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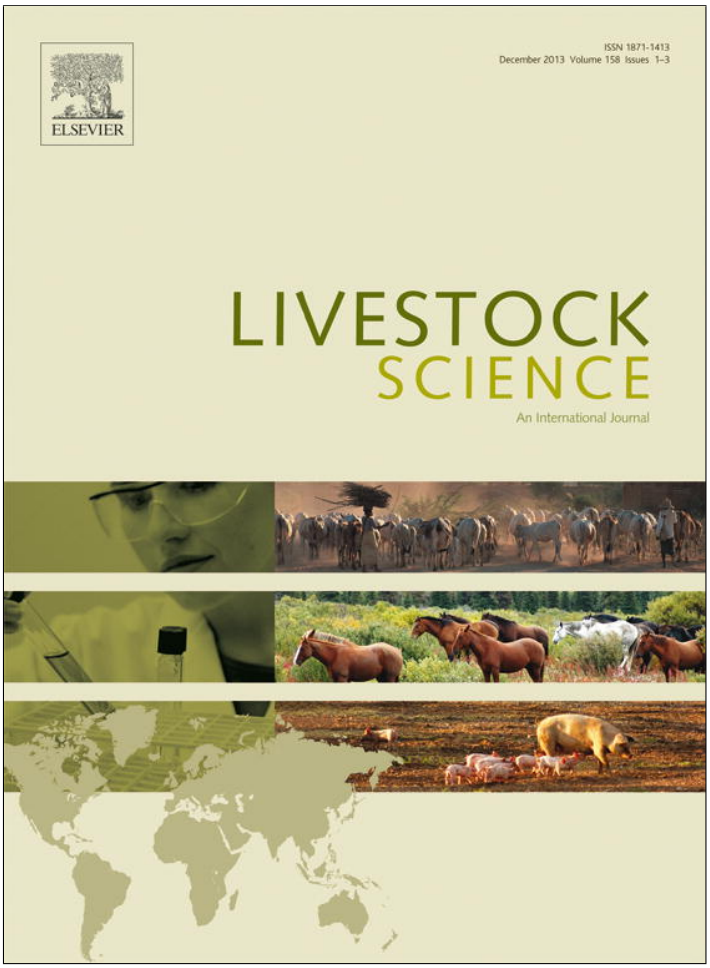

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The rob(1;29) chromosome translocation in endangered Andalusian cattle breeds

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ABSTRACT

In this study, we analysed the distribution of the *t*(1;29) Robertsonian translocation to determine whether this polymorphism contributes to the low reproduction efficiency observed in five endangered Andalusian (Spain) cattle breeds: Berrenda en Negro (BN), Berrenda en Colorado (BC), Cardena Andaluza (CA), Pajuna (PA) and Negra Andaluza (NA). All these breeds were reared exclusively in reproductive isolation under grazing conditions with natural mating. In total, we analysed the distribution of the rob(1;29) translocation in 714 animals: 192 BN, 235 BC, 156 CA, 56 PA and 75 NA. We also examined the translocation frequencies, *F* statistics and deviations from Hardy–Weinberg equilibrium among different herds and breeds and characterised the influence of geographical location and sex. The F_{ST} values ($P < 0.05$) revealed differences among the breeds and herds in BC, BN and CA. There were no significant geographical differences, except in the Cardena breed ($P < 0.001$). In addition to reproductive isolation, the differences observed among the herds might reflect the sporadic movement of bulls belonging to flocks with a high frequency of translocation, genetic drift and anthropic selection. The rob(1;29) frequency was reduced in some breeds, potentially reflecting the effects of human selection and breeding strategies implemented through official control programmes for this anomaly. In other breeds, the translocation remains present at high frequencies, reflecting crossbreeding with the Retinta breed, which has a high frequency of rob(1;29). No significant deviation in the expected percentage of heterozygotes was detected in any breed. The differences observed in the rob(1;29) frequencies in the endangered Andalusian cattle might be more attributable to internal factors than to differences between the breeds. Further studies regarding the mobility of sires among herds are warranted to determine the origin of the variations in the rob(1;29) frequencies observed in endangered Andalusian breeds.

