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Genetic markers for polled, African Horn and Scurs genes in tropical beef cattle

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Abstract

The objective of this project was to develop gene marker tests for the Polled, Scurs and African Horn genes. A microsatellite marker was found that is very closely associated with the polled locus in Brahman, Santa Gertrudis, Hereford, Droughtmaster and Limousin. In these breeds animals homozygous for the allele associated with polled almost never have horns or scurs, so no additional test for the scurs or African horn gene is required. In Tropical Composite cattle, those that are homozygous for the allele associated with polled are most likely to be polled, but also may be horned or scurred. This may be due to incomplete association, or due to another region of the chromosome such as the hypothesised African Horn gene. The marker test will 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. At the beginning of this project the inheritance of horns was reasonably well understood in *Bos taurus* breeds, with the Polled locus mapped to chromosome 1 and marker tests becoming available. However, these tests were developed using northern hemisphere cattle, and were not validated in Australian herds. Further, there was evidence that additional genes might affect horn development in cattle with Zebu or Sanga genetics, so the tests might be of limited value in many northern Australian beef herds. The objective of this project was to develop gene marker tests specifically for beef cattle in northern Australia, to facilitate breeding for polled, leading to the phasing out of the practice of dehorning.

The objective to develop gene marker tests for polled in northern beef cattle was approached through two major studies. First, a gene expression study, where tissue was sampled from the heads of calves at the time of differentiation between horned and polled. Gene expression levels for calves that went on to become polled were compared to levels for calves that went on to become horned in an attempt to discover which genes were responsible for the difference. Second, a mapping study where the genome of horned animals was compared to the genome of polled animals in an attempt to discover differences diagnostic of horn/polled status. Both studies were successful, and in the mapping study a marker was found with excellent potential for use as a test for polled in northern Australian beef herds.

The association between the marker and horn/polled status was discovered in Brahman cattle, and subsequently validated in a range of breeds. The Table below summarises the key results for the validation, and does not include animals used in discovering the association. Of animals found to be homozygous for the allele associated with polled (PP), in most breeds all or almost all are polled.

Breed	Total Tested	PP			
		Polled	Scurred	Horned	Power
Brahman	399	51	1	0	98%
Santa Gertrudis	185	8	0	0	100%
Hereford	191	45	0	0	100%
Droughtmaster	94	14	0	0	100%
Limousin	52	10	0	0	100%
Brangus	96	26	4	1	84%
Tropical Composite	247	43	8	9	72%

We also found that in Brahman, Santa Gertrudis, Hereford, Droughtmaster and Limousin, 90% of animals that were scurred were also heterozygous for the marker (77% in Brangus and Tropical Composite). The proportion of heterozygous animals were polled, scurred or horned depended

on the breed. If bulls that have been tested and shown to be homozygous PP are used in horned beef herds producing heterozygous progeny, in all breeds there will be a reduction in the proportion of horned calves, in some breeds there will be an increase in the proportion of scurred calves, and in all breeds there will be an increase in the proportion of polled calves. Over time, as the frequency of heterozygous and then homozygous PP cows increases, calves will be born overwhelmingly polled.

The existence of another gene, the African horn gene, has long been hypothesised for Zebu and Sanga cattle. In Brahman cattle we found that variation at the polled locus was sufficient to explain the presence or absence of horns, there was no evidence of another gene. The Tropical Composite cattle in our validation population contained Sanga genetics, and had a significant proportion of horned animals amongst those homozygous PP. This could be due to another gene, such as the African horn gene, or due to incomplete association between the marker and the locus causing the variation. This does not mean that the marker has no utility in Sanga breeds, but a higher proportion of animals that are homozygous for the allele associated with polled will be horned or scurred than in the other breeds tested.

The marker test developed in 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. The marker test could potentially be delivered for $20 to $40 per bull. If the test is adopted there will be an immediate reduction in the need for dehorning and, if and used widely and persistently, the whole herd could eventually be polled, and without scurs. It is recommended that a test be commercialised and made available to industry.

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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.

The inheritance of horns has long been of interest to cattle breeders and geneticists as an example of a sex affected dominant trait, and presumably due to a small number of genes. At the commencement of the project, in *Bos taurus* cattle the general mechanism was understood to be that variation was due to two loci, the Polled gene with alleles P and p, and the Scurs gene with alleles Sc and sc. To be horned required two copies of the p allele at the Polled gene, and animals with one or more copies of the P allele were either polled or scurred (loosely attached horns or small bud like structures), depending on sex and genotype at the Scurs gene. Most variation observed in the transmission of the horned phenotype could be explained by this model. However, exceptions to this pattern were observed, particularly in Zebu cattle and Sanga cattle, and in cattle with Zebu or Sanga ancestry. This lead to the hypothesis that a third gene, the African Horned gene, was segregating in these cattle, with alleles Ha and ha. Allele Ha, rare in *B. taurus* of British or European origin, caused horns in cattle that would be expected to be polled or scurred given their genotype at the Polled and Scurs genes. The genotype combinations and the resulting phenotypes described by Georges *et al* (1993) are in Table 1.1.

Genotype	Males	Females
Inheritance of the scurs (Sc) phenotype		
P/- Sc/Sc	Scurred	Scurred
P/- Sc/sc	Scurred	Polled
P/- sc/sc	Polled	Polled
p/p -/-	Horned	Horned
Epistatic effect of the African horn (Ha) gene		
P/- Ha/Ha	Horned	Horned
P/- Ha/ha	Horned	Polled
P/- ha/ha	Polled	Polled
p/p -/-	Horned	Horned

Table 1.1. Hypothesised genetic mechanism for the inheritance of horns, scurs and polled (Georges *et al.* 1993).

By the commencement of this project it was understood that DNA sequences differences responsible for genetic variation do not always occur in genes, so instead of Polled, Scurs and African Horn genes the terms Polled locus, Scurs locus and African Horn locus are used in this report. The location of the Polled locus had been mapped to the proximal part of bovine chromosome 1 by several groups (Brenneman *et al*. 1996; Brockmann *et al*. 2000; Georges *et al*. 1993) and was subsequently fine mapped to a 1Mb interval between two microsatellite markers, RP42-218J17_MS1 (at the centromeric end) and BMS6438 at the telomeric boundary of BTA1 (Drogemuller *et al*. 2005a; Drogemuller *et al*. 2005b). The Scurs locus was mapped to BTA19 in *B. taurus* animals (Asai *et al*. 2004). This study was based on a QTL mapping experiment involving only 6 families. Traditionally, the scurs expression was reported as sex-influenced. Consequently, male cattle need only one allele for scurs to exhibit the trait, whereas females need

two alleles (Long and Gregory 1978). During this project this mode of inheritance was questioned by researchers at INRA (Capitan *et al*. 2009). Their study conducted in French Charolais cattle found neither autosomal dominant mode of inheritance nor sex effect at the Scurs locus; instead they postulated that scurs may be recessively inherited, at least in Charolais. They also reported lack of evidence for any significant linkage between the scurs locus and the BTA19 region reported by Asai *et al*. (2004).

With the fine mapping of the Polled locus, gene marker tests for polled were coming onto the market. However, these were developed using northern hemisphere cattle and had not been validated in Australian herds. In particular their efficacy in cattle derived from *B. indicus* and African *B. taurus* was unknown. No gene marker tests for the Scurs or African Horn loci were available.

2 Project objectives

The objective of this project is to provide, for Australian cattle producers, a non-invasive welfare friendly alternative, to the practice of dehorning. This will be achieved by developing gene marker tests for the Polled, Scurs and African Horn loci, tests validated in Australian cattle herds. These tests will facilitate the introgression of polledness in tropical beef cattle, such that the practice of dehorning can be phased out.

3 Methodology

Three complementary approaches to developing an understanding of the inheritance of the various horn phenotypes were concurrently pursued.

