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Polled gene marker refinement: Refinement of the CSAFG29 Microsatellite Marker Test for Polled

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Abstract

The objective of this project was to improve on the gene marker tests for the Polled discovered in MLA project AHW.144. Potential improvements could arise from: better software to estimate marker associations, potentially involving pedigree information; better estimates of breed specific parameters used by the software; and the addition of other gene markers in the polled region to the test. While some progress was made with improved software and parameters, the biggest achievement in the project was through the use of additional gene markers. It was established that, in a test population, there was considerable linkage disequilibrium in the polled region, and that haplotypes of microsatellite markers showed stable associations with polled both within and across breeds. This opens the door to a gene marker test that is far more accurate than the test discovered in project AHW.144. The marker test will further facilitate the introgression of polledness in tropical beef cattle, such that the practice of dehorning can be phased out.

Executive summary

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 the 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.

The difficulty of distinguishing between homozygous polled animals and heterozygous carriers of the allele responsible for horns is an impediment to breeding for polled. In an earlier project, a gene test based on the CSAFG29 microsatellite was developed. The test is useful, but not perfect. For many animals it provides a clear result, distinguishing between homozygous and heterozygous animals. However, for other animals the test is unable to provide a clear result. The frequency with which this occurs depends on breed, and is particularly high in Brangus and Limousin, where for as many as 50% of polled animals the test cannot distinguish between homozygous polled and a carrier of the alleles responsible for horns.

The objective of this project was to improve the CSAFG29 test. A number of approaches were tried, and the most successful was the addition of other microsatellites markers to the test, markers that are closely linked to CSAFG29. Coupled with a haplotype based analysis method, in a discovery population these markers improved the test beyond our wildest expectations. Associations between haplotypes and polled appear to be robust across breeds, and therefore should have utility in crossbred and composite herds. The validation of the improved test in a larger population should now be a priority, and if validated, the release of the test to industry.

Contents

1 Background

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 the 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, 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 there were 6 microsatellite alleles 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.

On the basis of these results a pre-commercialisation trial of marker CSAFG29 was initiated. Early results from the Limousin breed were inconsistent with the validation population results, with 4 out of 7 polled bulls not carrying a 303 allele. All of the 4 had at least one 305 allele, leading to the hypothesis that their polledness was due to a 305P haplotype originating in Angus during the grading up process. The proportion

Angus for the 4 bulls ranged from 3.2% to 5.7%. After 142 samples from polled Limousin were tested, it appeared that allele 305 was at a high frequency in polled Limousin. In most cases there is no way of distinguishing the 305P haplotype from the 305H haplotype, but as samples accumulate we can make a prediction based on probability. If allele 305 is assumed to form a 305P haplotype 50% of the time, then for only half of the breed supplied polled Limousin samples can an unambiguous genotype call be made. In breeder provided samples from polled Brahman allele 305 is at a lower frequency. This permits a higher proportion of animals to be assigned a genotype with reasonable certainty, but we lack data on haplotype frequencies so these estimates may have high errors.

For both breeds the animals tested in the pre-commercialisation trial are with few exceptions polled. This ascertainment bias makes it difficult to estimate haplotype frequencies in the wider population. To assist in this, animals being genotyped under the Industry Influential Sires Project are also being tested for CSAFG29.

In summary, at the commencement of this project it was understood that:

- In breeds apart from Tropical Composite haplotype 303H is at low frequency.
- In breeds with no Angus background haplotype 303P is the only polled haplotype at high frequency.
- In Angus a number of polled haplotypes segregate, predominantly 305P and 303P.
- In breeds with recent infusions of Angus genetics haplotype 305P (and potentially other Angus polled haplotypes) may segregate.
- In breeds other than Angus, a 305H haplotype segregates.
- In the rare cases (outside Angus) where alleles other than the 303 or 305 base pair alleles are responsible for polled, the allele is generally close (in terms of base pairs) to allele 303. For example, there is some evidence that alleles 301 and 304, both at low frequency in the original validation populations, may sometimes be associated with polled.

2 Project objectives

The objective of the Project is to deliver a commercial test for polled/horned genotype to the Australian beef industry.

