The Value of Haplotyping

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Abstract

The relevant unit of inheritance is not the allele but the ancestral haplotype. These sequences are inherited faithfully over thousands of generations.

We have developed haplospecific markers for a region of Bota Chromosome 19 which influences fat metabolism. Haplotypes were identified by family segregation. Comparing haplotype frequencies between Wagyu, Angus and Simmental reveals breed specific ancestral haplotypes which allow the majority of Wagyu to be distinguished from Angus masquerading as Wagyu.

Breed specific haplotypes are associated with traits characteristic of a breed. These haplotypes can then be used for marker assisted selection to increase favorable characteristics within a breed or to blend desired characteristics from different breeds.

Key words: haplotypes

Introduction

Polymorphic genes are contained within frozen blocks as shown, by example, in the human Major Histocompatibility Complex (MHC) on chromosome 6p21.3.

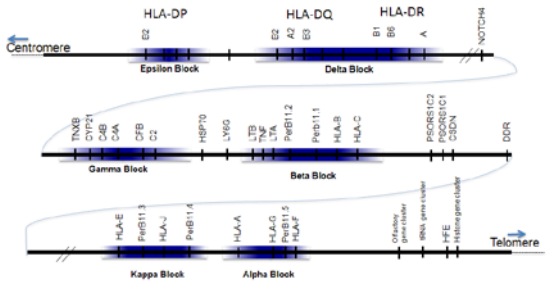

Figure 1. Each ancestral haplotype has its own map. Polymorphic Frozen Blocks are shaded. Not all genes are shown. PerB11 is now designated MIC. Adapted from (Dawkins *et al.*, 1999) and the MHC Map (**http://www.path.cam.ac.uk/~mhc/map/MainMapPage.html**)

Materials and Methods

Choosing the region of interest

This region of chromosome 19 was chosen because it contains several genes relevant to muscle growth and lipid metabolism. (Williamson *et al.*, 2011).

Table 1. Genes of Interest in Region.

Gene	Influences
SREBF1	Cholesterol and FA biosynthesis Regulation of intracellular lipids
STAT5A	Mammary tissue development Milk-fat percentage Fat cell formation and function
GH	Breakdown of lipids
Urotensin 2 receptor	Glucose metabolism Insulin resistance Skeletal muscle fat deposition Fatty acid metabolism
FASN	Fatty acid synthesis

Samples

Cattle sampled for the study were 65 Angus, 316 Simmental and 188 Wagyu. Animals were either full bloods registered with their respective breed societies in Australia, or had confirmed pedigrees tracing back to animals that were registered.

DNA was extracted from the blood, hair, semen or ear tissue using standard salting out methods.

PCR Methods

PCR tests following the method described in (Williamson *et al.*, 2011) were used for five haplotype markers in Chromosome 19: SREB1, NT5M, MRIP, TCAP and GH. An additional marker was developed near SECTM to extend the haplotype 2.2MB past GH towards FASN. Primer pair is shown in Table 2. Qiagen Fast Cycling Kit was used for this PCR with primer concentration of 0.5nM, DNA concentration of 4ng/µl. Cycling parameters were as follows: 95°C for 5 min; 30 cycles of 96°C for 10 s, 63°C for 30 s, and 68°C for 45 s; followed by a final extension at 72°C for 1 min. Polymorphic products were approximately 510 and 643 base pairs.

Table 2. Primers for SECTM marker.

SECTM F	CAGACTGATAAGGGGGGCAAAG
SECTM R	CTATAGAGTGCAGAAGGGGTGTC

Haplotypes

Segregation of these markers into haplotypes was determined from family segregation and/or pedigree or by homozygosity. For determination of breed haplotype frequencies in Figure 2, where two haplotype combinations were possible for an animal, both were included in the count at half value.

Animals for which insufficient information was available to determine haplotypes were excluded from haplotype frequency counts.

Results & Discussion

Haplotype frequency distributions between breeds (see Fig.2)

Haplotypes C1-C8 were found in all three breeds. Haplotypes W1-W8 were found in Wagyu but not in Simmental or Angus. Haplotypes A1 and A2 were found in Angus but not in Wagyu or Simmental. Haplotype S1 was found in Simmental but not in Angus or Wagyu. Haplotypes AS1-AS6 were found in Angus and Simmental but not Wagyu. Haplotypes WS1-WS4 were found in Wagyu and Simmental but not Angus. Haplotype WA1 was found in Wagyu and Angus but not Simmental.

Differences in haplotype frequencies between Wagyu, Simmental and Angus cattle.

