65 locus. Anim Genet 23: 85.

Calvo JH, Alvarez-Rodríguez J, Marcos-Carcavilla A, Serrano M, Sanz A, 2011. Genetic diversity in the Churra tensina and Churra lebrijana endangered Spanish sheep breeds and relationship with other Churra group breeds and Spanish mouflon. Small Rumin Res 95: 34-39.

Ciani E, Ciampolini B, D'Andrea M, Castellana E, Cecchi F, Incoronato C, D'Angelo F, Albenzio M, Pilla F, Matassino D, Cianci D, 2013. Analysis of genetic variability within and among Italian sheep breeds reveals population stratification and suggests the presence of a phylogeographic gradient. Small Rumin Res 112: 21-27.

Cinkulov M, Popovski Z, Porcu K, Tanaskovska B, Hodzic A, Bytyqi H, Mehmeti H, Margeta V, Djedovic R, Hoda A, *et al.,* 2008. Genetic diversity and structure of the West Balkan Pramenka sheep types as revealed by microsatellite and mitochondrial DNA analysis. J Anim Breed Genet 125: 417-426.

Earl DA, vonHoldt BM, 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conservation Genet Resour 4: 359-361.

Ede AJ, Pierson CA, Crawford AM, 1995. Ovine microsatellites at the OarCP34, OarCP38, OarCP43, OarCP49, OarCP73, OarCP79 and OarCP99 loci. Anim Genet 26: 130-131.

El Mousadik A, Petit RJ, 1996. High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L) Skeel) endemic to Morocco. Theor Appl Genet 92: 832-839.

Esteban C, 2003. Razas ganaderas españolas. II. Ovinas. Ed. FEAGAS y Ministerio de Agricultura, Pesca y Alimentación, Madrid. 470 pp.

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol Ecol 4: 2611-2620.

FAO, 2009. Preparation of national strategies and action plans for animal genetic resources. FAO Animal Production and Health Guidelines, No. 2, Rome. 70 pp.

Glowatzki-Mullis ML, Muntwyler J, Baeumle E, Gaillard C, 2009. Genetic diversity of Swiss sheep breeds in the focus of conservation research. J Anim Breed Genet 126: 164-175.

Goudet J, 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Updated from Goudet (1995). Available in http://www.unil.ch/izea/softwares/fstat.html. [25 April, 2012].

Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B, Pilling D, Negrini R, Finlay K, Jianlin H, Groeneveld E, *et al.,* 2010. Genetic diversity in farm animals-A review. Anim Genet 41: 6-31.

Handley LJL, Byrne K, Santucci F, Townsend S, Taylor M, Bruford MW, Hewitt GM, 2007. Genetic structure of European sheep breeds. Heredity 99: 620-631.

Hoffmann I, 2011. Livestock biodiversity and sustainability. Livest Sci 139: 69-79.

Hulme DJ, Silk JP, Redwin J, Barense WR, Beh KJ, 1994. Ten polymorphic ovine microsatellites. Anim Genet 25: 434-435.

Hulme DJ, Davies KP, Beh KJ, Maddox JF, 1996. Ovine dinucleotide repeat polymorphism at the McM218, McM150 and McM138 loci. Anim Genet 26: 57.

Kusza S, Nagy I, Sasvári Z, Stágel A, Németh T, Molnár A, Kume K, Bósze Z, Jávor A, Kukovics S, 2008. Genetic diversity and population structure of Tsigai and Zackel type of sheep breeds in the Central-, Eastern- and Southern-European regions. Small Rumin Res 78: 13-23.

Kusza S, Gyarmathy E, Dubravska J, Nagy I, Jávor A, Kukovics S, 2009. Study of genetic differences among Slovak Tsigai populations using microsatellite markers. Czech J Anim Sci 54: 468-474.

Langella O, 1999. Populations 1.2.31. CNRS UPR9034. Available in http://www.bioinformatics.org/~tryphon/populations/ [7 May, 2012].