For breeds where the testing of the influential sires provides confidence that no further validation is required, the test will be commercially released within months of the completion of the genotyping of the influential sires.

For all breeds, except for those where sufficient precision cannot be obtained, the test should be commercially released this financial year.

The test will be backed up with recommendations on moving a herd from horned to polled while minimising loss in genetic gain for other traits, and on reducing the need for genotyping by optimising use of the test in pedigreed populations.

The outcome of successful completion of the Project, followed by optimised, widespread breeding for polled, will be a shift in the predominant polled genotype from horned to polled in Australian Beef herds, resulting in a rapid reduction in the need for dehorning.

3 Methodology

Three complementary approaches to improving the accuracy of the test were pursued, and options for optimisation of the use of test were assessed.

3.1 Improved understanding of the association between CSAFG29 and polled

During the course of the project, additional CSAFG29 test results continually arrived. These were from animals tested as part of the project, as part of other research projects, and commercial samples tested by AGL. To maximise the information gained from these new results required that the data be stored and that methods to update breed specific estimates of associations be developed. For storage we implemented an SQL database, and software to estimate associations was implemented in the general purpose computer language DELPHI. A DELPHI application was developed to predict poll genotype from commercial samples, making use of the parameters estimated for each breed.

3.2 Development of a pedigree augmented test

For breeds where the strength of the association between polled and the gene marker test was inadequate to allow reliable prediction, the intention was to source or develop software to incorporate pedigree information into the analysis. As improvements to the test appear to allow reliable prediction, this activity became of less importance and was not pursued during the project.

3.3 Testing of markers in close proximity to marker CSAFG29

Eighty Limousin bulls were genotyped by AGL for 32 microsatellite markers located close to microsatellite marker CSAFG29. The microsatellites were sourced either from the literature, or from the marker discovery activity undertaken by M. Mariasegaram in an earlier phase of this project.

Limousin were chosen for this component of the study as, in Limousin, allele 305 at marker CSAFG29 is at a moderate frequency, and seems to form both 305P and 305H haplotypes in more or less equal proportion. In Angus, both alleles 303 and 305 are common, and presumed to always form 303P and 305P haplotypes. In other breeds, allele 305 commonly forms a 305H haplotype. This lead to the hypothesis that in Limousin, 305P came from Angus during grading up and 305H came from French Limousin. The Limousin animals to be genotyped were selected on the basis of the amount of information available on their genotype at the poll locus. In most cases the genotype was known almost with certainty through the phenotypes of progeny. That is, essentially these sires can be thought of as progeny tested for polled. Further, only animals with at least one 305 allele at CSAFG29 were genotyped, as we were seeking a marker that could discriminate between 305P and 305H.

In breeds such as Limousin, where polled is almost always dominant to horned, the CSAFG20 test is really only necessary for polled animals, as horned animals are almost certainly homozygous horned. Of the 80 bulls genotyped we found that the most useful contrast was in the 28 animals that were heterozygous at CSAFG29 with genotype 303, 305. These are all phenotypically polled, but from the progeny test data could be determined to be homozygous polled (13 bulls, assumed to have haplotypes 303P and 305P, coded 303P305P) or heterozygous (15 bulls, assumed to have haplotypes 303P and 305H, coded 303P305H).

We tested the significance of the association between CSAFG29 haplotype (303P305P or 303P305H) and haploid genotype at the 32 new markers using Fisher's exact test, as implemented in the software package R (R_DEVELOPMENT_CORE_TEAM 2007).

On the basis of the results from the Limousin data we tested 10 of the markers on 314 bulls from 9 breeds. The bulls were chosen on the basis of their CSAFG29 genotype and, apart from Angus (for which we had no CSAFG29 genotypes) had at least one copy of the 305 allele. There were also 3 Herefords included that were polled but had no copies of either the 303 or the 305 allele at CSAFG29. The phenotypes of the bulls were known (polled, horned or scurred), but the bulls were not progeny tested, so genotypes at the polled locus could only be inferred from phenotype. For each marker a test was conducted for an association between marker alleles and phenotype. The significance was estimated using Fisher's exact test, except for when all breeds were analysed together, when simulation (1,000,000 replicates) was used. All computations were performed in R.

