

D-LOOP MITOCHONDRIAL GENETIC ANALYSIS IN ABERDEEN ANGUS OLD TYPE FROM ARGENTINA

ANÁLISIS GENÉTICO DEL BUCLE D EN ABERDEEN ANGUS TIPO ANTIGUO DE LA ARGENTINA

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ABSTRACT

The massive use of reproductive breeding technologies (mainly Artificial Insemination and Embryo Transfer) resulted in an important decrease in the diversity in many breeds, and some genetic lines have been lost. Within the Angus breed, the “New Type” gain importance since 1970, leaving the “Old Type” to a reduced number of herds in the whole world. The objective of this work was to determine the genetic diversity in an “Old Type” herd in Argentina. DNA samples were analyzed for sequence variation in the hypervariable region of the mitochondrial DNA (D-loop). Sequence comparison and phylogenetic analyses revealed that haplotypes fell into European haplogroup (T3) in general, and in particular had high similarity with British haplotypes. Six distinct haplotypes were obtained, that differed from zero to four DNA bases with respect to the nodal sequence T3, with a nucleotide diversity of 0.442. Matrilineages genetic analysis suggested a Scottish origin of this herd. These results suggest that this herd could be a genetic reservoir of the Old Scottish Aberdeen Angus cattle.

Key words: mtDNA, *Bos Taurus*, Angus, matrilineages origin, genetic diversity

RESUMEN

La diversidad genética de numerosas razas se ha visto reducida por el uso masivo de las tecnologías reproductivas (principalmente la Inseminación Artificial y la Transferencia Embrionaria), incluso a llevado a la desaparición de algunas líneas genéticas. En la raza Angus, desde 1970 el tipo *New Type* se popularizó y el luego llamado *Old Type* quedó reducido a pocos rodeos en el mundo. El objetivo de este trabajo fue determinar la diversidad genética de un rodeo de Argentina considerado *Old Type*. La secuencia de la región Hipervariable del ADN mitocondrial (D-loop) fue analizada en muestras de ADN obtenidas de estos animales. Los resultados mostraron que todas las secuencias fueron incluidas dentro del haplogrupo europeo (T3) y presentaban una alta homología con los haplotipos reportados en animales británicos. En este rodeo se encontraron seis haplotipos distintos que presentaban cero a cuatro bases de ADN de diferencia con el consenso nodal (T3) y una diversidad nucleotídica estimada en 0,442. El análisis de los linajes maternos sugiere un origen Escocés de este rodeo en su genealogía materna, consistente con los registros genealógicos. Estos resultados son una evidencia para considerar a este rodeo como un reservorio del ganado Aberdeen Angus Escocés antiguo.

Palabras clave: ADN mitocondrial, *Bos Taurus*, Angus, linaje materno, diversidad genética

Fecha de recepción: 09/12/2014
Fecha de aceptación de versión final: 23/06/2015

INTRODUCTION

The Aberdeen Angus breed was developed at the beginning of the 19th century in Northeast Scotland. Due to its remarkable productive attributes, the Aberdeen Angus breed spread quickly in Great Britain, Ireland, United States, Canada, Australia and New Zealand. This type of polled hardy cattle, black or red coated, was characterized for its precocity, excellent fertility and maternal aptitude, high growth rate and excellent meat yield with insurmountable meat quality (Aberdeen Angus Cattle Society, 2014). In those years, the breed stood out because the animals were phenotypically short in height, short from head to tail, deep from back to ribs, compact and with large fat deposits. This animal type will be later called “Old Type” or “Traditional Type”, standing this for the original type that was brought from Scotland (Rare Breeds Conservation Society of New Zealand, 2014). Opposed to this, in the United States and Canada a “New Type” was developed in the decades of 1960 and 1970. The breed was selected attending to the needs of the feedlot industry in the USA, this “New Type” includes animals that are of a larger size, more muscular, more cylindrical in shape, with a higher birth weight and leaner carcasses in comparison with the “Old Type”. The “New Type” was spread all over the world, and has influenced almost every Angus herd, leaving Old Type animals in a reduced number of herds (Asociación Argentina de Angus, 2014).

