

Ancestry modelling of the Venezuelan JR-type composite breed

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Summary

The JR-type breed was created through the hybridization of Red Holstein (RH), Red Brahman (RB), and Criollo Limonero (CL) in an effort to obtain a composite breed that is capable of performing well under the conditions of the Venezuelan dry tropical forest. To date, the breed is in its third generation and demonstrating a promising improved yield and reproductive performance as compared to most native crossbred and purebred cattle (unpublished data). This study is the first to genetically characterize the JR-type breed using genome-wide ADMIXTURE, principal component analysis (PCA), and fixation index (F_{ST}) to define the population structure and genetic ancestry of the JR-type cattle based on the Illumina BovineHD 777K Beadchip. The PCA, ADMIXTURE and F_{ST} results showed that JR is a genetically unique breed distinguishable from *Bos indicus* and *Bos taurus* ancestral breeds. Further investigation is warranted to explain their unique genetic signature.

Keywords: admixture, criollo cattle, JR-type, PCA, fst

Introduction

The JR-type is a composite dairy cattle breed developed to produce well under the Venezuelan dry tropical environments. It was obtained by hybridizing purebred and crossbred Red Holstein (*Bos taurus*), Red Brahman (*Bos indicus*), and the native Criollo Limonero (tropically adapted *Bos taurus*). Red Holstein was included due to its recognized genetic potential for improved dairy traits (McDowell *et al.*, 1996). However, due to the limited ability of Holstein to perform well in the tropics (McDowell, 1985; Zambrano *et al.*, 2006), other tropically adapted breeds were also included. Brahman were introduced to improve adaptability and for the documented increase of heterosis in hardiness, growth, maternal effects, and reproductive traits (Cartwright *et al.*, 1964; Roberson *et al.*, 1986; Turner, 1980). Criollo Limonero, a Venezuelan native cattle that arose by natural selection from cattle brought to the New World by the Spaniards (Rouse, 1977; Buenaventura & Cortés Dueñas, 2007), were included to improve adaptation and fertility (Landaeta-Hernandez *et al.*, 2011; Zambrano *et al.*, 2006).

An understanding of the genetic diversity and population structure is useful for designing effective strategies for improving, managing and conserving farm animal genetic resources

(Edea *et al.*, 2015; Groeneveld *et al.*, 2010; Reist-Marti *et al.*, 2004). This is especially important in the development of emerging composite breeds such as the JR-type. To date, no scientific study has been conducted on the genetic characterization of the JR-type. Therefore, the objective of this study was to investigate admixture and ancestry within the JR-type breed and their relationship to other Criollo breeds, such as the Criollo Limonero, to improve mating selection strategies for the preservation and development of the JR-type.

Material and methods

Sample collection

Our study utilized 23 JR-type (JR) cattle from the original dairy farm where they were developed. Hair bulbs pulled from the tail tips of cattle were collected for DNA extraction. To assess the known ancestry of the JR cattle, genotypic data was available for 15 US Brahman (BR), 15 US Holstein (HO) including two Red Holstein (RH) from the HapMap project, and 15 Venezuelan Criollo Limonero (CL) from the preservation farm Estación Local Carrasquero in Zulia.

Genotyping & DNA extraction

Genomic DNA was isolated from hair root bulbs using the Qiagen Gentra® PureGene® Blood Kit following modifications optimized for hair root bulbs. All cattle were genotyped on the Illumina BovineHD 777K Beadchip (Illumina Inc., San Diego, CA, USA) at GeneSeek Inc. (Lincoln, NE, USA). GeneSeek Inc. also performed the raw data allele calls. Quality control measures using Golden Helix SVS v8 software (www.goldenhelix.com) assessed over 777 thousand single-nucleotide polymorphisms (SNPs), removing SNPs with genotyping call rates less than 95% and minor allele frequencies less than 5%. All individuals with less than 90% genotyping call rate across the remaining 665,803 SNPs were removed ($n = 2$). Three bulls identified as sires to the other JR genotyped samples were excluded from further analysis due to high degree of relatedness.

Genetic diversity and ancestry analysis

We ran unsupervised clustering algorithms to examine the population structure both within breed and based on known or anecdotal ancestry of the JR cattle. Model-based unsupervised clustering and supervised clustering using maximum likelihood population estimations was performed using the software ADMIXTURE (Alexander&Lange, 2011; Alexander *et al.*, 2009). The optimal K-value within ADMIXTURE was identified with the inferred number of populations producing the lowest cross-validation error, which is estimated during the clustering analysis.

ADMIXTURE analysis in the JR was completed in two separate groups, first to examine the influence of families and popular sires within the breed, and then with a subsequent analysis incorporating referenced breeds. Within breed, unsupervised ADMIXTURE analyses were run genome-wide utilizing all JR that passed quality control filtering ($N = 18$) with results compared to pedigree information. Structured and unstructured ancestry analyses were run genome-wide utilizing the same JR and 15 of each referenced ancestral breed including US HO, US BR, and

Venezuelan CL. Of the 15 HO, two individuals were identified as RH and included as they were more similar to the RH used in the development of the JR.