A better way to analyse closely linked markers is to combine the markers and estimate haplotypes, or haploid marker genotypes for individual animals, and attempt to estimate associations between haplotype alleles and genotype at the polled locus. A subset of markers that all show associations with polled are likely to be closely linked, and the number of haplotype alleles segregating in the population should be much smaller than the number of feasible haplotype alleles. We used the haplo.em function from the package in R to estimate haplotypes for the 10 markers and CSAFG29. No map information was used in this analysis - marker order is irrelevant as the intent is to develop a diagnostic test rather than to locate the causal mutation. Starting with the panel of 11 markers, an iterative process was applied to test the effect of dropping one marker at a time from the panel, to find a minimal panel with as few haplotype alleles as possible, but also in which haplotype alleles were associated only with either a polled allele at the polled locus or a horned allele at the polled locus. The method of testing the association between marker haplotype and polled was as follows:

1. Identify animals with a unique pair of haplotypes estimated (this was always the vast majority of animals)
2. Call all haplotypes in Angus P
3. Call all haplotypes in horned animals H
4. In polled or scurred animals that have an H, call the other haplotype P
5. In scurred animals that have a P, call the other haplotype H

3.4 Optimisation of the Test

A modelling study was conducted to determine, under a range of scenarios, how long it would take to eliminate polled from a herd. In particular, we considered the impact of the accuracy of the test being used. The study considered, not just the frequency of the polled alleles in the herd, but the frequency of horned animals, as they (and scurred animals) are the only ones that require dehorning.

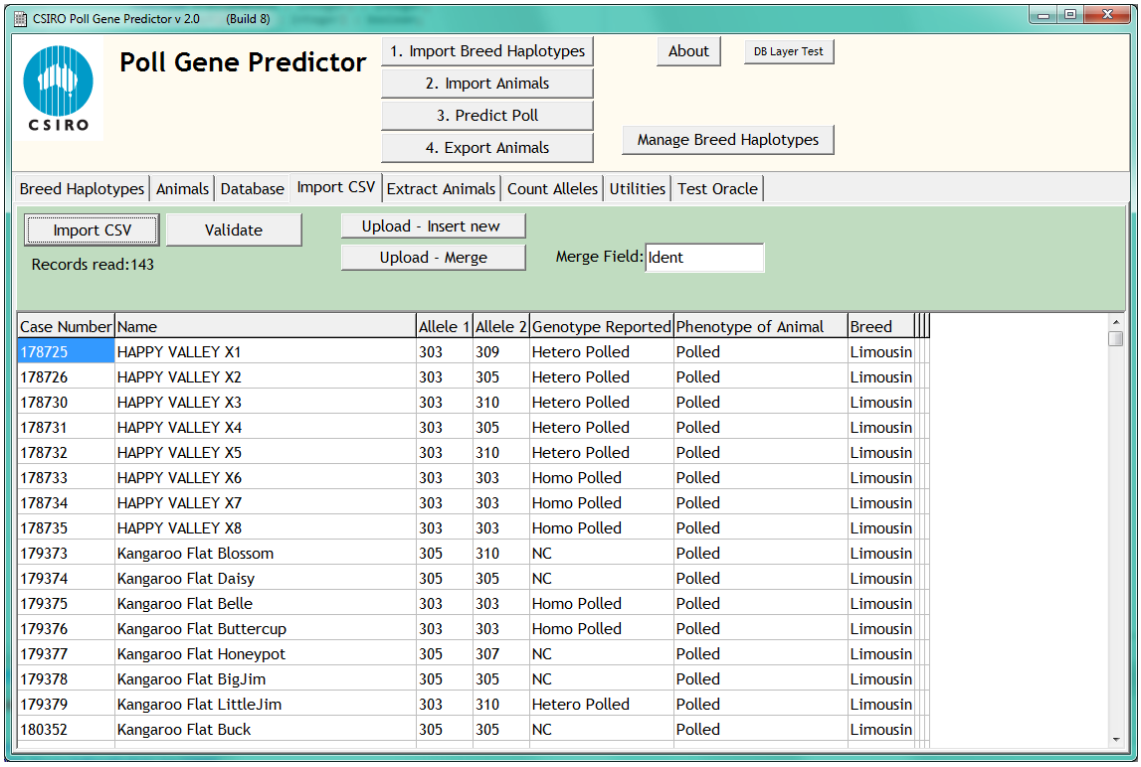
4 Results and discussion

4.1 Improved understanding of the association between CSAFG29 and polled

A database was written in the powerful "SQLite". This was chosen for its flexibility, and its ease of use for single user databases. The purpose of the database is to

facilitate the research phase of the project, but as an SQL database has been used the underlying structure can be easily ported to a larger, industrial strength database when required.

