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RECENT ANCESTRY FOR THE 821DEL11 DOUBLE MUSCLING ALLELE

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SUMMARY

Double muscling is an inherited condition in cattle that was first documented more than 200 years ago. Allelic heterogeneity has been found for the double muscling condition with each allele confined to the *myostatin* (*MSTN*) locus on BTA2. Genetic variability proximal to the 821del11 mutation in exon 3 of *MSTN*, was examined to determine the extent of haplotype homozygosity, and to estimate the time to the most recent common ancestor. Long homozygous segments (2.2 Mb) were observed for most 821del11 haplotypes, compared to short segments (130 kb) for cattle wildtype at the double muscling sites. These long homozygous segments are evidence for a recent common ancestor of the 821del11 allele, which existed between 230-380 years ago.

INTRODUCTION

Double muscling is an inherited condition characterised by large increases in muscle mass (Arthur 1995). The condition was first documented in cattle more than 200 years ago (Culley 1807), and the extensive research following this first report, has led to the discovery of 6 mutations responsible for double muscling in cattle (Grobet *et al.* 1998). These loss-of-function mutations are confined to the *myostatin* gene (*MSTN*), which encodes a potent negative regulator of skeletal muscle mass (McPherron *et al.* 1997).

Most of these mutations are confined to 1 or a few cattle breeds (Dunner *et al.* 2003). In contrast, the 821del11 mutation has been reported with moderate to high frequency in several cattle breeds (Dunner *et al.* 1997; Dunner *et al.* 2003; Gill *et al.* 2008; O'Rourke *et al.* 2009). This mutation is an 11 bp deletion at nucleotide 821 in exon 3 of the *MSTN* coding sequence; a frameshift mutation, which prematurely truncates the *MSTN* transcript. It is unlikely that this 11 bp deletion has arisen *de novo* in each cattle breed, but has probably disseminated from a common ancestor.

In a recent Australian study examining genetic variation at the *MSTN* locus (O'Rourke *et al.* 2007), it was found that the 821del11 mutation was confined to a single haplotype in an Angus cohort, which was also fixed in a Belgian Blue population. This lack of genetic variability associated with the 821del11 double muscling mutation in both breeds, provides further evidence for a common ancestor. It also implies that the common ancestor was recent. However, the region examined by O'Rourke *et al*. (2007) spanned less than 7 kb on *Bos taurus* autosome 2 (BTA2), which is too short to estimate the time since the most recent common ancestor (MRCA).

In this study, the extent of the haplotype homozygosity (HH) associated with the 821del11 double muscling mutation was determined. Cattle of different breeds were selected, and molecular distance representing the HH was used to estimate the time since the MRCA.

MATERIALS AND METHODS

DNA samples from 20 cattle were used in this study (Table 1). Seventeen cattle, representing a range of breeds, had at least 1 copy of the 821del11 double muscling allele. Three 3 Angus cattle were included as controls, which were homozygous wildtype at the known double muscling

Table 1. Breed and genotypes for cattle harbouring the 821del11

Breed	*N*[A]	Double muscling genotype[B]
Angus	3	*mh*/*mh*
Belgian Blue	3	*mh*/*mh*
Santa Gertrudis	2	*mh*/*mh*
Braford	2	*mh*/*mh*
Square Meater	1	*mh*/*mh*
Santa Gertrudis	5	*mh*/+
Murray Grey	1	*mh*/+
Angus	3	+/+

[A] *N*, number of cattle
[B] *mh*, muscular hypertrophy/double muscling allele; +, wild-type allele at the double muscling site

sites. Genetic relationships were not evident between the cattle with the 821del11 allele or the control Angus.

Genotyping was performed by DNA sequencing. Eight regions upstream and downstream of *MSTN* were targeted (Table 2). PCR primers were designed to flank a region containing at least 1 SNP annotated in either the BCM Bovine Genome Assembly SNPs or the BCM Interbreed SNPs database, to increase the likelihood of identifying changes in the HH. These target regions were amplified by PCR, and the PCR products were purified before DNA sequencing. DNA sequence data was analysed using Sequencher 4.10.1 (Gene, Codes, USA).

Haplotype phase was inferred from the genotypic data using PHASE v2.1.1 (Stephens *et al*. 2001). The haplotype phase was deduced only for the segment bounded by changes in HH between most animals in the double muscling group. Continued breakdown of the homozygosity was informative, but the accuracy of the inferred haplotype beyond this region could not be confirmed without related individuals. The number of generations to the most recent common ancestor was calculated using $g = \frac{1}{x}$, where g is the number of generations and x is the chromosomal distance (in Morgans) of the observed HH; assuming 1cM = 1 Mb. Minimum and maximum generations were calculated if the HH were different within the double muscling group. Generations were converted to years assuming a generation time of 5 years.

RESULTS AND DISCUSSION

MSTN is located between nucleotides 6532638-6539265 on BTA2. The 821del11 mutation in exon 3 of *MSTN*, spans nucleotides BTA2:6537462-6537472. In this study, genotypes were recorded at 34 SNP in the homozygous segment for the 821del11 cohort, which spanned from region 5 to region 14 (Table 2). Seven haplotypes harbouring the 821del11 allele were inferred.