To further investigate genetic population structure, principal component analysis (PCA) was conducted. To assess the genetic diversity of the sample populations, inbreeding coefficient, f , was calculated. To analyze the genetic ancestry of the JR cattle, the genetic divergence of the JR cattle was compared with other breeds in their anecdotal ancestry using the fixation index (F_{ST}). F_{ST} estimates were estimated using all markers from autosomal chromosomes. The PCA, f , and F_{ST} analyses used the same JR anecdotal ancestry populations as described in the ADMIXTURE analyses, and were all computed using the GoldenHelix SVS software.

Results

In order to determine the current genetic composition of JR after the third generation of inter-se breeding, we compared the JR's genome to genomes of its three founding breeds HO, BR, and CL. Strikingly, the JR defined their unique breed group with little admixture from or clustering with the other breeds (Figure 1). The optimal K-value in the unsupervised ADMIXTURE analysis was $K = 4$, where JR and all three reference breeds utilized formed distinct breed clusters (Figure 1). An average breed composition of JR cattle consisted of 99.41% unique JR genetic signature, 0.27% BR, 0.18% CL, and 0.14% HO (includes RH). Even at lower K values, JR distinctly clustered from the other breeds, as supported by the PCA (Figure 2a). PC1 (eigenvalue [EV] = 8.66) clearly separates the JR before separating *Bos indicus* and *Bos taurus* breeds. PC2 (EV = 7.25) markedly separates the BR from the *Bos taurus* breeds, and to a lesser degree separates the CL from the HO. Pairwise F_{ST} estimates showed JR has the least genetic divergence from RH, followed by the CL, and the greatest differentiation with BR (Figure 2b). The F_{ST} results are supported by the supervised ADMIXTURE analysis which was conducted in an attempt to decipher ancestral breed percentages within the JR. However, at $K = 4$ with only BR, HO (including RH), and CL populations defined, JR formed a cluster with CL, and at $K = 5$ with RH defined separately from HO, JR formed a cluster with RH. Supervised clustering was unable to distinguish foundational breed composition of the JR cattle. Within breed unsupervised ADMIXTURE was also unable to distinguish any family relationships. The mean inbreeding coefficient varied across breeds with the highest estimate in BR ($f = 0.372$, range 0.496 to 0.219), followed by HO – including RH – ($f = 0.219$, range 0.266 to 0.175), CL ($f = 0.198$, range 0.327 to 0.144), and JR ($f = 0.107$, range 0.145 to 0.040).

Discussion

The JR composite breed was created by mating RH bulls to half-blood Red Brahman x Limonero cows, and half-blood Brahman x Limonero bulls to graded Red Holstein cows to obtain a cross that is 50% Red Holstein, 25% Brahman, and 25% Criollo Limonero. Each subsequent generation utilized inter-se matings to maintain the foundation breeds' proportions. ADMIXTURE analysis allowed us to investigate whether we could detect these foundation breeds' genetic signatures within the third generation of inter-se JR animals. ADMIXTURE and PCA analysis identified JR as a genetically unique breed, distinguishing the JR before genetic segregation of the *Bos indicus* breeds from *Bos taurus* breeds. Separation of the JR from the other breeds is further supported by the F_{ST} values and inbreeding coefficient, with a moderate genetic divergence across all four breeds compared to the JR (average $F_{ST} = 0.35$) and a low

detectable amount of inbreeding ($f = 0.107$). These results were surprising, given the small sample size, single herd origin, and relatively new formation of the breed. Likely the three breed hybridization and small sample size from a single herd provided the JR with a strong common genetic signature. The incorporation of additional unrelated JR individuals from different farms would reduce this bias, but currently there are few unrelated JR individuals available. Interestingly, JR displayed only 0.15% HO/RH genetic ancestry (unsupervised) despite the heavy incorporation of the HO into the development of the JR. The underrepresentation of RH in our analysis could be biasing the true HO composition of the JR, and could change with the inclusion of additional RH. In all, these results suggest that the hybridization of the three foundational breeds including both *Bos indicus* and *Bos taurus*, in the development of the JR-type cattle has generated a very distinct genetic breed. Further investigation, incorporating more breeds and more JR animals is warranted to explain the unique genetic signature.

