

Using haplotypes to unravel the inheritance of Holstein coat color

T.J. Lawlor¹, P. M. VanRaden², D. Null², J. Levisse¹ and B. Dorhorst³

¹Holstein Association, USA, Brattleboro, Vermont, ²Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland, ³Virginia Tech., Blacksburg, Virginia, USA

ABSTRACT: Haplotype testing identifies SNPs that bracket a group of alleles from several different genes located upon a specific chromosomal section of DNA. For a trait with a limited number of genotypes and phenotypes one can determine rules of inheritance by matching up certain haplotypes with observed phenotypes. Our analysis identified two genes controlling Holstein coat color. A newly identified gene was found on chromosome 3, having 2 alleles, with the dominant allele coding for RED. Also identified was the well known MC1R gene located on chromosome 18. Four forms of the gene were identified, two causative alleles E^D and e , coding for eumelanin (black) and pheomelanin (red), respectively; E^{BR} believed to be linked to a regulatory mutation causing the Black/Red phenotype; and E^+ the ancestral form of the gene coding for a Red phenotype. In the traditional gene, MC1R, the RED alleles are recessive. The dominant red allele from the newly discovered gene on chromosome 3 is epistatic to all of the alleles at the traditional recessive red gene. A key advantage to breeders is that they can obtain haplotype results easily and at little to no cost from their participation in a genomic selection program.

Keywords: haplotypes; coat color genetics; accessibility

Introduction

The U.S. Supreme Court ruled on June 13, 2013 that identifying a DNA sequence change within a gene does not qualify for patent protection. Haplotype results can now be made widely available to the public, even for those genes where a royalty payment was previously required.

A haplotype identifies the SNPs that bracket a group of alleles from several different genes located upon a specific section of DNA. For example, the haplotype **HRR** brackets the gene MC1R, which is responsible for recessive RED. The biology of red coat color in Holsteins involves multiple genes with multiple mutations within the same gene and a phenotype that is prone to recording errors.

In Holsteins, there are three common coat colors Dominant Black, Black/Red and Red associated with the gene melanocortin 1 receptor (MC1R). Four known variants (E^D , E^{BR} , E^+ and e) are responsible for the phenotypes (Black, Black/Red, Red and Red, respectively). Until recently, a diagnostic test could only check for E^D and e , with E^{BR} and E^+ grouped together as the “wild-type” and labeled by most labs as E^+ .

Conveying genetic information to breeders, with regards to coat color has traditionally been done by adding “RED” to the end of an animal’s name and/or with the use of a limited number of genetic codes such as RC (carrier of a RED allele), TR (DNA tested free of a Red allele) or B/R (carrier of the Black/Red condition).

The color Black/Red is challenging from a recording point of view, as the animals are born with red hair that changes to black, usually by 6 months of age. Due to the timing of registration and naming of the animals, an incorrect –RED suffix could have been added and later a B/R code may be added.

Historically, the E^{BR} variant has been much more common than E^+ due to the popularity of the bull HANOVER-HILL TRIPLE THREAT and his sire ROYBROOK TELSTAR. Therefore, most breed associations would label an animal as B/R (Black/Red) if the animal’s pedigree indicated that it possessed a wild-type version of the MC1R gene.

Unraveling E^{BR} from E^+ animals has grown in importance with the recent popularity of AGGRAVATION LAWN BOY P-RED, who carries the rare E^+ . Breeders are troubled when some of his prominent sons and daughters consistently transmit RED coat color when breed to a RED mate, but, yet they are coded as B/R due to historical reasons.

An additional recording challenge is that the expression of red and white coat color is a sex-influenced trait. Males with the dominant red allele or the E^+ allele of the MC1R gene can become quite dark over time, to the point of being categorized as a black and white animal.

The objectives of this study were to 1) determine the rules of inheritance by matching up certain haplotypes with observed phenotypes for Holstein coat color 2) resolve some historical recording issues 3) compare the accuracy of this information with other molecular methods currently available, and 4) develop a delivery system for providing this information to dairy farmers for routine application in making selection and mating decisions.

