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Validation of a haplotype test for polled in Australian cattle

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Abstract

The marker test for polled discovered in MLA project AHW.144 (2010) and commercialised following validation in MLA project B.AWW.0209 (2012) performs well in some breeds, but in other breeds fewer than 50% of animals can be assigned a genotype. As part of the study in B.AWW.0209, to improve the assignment rates and improve the accuracy, a haplotype based test was developed and trialled in a small population. The objective of this project was to validate the haplotype test on a larger sample of animals from a wider range of breeds. We tested almost 2,000 cattle from a diverse range of breeds. Most haplotypes were at low frequency, and most animals carried common haplotypes. When the associations between haplotypes and underlying genotype at the polled locus were estimated we found that these associations were consistent across breeds. This means that a haplotype based test should work across breeds and also in crossbred animals. In the major breeds where we had sufficient data for a meaningful comparison, in no case did the haplotype test perform worse than the original single marker test, and in some breeds there were large improvements; assignment rates went from from 38% for Brangus to 97%, from 39% for Limousin to 95%, and from 34% for Shorthorn to 93%. Across all breeds the average assignment rate was 85%, and on the basis of these results this new improved test was launched commercially in November 2013.

Executive summary

As with previous MLA funded projects of this type, the major objective of this project was to decrease the need for dehorning in the Australian beef cattle herd. Currently, dehorning is routinely practiced, as horns are an important cause of bruising, hide damage and other injuries, particularly in yards, feedlots and during transport. Although it is advisable to dehorn at a young age, as a result of mustering practices and especially in northern Australia, dehorning is frequently carried out in older calves between 3 and 10 months of age. Dehorning in older calves is labour intensive and causes more pain to the animal. The wound takes longer to heal, is prone to secondary infection, with mortality rates in dehorned cattle estimated to be as high as 2% in extensively managed research herds [1], and potentially higher in commercial herds.

The best long term alternative to dehorning is to have naturally polled cattle. The transition to a 100% polled breeding herd will be quicker if it is possible to distinguish between homozygous polled animals and heterozygous carriers of the allele responsible for horns; cattle of both these genotypes are usually hornless. In previous projects we developed a single marker gene test that was able to make this distinction in many animals, but not all animals. To improve the assignment rate, we subsequently developed a test that used additional markers in the vicinity to the gene affecting polled, we call this test the haplotype test. In a small test population the haplotype test had far higher assignment rates than the single marker test; in Limousin 169 out of 198 samples that could not be assigned with the single marker test were assigned with the haplotype test. However, these animals were from a selected sample and so these results could not be generalised to the wider population.

The objective of this project was to validate the haplotype test in a much larger sample, across a wider range of breeds. This objective was completely satisfied. Haplotypes were estimated for almost 2,000 animals with poll/scur/horn records. Once haplotypes was observed in 3 or 4 animals we were usually able to determine whether the haplotype was associated with polled or horned. We observed that certain common haplotypes occurred across breeds, and that the associations between the haplotypes and polled were also consistent across breeds. This makes a haplotype test useful in crossbred animals and sometimes even in breeds where we currently have no data. In Brangus, Limousin and Shorthorn, where the original single marker test was able to assign genotype in fewer than 50% of animals, the haplotype test assigned genotypes in over 97%, 95% and 93% of animals respectively. Across all breeds the assignment rate was 85%.

On the basis of the results from this project, a new, improved test for polled was launched in November 2013.

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1 Background

The first four paragraphs of the background to this project are common to earlier MLA project B.AWW.0209.

Dehorning is routinely practiced in beef cattle, as horns are an important cause of bruising, hide damage and other injuries, particularly in yards, feedlots and during transport. Although it is advisable to dehorn at a young age, as a result of mustering practices and especially in northern Australia, dehorning is frequently carried out in older calves between 3 and 10 months of age. Dehorning in older calves is labour intensive and causes more pain to the animal. The wound takes longer to heal, is prone to secondary infection and leads to mortality in some cases. In the light of mounting animal welfare concerns about dehorning, In 2005 MLA commissioned a review on 'Genetic options to replace dehorning in Australian beef cattle'. This review identified the difficulty of distinguishing between homozygous polled animals and heterozygous carriers of the allele responsible for horns as an impediment to breeding for polled.