A suite of tools was developed to interface with the database, allowing data upload, data checking, initial data processing and data extraction (Figure 4.1.1). The interface is written in the compiled general purpose development language Delphi. This allows both a rapid development time and rapid execution times for computationally intensive numerical software. The syntax of Delphi is similar to that of other procedural languages (especially to Fortran90 and later Fortran variants) so final versions of the interface and processing tools can be quickly ported to whatever languages are required.



Case Number	Name	Allele 1	Allele 2	Genotype Reported	Phenotype of Animal	Breed
178725	HAPPY VALLEY X1	303	309	Hetero Polled	Polled	Limousin
178726	HAPPY VALLEY X2	303	305	Hetero Polled	Polled	Limousin
178730	HAPPY VALLEY X3	303	310	Hetero Polled	Polled	Limousin
178731	HAPPY VALLEY X4	303	305	Hetero Polled	Polled	Limousin
178732	HAPPY VALLEY X5	303	310	Hetero Polled	Polled	Limousin
178733	HAPPY VALLEY X6	303	303	Homo Polled	Polled	Limousin
178734	HAPPY VALLEY X7	303	303	Homo Polled	Polled	Limousin
178735	HAPPY VALLEY X8	303	303	Homo Polled	Polled	Limousin
179373	Kangaroo Flat Blossom	305	310	NC	Polled	Limousin
179374	Kangaroo Flat Daisy	305	305	NC	Polled	Limousin
179375	Kangaroo Flat Belle	303	303	Homo Polled	Polled	Limousin
179376	Kangaroo Flat Buttercup	303	303	Homo Polled	Polled	Limousin
179377	Kangaroo Flat Honeypot	305	307	NC	Polled	Limousin
179378	Kangaroo Flat BigJim	305	305	NC	Polled	Limousin
179379	Kangaroo Flat LittleJim	303	310	Hetero Polled	Polled	Limousin
180352	Kangaroo Flat Buck	305	305	NC	Polled	Limousin

Figure 4.1.1. Screen shot of interface to the database (animal names are pseudonyms).

All phenotype and CSAFG29 allele data held by the project team have been loaded into the database. In addition, genotypes at other markers in the vicinity of the polled locus have been loaded. Pedigree data have been loaded for the Limousin breed.

Associations between marker alleles and polled were estimated from the data and a stand-alone piece of software developed to estimate polled genotype on tested animals. The estimated associations are breed specific. The software written to estimate polled genotype from marker genotype has been made available to AGL, and is being used to estimate polled genotype for commercial samples. The software comes with breed specific parameter files.

4.2 Development of a pedigree augmented test

As noted in Section 3.2, this activity was not pursued.

4.3 Testing of markers in close proximity to marker CSAFG29

Table 4.3.1. Details of the 32 microsatellites used on the 80 Limousin bulls, along with the number of alleles and the significance level of Fisher's exact test for an association between marker genotype and poll genotype.