In 1879, the breed was introduced in Argentina when Mr. Carlos Guerrero imported the first pedigree bull (named Virtuoso) and two heifers (Asociación Argentina de Angus, 2014). Initially, the Angus breed had no further dissemination but, around 1950, the number of Angus breeders and animals increased in all temperate regions, being actually the most important breed in Argentina (60% of all animals in the country). It absorbed Shorthorn; Creole and other breeds due to its original characteristics that made it suitable for pastures, still the most important cattle production system in Argentina. Since 1970, as in the rest of the world, the “New Type” gained importance in Argentine despite the fact that this biotype was selected for a different production system. Furthermore, with the massive use of reproductive breeding technologies (Artificial Insemination and Embryo Transfer), the genetic diversity of many breeds has significantly decrease (Taberlet *et al.*, 2008) and some genetic lines have been lost. In this context, few Argentinean breeders closed their herds

(with great prevalence of Scottish blood) to the influence of the American “New Type”, preserving intact all the characteristics of the “Old Type”, and keeping them as true genetic reservoirs. Such reservoirs are currently used to restore the adaptability to pasture systems, lost due to the “New Type” influence. Even though it is quite difficult to trace them back to the original imports to Argentina, these herds have maintained the characteristics of the original Angus because no blood from any other herd has been introduced into them since the decade of 1960.

Mitochondrial DNA sequence polymorphisms have been used to examine genetic relationship within breeds (Giles *et al.*, 1980; Hauswirth and Laipis, 1982; Hill *et al.*, 2002), among breeds (Bradley *et al.*, 1996; Beja-Pereira *et al.*, 2006; Lirón *et al.*, 2006), and between domestic and wild populations (Oakenfull and Ryder, 1998). In order to establish a likely Scottish origin of some Old Type animals kept in Argentina, the D-loop mtDNA was analyzed and compared with previously reported Angus sequences from various countries.

MATERIALS AND METHODS

Animals and DNA extraction

Blood samples were collected from twenty Angus animals raised in a herd belonging to “Cabaña El Hinojo de Bru”, located in Coronel Suárez County (Buenos Aires Province, Argentina, 37° 26' 46" S 61° 53' 21" W). This herd was founded around 1960 with a pedigree of Angus Dams (Scottish origin) and an imported Scottish bull. Thereafter, the herd was kept close reproductively until the present, except for some Scottish semen or bulls used to reduce consanguinity. DNA was extracted from lymphocytes using DNAzol® method (Invitrogen, Carlsbad, CA, USA).

D-loop sequencing and sequence analysis

D-loop of mtDNA hypervariable region I (nucleotide positions 16,023 -16,262) was amplified using primers L15960 (5'-GGTAATGTACATAACATTAATG-3') and H16334 (5'-CGAGATGTCTTATTTAAGAGG-3') as suggested by Troy *et al.* (2001). All sequences were obtained for both DNA strands using the DYEnamic ET Dye Terminator Kit (GE Healthcare, USA) in a 10µl volume, containing approximately 40ng of purified DNA and 5pmol of primer. Sequencing was performed using a MegaBACE 1000 automated sequencer (GE Healthcare).

Raw sequences were edited and aligned using MegaBACE Sequence Analyzer (GE Healthcare). The resulting sequences of the D-loop were then aligned with other 67 published sequences from Argentina, Britain, Canada, China, Japan and USA (Loftus *et al.*, 1994; Bradley *et al.*, 1996; Troy *et al.*, 2001; Komatsu *et al.*, 2004; Shahid *et al.*, 2004; Qu *et al.*, 2006; Meng *et al.*, 2006; Ginja *et al.*, 2010), using CLUSTAL-X multiple alignment software (Thompson *et al.*, 1997).