3.1 Segregation Analysis

As described in section 1 and Table 1.1, the inheritance of the polled / horned condition in cattle has been hypothesised to be under the influence of three genes: Polled (P), African Horn (Ha) and Scurs (Sc) (Georges *et al*. 1993). Variations on this hypothesis exist, such as that of Long and Gregory (1978), which differs in that Sc/sc males express the scurred phenotype only when heterozygous P/p. The African horn gene, as the name suggests, is believed to be rare in British breeds but supposedly at a higher frequency in Zebu cattle. The African horn gene is believed to be segregating independently but with an epistatic effect on the Polled locus, and is sex–influenced in its inheritance. To test hypothesised modes of inheritance and to ascertain the presence of African Horn gene in the tropical beef cattle, segregation analyses of historical Belmont Research Station data on horns status was conducted. The full report on this analysis, prepared by Bruce Tier, AGBU, is attached (Appendix 1).

In a segregation analysis study, the phenotype (in this case horned, polled or scurs) is jointly examined for sire, dam, calf trios, and hypothesised modes of inheritance tested for consistency with observed phenotype. This study made use of historical records from the Belmont Research Station, covering the period from 1973 to 2000. There were 6,587 animals that had phenotype records on themselves and their sire and dam. Hence, this subset of the data was used for segregation analysis. Breeds (number of trios) included Hereford (905), Brahman (539), Africander (159), Belmont Red and Belmont Red cross (3863). It should be noted that although these were well phenotyped research farm cattle, at the time the research priority was not horns, and so they would have been dehorned at a young age. In young animals horns cannot always be distinguished from scurs, so it is likely that some phenotypes are incorrect. Furthermore, errors in the pedigree and in the assignment of breed codes may have occurred.

A range of hypothesised modes of inheritance were tested, including those of Georges *et al*. (1993) and Long and Gregory (1978). More complicated models, allowing more than 2 alleles at each locus, were also tested.

3.2 Gene Expression Study

Differential gene expression at the horn region of new born calves would information in the search for the genes affecting horns inheritance. Hence, one of the approaches to complement gene mapping studies is to explore the possibility of identifying the genes associated with horn development through differential gene expression among polled, horned and scurred animals. The key to the success of this study is the sequential tissue sampling from the horn region on the skull of the polled, horned and scurred calves early in their life. As horn growth starts during peri-natal period and the specific time of development is unknown and presumably variable, it is important to collect sequential samples for studying the gene expression differences.

3.2.1 Selection of animals

Six potential horned calves ('06 drop) from Beef CRC resource population (Project 4.1.3b - Life time reproduction) were included in the sampling. These calves were selected based on their mother's horn status information. However, selection of cows with a potential to produce polled and scurred calves is difficult solely based on the breeding history because of the multi-sire matings at Belmont Research Station. Hence, pregnant cows with a potential to produce polled and scurred calves were obtained from two polled Brahman breeders. Pregnancy was diagnosed on 10 cows from each property and these pregnant cows were transferred to Belmont Research Station during the month of September 2006. The calvings and the subsequent sample collection started from November 06 onwards.

3.2.2 Tissue sampling

The collection of tissue samples from the calves was started soon after their birth. Each calf was weighed and tagged at birth and the first sample was taken within three days of age in most cases. Then onwards, all the calves were mustered for sampling on an assigned day every week. The final samples were taken between 8 to 10 weeks of age providing around 8 sequential samples for each calf. In the horned calves, sampling was stopped as soon as the horn growth became apparent. After final tissue sampling of each calf, a blood sample (for DNA extraction) was taken from the jugular vein.

The tissue samples were taken after proper restraining of calves in a veterinary crush. A sub-cutaneous injection of local anaesthetic was given (~2 mL of lignocaine) and the sampling site (horn region on the skull) was prepared by shaving the area. When local anaesthesia is established (~10 min), a tissue sample was collected using a 'Paramount ® Sterile Dermal Biopsy Punch' (Fig 1). The sample from one side of the head was immersed in RNAlater® solution and transferred to the lab for storage at -20 degrees Celsius. The sample from the other side of the head was taken and immersed in 70% formaldehyde for histological studies. After the sampling, the sample site was sprayed with disinfectant solution and the calf was released to its mother.

3.2.3 Microarray experimental design

Sequential tissue sampling from skull region of new born horned (6), potential polled (9) and potential scurred (8) calves was conducted for microarray experimentation to identify the differential gene expression across the phenotypes. It was decided to concentrate on peri natal samples i.e. samples taken between the ages of 1 to 9 days after birth. A final list of 11 animals (13 samples with 2 control samples) was drawn based on quality check of all samples on the Agilent 2100 Bioanalyzer (Table 3.1).

	Male	Female
Horned	2	1
Polled	1	3
P-control		1
Scurred	3	1
S-control		1

Table 3.1. Number of samples included in the microarray experiment
*control refers to a sample taken from the central head region away from the horn region (horn region sample is also available from the same animal)

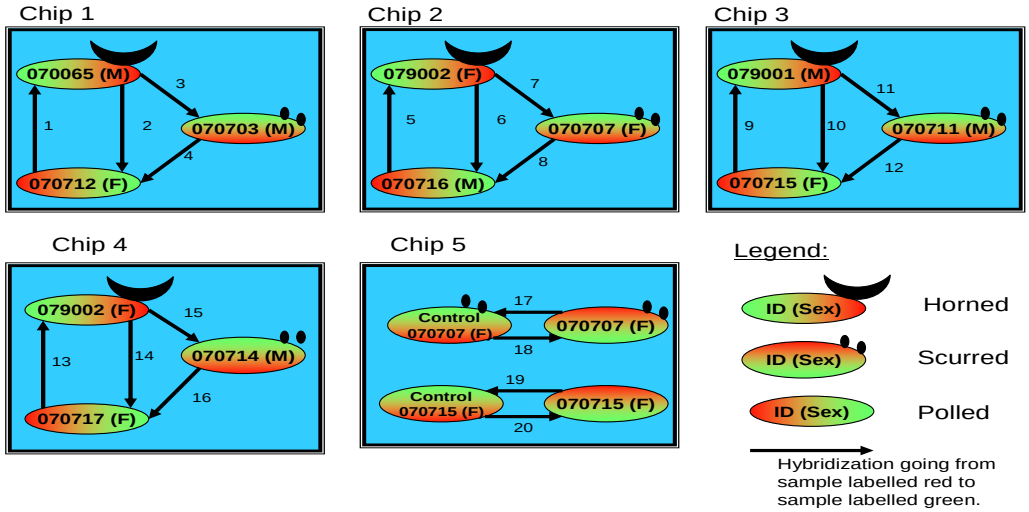

Fig 3.1. Experimental design for the gene expression study

The experimental design based on selected animals is shown in Figure 3.1. The design used 5 Agilent chips each containing 4 microarrays. For the first four chips, the conditions of horned, polled and scurred are be compared across the 2 sexes (ie. no confounding between sex and chip) and using RNA samples from independent individuals (ie. no confounding between individual and chip). There was a greater emphasis (ie. more hybridisations) in the more important contrast of horned versus polled. Also, care was taken to balance dye channel across samples so that no sample was labelled with the same dye of the two (case of scurred) or three (case of polled and horned) hybridisations in which it intervenes. Finally, the last chip was dedicated to the control versus horn site comparison within individual.

The proposed design will yield 8 pieces of information for each chip (coming from 4 arrays and 2 dyes) distributed across two sexes (all but chip 5 where all information comes from females) and three of the four possible conditions (Ho: horned; Po: polled; Sc: scurred; Co: control). The experimental design and the samples were submitted to the commercial provider (SRC Microarray facility, University of Queensland) for microarray services using the Agilent dual array platform. The results were obtained and analysed to identify significantly differentially expressed (DE) genes.

The analysis of the data was conducted in a twostep process. In the initial step, data from 16 arrays from first 4 chips were analysed and in the second step 4 arrays from 5th chip (control) were analysed to obtain various sets of differentially expressed genes (probes). The analysis was conducted using a mixed model approach with array, chip, dye and sex as fixed effects; probe, probe*treatment, probe*sex and residual as random effects. The BLUP solutions for each

probe*treatment interaction from this analysis were of greater interest and were used to generate necessary contrasts. The significance levels for identifying differentially expressed genes were determined based on model based clustering through mixtures of distributions. The DE probes from the control chip also provided information on biological significance of this identified differential expression.

3.3 Gene Mapping Study

The gene mapping study took place in 3 distinct phases, each with a different animal resource.