Materials and Methods

Data. Holstein genotypes are from the shared national resource maintained by the Council on Dairy Cattle Breeding (CDCB; Reynoldsburg, Ohio). Holstein animals and their dams, sires, and maternal grandsires (MGS) were

also available from the CDCB database. All haplotypes were identified using findhap.f90 software (VanRaden, 2011). The 75-marker haplotypes span about 5 Mbase and are fairly unique, often tracing back to often one or perhaps a few of the prominent sires in the 1950's or 1960's. Incomplete agreement of haplotype status with the actual DNA causative change is expected due to crossing over.

The breed associations have several sources of information as an indicator of animal's phenotype. As most Holsteins are Black and White, the Black phenotype was assumed if there was no indication of another color. A primary source of the phenotypes is the animal's name; the suffix -RED would indicate RED coat color. Animals with a genetic code of B/R and no suffix -RED in their name would be assumed to have the phenotype Black/Red.

Genotypic information was derived from the genetic codes. Of the 321,124 animals with an RC code, only 4,397 are based upon a genotype. The other (98.6% are based upon phenotypic observations of one of the parents being RED or the animal having 2 or more RED offspring. There were 1,472 animals with a genetic code of B/R, as no diagnostic test was available. Actual genotypic information on 6,646 animals Tested free of Red (TR) were available.

The search for the dominant red gene was aided in several ways. As the progenitor of dominant red is a Canadian animal, and her new form of red was observed to be consistently transmitted from one generation to the next, the Canadian Holstein Association recorded these animals as VRC (Variant Red Carrier). Additionally, we searched our database for animals whose phenotype would be consistent with a dominant mode of inheritance. For example, 80 RED animals were identified whose sire was TR, indicating the dam as a carrier of the dominant red allele. Similarly, there were 6 RED animals out of TR dams, implicating their sire. Another source of data was 942 Black offspring from two RED parents. Personal inspection and editing of this type of data was required to obtain the final group of animals confirmed to have the dominant red phenotype. Of those, only 44 males and 19 females had been genotyped. However, this was enough animals to accurately find the haplotypes associated with this trait.

Results and Discussion

Location of haplotypes. Knowledge of the gene traditionally associated with red coat color MC1R has been known for some time. Dominant inheritance of black over red hair color was suggested by Barrington and Pearson (1906), and later proven through a designed experiment by Lloyd-Jones and Evvard (1916) at Iowa State University. More recently, Joerg et al. (1996) mapped the causative deletion to be located on BTA18 at 14,758,207 on the UMD3.1 assembly. Having this prior information and finding our E^D and e haplotypes connected to black and red phenotypes in the same location provided confidence in our results.

A single haplotype common to 70% of all B/R carriers was also detected on BTA18 spanning the location of

the MC1R gene. This same haplotype has a frequency of only 0.3% amongst all genotyped animals. This suggests that within this expanse of DNA is the area of DNA responsible for the Black/Red phenotype.

Just recently, a diagnostic test for E^{BR} was discovered by Dr. Bertram Brenig at the Veterinary Institute of the University of Göttingen. Although it is not the casual condition itself, it is putatively linked to a regulatory mutation with the Black/Red phenotype. This test will be part of next chip from GeneSeek. Therefore, we now have two diagnostic tests (E^D and e) to detect changes in a coding region; a linked marker test (E^{BR}) to determine a change within an intron; and a more clearly defined indirect test for E^+ , the wild-type or ancestral form, when there are no changes detected.

A 75-marker haplotype common to 79% of all dominant red animals was detected at the 8-12 Mbase on BTA3. Most of the remaining dominant red animals had large sections of this haplotype, implying that some recombination had occurred during the 5 or more generations since the main progenitor Surinam Sheik Rosabel-RED. The primary source and one of the oldest animals with this haplotype is the Canadian bull, Seiling Rockman born in 1960, a maternal great grandsire of Rosabel. Our results indicate that the dominant red mutation occurred on BTA3 in her maternal gamete descending from Rockman. Thus 2 versions of Rockman's haplotype on BTA3 exist: the original and Rosabel's mutated version that causes dominant red. This contradicts an earlier study of Dreger and Schmutz (2010) that reported the mutation coming from BTA27 in Rosabel's paternal gamete from her sire, Shiek.