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1. Introduction

Gustavsson and Rockborn (1964) first identified rob(1;29), a Robertsonian translocation between chromosomes 1 and 29, in Swedish Red-and-White cattle. The hypothesis that this translocation originates from an ancient complex chromosome rearrangement has been widely supported (Di Meo et al., 2006). The rob(1;29) translocation has since spread to all European cattle breeds and, eventually, to the rest of the world through crossbreeding, without appearing *ex-novo* (Popescu and Cribiu, 1982). The frequency of a particular genetic alteration is typically maintained or even increased in a population only when carrier animals are provided with some adaptive advantage (Joly, 2011); however, this phenomenon has not yet been

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demonstrated for this particular trait (Popescu, 1989). In contrast, several authors have established that the rob(1;29) translocation reduces the reproductive efficiency of carrier animals (Refsdal, 1976; Popescu and Tixier, 1984; Moreno-Millán and Rodero, 2004).

The rob(1;29) frequency varies widely depending on the breed, the number of animals studied, geographic location, type of mating and production system (Ducos et al., 2008). Moreover, the frequency of this translocation is highly influenced through breeding programmes to reduce the primary effect of this trait; an economic derived from a reduced reproductive efficiency (Moreno Millan and Moreno, 2004; Iannuzzi et al., 2008; Ciotola et al., 2009). Previous studies have reported remarkably high translocation frequencies in several Andalusian minority breeds, such as Berrenda en Colorado (BC), Berrenda en Negro (BN), Cardena Andaluza (CA), Negra Andaluza (NA) and Pajuna (PA) (Rodero et al., 2010) and in the majority breeds Retinta (RE) and Charolais (CH) (Demyda-Peyrás et al., 2012). These results raise the question of how these percentages were achieved and preserved for several generations.

The phenotypic characteristics of Andalusian cattle have been systematically documented since the XVIIth and XVIIIth centuries (Rodero and Rodero, 2008b). However, these cattle have only been accepted as valid breeds since the end of XIXth century. To increase production, these authoctonous animals were crossbred with foreign cattle in the mid-twentieth century (Rodero and Rodero, 2008b). According to breeding associations, these populations have been reproductively isolated for the last 50 years. Nevertheless, we previously suggested the existence of crossbreeding between different Andalusian cattle populations, facilitating the spread of genetic traits, such as rob(1;29) (Rodero and Rodero, 2008a).

The relevance of these animals to the development of rural areas has led to the implementation of important conservation programmes, designed and formally conducted through breeders associations since 1992 (BOE, 2008). In addition, these breeds have been identified as fundamental components of the economy of small villages (Rodero and Rodero, 2008b).

The study aims to (1) determine the prevalence of rob (1;29) in indigenous Andalusian cattle breeds; (2) describe the distribution of the translocation among breeds and herds; and (3) evaluate rob(1;29) translocation frequencies, considering the genetic structure, reproductive association, geographic location and origins of the populations analysed.

2. Materials and methods

2.1. Sample animals

Overall, 714 animals belonging to five indigenous Andalusian cattle breeds, Berrenda en Colorado (BC), Berrenda en Negro (BN), Cardena Andaluza (C) Negra Andaluza (NA) and Pajuna (PA), were cytogenetically analysed in our laboratory. These breeds were recognised as endangered (BOE, 2008) and included in the Domestic Animal Diversity Information Service (FAO, 2011). These cattle originated from the Autonomous Region of Andalusia and are typically raised under extensive "*dehesas*" conditions, under natural mating conditions with sires produced at the same farms. Blood samples and information regarding the population structure of each breed were obtained from the breeders associations. Table 1 shows the population size, herd number and percentage of the animals of each breed studied.