Variations in the D-loop region were defined by direct comparison with the reference bovine mtDNA sequence (Accession No.V00654) published by Anderson *et al.* (1982). This haplotype corresponds to the predominant European T3 mtDNA haplotype as defined by Troy *et al.* (2001). A Median Joining Network was constructed using the methodology described in Bandelt *et al.* (1995) using Network 4.1.1.2 (Flexus-Engineering, 2014). Mean number of pairwise differences and nucleotide diversity were calculated using the algorithms implemented into Arlequin 3.5 analysis package (Excoffier and Lischer, 2010).

RESULTS

Sequence analysis of 240bp revealed seven polymorphic sites included, one transversion and six transitions (Table 1). Among them, one was a new polymorphic site on 16,257 (T/G) and three were only shared with the British reported animals (16,062 A/G, 16,200 G/A, 16,208 T/C), among all Angus sequences reported up to date. Nucleotide diversity was 0.004, and pairwise nucleotide difference was 1.026.

Six haplotypes were found that differed from zero to four bases respect to the nodal sequence T3 (Table 1). Nucleotide sequence comparisons and phylogenetic analysis assigned haplotypes to European group (T3) in general, and in particular had high similarity with British haplotypes. Three haplotypes were original and appeared in low frequency; among the rest, one was the nodal consensus (T3) and the other two had already been observed in British populations. None of these animals had showed haplotypes assigned to the African (T1), Near East (T2), or Cebuine (I) haplogroups.

Comparison of 67 published Angus sequences for D-loop and the Argentinean “Old Type” sequences are presented in Table 2. Reported sequences are grouped by taking into account only the region (240bp) studied here.

The Median Joining Network is also presented using that information (Figure 1).

DISCUSSION

The fact that this population remained completely close regarding mtDNA –no females were incorporated to the herd for almost 60 years– is revealed by the appearance of a low number of haplotypes. Probably the effective founder population was small and some haplotypes could have been lost. However, three of the haplotypes had two to four mutations when compared with T3, and appeared in a considerable frequency. This explained that both, nucleotide diversity (0.442) and mean number of pairwise differences (3.091), were higher than those calculated for Britain Angus (nucleotide diversity= 0.218, mean number of pairwise differences= 2.835), all Angus sequences reported (nucleotide diversity= 0.122, mean number of pairwise differences= 3.047) and European *Bos taurus* (nucleotide diversity= 0.022, mean number of pairwise differences= 2.529).

Most of the polymorphic sites and haplotypes found were original or shared with the British population. In particular, one out of seven polymorphic sites was original, and the rest also appeared in British Angus, and three of them were exclusive of these two populations. The haplotypes showed a similar pattern, the three new haplotypes had low frequencies; the other three –the most frequent– were the consensus (T3) and two other haplotypes had also been described by Troy *et al.* (2001) for the British populations. Again, one of the haplotypes was only shared by the “Old Type” and the British Angus. These observations support the hypothesis of a British origin of the population under study, at least from the maternal side, and the lack of American genetic influence in the herd.

The sequences were also compared with Creole reported sequences and haplogroups T1, T2 and I. None of the new original sequences were assigned to those haplogroups or this breed. Based on this observation, we discard the possibility that the animals in this herd have Creole ancestry in the maternal lineage. This is important because many actual older Angus herds were originally obtained and expanded, by absorbing other breeds with Angus bulls. Furthermore, the sequences were compared with reported sequences of Shorthorns (Ginja *et al.*, 2010), and neither of the haplotypes were found in those

Table 1. D-loop polymorphic sites founded in Argentinean Angus Old Type population

* The sequence position from BOVMT GenBank sequence is given above each column (Anderson et al., 1982).