Based on the results of the review, MLA funded a Beef CRC project (Beef CRC Project 3.1.3b, MLA Project: AHW.144) with the objective of developing gene marker tests for polled, African horn and scurs genes in Bos indicus and Sanga derived breeds. A key achievement for the Project was the discovery of a microsatellite marker (CSAFG29) that in Brahman was strongly associated with horned/polled phenotype. In the Brahman validation population all but 1 of 87 polled animals had at least one copy of the 303 bp allele. As CSAFG29 is a linked marker, this suggests that in Brahman allele 303 is in linkage disequilibrium with the cause of polled, forming a haplotype referred to here as 303P. There are a number of possible explanations for the polled animal not possessing allele 303P, including a phenotyping error, incomplete penetrance and the presence of another polled haplotype allele at low frequency in the population. Of the 229 animals with at least one copy of the 303 bp allele, only 21 were horned, and none of the 21 was homozygous for allele 303. Again, there are a number of possible reasons for the 21 horned animals, including phenotyping errors, incomplete penetrance, another locus affecting horns, and the presence of a 303H haplotype allele at low frequency in the population.

Subsequent validation in other breeds showed similar results for Limousin, Hereford, Droughtmaster and Santa Gertrudis. In particular, for Limousin all but 3 of 29 polled animals had at least one copy of the 303 allele, and only one of the 28 animals carrying at least one 303 allele was horned. The association between allele 303 and polled held to a lesser extent in Tropical Composite cattle with Sanga genetics, where a higher proportion of animals carrying a 303 allele were horned.

In the Angus validation population 6 microsatellite alleles were observed, each presumed to form a polled haplotype. The most common alleles were 303P, with a frequency of 30%, and 305P, with a frequency of 58%. In the Brahman validation population allele 305 is at a low frequency, and appears to form a 305H haplotype. In the Brangus validation population the 303 allele is at a higher frequency than the 305 allele (36% and 25% respectively), but 305 is often associated with polled, so the 305P haplotype is presumed to have originated in Angus.

In a second MLA funded project (B.AWW.0209) a pre-commercialisation trial on CSAFG29 was conducted, and on the basis of results from that trial, a marker test based on CSAFG29 launched. As linkage between polled and CSAFG29 was not complete, the test was only able to consistently assign polled status in some breeds. Limousin, and breeds with high proportions of Angus such as Brangus were particularly problematic. To address this, in B.AWW.0209 a concurrent activity was to explore the potential of a test that used markers in addition to CSAFG29, a haplotype test.

The results of that study were very encouraging in a small multi-breed test population of 314 head. For a haplotype comprised of 7 markers, 225 haplotype alleles were observed, of which 41 were associated with polled, 120 were associated with horned, and for 54 the association could not be estimated.

During the course of the above mentioned projects other knowledge about the genetics of horns was discovered. Scurs were shown to occur predominantly in animals that are heterozygous at the polled locus, but at different frequencies in different breeds. This suggests a genetic component for variation in scurs. There is also some data suggesting that homozygous polled animals can sometimes be horned, such as through the hypothesised African Horn Gene. The current polled test provides no information on these other forms of variation. This affects our ability to estimate associations between markers and polled, and reduces confidence in marker tests when progeny test results contradict test predictions.

2 Project objectives

The objective of the project is to confirm the power of the haplotype test for polled, resulting in identification and selection of homozygous bulls, leading to an acceleration of the shift of the Australian cattle herd to polled, and therefore a reduction in the incidence of dehorning. More specifically, the project will, by 1/4/2013 deliver:

1. A library of haplotypes, each with probabilities of association with polled or horned genotype.

2. Software that takes genotype results and, using the haplotype library, determines the probability that an individual will be horned or heterozygous or homozygous polled. As more genotypes are collected, the software will add new haplotypes to the library, and update the polled genotype probabilities for existing haplotypes.

3. A reliable haplotype based test to distinguish between heterozygosity and homozygosity for polled animals, that works in all Australian beef breeds as well as in cross bred and composite cattle (target: producing an unambiguous result in the majority of polled animals).

4. Increased knowledge regarding haplotypes in the region of polled in Australian beef herds, allowing immediate and comprehensive validation of SNP based tests that might become available.