2.2. Chromosomal analysis

Lymphocyte chromosome metaphase spreads were obtained according to the techniques of De Grouchy et al. (1964), with minor modifications. Briefly, 7 ml of jugular blood samples were collected from all animals in sterile sodium-heparin Vacutainers™. The samples were centrifuged at 800 g for 10 min. The white cell interphase and 1 ml of autologous serum were inoculated into 10-ml sterile tubes containing 8 ml of RPMI 1640 medium supplemented with 5 μg/ml Pokeweed Lectin, 100 IU penicillin/ml, 100 μg/ml streptomycin and 250 ng/ml amphotericin B. The cultures were incubated at 38 °C for 72 h. At one hour before harvesting, 1 μg/ml of colcemid was added to each tube. The cells were harvested and incubated for 25 min in 0.075 M KCl hypotonic solution. Subsequently, the cells were fixed in a cold methanol:acetic acid (3:1) solution for 30 min and stored for 24 h at 4 °C. The chromosome spreads were obtained after dropping 120 μl of the cell suspension onto pre-frozen wet slides, air dried for 20 min and stained for 15 min in a 10% Giemsa water solution. The samples were assessed using a Reichert Polyvar microscope at 1250 × magnification. Approximately 30 analysable metaphase samples (those with intact and non-overlapping chromosomes) from each animal were evaluated and counted. The chromosome spreads showing abnormalities were G banded, according to the method of Iannuzzi (1996), to confirm the identity of the abnormal chromosomes.

2.3. Herd and buffer georeferencing procedure

To evaluate the influence of the geographical location on the distribution and frequency of rob(1;29), the herds were grouped into geographic nuclei called buffers. If a geographical influence exists, the exchange of reproductive animals will be larger within buffers than between buffers, and consequently, differences in rob(1;29) will be larger between buffers than

Table 1
Total population census and sample size of each studied breed by sex. *Data sources*: 1: Official records from Spanish government; 2: breeders associations and 3: Pastor (2010).

Breed	Total Census[1]		Herds[2]	Sampled animals	Sampled herds
	Females	Males			
BC	3205	589	149	235 (6.2%)	37 (24.8%)
BN	2151	244	104	192 (8%)	41 (39.4%)
CA	861	9	14	156 (17.9%)	6 (42.4%)
NA[3]	1010	82	24	75 (6.9%)	4 (16.7%)
PA	647	41	36	56 (8.1%)	6 (16.7%)

Breeds: BC: Berrenda en Colorado; **BN:** Berrenda en Negro; **CA:** Cardena Andaluza; **NA**: Negra Andaluza and **PA:** Pajuna.
In brackets: Percentage of sampled animals/herds.

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within buffers. To examine this idea, we adapted the methodology of Pastor (2010) in Andalusian breeds.

First, all known flocks of each breed were geo-positioned according to the GPS coordinates of the government system for recording farms (SIGGAN, 2008) and displayed in a cartographic map of the Andalusian region using the Carta Digital™ software.

Second, we represented the influence area of each herd using a circle with a radius of 30 km, to limit the area and increase the potential for reproductive exchange between the herds, due to assisted reproductive techniques were not yet implemented in these breeds.

Subsequently, the buffers were represented as a circle with a radius of 30 km from the geometrical centre, located in the confluent areas of the herds analysed. However, for consideration in this study, the buffers must contain rob(1;29) carriers in at least two different farms located closer than 60 km. Using this approach, buffers were determined in only three breeds (BC, BN and CA; Fig. 1). Because of the dispersion of the herds in the other two breeds (NA and PA), the flocks were analysed without considering the geographical buffer (Fig. 1).

2.4. Statistical analysis

All statistical analyses were performed using the Statistica for Windows 7.0 (Statsoft, Inc.® Tulsa, OK, USA) software package. The translocation frequencies of each genotype (homozygote and heterozygote) were determined according to breed, sex, herd and geographical buffer. The statistical significance of these factors ($P \leq 0.05$) was analysed using a χ^2 homogeneity test. We also examined the allelic frequencies and the Hardy–Weinberg equilibrium, considering rob(1;29) as a non-neutral genetic trait that behaves as a single locus with Mendelian segregation. The genetic structure within and among breeds was examined using *F* statistics (F_{IT}, F_{ST} and F_{IS}), according to Weir and Cockerham (1984), through Genetix v 4.02 software. *F*-statistics are defined as a function of observed and expected heterozygosities, where negative values indicate more observed heterozygosity of individuals (F_{IS}, F_{IT}) or subpopulations (F_{ST}) than expected based on the frequency in the total population (F_{IT}, F_{ST}) or in the sub-population (F_{IS}).