3.3.1 First Whole Genome Scan (WGS)

The animals for WGS were selected (Table 3.3.1.1) based on their unrelatedness from two breeds, Brahman (*B. indicus*) and Hereford (*B. taurus*). Even though the primary interest in the study was *B. indicus* cattle and the African horn gene, a small number of Herefords were included to provide necessary contrast for analyses. The logic for using more Brahmans than Herefords is because of the additional complexity in the inheritance of horns status in Brahmans. Due to the sex influenced expression of African horn gene, the design confounded sex and horns status in Brahmans to reduce the number of possible genotypes. However, in Herefords only a single gene hypotheses is presumed and thus both sexes were represented in equal numbers.

Breed	Sex	Horn status	No.	Source
Brahman	Female	Horned	34	CRC 2.3
Brahman	Male	Polled	34	Various sources (CRC, Hillgrove, Kenilworth)
Hereford	Male	Polled Horned	5 5	From various farms around Brisbane
Hereford	Female	Polled Horned	5 5	
Total			88	

Table 3.3.1.1. Breed and sex specific details of animals involved in the initial WGS

Several Hereford samples were collected from farms around Brisbane and were checked against Hereford database to short list as many unrelated individuals as possible. Consequently 20 Hereford polled and horned samples were selected for WGS. Blood samples from polled male Brahmans were collected from across the industry and research herds. Beef CRC resource population consisted of predominantly horned animals and hence the required numbers (34) of horned Brahman female samples were sourced from this population. These samples were selected based on their unrelatedness (from different sire families).

Isolated DNA from all animals was quantified using the Nano Drop and was diluted to an even concentration. Where the quantity of DNA was insufficient to meet Affymetrix's requirements for the SNP assay, additional round of extraction was carried out on the necessary samples. Quality was checked for all samples for even concentration by a PicoGreenTM assay and also tested for PCR amplification through TaqmanTM assay. As per Affymetrix guidelines, samples were plated on to a 96 well plate and shipped to genotyping lab, Affymetrix Inc., California, USA for whole genome amplification and subsequent genotyping with Affymetrix GeneChip Bovine 25 K panel and 11.5K panel. The submitted samples also included 3 additional samples as controls.

The genotyping results were obtained in the last week of September 2007. The SNP genotypes were obtained for 89 samples on the 25K panel and for 91 samples on the 11.5K panel. On the 25K panel, 23571 assays passed out of 25068 assays attempted and on the 11.5K panel, 10626 assays passed out of 11180 assays attempted. Further, sex as determined by X-chromosome

markers for 2 samples did not match with the recorded sex and hence were not included in the analyses. This could be due to contamination of samples.

3.3.2 Fine mapping for Polled locus in Brahman and other breeds

This study represents the first attempt to evaluate the polled haplotype in *B. indicus* cattle. The intention behind the study was to gain a better understanding of the Polled locus in Brahman cattle, thus facilitating the mapping of African Horn locus, Scurs locus, or both.

The polled locus has been mapped to the proximal part of bovine chromosome 1 by several groups (Brenneman *et al*. 1996; Brockmann *et al*. 2000; Georges *et al*. 1993) and was subsequently fine mapped to a 1Mb interval between two microsatellite markers, RP42-218J17_MS1 (at the centromeric end) and BMS6438 at the telomeric boundary of BTA1 (Drogemuller *et al*. 2005a; Drogemuller *et al*. 2005b). Published microsatellite markers (Drogemuller *et al*. 2005a) in the region were ordered and genotyped in the WGS animals. Additional SNP information for markers in this region were used to supplement the microsatellite markers as illustrated in Figure 3.3.2.1 giving a total of 17 microsatellites and 37 SNP across a 4.9 Mb region. Haplotypes were derived using PHASE (Stephens *et al*. 2001).

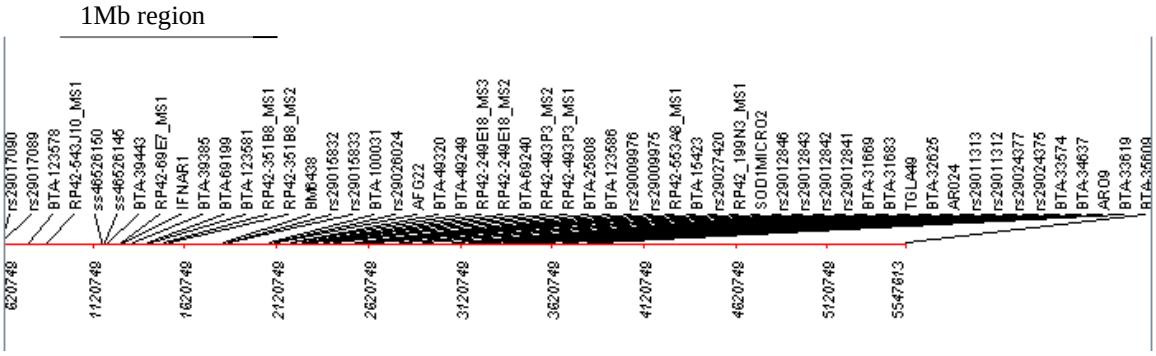

Figure 3.3.2.1. Markers used to fine map the polled region between Btau4.0 coordinates 620478 and 5547613 (4.9 Mb) at the centromeric and telomeric ends respectively.

The first use of the markers was on animals used in the Whole Genome Scan study (Section 3.3.1), referred to here as the discovery population. Two further animal resources were developed to validate the results and one historical population was also used for validation.

For the first validation population, referred to here as the Hillgrove Resource population, a breeding program was designed to generate ~200 to 250 Brahman calves from 15 sires through AI and subsequent use of mop-up bulls. This breeding program was conducted on a collaborating breeder's property, Hillgrove Pastoral Company (Charters Towers, QLD) in conjunction with a University of Queensland research project aiming to test new synchronisation protocols under the leadership of Dr. Michael McGowan. In November 2006, 400 Brahman heifers (243 horned, 102 polled and 55 scurred) were selected based on condition score and reproductive tract condition. These heifers were assigned to 10 polled AI sires and 5 polled mop-up bulls to produce various phenotypes and possible combinations of various alleles at the hypothesised loci. These bulls and semen were sourced from Kenilworth Brahmans and from Belmont Research Station. A total of 234 calves were produced and accurately phenotyped for horns status. Originally it was intended to perform a whole genome scan on this population, but it was decided that this was unnecessary given the results of the fine mapping study.

The second validation population targeted the following breeds: Brahman, Santa Gertrudis, Hereford, Droughtmaster, Limousin, Brangus and Angus. The number of animals sampled varied across breeds depending on the relative frequency and importance of polled condition, ease of sourcing animals and the ability to obtain accurate phenotypes. In total, 1045 animals (Table 3.3.2.1) were genotyped and marker genotypes were matched against their recorded phenotypes.

	Properties	Horned	Polled	Scurred	Total
Brahman	4	176	87	136	399
Santa Gertrudis	2	102	52	31	185
Hereford	4	70	95	26	191
Droughtmaster	2	33	38	23	94
Limousin	1	22	29	1	52
Brangus	1	32	37	27	96
Angus	NA	-	91	-	91
					1045

Table 3.3.2.1. Breeds and animals involved in the field validation study

The third validation was conducted using stored DNA from cattle from the Belmont Research Station. There were 247 stored samples from male animals on which horn phenotype was known. Of these, 98 had breed recorded as "AX Belmont Red" and 149 had breed recorded as "ABAB F2", both breeds having some component of Africander, and hence some chance of having a different poll/scur/African horn allele.

3.3.3 Marker association study to map scurs locus on chromosome 19

A pilot study was conducted to ascertain the presence or absence of a scurs locus on BTA19 in Australian populations. The previously reported scurs locus region is flanked by two microsatellite markers BMS2142 and IDVGA46 at the proximal and distal ends (Asai *et al*. 2004). This enveloping region (26 to 29 Mb on BTA19) was saturated with 12 new microsatellite markers, identified from the most recent assembly of the bovine genome. An efficient strategy called M13 tailing was used to reduce the cost of labelling by using a universal fluorescently labelled primer to amplify microsatellite specific sequences (Schuelke 2000). The markers were genotyped in three groups (scurred, polled and horned categories) of 16 Brahman females each. Selection of scurred and polled animals for this study were based on observed heterozygosity at the Polled locus indicated by the marker test developed elsewhere in this project. Two out of the 12 markers, namely S1 and S4 failed to amplify. Genotypes for the ten markers were scored for these 48 animals and the data was analysed in PHASE (Stephens *et al*. 2001) to impute haplotypes for comparison across phenotype categories.