5. Increased knowledge of the mechanism of inheritance of scurs in animals that are heterozygous polled.

6. A tool to assist breeders in evaluating strategies for reducing the frequency of horns and dehorning in their herds.

7. A predictor for probability of polled genotype in pedigreed populations.

8. The project team will engage in webinars and workshops with key stakeholder groups during the course of the project.

3 Methodology

We use lower case words to describe phenotypes, that is, what is observed on the animal (polled, scurred, or horned), and assume alleles with two genotypes at the polled locus, with haploid genotypes described by the upper case words POLLED and HORNED. To simplify the language we use "POLLED haplotype" and "HORNED haplotype" to refer to haplotypes linked to POLLED and HORNED respectively. Diploid genotypes are described by pairs of upper case letters (PP, PH and HH). Diploid genotypes derived from progeny testing or other pedigree data have the word "test" appended (i.e. PPtest, PHtest and HHtest).

3.1 Animals

Animals used in the study were from a wide range of breeds and crosses, and had a range of phenotypes, as summarised in Table 3.1. Animals were selected for the study on the basis of availability of a stored sample of DNA for genotyping, linked to a phenotype record (polled, scurred and horned). For 85 Limousin bulls we had progeny test data, and under the assumption that POLLED is fixed in the Angus breed, we assigned a genotype of PPtest to all samples from Angus. Even though these pedigree test results are measures of genotype rather than of phenotype we do not assume that they are without error, and in our analyses we treat them as a special class of phenotype, replacing the actual phenotype for relevant animals. Accordingly, in descriptions that follow, "phenotype" includes pedigree based estimates of genotype. Samples without phenotype records were included for the Brahman and Santa Gertrudis breeds to assist in building haplotypes of B. indicus origin. Stored DNA was available from animals used in research projects and from animals submitted for testing with the existing CSAFG29 marker test. While the animals from research projects are likely to be a representative sample of the breed in Australia, animals submitted for testing with CSAFG29 are clearly from a selected set, almost certainly polled, and likely to have a significant probability of carrying a HORNED allele at the polled locus. Where possible, samples within breed were chosen to be balanced across phenotypes, and from a broad spread of the genetics available in Australia for that breed. However, for some breeds the representation was limited by sample availability. Although not of primary interest in this study, samples from the Holstein Friesian breed were included to allow a comparison with the results of other published studies.

	Phenotype				*Progeny Test*		
Breed	polled	scurred	horned	unknown	PPtest	PHtest	HHtest
Angus*	0	0	0	0	66	0	0
Belmont Red	2	0	0	0	0	0	0
Blonde d'Aquitaine	19	0	9	0	0	0	0
Brahman	172	23	101	22	0	0	0
Brangus	65	40	13	0	0	0	0
Charbray	8	8	7	0	0	0	0
Charolais	38	3	25	0	0	0	0
Dexter	15	0	14	0	0	0	0
Droughtmaster	57	21	30	0	0	0	0
Hereford	119	0	59	0	0	0	0
Holstein-Friesian	0	0	28	0	0	0	0
Limousin	152	1	63	0	27	56	2
Santa Gertrudis	96	5	32	114	0	0	0
Senepol	1	0	0	0	0	0	0

Shorthorn	101	0	70	0	0	0	0
Simmental	59	1	60	0	0	0	0
Wagyu	0	0	10	0	0	0	0
Wagyu X Hereford	13	0	11	0	0	0	0

Table 3.1. Counts of animals by breed and polled phenotype. *The 66 Angus animals were phenotypically polled, but under the assumption that they are homozygous PP they were assigned a PPtest genotype.