3. Results and discussion

In total, the frequencies of the rob(1;29) translocation showed a high incidence in both sexes among all animals studied (Table 2). The percentages of carrier animals among all breeds did not differ significantly ($P > 0.05$) between males (23.64%) and females (24.04%), and the populations was in Hardy–Weinberg equilibrium (Table 3). The total allelic frequencies were 0.1197, with a F_{IS} value of −0.036 when the entire population was divided in breeds, and of −0.125 when it was divided in herds. These data are consistent with those from previous studies in other European cattle breeds (Ducos et al., 2008). The rob(1;29) translocation is present in almost all major breeds worldwide, with the exception of the Holstein-Friesian breed (Seguin et al., 2000) and some African and south American lineages. In the Holstein-Friesian breed, only sex chimerism (Citek et al., 2009) and a single rare case of Y-autosome reciprocal translocation (Switonski et al., 2011) have been reported.

The translocation frequency was lower in males compared with females, except for the CA breed. However, these results might not be conclusive due to the smaller sample size of males analysed. Notably, this sample size is low because the breeders associations do not permit the registration or use of new rob(1;29) carriers as sires to reduce translocation frequencies over time.

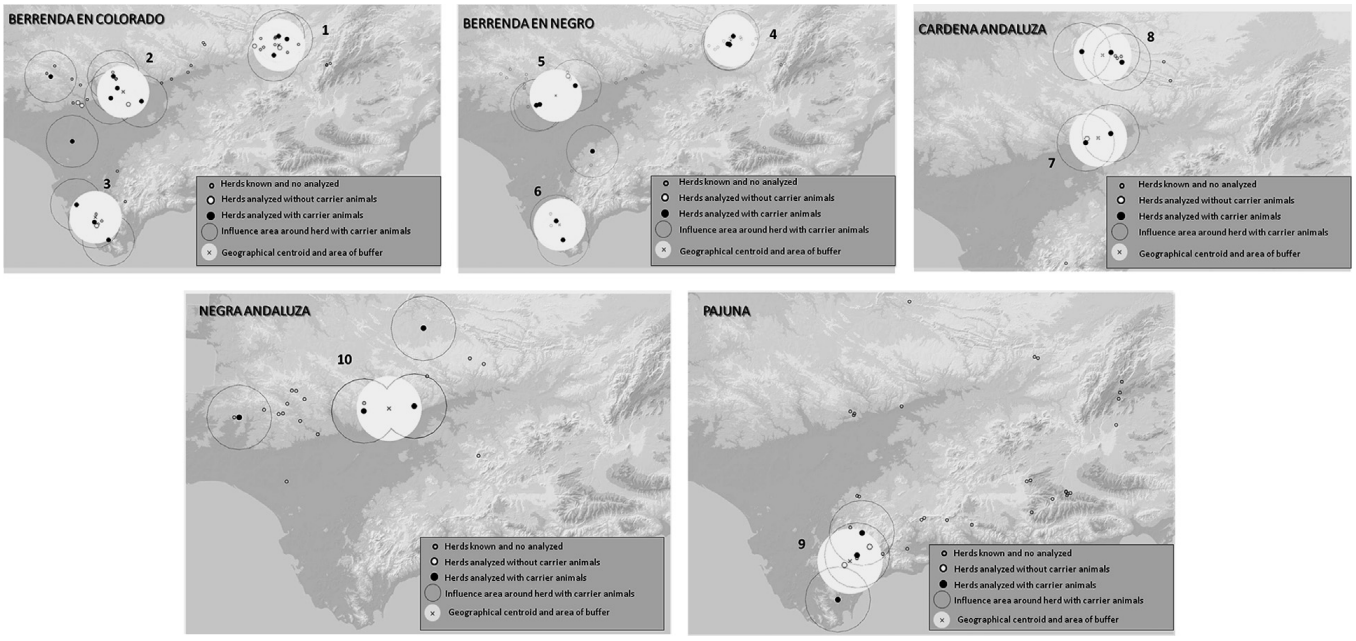

Fig. 1. Geographical location of the buffers and flocks of the five endangered Andalusian cattle breeds analysed in the present study. Map of the Andalusia region depicting the geographical distribution the five cattle breeds analysed. In each breed, the crossed circles show all known flocks, the white dots show rob(1;29)-free flocks and the black dots show rob(1;29)-positive flocks. In both Berrenda and Cardena breeds, the crossed circles represent the determined buffers with a radius of 30 Km. More than two carrier herds of each breed were permitted within these buffers.