Sequence	POLYMORPHIC SITES *							Number of animals	Reference
	16062	16109	16200	16208	16247	16255	16257		
T3	A	T	G	T	C	T	T		Consensus
AA1	-	-	-	-	-	-	G	1	This paper
AA2	-	C	-	-	-	-	-	2	This paper
AA3	G	-	A	C	-	-	C	3	This paper
AA4	G	-	A	C	T	-	-	4	Troy <i>et al.</i> 2001
AA5	-	-	-	-	-	-	-	5	Anderson <i>et al.</i> 1982
AA6	-	C	-	-	-	C	-	5	Troy <i>et al.</i> 2001

Table 2. Comparison of 67 published Angus sequences for D-loop with the Argentinean “Old Type” sequences

Sequence Pubmed ID	Population	Differences with T3	POLYMORPHIC SITES *																																								
			16039	16042	16050	16053	16056	16057	16058	16062	16067	16068	16083	16094	16095	16096	16109	16112	16113	16119	16121	16122	16127	16135	16138	16139	16141	16143	16147	16167	16200	16208	16219	16229	16231	16232	16247	16250	16255	16257			
Consensus (T3)	Europe		A	T	C	T	A	G	C	A	A	T	A	C	T	G	T	T	T	G	T	C	T	T	C	T	A	T	C	G	T	C	A	C	C	C	A	T	T				
EF990788-EF990789-EF990790-GU338237-GU338238	“Old Type”	0																																									
EF990787	“Old Type”	1																										G															
GU338242-GU338243	“Old Type”	1																				C																					
EF990791-EF990792-GU338239-GU338240-GU338241	“Old Type”	2																																								C	
EF660565-EF660566-EF660567-EF990786	“Old Type”	4																																								G	
GU338244-GU338245-GU338246	“Old Type”	4																																									G
AF336464-AF336465-AF336472-L27712-U51816	Britain	0																																									
AF336471	Britain	1																																									
AF336466-L27713	Britain	2																																									
AF336470	Britain	2																																									
AF336473	Britain	2																																									
AF336467-AF336468-U51819	Britain	3																																									
AF336469	Britain	4																																									
FJ815941-FJ815943-FJ815945-FJ815946-FJ815947-FJ815950	Argentina	0																																									
FJ815940	Argentina	1																																									
FJ815948	Argentina	1																																									
FJ815949	Argentina	1																																									
FJ815951	Argentina	2																																									
FJ815944	Argentina	5																																									
FJ815942	Argentina	6																																									
AFD34445	Canada	3																																									
DQ520587-DQ520588-DQ660307	China	0																																									
DQ520590	China	4																																									
DQ520591	China	4																																									
DQ520589	China	5																																									
AB079347-AB079348	Japan	0																																									
AB079345	Japan	2																																									
AB079346	Japan	2																																									
AB079344	Japan	3																																									
AY676863-AY676864-AY676871-FJ815927-FJ815928-FJ815931-FJ815933-FJ815934-FJ815937-FJ815938-FJ815939	USA	0																																									
AY676859	USA	1																																									
AY676865-FJ815929	USA	1																																									
AY676866	USA	1																																									
AY676872	USA	1																																									
FJ815930	USA	1																																									
FJ815936	USA	1																																									
AY676857	USA	2																																									
AY676852	USA	2																																									
AY676867-AY676870-FJ815926-FJ815932	USA	2																																									
AY676873	USA	2																																									
FJ815925	USA	2																																									
FJ815935	USA	2																																									
AY676868-AY676869	USA	3																																									