3.2 Markers and Genotyping

The microsatellite markers used in this study were all discovered in the study of Mariasegaram et. al. [2]. In an initial screen on a small population (n ~ 300), the 10 markers that were most polymorphic and that showed the highest associations with polled phenotype were identified. These are listed in Table 3.2. Note that although these showed the strongest association with polled, none apart from CSAFG29 showed any consistent association across breeds. Primers for the markers are listed in Table 3.3. Of 1,838 animals in total, 1,759 had a record for all 10 markers, and of these 1,625 also had either a phenotype record or a progeny test genotype. DNA was extracted from hair follicles using a proteinase-K digest by incubating the sample at 60°C for 45 min and then at 95°C for 45 min. DNA samples utilised from previous research projects had been extracted from semen samples using a standard phenol-chloroform method. PCR was performed in a total volume of 12 µl containing 10-20 ng DNA, 10 µM forward and reverse primers, 0.12 µl Kappa Taq (GeneWorks, Adelaide, Australia) and standard PCR cycling conditions on ABI3500 thermocycler. Capillary fragment separation was performed on an ABI3700 Genetic Analyser and genotypes analysed with GeneMapper (Applied Biosystems) using the Liz-500 as a size standard reference (Applied Biosystems).

Marker	*Location*	*Alleles*
CSAFG28	1495504	9
CSAFG29	1507991	36
CSAFG30	1537311	11
CSAFG31	1558614	9
CSAFG33	1614784	17
CSAFG34	1677140	8
CSAFG35	1690127	9
CSAFG37	1809547	22
CSAFG38	1840774	7
CSAFG22	2119315	12

Table 3.2. Microsatellites with map location in base pairs (Bta4.0) and number of alleles.

Marker	*Primer Sequence Forward*	*Primer Sequence Reverse*
CSAFG28	GCAGCTGAATAGCAGCAACA	AACCTGCCTGCACTAAAGGA
CSAFG29	AAAATCTTCATTGAATTTGTTAC	AGACTTCGCAGCCAAAAA
CSAFG30	ACAGGGAACTGTGGCTTGAG	CAGAGCATCCTCCTCTCACC
CSAFG31	GTGACTGCCGTCCTTGTGT	ACGGGCAGGTAATACCCAAC

CSAFG33	GGTGTACAGTTCAGTGGTGTTAATG	TGTTCCGGCAATCAGTTGTA
CSAFG34	CTGCCCATTGCCCCTAAAG	CGCGTATGTGTGTGCCTCT
CSAFG35	GCATTCGGCTGACTTCAAA	TGTGGCAGAAAATTCACACC
CSAFG37	TGTGCTATTGCTGTACACCTGA	CCTGCCAGTTTCCTCTGTTT
CSAFG38	AGTGGGGTCACCCTAAGGAC	CGGGCTACAGTACACGAGGT
CSAFG22	TGATGTCTCTGCAAGCCAAG	CATTGCAGGTGACACAGGTT

Table 3.3. Primer sequences.

3.3 Statistical methods.

As in MLA project B.AWW.0209, we used the haplo.em function from the haplo.stats [3] package in R [4] to estimate haplotypes from the diploid genotypes. Haplotype alleles identified by haplo.em take arbitrary integer identifiers, which are dependent on the order in which samples occur in the input file. This means that the integer identifier for a haplotype allele might change if the software is run on different samples. To avoid the potential for confusion, we standardised the naming of the haplotype alleles such that haplotype allele 1 was the allele with the highest frequency, haplotype allele 2 was the allele with the second highest frequency, and so on. In addition to reducing confusion, this will allow interested breeders to become familiar with the alleles present in their populations, and the size of the integer gives an immediate indication of whether the allele is common.

The association between estimated haplotypes and POLLED or HORNED was estimated using a second piece of software written in R. The software applies a Metropolis Hastings algorithm to sample solutions based on genotypes, phenotypes, and a penetrance function. The penetrance function contains our understanding of the relationship between diploid polled genotype and phenotype. The penetrance function we used appears in Table 3.4. Each row sums to one, and contains the probability of diploid genotypes (in columns) given observed phenotype (in rows). For example, if an animal is scored as polled, then we assume a priori that there is a 49% chance it is homozygous PP, a 49% chance that it is heterozygous PH, and a 2% chance that it is homozygous HH. Penetrance values for progeny tested animals are given more weight than for animals with only a phenotype, but even then we do not make any probability equal to zero, as we acknowledge the possibilities of phenotyping errors, of genotyping errors, and of sample mislabelling errors. We recognise that the values we have used in the penetrance function are not known with any precision, and are likely to vary between breeds. For example, in breeds where the HORNED allele is rare, polled animals are more likely to be homozygous PP than heterozygous PH. As data accumulate we may modify the penetrance function, probably first by applying a different function for Bos indicus cattle. Strictly speaking, a penetrance function is the proportion of individuals in each phenotype class given the genotype, but as we have progeny tested individuals and known genotypes we express it here as the proportion of animals in each genotype class given the phenotype. The test is flexible and more phenotype classes can be added if required.