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Table 2
Relative and absolutes frequencies of normal and rob(1;29) genotypes detected by breed and sex in Andalusian endangered Cattle breeds.

Breed	Males					Females					χ^2 [a]	Total
	N	Normal genotype	*t*(1;29) genotype	*t*(1;29); *t*(1;29) genotype	*t*(1;29) total frequency	*N*	Normal genotype	*t*(1;29) genotype	*t*(1;29); *t*(1;29) genotype	*t*(1;29) total frequency		Allele frequency
BC	72	0.8056 (58)	0.1806 (13)	0.0138 (1)	0.1944	163	0.7669 (125)	0.1963 (32)	0.0368 (6)	0.2331	1.17 n. s.	0.1255
BN	45	0.7111 (32)	0.2222 (10)	0.0667 (3)	0.2889	147	0.6735 (99)	0.3061 (45)	0.0204 (3)	0.3265	2.96 n. s.	0.1588
CA	18	0.7222 (13)	0.2778 (5)	0.0000 (0)	0.2778	138	0.8406 (116)	0.1594 (22)	0.0000 (0)	0.1594	1.39 n. s.	0.0865
NA	3	1.0000 (3)	0.0000 (0)	0.0000 (0)	0	72	0.8055 (58)	0.1806 (13)	0.0139 (1)	0.1945	1.27 n. s.	0.0933
PA	27	0.7407 (20)	0.2593 (7)	0.0000 (0)	0.2593	29	0.6552 (19)	0.3103 (9)	0.0345 (1)	0.3448	1.59 n. s.	0.1518
Total	165	0.7636 (126)	0.2121 (35)	0.0242 (4)	0.2364	549	0.7596 (417)	0.2204 (121)	0.0200 (11)	0.2404	0.16 n. s.	0.1197
χ^2 [b]			7.79 n. s.					19.94*				
χ^2 [c]							19.49*					
χ^2 [d]							0.15 n.s.					

Breeds: BC: Berrenda en Colorado; **BN**: Berrenda en Negro; **CA**: Cardena Andaluza; **NA**: Negra Andaluza and **PA**: Pajuna. **N**: total analyzed animals.
In each value: Relative frequencies (without brackets) and total detected cases (with brackets). **n. s.**: no significant differences.
* $p < 0.05$.
[a] Comparison among sex and within breed.
[b] Comparison among breeds by sex.
[c] Comparison among breeds for without considering sex.
[d] Comparison among sex without considering breeds.

Table 3
Wright *F*-statistic values for rob(1;29) in five Andalusian Cattle minor breeds and the total of population by herds and buffers.

Breed	Total		**Subpopulations (herds)**			**Subpopulations (buffers)**		
	Observed heterozygosity (%)	**Expected heterozygosity (%)**	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Berrenda en Colorado (*N*=235)	19.1	21.9	−0.033	0.136	0.159	0.175	0.250	0.092
Berrenda en Negro (*N*=192)	28.6	26.7	−0.221	0.007	0.186	−0.108	−0.116	−0.007
Cardena Andaluza (*N*=156)	17.3	15.8	−0.225	−0.066	0.129	−0.129	−0.056	0.065
Negra Andaluza (*N*=75)	17.3	16.9	0.054	0.040	−0.015			
Pajuna (*N*=56)	28.6	25.7	−0.105	−0.037	0.062			
Total of population (*N*=714)	21.4	21.1	−0.123	0.034	0.140	−0.033	0.008	0.039

In bracket animals sampled.

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3.1. Differences across breeds

Significant interbreed differences ($P<0.05$) in the rob (1;29) frequencies were observed (Table 2). Higher frequencies were observed in the BN breed, and lower frequencies were observed in the NA breed. We also detected differences in the frequencies of this translocation between CA and NA females. The Wright *F*-statistics obtained analysing the population by herds or by breeds as sub-populations did not show a significant deficit in the heterozygous genotype for rob(1;29) (Table 3). The results (-0.036 for F_{IS}, -0.053 for F_{IT} and -0.017 for F_{ST}) are showing an excess of heterozygotes, which may indicate selection. The F_{ST} value (-0.017) indicates that there was a little differentiation between the breeds. It suggests the existence of other factors, such as origin coincidence, breeder´ exchange or gene flow.