Ligda C, Altarayrah J, Georgoudis A, Econogene Consortium, 2009. Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. Small Rumin Res 83: 42-48.

Luikart G, Alendorf F.W, Sherwin B, Cornuet JM, 1998. Distortion of allele frequency distributions provide a test of recent population bottlenecks. J Hered 12: 238-247.

Nei M, Tajima R, Tateno Y, 1983. Accuracy of estimated phylogenetic threes from molecular data. J Mol Evol 19: 153-170.

Parés PM, 2008. Caracterització estructural i racial de la raça ovina Aranesa. Doctoral thesis. Universitat Autònoma de Barcelona, Barcelona. [In Catalan].

Parés PM, Perezgrovas R, Jordana J, 2011. Comparison of four European endangered red sheep based on fleece characteristics. Anim Genet Resour 48: 85-91.

Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt G, Econogene Consortium, 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. Anim Genet 38: 37-44.

Piry S, Luikart G, Cornuet J.M, 1999. Bottleneck: A computer program for detecting recent reductions in effective population size from allele frequency data. J Hered 90: 502-503.

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

Salamon D, Gutiérrez-Gil B, Arranz JJ, Barreta J, Batinic V, Dzidic A, 2014. Genetic diversity and differentiation of 12 eastern Adriatic and western Dinaric native sheep breeds using microsatellites. Animal 8: 200-207.

Sánchez-Belda A, Sánchez-Trujillano MC, 1986. Razas ovinas españolas. Ed. FEAGAS y Ministerio de Agricultura, Pesca y Alimentación, Madrid. 877 pp.

Santos-Silva F, Ivo RS, Sousa MCO, Carolino MI, Ginja C, Gama LT, 2008. Assessing genetic diversity and differentiation in Portuguese coarse-wool sheep breeds with microsatellite markers. Small Rumin Res 78: 32-40.

Schneider S, Kueffer JM, Roessli D, Excoffier L, 1997. Arlequin: a software for population genetics data analysis, vers. 1.1. Genetics and Biometry Laboratory, Dept. of Anthropology, University of Geneva, Switzerland. 174 pp.

Scott PC, Maddox JF, Gogolin-Ewens KJ, Brandon MR, 1991. Inmunogenetics 34: 80-87.

Reynolds J, Weir BS, Cockerham CC, 1983. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. Genetics 105: 767-779.

Takezaki N, Nei M, 1996. Genetic distance and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389-399.

Tapio M, Tapio I, Grislis Z, Holm LE, Jeppsson S, Kantanen J, Miceikiene I, Olsaker I, Viinalass H, Eythorsdottir E, 2005. Native breeds demonstrate high contributions to the molecular variation in northern European sheep. Mol Ecol 14: 3951-3963.

Tapio M, Ozerov M, Tapio I, Toro MA, Marzanov N, Ćinkulov M, Goncharenko G, Kiselyova T, Murawski M, Kantanen J, 2010. Microsatellite-based genetic diversity and population structure of domestic sheep in northern Eurasia. BMC Genetics 11: 76.

Tolone M, Mastrabgelo S, Rosa AJM, Portolano B, 2012. Genetic diversity and population structure of Sicilian sheep breeds using microsatellite markers. Small Rumin Res 102: 18-25.

Vainman D, Mercier D, Moazami-Goudarzi K, Eggen A, Ciampolini R, Lépingle A, Velmala R, Kaukinen J, Varvio SL, Martin P, 1994. A set of 99 cattle microsatellites: characterization, synteny mapping and polymorphism. Mammalian 5: 288-297.

Weir BS, Cockerham CC, 1984. Estimating F statistics for the analyses of population structure. Evolution 38: 1358-1370.

Zimin AV, Delcher AL, Florea L, Kelley DR, Schatz MC, Puiu D, Hanrahan F, Pertea G, Van Tassell CP, Sonstegard TS, *et al.,* 1999. A whole-genome assembly of the domestic cow, Bos taurus. Genome Biol 10: 42.