4 Results and discussion

4.1 Segregation Analysis

The full report on this analyses prepared by Bruce Tier, AGBU is attached (Appendix 1). Table 4.1.1 summarises the trio phenotypes across breeds. There is an overall lack of fit for the data for all of the hypothesised modes of inheritance. That is, for any hypothesis, of the 6,587 sire, dam, calf trios, horn inheritance patterns unexpected under the hypothesis were more frequent than would be expected unless the pedigree or phenotyping error rate was high.

Parents		Male Progeny		Female Progeny	
Sire	Dam	Poll	Horn	Poll	Horn
Poll	Poll	592	158	572	199
Poll	Horn	379	293	400	393
Horn	Poll	430	331	326	438
Horn	Horn	94	957	54	971

Table 4.1.1: Numbers of trios by phenotype status of parents, progeny and gender, all breeds.

Accepting that the unexpected phenotypes might be due to pedigree or phenotyping errors allows the estimation of allele frequencies under each hypothesis. None of the models tested provided results that were uniformly good across breeds, so in Table 4.1.2 we present estimates of allele frequency for the hypothesized modes of inheritance of Georges *et al*. (1993) and Long and Gregory (1978). It must be noted that there these estimates are conditional on the model being correct, and there is considerable evidence that the model is not correct. In general, allele P at the Polled locus is at moderate frequency in all but Brahman, where it is at a very low frequency, and allele Ha at the African Horn locus increases with increasing Zebu or Sanga genetics, and is at a high frequency in Brahman and Africander. Allele Sc at the Scurred locus is at low to moderate frequency in all but Hereford and Brahman, where it is at very low frequency, and Africander where it is at a moderately high frequency. We note again that these results must be taken with extreme caution, as there is considerable evidence that the hypothesised models are not correct.

Population	Hypothesis	f(P)	f(Ha)	f(Sc)
All	Georges	0.34-0.46	0.34-0.40	0.20-0.27
	Long	0.25-0.31	0.38-0.49	0.21-0.62
AX Belmont Red	Georges	0.25-0.37	0.0-0.05	0.25-0.36
	Long	0.25-0.34	0.0-0.06	0.34-0.46
BX Belmont Cross	Georges	0.18-0.27	0.20-0.37	0.34-0.41
	Long	0.18-0.25	0.51-0.64	0.50-0.61
HSS Selected Hereford	*Georges*	0.31-0.46	0.0-0.07	0.0-0.07
	Long	0.31-0.43	0.0-0.07	0.0-0.10
HSR Random Hereford	*Georges*	0.33-0.50	0.0-0.10	0.0-0.05
	Long	0.31-0.47	0.0-0.07	0.0-0.05
GA Grade Africander	Georges	0.25-0.40	0.82-0.87	0.70-0.80
	Long	0.45-0.54	0.82-0.87	0.67-0.79
GB Grade Brahman	*Georges*	0.0-0.05	0.80-0.87	0.00-0.15
	Long	0.0-0.05	0.79-0.86	0.00-0.15
ABBA F2 (F2AB0)	Georges	0.34-0.44	0.31-0.37	0.23-0.31
	Long	0.38-0.47	0.34-0.43	0.27-0.36
ABBA F2 (F2AB0)	Georges	0.31-0.43	0.0-0.05	0.18-0.28
	Long	0.30-0.41	0.0-0.05	0.21-0.34

Table 4.1.2: Range of least unlikely gene frequencies for three loci (polled (P), African horn (Ha) and scur (Sc)) under different hypotheses.
(Bold indicates the statistically least unlikely hypothesis, italics indicates indifference)

4.2 Gene Expression Study

A total of 733 probes were found to be differentially expressed between the three contrasts of interest, namely Poll versus Horn, Poll versus Scurs and Horn versus Scurs. This converted to 573 genes identifiers after considering only those probes that resulted in unambiguous assignment from BLAST and for which ENTREZ gene identities were available (Figure 3.2.1). For all of the differentially expressed probes, the corresponding human GeneIDs were analysed in the DAVID database and in Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com). This allowed for the identification of enriched biological processes and cellular components specific to the three contrasts of interest. The IPA software was useful for establishing gene network connections in pathways of interest.

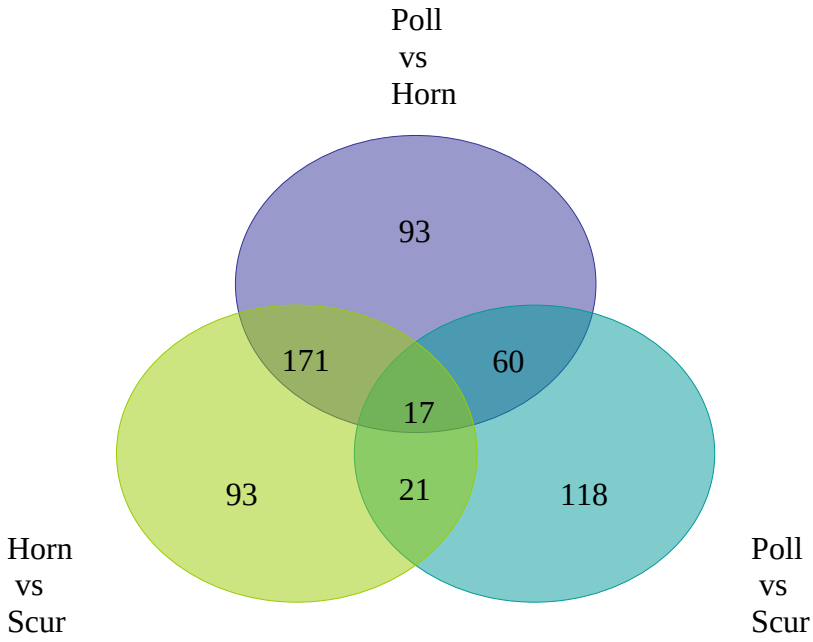

Figure 3.2.1. Venn diagram showing differentially expressed gene identifiers across three contrasts of interest namely: Poll vs. Horn; Poll vs. Scur; Horn vs. Scur

The most interesting pathways differentially regulated between polled and horned animals appear to be involved in regulation of keratinocyte differentiation in the epidermis. Interestingly several genes related to cell adhesion, particularly those of the desmosomal complex are down regulated in horned animals compared to polled. This pattern of down regulation has strong similarities to the process of epithelilal to mesenchymal transformation (EMT) wherein epithelial cells shed their attachments to neighbouring cells and become motile. Interestingly genes related to the cytoskeleton remodelling also appear to be down regulated. In the context of horn development, the down regulation of cell junction complexes might set the stage for kertainocyte migration from the subrabasal layers to the surface for incorporation into the hardened horn.

Genes involved in the formation of proteinaceous extracellular matrix is the most enriched cellular component in scurred animals in comparison to polled (Table 3.2.1). Notably matrix metalloproteinases are highly up regulated in scurred animals in comparison to polled indicating a role in extracellular matrix remodelling. Other genes include extracellular matrix related proteins including type I and II collagens, tenasin and lumican.

Contrast	Enriched gene category	Gene number[1]	p-value[2]
PvH	epidermis development	17	1.00E-10
PvH	cell junction	20	1.90E-06
PvH	actin cytoskeleton	14	6.90E-05
PvH	cell communication	12	5.50E-05
PvS	tissue development	13	4.80E-06
PvS	inorganic (anion) transport	8	1.50E-04
PvS	epidermis development	6	4.10E-03
PvS	extracellular matrix	22	5.20E-14
PvS	fibrillar collagen	5	1.20E-06
PvS	ECM-receptor interaction	10	8.80E-07
PvS	focal adhesion	11	1.20E-04

Table 3.2.1. Enriched gene categories from functional annotation clustering in DAVID (enrichment fold greater than 3.0 are shown)
[1]Gene number indicates the number of genes in the original list of differentially expressed genes that are represented in a given GO term category
[2]p-value indicates the significance of the GO term finding in relation to overall genome

The gene network connections in PvH comparison are also depicted in Fig 3.2.2. Red refers to down regulation and green refers to up regulation with the colour intensities pointing towards the levels of expression.