Upstream of *MSTN* on BTA2, the homozygosity for each haplotype ceased in region 5 at the BTA2:5800179(C>G) polymorphism. The homozygosity between 5 of the 821del11 haplotypes extended to the BTA2:7966491(A>T) polymorphism in region 14. For these haplotypes the total molecular distance of the homozygous segment was 2.2 Mb. A shorter homozygous segment was determined for the other two 821del11 haplotypes. The shortest haplotype ended at the BTA2:71110674 (G>A) polymorphism in region 10, spanning 1.3 Mb. The other 821del11 haplotype, ended at the BTA2: 7309658(A>C) in region 12 and spanned a total of 1.5 Mb.

In contrast, the HH for the wildtype controls (+/+) ended at the first informative polymorphism either side of *MSTN*. The molecular distance observed for the homozygous segment was 130 kb. This segment may be shorter, but requires examination of informative polymorphisms closer to

Table 2. BTA2 regions examined to determine the extent of homozygosity

Region	Amplicon size (bp)	Genomic location (BTA2)[A]	Incorporated SNP[B]
1	621	4027100..4027720	BTB-01076675 - BTB-01076676
2	697	4527168..4527864	BTB-00077984 - BTB-00077985
3	698	5015262..5015959	BTB-00079061 - BTB-00079062
4	668	5596834..5597501	BTB-00077602 - BTB-00077603
5	603	5799653..5800255	BTA-47420 - BTA-47424
6	686	6050650..6051335	BTB-00078524 - BTB-00078525
7	678	6266245..6266923	BTB-00079578
8	607	6480493..6481099	BTB-01391593 - BTB-01391594
MSTN	-	6532638..6539265	-
9	603	6614801..6615403	BTB-01391592; BTA87786 - BTA87787
10	670	6834655..6835324	BTB-01923604 - BTB-01923605
11	581	7110408..7110968	BTB-01046029
12	653	7309408..7310038	BTB-01843518 - BTB-01843519
13	684	7469851..7470515	BTB-00078542 - BTB-00078543
14	580	7966409..7966966	BTB-00078703 - BTB-00078704
15	602	8553093..8553675	BTB-01111224 - BTB-01111225
16	698	9022298..9022973	BTB-00079203 - BTB-00079204

[A] All regions are located on *Bos taurus* autosome 2 (BTA2); nucleotide position on Btau_4.0 is provided
[B] BTB, from the BCM Bovine Genome Assembly database; BTA, from the BCM Interbreed SNPs database

MSTN. However, previous studies have also found a low density of polymorphic markers adjacent to *MSTN*, and have relied on more distant microsatellite markers (Charlier *et al.* 1995; Dunner *et al.* 1997; Wiener *et al.* 2003; Wiener and Gutiérrez-Gil 2009). Wiener and Gutiérrez-Gil (2009) genotyped annotated SNP in closer proximity to *MSTN*, but found most to be monomorphic. The advantage of the DNA sequencing approach employed in this study, was that genotypes were not restricted to the annotated SNP. This approach increased the likelihood of identifying informative polymorphisms, and may be useful to examine other regions closer to *MSTN*.

The accepted approximation was used to convert molecular distance to genetic distance (Moisio *et al*. 1996). For the cohort with the 821del11 allele, the homozygous segment ranged in genetic distance from 1.3-2.2 cM (Table 3). This observed HH for the 821del11 mutation is supported by previous studies. Wiener and Gutiérrez-Gil (2009) found the average conserved segment for the 821del11 allele was 2.3 cM in Belgian Blue cattle, and that this same region was conserved in South Devon cattle. Dunner *et al*. (1997) predicted a 2-3 cM ancestral segment for the Belgian Blue and Asturiana cattle they used to fine map the double muscling locus.

Time to the MRCA can be determined from the chromosome segment that has been inherited without recombination in the descendants (Goddard and Meuwissen 2005). The genetic distance estimates were used to calculate that the MRCA of the 821del11 mutation, occurred between 46-76 generations ago, or between 230-380 years ago (Table 3). This estimate is consistent with the first report of double muscling (Culley 1807).

The definition of time to MRCA by Goddard and Meuwissen (2005) implies that the ancestral segment has been inherited identical by descent (IBD). In this study, the IBD segment harbouring the 821del11 allele is assumed to be equivalent in length to the HH, which could under-estimate the time to the MRCA if the HH extends beyond the ancestral segment. Moreover, the MRCA estimates in this study were calculated using the minimum HH overlap between haplotypes. The minimum overlap that was used, rather than random overlap, is likely to under-estimate the length

Table 3. Estimates of time to the MRCA for the 821del11 double muscling allele

MSTN allele	HH length (Mb)[A]	HH distance (Morgans)[B]	N_g[C] range	Common ancestor (years)
821del11	1.3-2.2	0.01-0.02	46-76	231-382
Wildtype (+/+)	0.13	0.001	-	-

[A] Minimum and maximum haplotype homozygosity
[B] Distance was calculated assuming 1 Mb = 1 cM (Moisio *et al*. 1996)
[C] N_g, Number of generations

of the HH.

It is noteworthy that the MRCA estimates may not correspond to the age of the double muscling mutation. Selection pressure on the double muscling phenotype prior to the MRCA cannot be predicted from our results or from the available literature. However, this study has exploited the genetic variability proximal to *MSTN* to provide an estimate of the MRCA historical account of the 821del11 double muscling mutation. The long homozygous segment associated with this allele provides evidence for a recent common ancestor. Given that this mutation has been reported with moderate to high frequency in several cattle breeds, we can speculate that the MRCA for the 821del11 mutation existed before the diversification of modern cattle breeds.

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