Genotype Phenotype	PP	PH	HH
Horned	0.05	0.10	0.85
Polled	0.49	0.49	0.02
Scurred	0.20	0.79	0.01
Progeny test PP (and Angus)	0.97	0.02	0.01
Progeny test PH	0.03	0.94	0.03

Progeny test HH	0.01	0.02	0.97

Table 3.4. Penetrance function relating diploid polled genotype to phenotype.

We ran 4 replicates of the software, each with 10,000 samples accumulated after a 10,000 sample burn in period. The average of the 40,000 samples (4 x 10,000) was used to generate the probability of POLLED for each haplotype.

3.4 Discovery of regions of the genome affecting scurs

As scurs occur predominantly in heterozygous PH individuals, we identified the animals using our polled test that were most likely to be heterozygous. From this list of animals we then found those that also had Bovine SNP50 data in the database. For these individuals a dataset was assembled containing the Bovine SNP50 genotypes and polled phenotype. The plan was to conduct a genome wide association study on this dataset. Unfortunately, very few of the animals that were heterozygous polled had a Bovine SNP50 genotype, and amongst these there was almost no variation in polled phenotype. Consequently, as no money was budgeted for additional SNP genotyping, we were unable to complete this activity.

3.5 Strategies for reducing the incidence of horns

In previous projects a report was prepared on strategies for reducing the incidence of horns. An objective of this project is to prepare this information in this report in a form more accessible to breeders. Over the course of the project team members have had on-going communication with breeders using original test, have presented at various forums, and have contributed to “fact sheet” style material to describe the old test, the new test and application of the tests. Now that results for the new haplotype test are available we will soon be in a position to make available parameters and implementation strategies for breeds currently running analyses on single gene disease traits, allowing them to allow the inclusion of polled in these analyses.

4 Results and discussion

4.1 Validation of the haplotype test

In the initial sample set, 465 haplotype alleles were observed. The distribution of allele frequencies is displayed in Figure 4.1. In panel A it can be see that while the most frequent allele was seen over 300 times, over 200 alleles were seen only once. In panel B it can be seen that the first 100 alleles account for around 80% of observed haplotypes. The 200 alleles that were seen only once account for less than 10% of observed haplotypes. This suggests that in something less than 20% of new samples submitted from commercial herds we will see a new haplotype allele that we have not seen before. The number could be significantly less than that, as our current data set contains samples from many breeds, and is therefore probably more diverse in proportion to its size than it will be at any time in the future.

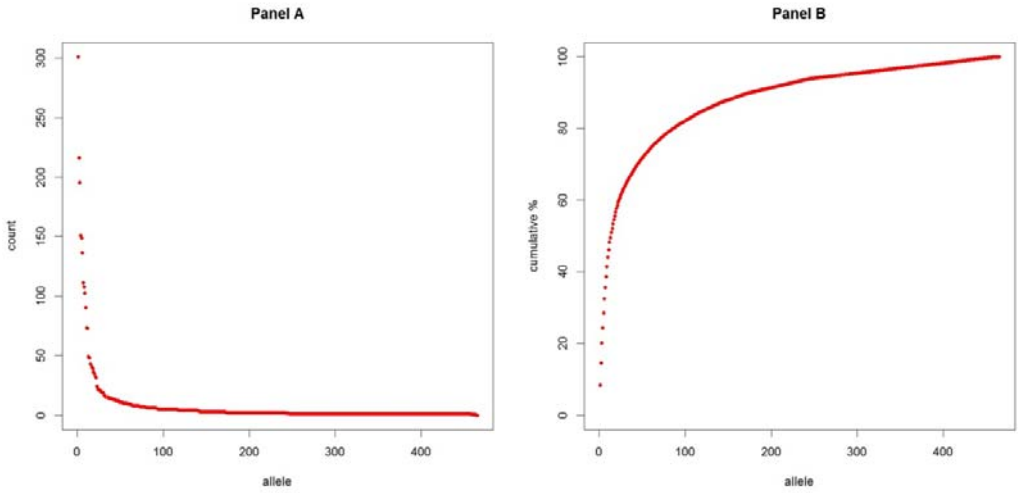

Figure 4.1. Frequencies of observed haplotype alleles in the 1838 animal calibration population.