Plastow et al (2013) conducted a similar study focusing solely on identifying the causal mutation behind dominant red and confirmed its location on BTA3 in a gene not previously known to have a direct role in pigmentation biology. A diagnostic test is now present on the newest version of GeneSeek's GGP-LD SNP chip. Expression of dominant red is a sex-influenced trait, whereby the males may darken in color over time to the point of being categorized by many breeders as being black.

Order of Dominance. The order of dominance was established as $E^D > E^{BR} > E^+ > e$ for the different variants of the MC1R gene. The dominant allele D from the dominant red gene on BTA3 is dominant or epistatic to all other known Holstein coat color alleles.

Accuracy of test. A haplotype test does not check for the presence or absences of the causative mutations, i.e., the precise genetic bases that are directly responsible for a trait are not checked. The haplotype test tries to determine if a small segment of an animal's DNA is identical to the same chromosome segment of a close relative. If it is, and we have a gene test result on the relative, we can infer that the animal would have the same DNA sequence, and subsequently a similar gene test.

The key to having an accurate haplotype test is to be able to keep the length of the sections short enough to

lower the risk of a crossover, while long enough so that each haplotype remains somewhat unique within a family. We currently track approximately 730 different chromosome segments in Holsteins, each about the same size, 75 SNPs per haplotype. The number of haplotypes per chromosome varies depending upon the size of the chromosome. There are 40 haplotype segments being tracked on the longest chromosome and 11 haplotype segments for the shortest chromosome.

The frequency of chromosome breakages within cattle has been well studied. On average, there is one breakage point per pair of chromosomes, per meiosis. By tracking the smaller DNA sections, the percentage of crossovers within each Holstein haplotype segment is about 2%. Or stated another way, the probability that the offspring would have received an intact Holstein haplotype from one of its parents is 98%. A quick check of the genetic markers of the individual and its relatives pushes the probability of determining if a recombination has occurred to greater than 98%.

Amongst the 474,000 genomic tested Holsteins, we looked for inconsistencies between the phenotype expected and the derived haplotype. For example, we found 11 animals whose new HHR haplotypes indicated that they are homozygous for E+ E+ and should be RED. All, but one was registered with “RED” in their name. Confirmation was sought and obtained that the one potential discrepancy was indeed RED as predicted. Several large herds were contacted regarding concurrence between haplotype information and their own observations; this resulted in a concurrence rate of over 99%. High genetic value animals and animals where the haplotype results seem questionable are encouraged to obtain a diagnostic test.

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Table 1. Conveying information on HDR to the industry.

HDR	Holstein Dominant Red		
Haplotype	Genotype	Phenotype	Genetic Code
0	d d	Black	
1	D d	Red	DR1
2	D D	Red	DR2

D symbolizes the dominant allele and d the recessive allele

Table 2. Conveying information on HRR to the industry.

HRR	Holstein Recessive Red				Frequency and Number*
Haplotype	Genotype	Phenotype	Designation	Genetic Code	
0	E ^D E ^D	Black		TR	91%
1	E ^D E ^{BR}	Black		B/R	10%
2	E ^D E+	Black		RC	4%
3	E ^D e	Black		RC	62%
4	E ^{BR} E ^{BR}	Black / Red		B/R	<1% (16)
5	E ^{BR} E+	Black / Red		B/R RC	<1% (8)
6	E ^{BR} e	Black / Red		B/R RC	1% (299)
7	E+ E+	Red	RED		<1% (11)
8	E+ e	Red	RED		3%
9	e e	Red	RED		20%

* Frequency of the primary haplotype is 91%, all other percentages are expressed within the remaining 9%.