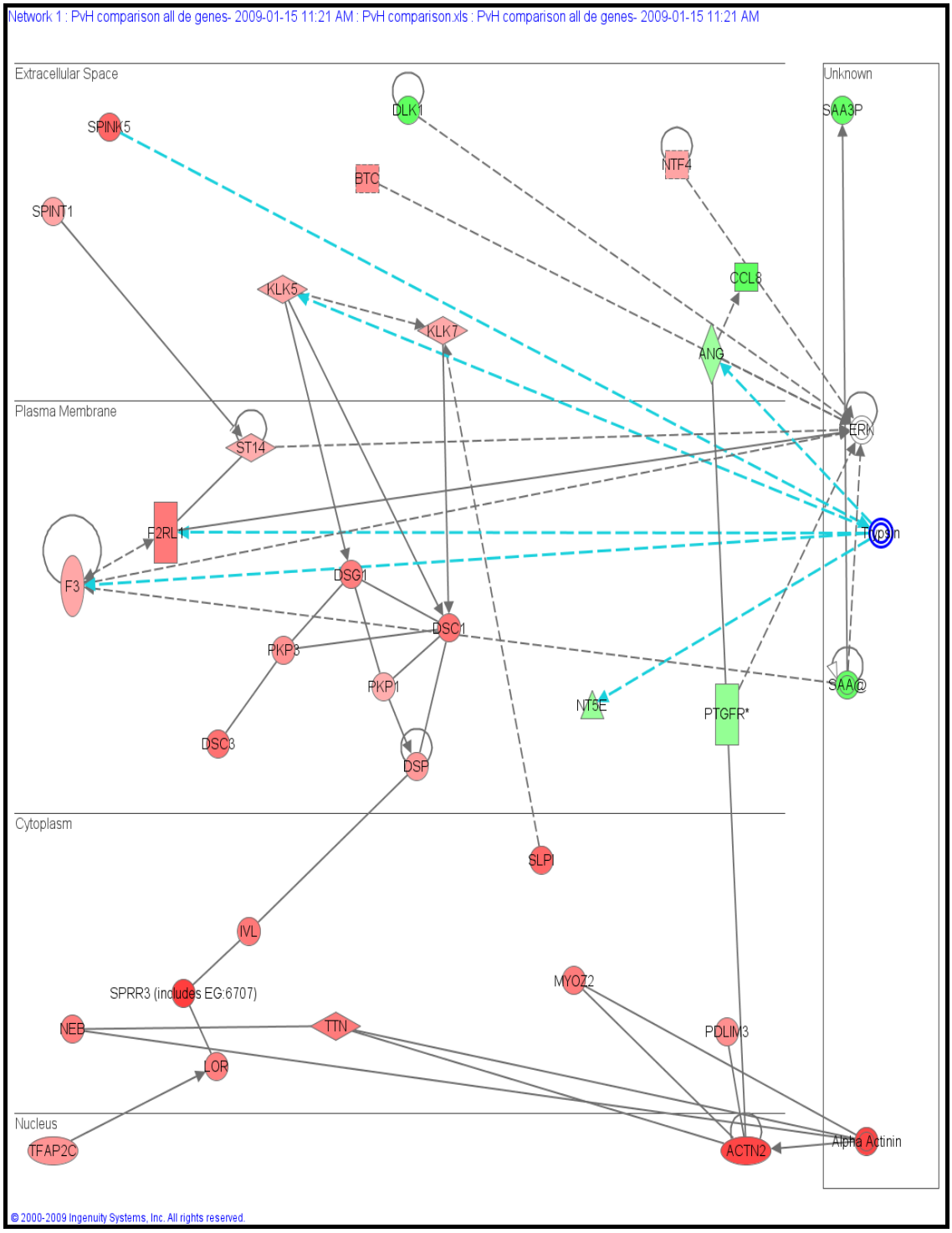

Figure 3.2.2. PvH comparison of networked genes showing the down regulation of genes involved in cell adhesion and those related to epidermal differentiation.

4.3 Gene Mapping Study

4.3.1 First Whole Genome Scan

An analysis of the SNP data was conducted with an aim to identify SNPs associated with horn status (polled or horned). Necessary quality control measures on the SNP genotypes were implemented. Given the design of the experiment with uneven distribution of number of animals

within each phenotype-sex class and the confounding of sex with horn status (in Brahmans), data from each breed were analysed separately.

Three cascading hypotheses were of interest in the analyses:

- Variation at the Polled locus on BTA 1 is sufficient to explain phenotypic variation in horn status in Brahman cattle. If disproved then;
- A locus located anywhere on the genome, other than the polled locus on BTA 1, is sufficient to explain variation in horn status in Brahman cattle. If disproved then;
- In conjunction with the polled locus on BTA 1 a second locus, located anywhere on the genome, is sufficient to explain variation in horn status in Brahman cattle.

In each breed, for each SNP, the observed frequencies (O) of the 3 SNP genotype classes (homozygous, heterozygous and homozygous) were compared to the expected frequencies (E), for polled and horned phenotypes. The null hypothesis is that the frequencies of genotypes are not affected by the horn status, and the test statistic $\sum \frac{(O-E)^2}{E}$ has a chi-squared distribution under the null hypothesis. In the case of Herefords, caution is needed in interpreting the results because of the small number of animals.

Only SNPs on BTA 1 were included in the analyses of Hereford data as the polled locus is widely known to be on BTA 1. Thus it was appropriate to account for the multiple testing of 1129 SNP. In Brahmans, hypotheses 1 was tested against only the most significant SNP from the Hereford experiment, so there is no multiple testing. For the Brahman hypotheses 2 and 3 it was appropriate to account for the multiple testing of 17746 SNP (excluding SNPs on the X chromosome as there was deliberate confounding of sex and horn status in Brahmans). SNP with minor allele frequencies less than 0.1 were omitted from consideration.

Hereford: Eighteen animals were included in this analysis. One SNP on BTA 1 was completely in accordance with the hypothesized penetrance function of the polled locus. Genotype counts for this SNP are in Table 4.3.1. The value of the test statistic for this SNP was 18, with a P-value of 0.0001. The next most significant SNPs on BTA 1 were located close by, with P-values of 0.003, not an unusual result given the number of SNP tested.

	bb	bB	BB
Horned	8	0	0
Polled	0	6	4

Table 4.3.1. Genotype counts for the most significant SNP for horn phenotype in the Hereford data.

Brahman: The significant SNP on BTA 1 (in Herefords) was not consistent with the hypothesized penetrance function of the polled locus in Brahmans. The observed numbers of individuals in the different classes showed some departure from the expected numbers (P = 0.05).

Over the whole genome, eight of the 12 most significant SNP were located on BTA 1. Genotype counts for the most significant SNP of these, which was also the most significant SNP, appear in Table 4.3.2. The value of the test statistic for this SNP was 29, with a P-value of 4.2E-07, highly significant even with the multiple testing.

	bb	bB	BB
Horned	0	9	21
Polled	13	13	2

Table 4.3.2. Genotype counts for the most significant SNP for horn phenotype in the Brahman data.

Linkage disequilibrium measures (D' and r) were calculated for the eight most significant SNP for the Brahman cattle on BTA 1 and the SNP that was significant in Hereford. According to the physical map this group of eight SNP were spread over a 6.3 Mbases, which included the location of the significant SNP from Hereford data. There were a further 25 SNPs located (according to the physical map) within this region of BTA 1. Some of these had significant departures from random association between genotypes and phenotypes.

4.3.2 Fine mapping the Polled locus in Brahman and other breeds

Table 4.3.2.1 shows the haplotypes in both horned and polled Brahmans covering the region of interest and flanking it slightly on the centromeric and telomeric ends. A conserved haplotype block starting at SNP BTA-39385 and extending to microsatellite BM6438 was evident in all polled animals including a small proportion of the horned Brahmans (36%).