marker	alleles	p.value
CSAFG33	10	1.11E-06
RP42-553A8_MS1	9	3.35E-04
CSAFG31	2	1.34E-03
CSAFG37	8	1.69E-03
BMS6438	4	5.22E-03
CSAFG38	4	6.00E-03
RP42-67E7-MS1	3	7.11E-03
TGLA49	6	1.35E-02
Unknown	6	1.35E-02
RP42-199N3_MS1	8	1.84E-02
RP42-493P3-MS2	5	4.51E-02
CSAFG34	5	4.95E-02
ARO9	8	7.53E-02
CSAFG22	4	1.29E-01
RP42-249P3_MS1	2	1.49E-01
CSAFG24	2	1.54E-01
RP42-249E18_MS2	7	1.71E-01
CSAFG28	4	2.06E-01
RP42-543J10_MS1	2	2.11E-01
CSAFG26	3	2.77E-01
CSAFG27	3	2.77E-01
AR024	3	4.40E-01
CSAFG25	1	1.00E+00
CSAFG30	1	1.00E+00
CSAFG32	1	1.00E+00
CSAFG35	4	1.00E+00
CSAFG36	2	1.00E+00
IFNAR1	2	1.00E+00
RP42-249E18_MS1	4	1.00E+00
RP42-249E18_MS3	2	1.00E+00
RP42-351B8-MS2	2	1.00E+00
RP42-351B8_MS1	4	1.00E+00

In Figure 4.3.1 the associations between diploid marker genotypes and polled haplotype for the 11 most promising markers are shown graphically. There is one row for each bull and two columns for each microsatellite. Bulls with polled haplotype 303P305H (i.e. heterozygous at the polled locus) appear in the top 15 rows, and bulls with polled haplotype 303P305P (i.e. homozygous polled) appear in the lower 13 rows. Within the two columns for each marker, alleles usually associated with polled are shaded yellow or green and alleles usually associated with horns are shaded blue or pink. The greater allelic diversity in the 303P305H bulls is consistent with the recent Angus origin of 305P hypothesis, but in general the results are not particularly easy to interpret if we assume that all of these bulls carry a 303P haplotype. For many of the markers, such as for CSAFG33, the most significant marker found with Fisher's exact test, no allele is common in both groups. At others, such as CSAFG31, one allele (203) is present in all animals, and therefore potentially linked to the 303P haplotype, but it is the animals that are homozygous polled (haplotype 303P305P) that are heterozygous at the marker.

Figure 4.3.1. Diploid alleles for the most promising 11 microsatellite markers, along with CSAFG29 haplotype (303P305H or 303P305P). Each bull appears in one row, and there are two columns for each marker. Shading is consistent only within marker, and is to illustrate allele differences between CSAFG29 haplotype.

CSAFG29 haplotype	BMS6438		CSAFG31		CSAFG33		Unknown		CSAFG37		CSAFG38		RP42-199N3_MS1		RP42-493P3-MS2		RP42-553A8_MS1		RP42-67E7-MS1		TGLA49	
303P305H	255	257	203	203	76	85	197	199	241	241	312	319	160	160	165	174	222	226	116	116	166	168
303P305H	255	257	203	203	76	85	199	199	241	241	312	319	158	160	165	176	213	222	116	116	168	168
303P305H	255	257	203	203	76	85	199	199	241	241	312	319	160	160	165	165	222	226	114	116	168	168
303P305H	255	257	203	203	85	89	199	199	241	242	312	319	158	160	165	176	213	232	116	118	168	168
303P305H	255	257	203	203	85	89	199	199	241	242	312	319	160	160	165	165	226	232	114	118	168	168
303P305H	255	257	203	203	85	93	199	199	240	240	312	319	160	162	165	165	222	226	114	116	168	168
303P305H	255	257	203	203	85	93	199	199	241	242	312	319	160	162	165	165	220	226	114	116	168	168
303P305H	255	257	203	205	76	85	195	197	239	241	312	319	151	160	174	174	213	226	116	116	164	166
303P305H	257	257	203	203	76	89	197	199	242	242	312	312	160	160	165	174	226	232	116	118	166	168
303P305H	257	257	203	203	85	93	199	199	241	242	312	321	158	160	165	176	220	226	116	116	168	168
303P305H	257	257	203	203	89	91	197	197	240	242	312	312	160	164	165	174	224	226	114	116	166	166
303P305H	257	257	203	203	89	91	197	199	242	242	312	312	158	164	165	177	213	224	114	114	166	168
303P305H	257	257	203	203	89	91	197	199	_	_	312	312	158	164	165	177	213	224	114	114	166	168
303P305H	257	257	203	203	91	93	199	199	240	240	312	312	160	160	165	176	222	228	116	116	168	168
303P305H	257	257	203	205	76	89	199	199	242	243	312	312	160	160	165	165	220	232	116	118	168	168
303P305P	257	257	203	203	76	76	199	199	241	242	312	312	153	160	165	174	222	224	116	116	168	168
303P305P	257	257	203	205	76	76	195	199	239	242	312	312	153	158	165	174	222	224	114	116	164	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	312	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	312	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	312	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	312	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	312	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	321	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	76	199	199	241	243	312	321	160	160	165	165	220	222	116	116	168	168
303P305P	257	257	203	205	76	89	199	199	242	243	312	312	160	160	165	165	220	232	116	118	168	168
303P305P	257	257	203	205	76	93	192	199	242	243	312	312	153	160	165	165	220	226	116	116	162	168
303P305P	257	257	203	205	76	93	199	199	242	243	312	312	160	160	165	165	220	220	116	116	168	168
303P305P	257	257	203	205	76	93	199	199	242	243	312	313	160	160	165	165	220	220	116	116	168	168