The results from running the sampling software on the 1,838 animal calibration population are displayed graphically in Figure 4.2. In the top panel the 465 haplotype alleles are plotted on the X axis, with the probability that they are associated with POLLED on the Y axis. The haplotype alleles are sorted in order of the probability that they are POLLED. The colours indicate the number of times that the haplotype allele was observed. It can be seen that around 250 alleles have very low probabilities of being POLLED, around 65 alleles have very high probabilities of being POLLED, and around 150 alleles have an intermediate probability. These alleles are predominantly alleles that were only seen once, and we can infer that they were seen in a polled animal, coupled with an allele that was not known to be HORNED. That 150 out of 465 haplotype alleles could not be assigned to be either POLLED or HORNED sounds a lot, but as illustrated in the bottom panel, as a proportion of the haplotypes observed in the population (1,838 individuals with two haplotypes each) it is not so great. As common haplotype alleles can easily be assigned to be either POLLED or HORNED, and as most animals carry common haplotype alleles, most animals can be assigned a genotype of PP, PH or HH based on their marker genotype.

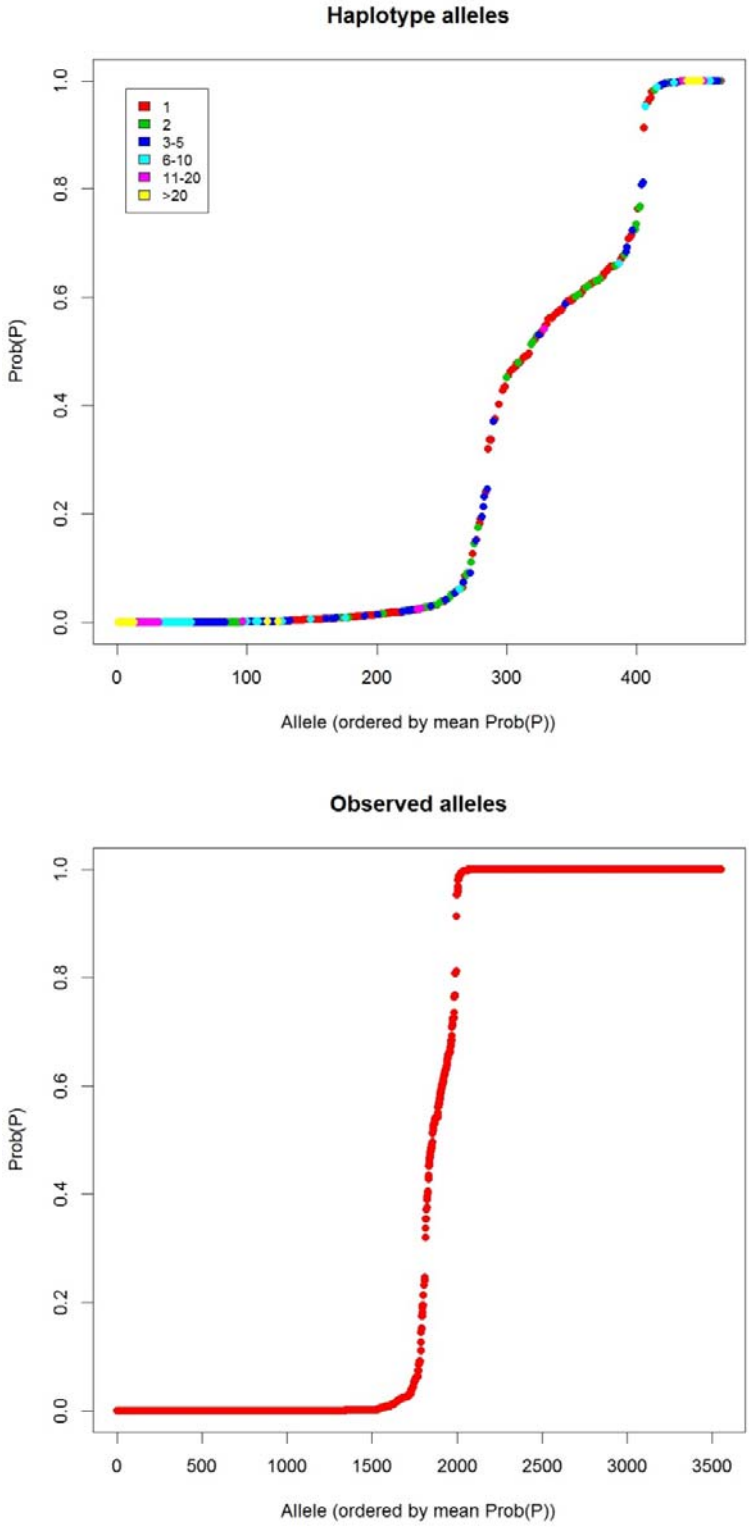

Figure 4.2. Estimated probability that haplotype alleles are associated with POLLED. In the top panel the X axis contains the 465 haplotype alleles, and in the bottom panel the X axis contains the observed alleles in the population (i.e., two alleles for each of the 1838 animals).

The associations estimated with the sampling software proved to be consistent across breeds. For example, the most abundant POLLED haplotype in our sample was common in Hereford, Santa Gertrudis, Shorthorn and Brahman. A second common POLLED haplotype in our sample was seen in Angus and is the most frequent POLLED haplotype in Limousin. As there are more HORNED haplotypes, with each one seen fewer times, they were more likely to appear within a breed, but many HORNED haplotypes do appear in multiple breeds, and these were consistent with the known history of the breeds. For example, the most abundant HORNED haplotype in our sample was common in Santa Gertrudis, Droughtmaster and Brahman, and the second most abundant HORNED haplotype was common in Charolais, Limousin and Simmental.