BTA-39385	1338205	1	1	0	0	1	1	1	0	1	0	0	1
BTA-69199	1417209	1	1	1	1	1	1	1	1	1	1	1	1
BTA-123581	1437006	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
RP42-351B8_MS1	1512221	***142***	***142***	***142***	***142***	***142***	***142***	***142***	***142***	***142***	***138***	***142***	***142***
RP42-351B8_MS2	1534197	***149***	***149***	***149***	***149***	***149***	***149***	***149***	***149***	***149***	***149***	***149***	***149***
BM6438	1833339	257	257	257	257	257	257	257	257	257	257	257	267
rs29015832	1857578	1	0	0	0	0	0	0	0	0	0	0	0
rs29015833	1857618	0	0	0	1	0	0	1	1	1	1	0	0
BTA-100031	2087014	0	1	0	0	0	1	1	1	0	1	1	0
rs29026024	2114048	1	1	1	1	1	0	0	0	1	0	1	1
	Polled (frequency)	0.0	0.0	7.4	5.9	17.6	1.5	4.4	1.5	38.2	0.0	1.5	0.0
	Horned (frequency)	2.6	2.6	0.0	0.0	0.0	0.0	2.6	21.1	2.6	2.6	0.0	2.6

Table 4.3.2.1. Conserved haplotypes in suspected polled region

A possible explanation for the presence of horns in the small group of Brahman animals possessing the "polled" haplotype was to attribute it to the effect of another locus which overrides the effect of the Polled locus (Georges *et al*. 1993). Interestingly the same "polled" haplotype was observed in both horned and polled Hereford (data not shown) which suggested a case for marker un-informativity as potential explanation. The only way to rule out this possibility as a first exercise was through more targeted marker development in the region of the conserved haplotype.

Consequently, 15 new microsatellites were identified from the most recent assembly of the bovine genome to cover the region between BTA-39385 (at 1.3 Mb on BTA1) and BM6438 (1.83 Mb) at the telomeric end, providing average spacing of 23 Kb between markers in this 0.5 Mb region. Primers were designed to allow for multiplexing in three groups to increase efficiency of genotyping. These new markers were genotyped in the discovery population.

One microsatellite marker, CSAFG29, was identified to be of interest. The results from comparison of predicted and actual phenotype for horn status based on marker CSAFG29 are presented in Table 4.3.2.2. For this microsatellite, in what follows we refer to an allele P, being the allele associated with polled, and an allele H, being all of the other alleles. All the polled

animals were predicted to be polled by this marker. Although, 2 out of 40 horned animals were predicted to be heterozygous polled, it is possible that these animals actually were scurred, which is plausible in the heterozygous polled status.

	Marker Genotype			
Observed phenotype	PP	PH	HH	Total
Polled	25	22	0	47
Horned	0	2	40	42

Table 4.3.2.2 Observed and predicted horn phenotype for marker CSAFG29 in the discovery population.

For the first validation, all the bulls, cows and calves were genotyped for CSAFG29 in the Hillgrove Resource population. A summary of these results are presented below in the Tables 4.3.2.3 and 4.3.2.4. Out of the total number of calves produced from this breeding program, 234 calves were parentage assigned but two calves with missing genotype and four calves with missing horn status were removed from the analyses.

	Cow Polled genotype			
Cow Horn Status	HH	PH	PP	Total
Dehorned	5			5
Horned	151			151
Polled	3	40	1	44
Scurred	5	29		34
Total	164	69	1	234

Table 4.3.2.3. Cows horn status and polled locus genotypes

		Calf polled genotype				
Calf Horn Status	Calf Sex	HH	PH	PP	sub-total	Grand Total
Horned	F	1	1		2	
Horned	M		3		3	5
Polled	F	1	28	21	50	
Pooled	M		19	19	38	88
Scurred	F		66		66	
Scurred	M		69		69	135
Total		2	186	40		228

Table 4.3.2.4. Calves horn status and polled locus genotypes

Out of the 147 horned cows, 146 calves were PH and only one HH (deviation from expectation). These are predominantly scurred (107/147 – 73%) or polled (35/147 – 24%). 4 out of this 147 calves are deviations from expectation that support either another gene (Ha) or a variation at the scurs locus. It is also interesting to note that all the scurred calves are heterozygous (PH) for the polled marker. Out of 40 polled and PH cows, there were 24 PP calves (60%) that are polled. All

PP calves are polled. In this population, horns inheritance can be explained by the polled locus in a vast majority of cases. There is residual variation in scurred vs polled, but only in animals heterozygous at the polled locus. There is no evidence in support of the existence of African horn gene in this population.

The second validation used the 247 animals sampled from a range of breeds (Table 3.3.2.1). In Table 4.3.2.5 the results for Brahman, Santa Gertrudis, Hereford, Droughtmaster and Limousin are presented. In general, the marker was validated in most of these breeds with accuracy levels varying between 87 and 100%. Shaded cells represent deviations from expectation. For example, in Brahmans, 21 horned animals were genotyped as PH; 1 polled animal genotyped as HH; 14 scurred animals were genotyped as HH. Scurred individuals are expected to be either PH or PP to enable the expression of scurs locus. Of most importance is the fact that in only one case is a PP animal other than polled, that being a Brahman with scurs. As with the Hillgrove population, the vast majority of scurred animals are heterozygous.

Horn Status	HH	PH	PP	Total	Accuracy%
Brahman					
Horned	155	21		176	88
Polled	1	35	51	87	99
Scurred	14	121	1	136	90
				399	*92*
Santa Gertrudis					
Horned	95	7		102	93
Polled	3	41	8	52	94
Scurred	1	30		31	97
				185	*95*
Hereford					
Horned	67	3		70	96
Polled	7	43	45	95	93
Scurred	2	24		26	92
				191	*94*
Droughtmaster					
Horned	33			33	100
Polled	3	21	14	38	92
Scurred	3	20		23	87
				94	*93*
Limousin					
Horned	21	1		22	95
Polled	3	16	10	29	90
Scurred		1		1	100
				52	*95*

Table 4.3.2.5. Field validation results – Horn status and their respective polled locus marker genotypes in Brahman, Santa Gertrudis, Hereford, Droughtmaster and Limousin breeds. Accuracy% represents the percentage of animals in each phenotype category that agree with the marker genotype**.**

Table 4.3.2.6 presents results from Brangus and Angus breeds. In Angus and Brangus breeds, it was observed that in addition to the original allele, another allele was also in strong association with the polled condition. Thus unlike other breeds, the presence of either of these two alleles was

indicative of polled status. However, this was not the case in other *B. taurus* breeds. For example, this additional allele in Hereford was associated with the presence of horns. There could be a breed specific mutation at this microsatellite in Angus and Brangus. Further, the accuracy in horned Brangus was only 63%. However, the marker still has predictive power, 84% of animals with genotype PP are polled, and only 3% horned. Again, almost all scurred animals are heterozygous.

Horn Status	HH	PH	PP	Total	Accuracy%
Brangus					
Horned	20	11	1	32	63
Polled		11	26	37	100
Scurred		23	4	27	100
				96	*88*
Angus					
Horned					
Polled	1	20	70	91	99
Scurred					
				91	*99*

Table 4.3.2.6. Field validation results – Horn status and their respective polled locus marker genotypes in Brangus and Angus breeds. In these two breeds, an additional allele of the marker was found to be in association with polled condition.

The third validation study was conducted using stored DNA from cattle from Belmont. Results from testing the marker are in Table 4.3.2.7. The proportions for the two breeds are similar, and differ from those of the other breeds tested in that more animals with genotype PP have horns or scurs and more animals with genotype PH have horns. However, the test still has merit in these breeds. Adding copies of the P allele reduces the probability of being horned from 92% (no copies of the P allele), to 29% (one copy of the P allele) to 15% (two copies of the P allele). There are a number of possible explanations for the test being less effective in these animals. It could be that the linkage between the microsatellite and the causal mutation is not complete. Alternatively there may be other regions of the genome affecting horn growth in these breeds, such as the hypothesized African horn gene. However, it is also possible that the cause, whether incomplete linkage or another gene, did not originate in the component of Africander genetics but in another of the founder breeds. The results are consistent with the hypothesis of an African horn gene but are not evidence for the existence of such a gene.