Breed	H	P	S	CSAFG29	BMS6438	CSAFG31	CSAFG33	CSAFG37	CSAFG38	RP42.199N3_MS1	RP42.493P3.MS2	RP42.553A8_MS1	RP42.67E7.MS1	TGLA49
All	121	186	7	2.79	4.38	3.22	6.00	6.00	5.70	3.39	1.13	1.93	2.65	2.61
Angus	0	55	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brahman	5	7	1	0.59	0.60	1.52	0.25	1.76	0.23	0.23	0.23	1.11	0.53	0.02
Charolais	36	2	1	0.45	0.64	3.38	1.65	0.94	1.02	0.03	0.38	0.27	0.52	0.15
Droughtmaster	5	5	2	0.17	0.00	0.07	0.37	0.02	0.70	0.08	0.84	0.33	0.09	0.04
Hereford	40	8	0	0.55	2.06	0.51	3.31	5.04	4.75	0.54	0.24	3.12	0.35	4.37
Limousin	1	54	1	0.46	1.56	0.93	1.49	0.32	0.80	2.05	2.32	1.09	0.47	0.60
Santa Gertrudis	5	7	2	0.00	0.89	0.68	0.83	0.55	0.62	0.47	1.22	0.18	0.00	0.02
Shorthorn	0	45	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Simmental	29	3	0	0.00	0.50	3.21	3.19	3.39	0.50	0.00	0.00	1.08	0.24	0.00

Table 4.3.2. Counts of horned (H), polled (P) and scurred (S) bulls from 9 breeds, and significance levels for a test of association between phenotype and genotype at 11 microsatellite markers. Significance levels are expressed as –log10(p.value), where p.value was estimated using Fisher's exact test, except for when all breeds were included, when p.value was estimated through simulating 1,000,000 random replicates under the null hypothesis. Cells are shaded when the test is significant at the 1% level (-log10(p.value) = 2).

Table 4.3.2 contains a summary by breed, with a test (expressed as –log10(p.value)) of significance for an association between marker allele and phenotype for the 314 bulls from 9 breeds. Most of the markers show significance when all breeds are analysed together, but this may be due to a confounding of breed and phenotype in these data. For example, a difference in allele frequency between Angus and Charolais will cause a significant association between phenotype and marker, as all of the Angus are polled and most of the Charolais are horned in these data. Within many of the breeds there is insufficient variation in phenotype to detect associations with genotype: this was deliberate. As Angus is a possible source of polled in any breed that has recently bred up from an Australian cow base, an understanding of the alleles segregating in Angus can assist with the interpretation of the results. In other breeds it was our choice of animals with at least one 305 allele at CSAFG29 that skewed the distribution of phenotypes. For 4 breeds at least one marker was significant at the 1% level (-log10(p.value) = 2), but none were significant at the 1% level for more than two breeds.