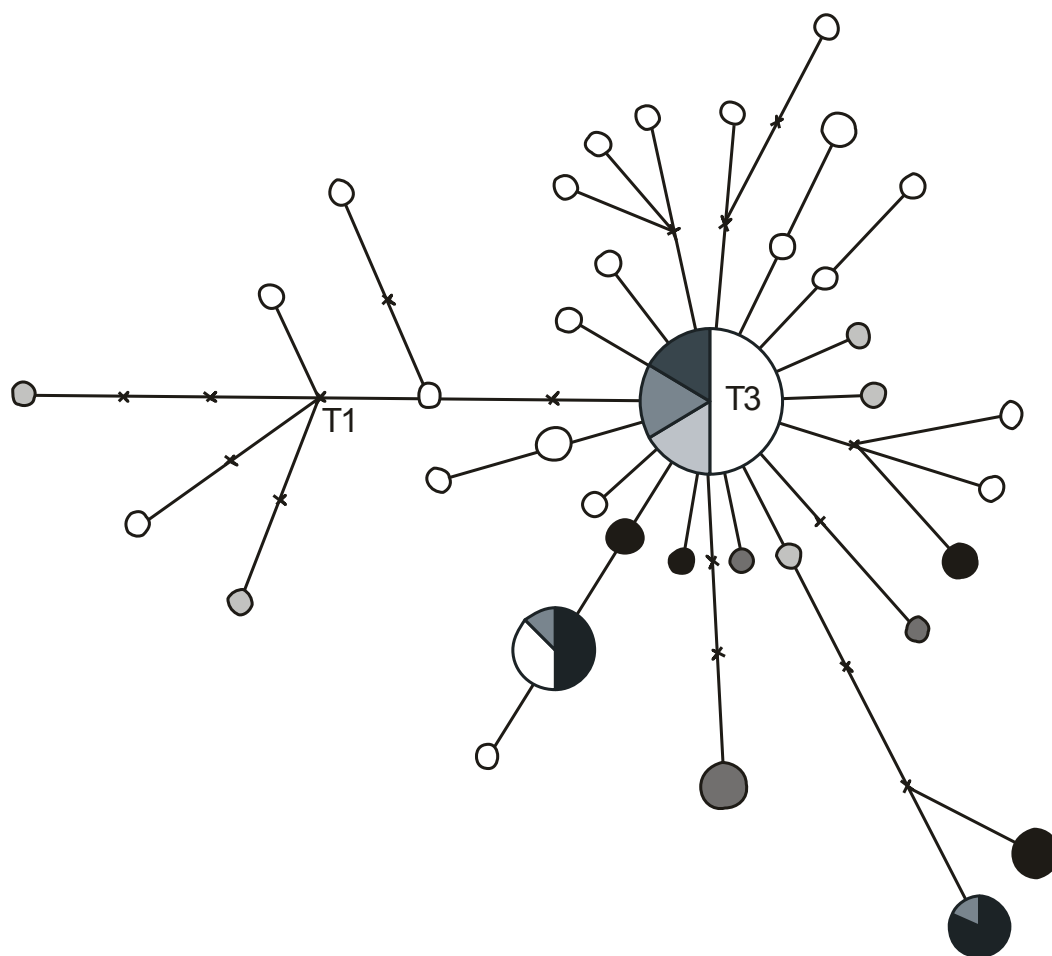


Figure 1. A reduced Median Joining Network (Bandelt et al., 1995) featuring mtDNA sequences from 87 Angus. Circles represent sequences haplotypes: the area is proportional to the frequencies of the haplotype. Black represents “Old Type” Angus sequences, dark gray represents British Angus, light gray represents Argentine Angus, and white represents remaining Angus sequences. The consensus nodes T1 (African) and T3 (European) are indicated.

sequences, excluding also a male mediated absorption from the Shorthorn breed, which was the most numerous breed in the first half of the XXth century in Argentina.

Previous SNP genetic studies of this herd, in autosomic genes involved in fat metabolism and growth (Villegas-Castagnasso *et al.*, 2006), showed important frequency differences between this population and a sample of Argentinean Angus obtained from several herds, most of them classified as “New Type”. In addition, the phenotype of this herd remains almost unchanged from

the Traditional Aberdeen Angus phenotype described by Rare Breeds Conservation Society of New Zealand (2014). The phenotypic and genetic data analysed suggest that this herd could be considered as Angus “Old Type”, and could be a reservoir of the Old Scottish Aberdeen Angus. Even though there was an absence of “New Type” influence with the markers employed, further studies with other markers should be carried out to be able to consider that this herd is of “pure Old Type” descendent.

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ACKNOWLEDGMENTS

The research for this paper was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC) and Universidad Nacional de La Plata (UNLP). The authors acknowledge Cabaña El Hinojo de Bru (Coronel Suárez, Provincia de Buenos Aires) for facilitating the animals for the research.