The most endangered breed, CA, (Table 1) showed the biggest deficit in heterozygosity, and BC, the largest census, presented an excess of 1.8%, showing a major subdivision and maintaining the levels of heterozygosity. However, despite the small sample size, the analysis of Wright *F*-statistics did not clearly demonstrate a heterozygous deviation in BC and NA breeds.

The CA breed has also been identified outside Andalusia. Furthermore, this breed coexists with the Lidia breed as assistants to manage fighting bulls (Gonzalez et al., 2012). Notably, the rob(1;29) alteration has been detected in only one case in more than 100 studied Lidia samples (Zarazaga and Arruga, 1982). Thus, we propose that uncontrolled crossbreeding between these two populations in the past might have contributed to the reduction in the rates of the rob(1;29) translocation observed in CA cows. In the NA breed, investigated for first time in the present study, no carrier bulls were detected. Moreover, the frequency observed among the females was 19.45%. The males of the NA breed could not be analysed due to the low sample size ($n=3$).

Differences observed between the Berrenda breeds are difficult to explain, as both breeds had been reared as a single population for many years before the recent split into two different populations. We suggest that breeding strategies without considering rob(1;29) such as the use of the Retinta breed in uncontrolled crossbreeding for more than 10 generations might be the major causes of these differences. Consistently, the influence of the Retinta breed in the BC and PA breeds has been previously demonstrated (Luque et al., 2009). The rob(1;29) prevalence detected in this major Spanish breed was higher than 32% (Moreno Millán, 2003; Demyda-Peyrás et al., 2012). Consequently, the influence of Retinta cattle might also influenced the frequencies observed in these two Andalusian populations.

3.2. Differences within breeds

The frequencies observed among the males within the breeds differed slightly only in the BC breed. This is underlined by the F_{ST} values which indicate that over 99% of the variability observed for rob(1;29) primarily reflects differences within the breeds and not between them, particularly in BC and CA. With the exception of NA, the F_{IS} indices within the breeds were negative

Table 4
Comparison of relative frequencies of rob(1;29) genotypes determined different geographic nucleus/herd in Andalusian Cattle minor breeds.

Cattle breed	Geographic nuclei (Buffers)							Herds									
	Berrenda en Colorado			Berrenda en Negro			Cárdena Andaluza	Negra Andaluza				Pajuna[c]					
Nucleus/Herd	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***	
Sampled animals	55	41	19	59	40	10	71	85	9	14	30	22	20	10	8	9	
Normal	0.9273	0.6341	0.8421	0.6441	0.7500	0.8000	0.9437	0.7294	0.8889	0.7143	0.8000	0.8636	0.6500	0.6000	0.3750	0.8889	
t(1;29)	0.0727	0.2927	0.1053	0.3559	0.2250	0.2000	0.0563	0.2706	0.1111	0.2857	0.1667	0.1364	0.3500	0.4000	0.5000	0.1111	
t(1;29) *t*(1;29)	0	0.0732	0.0526	0	0.0250	0	0	0	0	0	0.0333	0	0	0	0.1250	0	
χ^2 [a]	15.20**			4.31 n. s.			13.71****										
χ^2 [b]	74.28****			36.94*			41.32****		3.42 n. s.				15.13 n. s.				

n.s.: no significant differences. Relative frequencies are calculated in relation with the total sampled in each geographical nucleus/herd.
* $p<0.05$;
** $p<0.01$;
**** $p<0.001$.
[a] χ^2 test between buffers of Berrenda en Colorado, Berrenda en Negro and Cardena Andaluza breeds.
[b] χ^2 test between all analyzed herds from each breeds.
[c] Only shows herds with animals carriers. **On the same row:** relative frequencies determined in each geographic buffer/herd.

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(Table 3), suggesting a HW deviation. However, the differences between the heterozygosities (Ho–He) were not significant, suggesting that these populations are in HW equilibrium. The analysis of these results and the negative or close to zero F_{IS} values suggest the absence of an heterozygous deficit despite the fact that these breeds are isolated reproductively, with a small population size and that they also may have experienced a Wahlund effect.