It is clear that no marker alone (apart from CSAFG29) is highly predictive of polled. Combining the markers into haplotypes produced much better results. After dropping markers, 7 remained that produced 41 P haplotypes, 120 H haplotypes and 54 haplotypes of unknown association. Note that our prior knowledge about CSAFG29 was ignored in this analysis. Most P haplotypes had 303 or 305 at CSAFG29, but some had 299, 307, 310, 311, 313, 314. Almost all of the unknown haplotypes are at very low frequency. Only one P haplotype ever occurs in a horned animal (a Droughtmaster – this could be a phenotyping error). There are 2 polled Herefords that are homozygous HH, again these could be phenotyping errors or it could be that too many markers were removed from the panel.

In Limousin, 3 haplotype alleles, each having allele 305 at CSAFG29, were common. Two of these only occurred in polled animals (79 observed haplotypes, never observed in a horned animal) and one occurred 19 times in polled animals and 18 times in horned animals. Together these three haplotype alleles represented 66% of the haplotypes seen in Limousin with a 305 allele at CSAFG29. Of the 176 haplotypes with 305 at CSAFG29 observed in Limousin, only 8 could not be assigned to an allele either associated with polled or with horns, these 8 only appearing in polled animals.

The associations between haplotype alleles and polled genotype are consistent across breeds. For example, the most common haplotype allele that is associated with polled occurred 74 times, in Angus (8), Brahman (2), Charolais (2), Droughtmaster (6), Limousin (52), Santa Gertrudis (1) and Simmental (3). In one case (the Droughtmaster noted above) this was in an animal with a phenotype of horns, in 4 cases in animals with a phenotype of scurred, and in the remaining 69 cases, in polled animals.

The 314 animals in this population were chosen to be those where the CSAFG29 marker was inconclusive, i.e. in animals carrying a 305 allele. As such they are not a random sample, limiting the conclusions that can be drawn from these data. However, this is still an outstanding result. Most haplotypes that carry 305 at CSAFG29 are resolved, only 29 out of 198 Limousin haplotypes remain ambiguous, and the haplotype associations are consistent across breeds.

4.4 Optimisation of the Test

The main result of this study was to show that, without progeny testing, unless the test is 100% accurate, the herd will never become free of the horned allele. If the accuracy of the test is a%, then the frequency of the polled allele will asymptote at ((100 + a)/2)%. So, unless the test is close to 100% accurate, a progeny test may be required for bulls that are not known to be homozygous polled by pedigree. Thus there are considerable potential savings to industry if the test can be made more accurate. The full report is appended (Appendix 1).

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.

If the haplotype based test performs as well in a validation population, then it is far superior to the test based on CSAFG29 alone. The hypotheses taken into the project – that 305 alleles at CSAFG29 show associations with both polled and horned, and that these associations can be explained by the recent origin of the allele, is supported, but the picture is much more complicated, and no other marker on its own or with CSAFG29 will be enough to provide a robust test, a haplotype is required.

If pedigree information is available it will be possible to reduce the number of animals that require testing, as the genotypes of some animals will be able to be inferred from pedigree, phenotype and test results on other animals. If validated, this test will work successfully in all breeds 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, if validated, will be far 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 could eventually be polled, and without scurs.

7 Conclusions and Recommendations

In Australian beef cattle, a haplotype of microsatellite markers appears to offer a highly predictive test for polled in the breeds tested. An immediate priority will be to validate the test on a larger population. Further, as the analysis is more complicated than for a single marker test, it will be necessary to develop software to maximise the information obtained from the marker test.

If validated, it is recommended that a test be commercialised and made available to industry.

8 Bibliography

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9 Appendices

9.1 Appendix 1 Introgressing the Polled Locus into a commercial herd: the affect of the accuracy of a test. Bruce Tier

Polled animals are preferred to horned animals for many reasons. In most European breeds there is a single locus that controls hornedness in cattle, and the allele for horns is recessive. This means that only animals that are homozygous for the horned allele will have horns and animals that are heterozgyous will not. The situation is not as clear in Tropical cattle, and some speculation remains about the control over hornedness. With the financial support of MLA, the Beef CRC has developed a test for the polled locus which is based on a locus tightly coupled to the controlling gene. It is highly reliable in most breeds, but not in all. For some breeds, including Limousin and Brahman, the test provides only a probability that the allele is horned. The recent development of haplotypes may provide a more reliable test for the ambiguous breeds. Increasing reliability of the test will influence the rate at which a herd can change being completely horned to being polled.