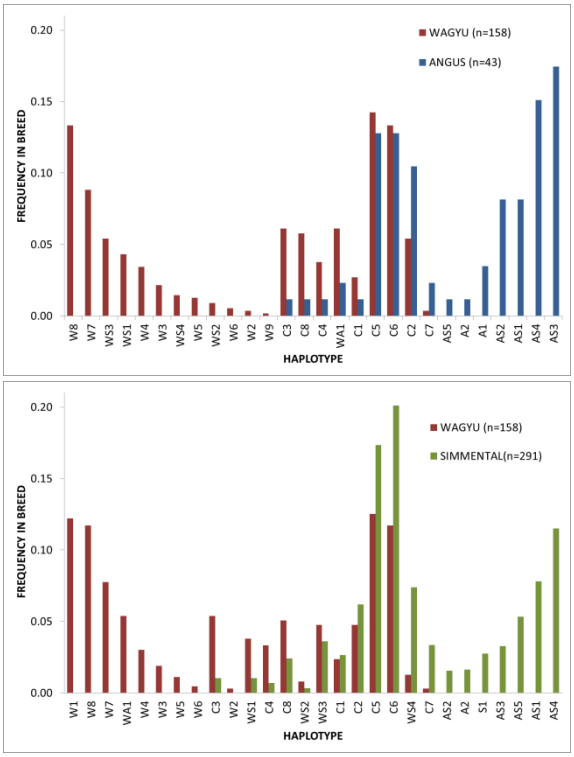

Figure 2. Frequencies of TCAP-SECTM haplotypes on Bota chromosome 19 between MRIP and FASN in different breeds.
Haplotype names reflect the occurrence in the breeds (W=Wagyu; S=Simmental; A=Angus; C = present in all breeds; n=number of cattle).

Testing for breed

The region was broken into two adjacent haplotype block. SREB1, NT5M, MRIP, TCAP markers define "MRIP" haplotypes over a block of approximately 6MB. GH and SECTM markers define "SECTM" haplotypes of approximately 2.5MB. The blocks are separated by approximately 8MB.

MRIP Haplotype frequencies differ between Angus and Wagyu.

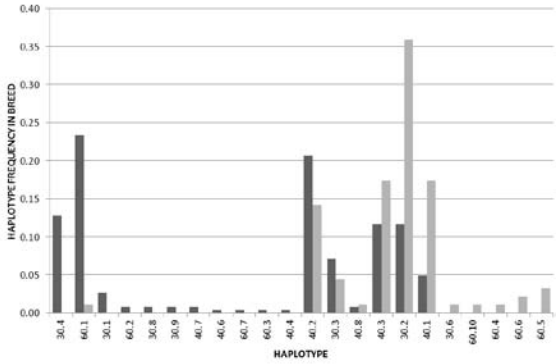

Figure 3. Frequencies of SREBF1-TCAP haplotypes in Wagyu (dark grey, n=133 animals) and Angus (light grey, n=46 animals).

SECTM Haplotypes frequencies differ between Wagyu and Angus.

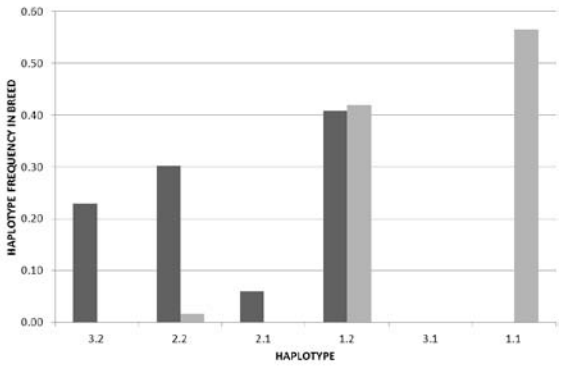

Figure 4. Frequencies of GH-SECTM haplotypes in Wagyu (dark grey, n=109 animals) and Angus (light grey, n=31 animals).

Table 3. Wagyu Breed Determination.

	Wagyu	Angus	Predictive Value
Positive Test[1]	120	2	PPV=98%
Negative Test	15	41	NPV=73%
Total Animals	135	43	

[1]animal tests positive if it has at least one of the haplotypes: 60.1, 30.4, 60.2, 30.8, 30.9, 3.2, 2.2, or 2.1 and none of the haplotypes 60.5; 60.6; 60.4, 60.10: 30.6 or 1.1.

Conclusions

An Alternative Strategy for Selective Breeding

1. Define polymorphic frozen blocks and their ancestral haplotypes in regions of interest.

2. Develop robust haplospecific markers.

3. Identify those haplotypes which are relatively frequent in breeds with desirable (or interesting) traits whilst excluding those which are common to multiple breeds.

4. Develop minimal requirements for reliable determination of breed and parentage.

5. Compare haplotype frequencies in elite versus poor performers within a breed, and in cross breeds, allowing for vagaries of penetrance.

6. Compare candidate haplotypes to determine whether these have arisen by ancestral recombination events which can help to localise the operative components.

7. Consider functional explanations for observed haplotype associations.

8. Test the hypotheses of specific haplotype associations and interactions using AI, ET and Cloning experiments.

9. Use Marker Assisted Selection to blend preferred haplotypes into already successful herds or to increase the frequency of haplotypes already present.

Acknowledgements

Manuscript 1314 of the CY O'Connor ERADE Village Foundation.

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