Horn Status	HH	PH	PP	Total	Accuracy%
AX Belmont Red					
Horned	29	10	4	43	67
Polled	1	12	22	35	97
Scurred	4	13	3	20	80
				98	
ABAB F2					
Horned	48	20	5	73	66
Polled	1	27	21	49	98
Scurred	1	21	5	27	96
				149	
Total Tropical Composite					
Horned	77	30	9	116	66
Polled	2	39	43	84	98
Scurred	5	34	8	47	89
				247	

Table 4.3.2.7. Validation results – Horn status and their respective polled locus marker genotypes in AX and ABAB F2 cattle from Belmont.

4.3.3 Marker association study to map scurs locus on chromosome 19

There were no significant differences (P=0.96) in the haplotype frequencies of various phenotypes of interest based on a case-control permutation test. Thus our initial pilot study was inconclusive at best and there is no significant association between the markers tested on chromosome 19 and the scurs phenotype.

5 Successes in Achieving Objectives

This project has met all of its objectives, including the most important one: the development of a validated marker test to facilitate the breeding of polled cattle. The test itself provided valuable information about the mode of inheritance of horn phenotypes. For example, the discovery that scurred animals are almost always heterozygous at the polled locus means that there is no need to develop a test for a separate Scurs locus, if indeed one exists, as breeding for homozygous polled will eradicate scurs. Similarly, as no evidence in support of the existence of the African Horn locus in Zebu cattle was found there is no need for an additional test for Brahman cattle. The polled locus on chromosome 1 explains most of the variation between horned and polled animals. There is still some question regarding an additional locus affecting horns in Sanga derived cattle, but the test developed in this project still explains most of the variation, and is probably sufficient to breed for polled if augmented with pedigree information.

The test is not perfect, and works better in some breeds than others, but in all breeds in which it has been validated, breeding for the allele associated with polled will immediately lead to a decrease in the number of horned animals, and once the allele frequency exceeds 50%, to a decrease in the number of scurred animals. The northern beef industry will be able to phase out the practice of dehorning.

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. This project has delivered a marker test that could be delivered at $20 to $40 per animal, a test that can discriminate between animals that carry two copies of the allele responsible for polled and animals that carry one copy of the allele responsible for polled and one copy of the allele responsible for horns and scurs. If the test is adopted there will be an immediate reduction in the need for dehorning and, if and used widely and persistently, the whole herd could eventually be polled, and without scurs.

7 Conclusions and Recommendations

In Australian cattle, microsatellite marker CSAFG29 is very closely associated with the polled locus in Brahman, Santa Gertrudis, Hereford, Droughtmaster and Limousin. Animals homozygous for the allele associated with polled are almost always polled. Animals heterozygous at the marker may be polled, scurred or horned, and the ratio of the phenotypes differs between breeds. Animals that are homozygous for the non-polled allele are most likely to be horned, but occasionally may be polled or scurred. Again the ratio of the three phenotypes probably depends on breed.

In Sanga breeds microsatellite marker CSAFG29 is either less closely associated with the polled locus, or other regions on the genome also affect polled status. This does not mean that the marker has no utility in Sanga breeds, but a higher proportion of animals that are homozygous for the allele associated with polled will be horned or scurred than in the breeds already mentioned. This may also be the case for Brangus.

Microsatellite marker CSAFG29 provides an inexpensive test for genotype at the polled locus, and has immediate utility in breeding for polled. It is recommended that a test be commercialised and made available to industry.

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9 Appendices

9.1 Appendix 1

Segregation Analysis of Horn/Scur/Poll status of Belmont herd 1950-2000

Introduction

Following a study (Prayaga, 2005) to ascertain the importance of hornedness in tropical cattle in Australia, MLA funded a project through the Beef CRC to develop a tests for genes causing cattle grown in Northern Australia. Such tests will enable the polled genes to be introgressed rapidly in to these populations of cattle so that dehorning of young cattle will no longer be necessary. The first stage of this project was to examine, using segregation analysis, the CSIRO data on their tropical herd grazed at Belmont research station near Rockhampton.

Two loci are believed to be associated with hornedness in cattle. One locus relating to hornedness in European cattle has been mapped to a small section of chromosome 1 (). It has two effective alleles – an allele for poll (P) and an allele for horn (p). The gene for horn is recessive and European cattle with horns must be homozygous horned (pp); other genotypes are polled. In tropical cattle, such as Brahmans and their relatives, it is believed that horns are primarily caused by the African Horn gene. It is believed that this locus also has two effective alleles – one for horns (Af) and one for poll (af). The expression of this locus is believed to be different to be that of the European horned locus in that the the horned allele is dominant in males, but recessive in females.

Segregation analysis is used to ascertain the method of inheritance of a trait when it is controlled by one or a small number of loci. The phenotypes of parents are used to determine the proportions of different gametes that are available and consequently the frequencies of the phenotypes of progeny resulting mating parents of different phenotypes. Penetrance functions define the proportion of possible phenotypes expressed by particular genotypes. Segregation analysis is most commonly used on experimental data arising from crosses of known genotypes. When it is used for the analysis of field data two important assumptions are that the population (parents and offspring) is in equilibrium and the phenotypes have been observed correctly. Alternative penetrance functions can be used to accommodate potential errors in phenotypes.

Development of the herd at Belmont dates back to the 1950s. However, phenotypes for horned status have only been collected since the early 1970s. This herd combines animals from both

temperate (European) and tropical breeds. Consequently it is thought that both the Poll and African Horn gene are thought to be segregating in this population. There is another gene – the Scur gene (S) – which gives rise to the scurred phenotype. The horned genes dominate the scur gene in that only animals that would be polled given their genotypes at the horned loci exhibit scurs. The scurred phenotype generates additional complexity as scurs can be mistaken for horns (and vice-versa) early in life. Since animals were generally dehorned at an early age, and thus do not express their true phenotype, it is likely that there are errors among the phenotypes in this dataset.

Two hypotheses currently exist for the mechanism of inheritance in such populations. These are shown in Table 1. There are some key observations relating to both hypotheses. First horns arise when the Poll gene is homozygous recessive (pp) or the African Horn gene is homozygous (HaHa), or in males when the African Horn gene is heterozygous (Haha). The implications of this are that there is a clear difference in the mechanism of inheritance between the two loci which can be exploited by segregation analysis. As scurs are only expressed when the horned genes are in polled status it is possible to consider a model with only two horned loci by reclassifying scurred individuals to be polled for the horned loci.

Table 1. Inheritance of scurred and African horn genes in beef cattle (Georges *et al*. 1993)
Polled gene – 'P' is favourable and 'p' is unfavourable allele; Scurs gene – 'Sc' is unfavourable and 'sc' is favourable allele; African horn gene – 'Ha' is unfavourable and 'ha' is favourable allele

Genotype	Males	Females
Inheritance of the scurred phenotype		
P/- Sc/Sc	Scurred	Scurred
P/- Sc/sc	Scurred[a]	Polled
P/- sc/sc	Polled	Polled
p/p -/-	Horned	Horned
Epistatic effect of the African horn gene on the polled locus		
P/- Ha/Ha	Horned	Horned
P/- Ha/ha	Horned	Polled
P/- ha/ha	Polled	Polled
p/p -/-	Horned	Horned

[a]Sc/sc males express the scurred phenotype only when heterozygous P/p according to Long and Gregory (1978).

The primary goal of this analysis was to examine the data to ascertain if it contained evidence of the African Horned gene segregating in this population. This was done in two ways. Firstly both three locus models outlined in Table 1 were examined with segregation analysis. Secondly these models were considered for models with the two loci responsible for hornedness.

Materials and Methods

Data

Data were collected at Belmont by a number of operators over the years. In addition to almost complete pedigree recording, the horned status, the breed, grade, line and gender of each individual was recorded. While great care was taken in recording these data some phenotypes may be incorrect. Furthermore, errors in the pedigree and in the assignment of breed codes may have occurred.