To illustrate the effect of different accuracy of a test on the introgression of the polled gene a model herd of 1000 cows was used. The age structure of the herd was held constant and is shown in Table 1. The calving rate shown in Table 1 relates to heifer calves only, and only those that survive until one year old. Each year 80% of the females in one age group are transferred to the next age group, with all cows being culled after 7 years.

Table 1: Age structure of cows in model herd.

Age group (years)	1	2	3	4	5	6	7
Cows	342	273	218	175	139	112	83
Proportion	0	0.27	0.22	0.17	0.14	0.11	0.08
*Heifer Calving rate	0	0.28	0.37	0.37	0.37	0.37	0.37
Numbers heifers born	0	75	80	64	51	41	31

* Heifers only of interest.

Three scenarios were modeled where selection of polled sires was based on a gene test that was either 80, 90 or 100% accurate. No selection of polled cows was undertaken. In all cases bulls were purchased from other herds.

Young bulls sired half the calves each year, with (progeny tested) homozygous poll older bulls siring the other half. For the cases where the test was less than 100% accurate, young polled sires could be either homozygous or heterozygous. The frequency of the genotypes in the population was assumed to be p^2 and 2pq (respectively, where p is the frequency of the polled allele). This implies that the producer is acquiring young bulls from seedstock herds with a similar objective. Thus homozygous polled bulls are a $p^2/(p^2+2pq)$ proportion of all polled bulls. Table 2 illustrates the proportion of homozygotes amongst all polled animals given different allele frequencies. Unsurprisingly, the frequency of the polled gamete is high in polled animals and increases with the increasing frequency in the population. The proportion of polled animals that are homozygous is very low when the polled allele is at low frequency when most polled animals will be heterozygotes. Consequently, when the desirable allele is at low frequency, a two-stage selection strategy is desirable so that homozygous polled animals can be selected in the second stage. Whenever the test is less than 100% accurate, progeny testing will be necessary to eliminate the horned allele from the population. Without progeny testing, the less accurate tests

will result in a low frequency of the horned allele so that only a handful of horned calves are born each year (see Table 2). Without a two-stage selection process the frequency of the polled gene in the population is limited to the accuracy of the test.

The most efficient design of a progeny test for polled bulls would be to mate bulls so that they had n progeny from horned cows. When all progeny are polled, the probability of the sire being homozygous is $1-(1/2)^n$. If any of the progeny are horned then the bull must be heterozygous.

Table 2: Relationship between allele frequencies, the proportion of homozygous polled individuals in the polled population and proportion of horned animals with allele frequency in the population.

	Frequency of polled locus (p) in the population								
P	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
P in Polled animals	0.53	0.56	0.59	0.63	0.67	0.71	0.77	0.83	0.91
Proportion of polled animals that are homozygotes	0.05	0.11	0.18	0.25	0.33	0.43	0.54	0.67	0.82
Proportion of horned animals	0.81	0.64	0.49	0.36	0.25	0.16	0.09	0.04	0.01

Table 3 Allele frequencies for cows and calves for the three different cases of introducing polled sires into a fully horned herd.

Years since first introduction	Accuracy of test					
	80		90		100	
	Calves	Cows	Calves	Cows	Calves	Cows
1	0.45	0	0.47	0	0.5	0
2	0.45	0	0.47	0	0.5	0
3	0.5	0.1	0.53	0.1	0.55	0.11
4	0.55	0.2	0.58	0.22	0.61	0.23
5	0.6	0.3	0.63	0.32	0.67	0.33
6	0.64	0.39	0.68	0.41	0.72	0.43
7	0.69	0.48	0.73	0.5	0.76	0.53
8	0.73	0.55	0.77	0.59	0.81	0.62
9	0.75	0.6	0.79	0.63	0.83	0.66
10	0.77	0.64	0.81	0.68	0.86	0.71
15	0.84	0.79	0.89	0.83	0.94	0.88
20	0.88	0.85	0.92	0.9	0.97	0.95
25	0.89	0.88	0.94	0.93	0.99	0.98
30	0.9	0.89	0.95	0.94	0.99	0.99
35	0.9	0.9	0.95	0.95	1	1