We also observed differences in the frequencies of CA and NA females, potentially reflecting animal census and breeding strategies, consistent with the large frequency variation observed among herds (Table 4). Furthermore, in small flocks, an erroneous breeding decision might strongly influence the rob(1;29) frequencies. Another possible explanation is the occurrence of naturally and frequent uncontrolled crossbreeding between Andalusian cattle and "foreign" breeds (Rodero et al., 2005; Martín-Burriel et al., 2011). Moreover, the resulting animals from these crossbreeding might have been included in the official herd books based only on phenotypic classification (Rodero and Rodero, 2008a), allowing the rob(1;29) translocation to extend to Andalusia.

3.3. Comparison between geo-positioned buffers and herds

The influence of the closest herds with carriers was determined using different buffers only for the BC, BN and CA breeds (Table 4). In these breeds, the fixation indices clearly differed from those obtained without geographical clustering. In BC, the F_{IS} value is positive among buffers (0.175) but negative among herds without considering geo-referencing (−0.033). In BN, the F_{IS} value remains consistent, but the F_{IT} value is positive among herds (0.007) and negative among buffers (−0.116). Furthermore, the F_{ST} indices (Table 3) were consistent with the χ^2 results (Table 4). The F_{ST} shows no or a small influence of buffers (geography) since they range from −0.007 to 0.092, whilst the χ^2 test showed a clear geographical influence on the rob(1;29) frequency distribution. Higher F_{ST} indices among buffers were observed in the BC breed, suggesting an effective geographical isolation and small flow of breeder animals or a Wahlund effect due to small buffer size.

In both Berrenda breeds, the location of the three population buffers and the herd dispersion were similar (Fig. 1). Interestingly, significant differences ($P \leq 0.05$) were observed within the BC buffers, particularly in buffer 2, but not within the BN buffers, suggesting the existence of a genetic flow between the herds of this last breed, independent of geographic location. This idea is also consistent with the results obtained from the comparison of the two close buffers of CA, which showed large differences ($P \leq 0.001$) in the frequencies of rob(1;29) heterozygous individuals (0.0563 and 0.02706, respectively). We suggest that the extremely small sample size of males ($n=6$) demonstrates a clear "founder effect" that could explain the lower F_{IS} value (−0.129) observed (Table 3). F_{IS} and F_{IT} values obtained in the BC breed suggest that the observed genetic variability reflects inter-herd instead intra-herd differences, without a clear deviation from observed (Ho) to expected (He) heterozygotes.

The positive F_{ST} values obtained in BC, BN and CA suggested the existence of inter-buffer differences, generating a positive overall F_{ST} value. In the second analysis, considering herds as subpopulations, higher F_{ST} values were obtained. These results are showing a clear herd effect in these breeds, but it is not caused by the geographical localization of the animals (buffer effect). These F_{ST} values obtained in the breeds may suggest that genetic selection performed by the farmers could be different among herds. In addition, the herds and geographical factors might affect the subdivision of the breeds. In these farms it is very common that producers also breed their own sires or even, in some cases, share sires with neighbors. The comparison between herds or buffers for each breed conducted using χ^2 confirmed the results of the F_{ST} analysis (Table 4). The breed with the lowest F_{ST} (−0.007) between buffers is BN, which is not significantly different. The other two breeds (BC and CA), which were indeed significantly different, showed higher F_{ST} values. The same trend was observed when the analysis was performed over herds. The NA breed, which showed the lowest F_{ST} value was the only one with no significantly differences. This population, the most endangered Andalusian breed, also demonstrates a founder effect, as the 14 existing herds are actually descendants of only 3 founder herds (Gonzalez et al., 2012). Therefore, although the estimated heterozygote deficiency, observed through DNA microsatellites, was not high (Rodero et al., 2005), the conservation programme should carefully plan mating using sires free of rob(1;29) polymorphisms.