Data consisted of horn status phenotypes of 8 breeds / composite lines recorded from 1973 to 2000. Out of a total of 19,842 animals, only 10,702 animals had records on horn status phenotype; and only 6,587 animals had phenotype records on themselves and their sire and

dams. Hence, this subset of the data was used for segregation analysis. Table 2 illustrate the total and recorded numbers of individuals and the numbers of trios – when parents and progeny were recorded and of the same breed – by breed. The complete set of 6,587 trios and all subsets of trios were analysed.

Segregation Analysis

The hypotheses on horns inheritance (Table 1) presented by Georges *et al*. (1993) and a slight variation of it as per Long and Gregory (1978) were tested by postulating three bi-allelic loci: Poll (P / p), African horn (Ha / ha), and Scurs (Sc / sc). The observed number of individuals in each of the possible phenotypes were compared with expected number of individuals under two hypotheses for all possible gene frequencies. The numbers of genotypes for each class of phenotypes (parents and progeny) were calulated and compared with the observed numbers. The least unlikely (most likely) ranges of allele frequencies are reported after a complete grid search of the parameter space. A second series of analyses was undertaken using a model with only the two horned loci. For these analyses all scurred individuals were reclassified as polled.

Table 2: Total and recorded numbers of animals and trios (parents and progeny recorded if more than 100) by breed.

Number	Breed type	N	Recorded	Trios*
1	AX Belmont Red	4546	2035	1349
2	BX Belmont Cross	3525	1635	906
3	HSS Selected Hereford	1607	1175	720
4	HSR Random Hereford	1673	434	185
5	GA Grade Africander	840	483	159
6	GB Grade Brahman	2042	1348	539
7	SAH Sahiwal	303	135	
8	CG Chance Get?	278	152	
9	Call Callipe Hereford	1670	1456	696
10	ABAB F2 (F2AB0)	1899	1676	912
11	ABBA F2 (F2AB)	0	0	
12	ABBX F1 (AXBX)	130	104	
13	F3AB F3 (AXBX)	840	25	
14	F4AB F4 (AXBX)	369	0	
15	FAAB (AXBX)	48	41	
34	Chimera	3	0	

Results and Discussion

Horned Loci

The results from the three sets of analyses are presented in Tables 3-5 below. Tables 3 and 4 show the results from the two locus models without and with the phenotypic error term included respectively. The two locus models provide the most insight into the frequency of the horned genes. Table 5 illustrates the results from the two alternative hypotheses regarding the action of the scurred locus in the three locus analyses. It is most important to note that the analyses of the complete set and all subsets revealed departures from expected numbers of phenotypes in all cases – no set of data fitted any of the hypotheses tested very well. Possible reasons for this are discussed below.

Table 3: Range of least unlikely gene frequencies for the two horned loci (Polled (P) and African Horn (A)) assuming no errors in the data.

Population	N males	N females	f(P)	f(AHa)
All	3234	3353	0.20-0.37	0.01-0.18
AX Belmont Red	649	700	0.28-0.37	0.0-0.14
BX Belmont Cross	440	466	0.11-0.31	0.10-0.50
HSS Selected Hereford	365	355	0.31-0.50	0.0-0.15
HSR Random Hereford	82	103	0.31-0.58	0.0-0.20
GA Grade Africander	69	90	0.15-0.40	0.47-0.81
GB Grade Brahman	269	270	0.07-0.25	0.40-0.77
CALL Calliope Hereford	377	319	0.25-0.36	0.0-0.11
ABAB F2 (F2AB0)	427	485	0.31-0.46	0.0-0.11

The range of least unlikely frequencies for the dominant allele (P, Ha or Sc) shown in these tables suggests that both alleles for each locus are present in the complete population. The frequency of the African horn gene in European breeds is very low, but the frequency of the polled gene in Tropical breeds is moderate. The relatively low frequency of the African horned gene in the total population is due to the low proportion of trios from tropical breeds of cattle in the whole population. Introducing an additional term to model errors in the phenotypes improves the fit of most models where the African horn gene is in low frequency. However, it does not alter the general picture regarding the least unlikely frequencies of the alleles.

Table 4: Range of least unlikely gene frequencies for two horned loci (Polled (P) and African Horn (Ha) with variable (0-20%) error rates (e).

Population	N males	N females	f(P)	f(Ha)	e
All	3234	3353	0.23-0.30	0.01-0.07	0.03-0.17
AX Belmont Red	649	700	0.28-0.34	0.0-0.05	0.03-0.18
BX Belmont Cross	440	466	0.15-0.23	0.17-0.40	0.0-0.03
HSS Selected Hereford	365	355	0.31-0.40	0.0-0.05	0.03-0.20
HSR Random Hereford	82	103	0.34-0.43	0.0-0.07	0.09-0.20
GA Grade Africander	69	90	0.15-0.40	0.47-0.81	0.00*
GB Grade Brahman	269	270	0.09-0.20	0.41-0.63	0.0-0.02
CALL Calliope Hereford	377	319	0.23-0.30	0.0-0.05	0.02-0.18
ABAB F2 (F2AB0)	427	485	0.31-0.38	0.0-0.03	0.03-0.18

Scurred Locus

The addition of the scurred gene increases the numbers of different classes of phenotypes and reduces the power of the analyses. However the three locus models are consistent with the two locus models. Generally, Long's hypothesis was preferred to Georges' at higher frequencies of the scurred gene, and Georges' preferred over Long's at lower frequencies of the scurred gene. This difference arises from the males having to be homozygous for the scurred gene under Long's hypothesis. Generally the distribution of female calves fitted both hypotheses much less well than the male calves.

Table5 : Range of least unlikely gene frequencies for three loci (Polled (P), African Horn (Ha) and Scur (Sc))under different hypotheses
(Bold indicates preferred hypothesis, italics indicates indifference).

Population	Hypothesis	N males	N females	f(P)	f(Ha)	f(Sc)
All	Georges	3234	3353	0.34-0.46	0.34-0.40	0.20-0.27
	Long			0.25-0.31	0.38-0.49	0.21-0.62
AX Belmont Red	Georges	649	700	0.25-0.37	0.0-0.05	0.25-0.36
	Long			0.25-0.34	0.0-0.06	0.34-0.46
BX Belmont Cross	Georges	440	466	0.18-0.27	0.20-0.37	0.34-0.41
	Long			0.18-0.25	0.51-0.64	0.50-0.61
HSS Selected Hereford	Georges	365	355	0.31-0.46	0.0-0.07	0.0-0.07
	Long			0.31-0.43	0.0-0.07	0.0-0.10
HSR Random Hereford	Georges	82	103	0.33-0.50	0.0-0.10	0.0-0.05
	Long			0.31-0.47	0.0-0.07	0.0-0.05
GA Grade Africander	Georges	69	90	0.25-0.40	0.82-0.87	0.70-0.80
	Long			0.45-0.54	0.82-0.87	0.67-0.79
GB Grade Brahman	Georges	269	270	0.0-0.05	0.80-0.87	0.00-0.15
	Long			0.0-0.05	0.79-0.86	0.00-0.15
CALL Calliope Hereford	Georges	377	319	0.34-0.44	0.31-0.37	0.23-0.31
	Long			0.38-0.47	0.34-0.43	0.27-0.36
ABAB F2 (F2AB0)	Georges	427	485	0.31-0.43	0.0-0.05	0.18-0.28
	Long			0.30-0.41	0.0-0.05	0.21-0.34

Implications

The overall lack of fit for any set of data with any of the hypothesised modes of action suggests that either the hypotheses are incorrect or that there are errors in the data which disrupt the equilibrium between parental and offspring phenotypes. Although the contrasting modes of action of the two horned loci provide only a limited set of options for the data to choose between other modes of inheritance are less likely. Detailed examination of some paternal half-sib families reveals that it would be difficult or impossible to infer the genotypes or to modify the phenotypes of many individuals predicated on their progeny distribution.

There is some evidence for the presence of the African Horned gene at low frequency in this population. In the Belmont Cross breed horned phenotypes are more likely to arise from the African horned gene than the recessive polled gene. Nevertheless it is essential in any molecular studies searching for the African Horned gene that we should only deal with individuals that are not horned because of the polled gene. This will require identifying haplotypes for the polled gene in the Brahman population before attempting to map the African horned gene.