As shown in Figure 4.2, for a proportion of haplotypes the association with POLLED could not be estimated from the data in the validation trial. This means that for a proportion of animals genotype cannot be assigned. Assignment rates vary across breeds, and are displayed in Table 4.1, for a threshold of 90% (i.e., an animal is classed as “assigned” if the most likely genotype has a probability of over 90%). These results include the animals used in the validation trial, and also all animals of these breeds that were submitted for testing along with a phenotype record since the launch of the new test in November 2013. In all cases assignment rates are as good as or better than results using the single marker test, and in the case of Brangus, Limousin, Simmental and Shorthorn the assignment rates are much higher.

	% Informative Result		
Breed	**Number Tested**	**Haplotype Test**	**Old Test**
Brahman	434	89%	89%
Brangus	115	97%	38%
Charolais	71	86%	72%
Droughtmaster	136	82%	73%
Hereford	183	96%	72%
Limousin	360	95%	39%
Santa Gertrudis	225	92%	77%
Simmental	118	88%	56%
Shorthorn	167	93%	34%

Table 4.1. Percentages of informative results (those achieving a 90% threshold) with the haplotype test and the original single marker test. The results for the haplotype test include commercial samples submitted with phenotypes since the launch of the test.

Where a POLLED or HORNED genotype cannot be assigned to a haplotype it is because we have not seen that haplotype before in an animal that provides useful information. Information comes from:

1. Progeny tested animals.
2. Animals that are horned: both haplotypes are likely to be HORNED.
3. Animals that are polled or scurred carrying a haplotype known to be HORNED: the other haplotype is likely to be POLLED.
4. Animals that are scurred carrying a haplotype known to be POLLED: the other haplotype is likely to be HORNED.
5. Animals that are polled and that are homozygous for the haplotype: the haplotype is likely to be POLLED.

Information on HORNED haplotypes comes mainly from horned or scurred animals, and while a lot of these were tested in the validation trial, we would not expect many samples from these animals to be submitted for commercial testing, as the breeder knows that the genotype is almost certainly not homozygous PP. Accordingly, for breeds not in the validation population or for breeds with few horned animals in the validation population, unless the horned haplotypes are common to other breeds assignment rates in other than homozygous PP individuals will be low. Assignment rates for a range of breeds for which we had fewer samples are shown in Table 4.2. The two polled Belmont Reds were easily assigned as their haplotypes had been seen in other breeds, but assignment rates in other breeds were lower as there was insufficient data to resolve haplotypes unique to those breeds. Note that even when all of the animals in a breed were horned, such as Holstein Friesian, not all haplotypes are assigned to be HORNED. Our penetrance function (Table 3.4) gives a probability of HH of 85% to a horned animal, which is not enough to exceed the 90% threshold if the haplotype is seen only once.