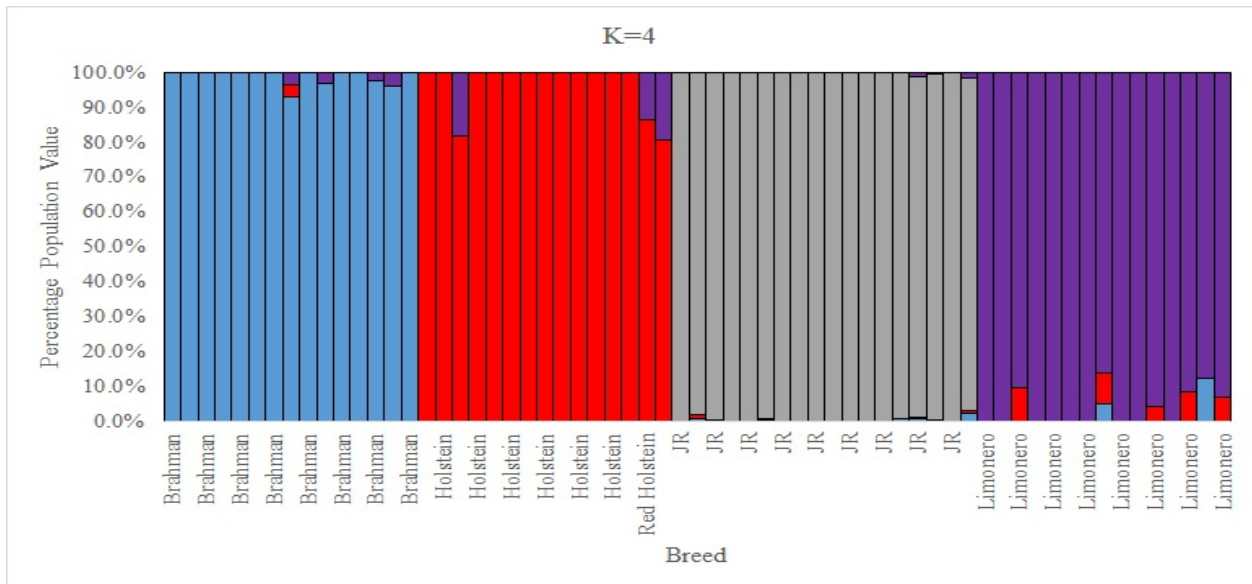


Figure 1. Genetic ancestry of composite JR-type cattle assessed by ADMIXTURE. Unsupervised ADMIXTURE at $K = 4$ where bar colors reflect breed identification; blue is Brahman, red is Holstein, grey is JR-type, and purple is Criollo Limonero.

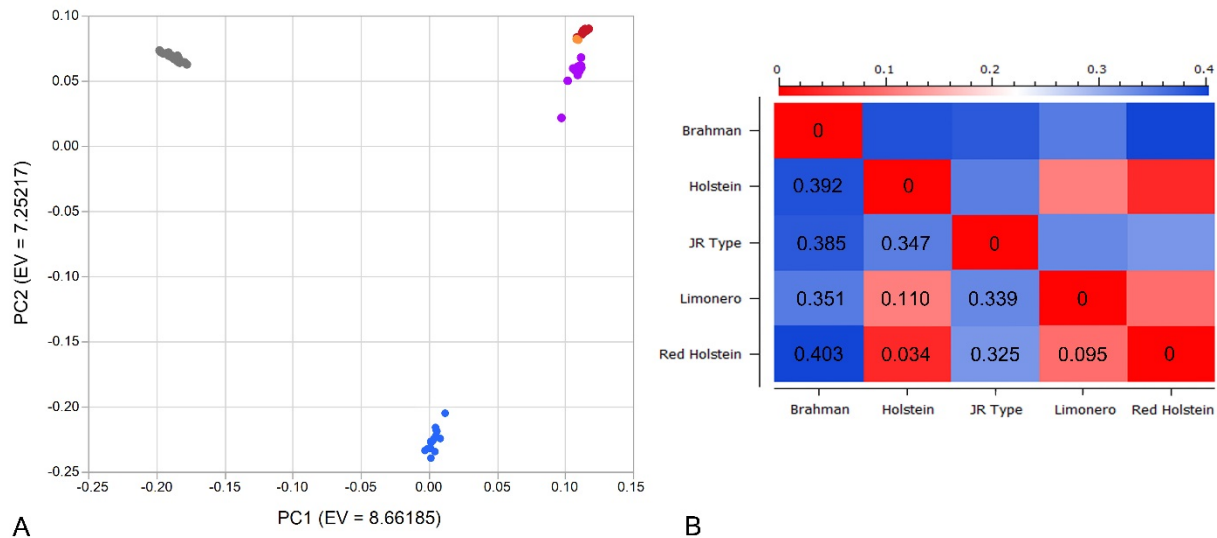


Figure 2. Genetic diversity of composite JR-type cattle assessed by a) principal component analysis and b) F_{ST} analysis. Principal component analysis depicts individuals colored by breed; Brahman are blue, Holstein are red, Red Holstein are orange, Limonero are purple, and JR-type are grey. In the F_{ST} matrix, pairwise F_{ST} estimates are represented on a sliding scale of dark red to dark blue. Red squares represent a value of 0, white squares a value of 0.22, and dark blue squares a value greater than 0.4.

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