3.4. Breed effect and phylogenetic relationships

In a recent study using microsatellites, Martín-Burriel et al. (2011) demonstrated that Berrenda en Negro and Negra Andaluza breeds are split into different subpopulations as a consequence of reproductive and geographical isolation. Due to this phenomenon, crossbreeding between these subpopulations increases the levels of heterozygosity. In the present study, they cannot be distinguished based on rob(1;29) frequencies in buffers or herds, suggesting that geographical location did not produce reproductive isolation, generating variability in rob(1;29) frequencies among the herds. However, the calculated *F*-statistic values and observed heterozygosity levels are consistent with the possibility of animal exchange between the herds. These results suggest that genetic drift, "bottle necks" and the "founder effect" might also contribute to the observed rob(1;29) frequencies. This result is also consistent with those obtained through other genetic traits in minority breeds (France and Rigg, 1998; Laloë et al., 2010; Cañón et al., 2011).

South American Creole breeds have a common Iberian origin and are most likely descendants of Andalusian breeds (Rodero and Rodero, 2008b). However the prevalence of the rob(1;29) translocation is extremely variable in these populations. Munoz et al. (1994) observed breeds with a high prevalence of rob(1;29) carriers in Venezuela (21%), whereas De Luca et al. (2002a) observed these carriers in Bolivia (28%; Chaqueño biotype), Sanchez et al. (2005) observed carriers in Colombia (22%) and

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Schifferli et al. (2003) observed carriers in northern Argentinean Creole breeds. On the contrary, this translocation has not been detected in Patagonic biotype Creole breeds in Argentina (Genero et al., 1999) and in the Blanco Orejinegro, Sanmartinero, Harton del Valle and Costeño con Cuernos biotypes in Colombia (Sanchez et al., 2005). Furthermore, the rob(1;29) translocation has only been detected at a prevalence of 1% in the Saavedreño biotype in Bolivia (De Luca et al., 2002b). These "translocation-free" biotypes have been reproductively isolated without anthropic selection for hundreds of years. The nearly complete disappearance of rob(1;29) from Bolivian breeds and the total disappearance of this translocation in Patagonian and some Colombian Creole breeds reflect genetic drift, natural selection and gene introgression. The results obtained in the present study are more similar to those of northern Creole cattle, showing a high rob(1;29) frequency and suggesting that the cattle populations examined here were not reproductively isolated or influenced through uncontrolled breeding strategies, "bottle necks" or "founder effects".

3.5. Andalusian cattle diversity and rob(1;29) origin

A study by Edwards et al., (2011) suggested that the high diversity of breeds observed in Spanish and Portuguese cattle reflects the geographical location of the Iberian Peninsula. African genes might have introgress via migrating animals through the Gibraltar strait, as previously suggested from the mtDNA analysis (Ginja et al., 2010). A low rob(1;29) frequency has been previously described in only one minority breed of African *Bos taurus* (Nel et al., 1985). However, this lineage was most likely "contaminated" in the recent past with European breeds introduced through Dutch merchants. Nel et al. (1991) demonstrated that randomly tested bulls used in artificial insemination in Africa were free of this translocation. Therefore, the rob(1;29) frequencies determined in the studied breeds are not associated with this origin. Accordingly, the breeds analysed in the present study were reproductively isolated until recently, preventing "contamination" through a genetic stream from Continental European breeds. This hypothesis supports the large differences observed among herds of the same breed (Table 3). Reproductive isolation, breeding strategies using untested sires, uncontrolled animal mobility and the influence of official control programmes against rob (1;29) are likely the major causes of this variability.

4. Conclusions

High variability in the prevalence of the rob(1;29) translocation has been detected among Andalusian cattle breeds, herds and geographic locations. The rob(1;29) frequency might have been reduced in some breeds through the effects of genetic drift, human selection, adaptation to the environment and breeding strategies. Accordingly, control programmes performed through breeders associations are currently in development for BC, BN, PA and CA breeds and includes testing all males to avoid the use of positive animals as breeders. Nevertheless, this translocation remains present in other breeds, primarily reflecting introgression from other breeds. According to our results, these high and variable frequencies might be attributed to differences in the reproductive isolation of farms and geographical areas, genetic drift and/or human selection strategies. Therefore, rob(1;29) analysis should be performed, considering the reproductive management of each farm. However, the rob (1;29) frequency was not influenced through the geographical location of the different herds of the same breed. Further studies involving the mobility of sires among herds are necessary to determine the origin of the rob(1;29) frequencies observed in the analysed endangered Andalusian breeds.

Conflict of interest statement

None.

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