Breed	Number Tested	% Informative Result
All (including breeds in Table 4.1)	1957	85%
Belmont Red	2	100%
Blonde d'Aquitaine	26	65%
Charbray	23	96%
Dexter	25	72%
Drakensberger	80	85%
Holstein Friesian	27	93%
Senepol	1	0%
Wagyu	8	88%
Wagyu X Hereford	23	100%

Table 4.2. Assignment rates for other than the main breeds. Results for the old test are not available for these breeds.

4.2 Commercialisation of the haplotype test

Software has been written to use the associations estimated using the sampling software to predict polled genotype on commercial samples. This software, which is written in Fortran, maintains a library of genotypes and associated haplotypes, of phenotypes, and of the associations between haplotypes and POLLED or HORNED. Protocols have been developed to facilitate the submission of marker genotypes and the return of polled predictions. The improved test was launched in November 2013, and it will be offered by the same providers who offer the original single marker test.

4.3 Ongoing Refinement of the Commercial Test

It is important to note that assignment rates for this test increase as more data is added to the phenotype and genotype library. For new breeds it may sometimes be necessary to seed the database by genotyping some horned animals, as HORNED haplotypes that are specific to that breed will be difficult to identify if only polled cattle are genotyped commercially.

Review and refinement should be an integral part of any test roll-out, but especially for a test based on statistical associations, such as the haplotype test. Confidence in the test would be undermined were we to persist in the light of data inconsistent with the assumptions on which the test is based. With the new data the opportunity will be there to refine the delivery software, to refine the parameters used in estimating associations, and to develop additional checks to guard against incorrect assignments in non-standard situations. Our penetrance function (Table 3.4) is based on hypothesised modes of inheritance of polled and the likely phenotyping error rate. These probabilities were consistent with the data available at the time the test was launched. If the penetrance function is correct, then, given 100 bulls with genotype probabilities of 90% PP and 10% PH, with the addition of new data (such as a progeny test, or more tested animals), 90 would turn out to be PP and 10 would turn out to be PH. Over the next 12 months, as data accumulate, we can track movements in predicted genotype. We can then tune and extend the penetrance function; essentially we can find the set of penetrance probabilities that produce outcomes at the appropriate probabilities when new data are added.

5 Successes in Achieving Objectives

This project has met most of its objectives, including the most important one: the development of an improved marker test to facilitate the breeding of polled cattle.

The haplotype based test is superior to the test based on CSAFG29 alone, especially in the breeds where the original test was often inconclusive, such as Limousin. As the haplotypes show consistent associations with POLLED across breeds, the new test will often work successfully in breeds other than those in the validation population, and in crossbred cattle, regardless of whether pedigree information is available. This will give it particular utility in northern beef herds, where composite cattle are common, and pedigree not available on commercial animals.

6 Impact on Meat and Livestock Industry – Now and in 5 Years Time

This project has the potential to have a profound effect on the beef industry over the next 10 years. The practice of dehorning is likely to be under increasing scrutiny, particularly when practiced in older calves. Building on the results of earlier projects, we now have a test that is more useful, especially in composite herds and herds where pedigree is not available. If the test is adopted there will be an immediate reduction in the need for dehorning and, if used widely and persistently, the whole herd will eventually be polled, and without scurs.

7 Conclusions and Recommendations

In Australian beef cattle, a haplotype of microsatellite markers offers a highly predictive test for polled in the breeds tested. The test has been commercialised and several hundred commercial samples have already been tested.

It is highly desirable that the software and parameters used in the commercial delivery of the test are refined as data accumulate over the first 12 months of its operation (see section 4.3). There is a risk that confidence in the test (and through association, other gene tests) is undermined should we fail to ensure that results are estimated using appropriate sets of parameters. A mechanism for supporting this activity should be found.

The project was unable to deliver a test for scurs, and further research in that area should be a priority.

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