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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<sup>™</sup>. 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  $\mu$ l 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 Jannuzzi (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 <sup>1</sup>		Herds <sup>2</sup>	Sampled animals	Sampled herds
	Females	Males		ammais	iicius
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 <sup>3</sup>	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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