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Gene discovery for post partum reconception and age at puberty in tropically adapted beef cattle

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Abstract

The aim of this project was to develop DNA-based tools to help the northern Australian beef industry achieve reduced age at puberty and improve postpartum re-conception in first lactation cows. Genome-wide association studies were conducted on the Beef CRC Northern Breeding Project resource population. A total of 1,709 female cattle (843 Brahman and 866 Tropical Composites), with phenotypic records for lifetime reproductive performance and other production traits, were genotyped with the Illumina Bovine SNP50 chip. The study was able to identify significant associations between many single nucleotide polymorphisms (SNP) and the two traits in the two populations studied. These SNP associations were used as the basis for genomic prediction equations and incorporated into genomically-enhanced Breedplan Estimated Breeding Values (gEBV). To test whether smaller panels of markers could be used to aid genetic selection for reproductive traits, a panel of 10 SNP per breed and per trait was tested for its effectiveness. Genome regions with significant SNP associations were further investigated for possible causative genes. Significant SNP and possible causative genes could be used to enhance the accuracy of genomic selection. The results have built a platform for future delivery of DNA-based predictions of genetic merit for female reproduction in tropically adapted breeds.

Executive summary

Reproductive performance is an important trait for beef production in tropical and subtropical environments of Australia. The major contributors to reproductive inefficiency in tropically-adapted beef cows are age at puberty and extended postpartum anestrus intervals. Beef CRC research has shown that these components of reproductive performance are moderately to highly heritable, making genetic selection a promising strategy for improving these traits. Reproductive traits are expensive to measure. The project goal was to identify diagnostic DNA markers for components of reproductive performance in female beef cattle. Genome-wide association studies were conducted on the Beef CRC Northern Breeding Project resource population, with a total of 1,709 female cattle (843 Brahman and 866 Tropical Composites) genotyped with the Illumina Bovine SNP50 chip. Related animals were also genotyped with the high density chip, containing over 700,000 single nucleotide polymorphisms (SNP). From this, genotypes for the remaining population were imputed so that all animals had information for over 700,000 SNP.

In addition, the database for the Beef CRC Northern Breeding Project resource population contains records for at least 25 reproduction and other performance traits. The analysis conducted in this report deals with 20 of these traits (at least 35,000 phenotypic records in total), and their associations to almost 12 million marker genotypes (700,000 per animal).

We present the results for two female reproduction traits: (1) Age at puberty, defined as age in days at first observed *corpus luteum* following frequent ovarian ultrasound scans (AGECL); and (2) the postpartum anestrous interval, measured as the number of days from first calving to first ovulation postpartum (first re-breeding interval, PPAI). In addition, correlated traits such as weight, height, insulin-like growth factor-1 (IGF1) concentration in peripheral blood, condition score and fatness were also examined. In Brahman, 41% of the significant markers for AGECL mapped to a single chromosomal region on *Bos taurus* autosome (BTA) 14. In Tropical Composites, 16% of these significant markers were located on BTA5. In Tropical Composites, the largest concentration of PPAI significant markers were located on BTA5 (19%) and BTA16 (17%). In Brahman cattle the largest concentrations of significant markers for PPAI were located on BTA 3 (14%) and BTA14 (17%).

Few of the significant markers for female reproduction traits for the Brahman and Tropical Composite breeds were located in the same chromosomal regions. However, fatness and live weight traits as well as serum IGF1 concentration were found to be associated with identical genome regions within and between breeds. Clusters of SNP associated with multiple traits were located on BTA14 in Brahman and BTA5 in Tropical Composites.

The genome-wide association results were used to derive genomically-enhanced predictions for reproductive traits that will be incorporated into Breedplan and made available to industry as genomically-enhanced EBV (gEBV). These gEBV will provide predictions of genetic merit on very young animals. By providing more accurate predictions at an earlier age, breeders will be able to make timely selection decisions and thereby increase the rate of genetic progress on reproductive traits. The progress towards reproduction gEBV is described in detail in the Beef CRC's final report (Program 7).

In order to explore potential additional applications for the genomic data, the genome-wide association results were also used to select the top 10 SNP markers to predict AGECL and PPAI traits in each breed. Different sets of SNP markers emerged as the predictive markers for the two traits, and for each breed. Within the

same population that was used to discover the top 10 markers, these markers were able to predict between 23 % and 78 % of the genetic variance for the traits. The effect of the chosen SNP markers on 19 other traits was explored. Using the same significance threshold that was applied to the selection of the markers, only one of the SNP markers was significantly linked to growth and carcass traits in Brahman cows. The top ten genetic markers for each trait were able to explain a large portion of the heritable variation for each trait, and showed no detrimental or antagonistic associations with other production traits.

The use of molecular genetics methods to predict the genetic merit of young bulls and replacement heifers for reproduction traits is an important goal for the industry. Exploring the effects of the top 10 markers shows that the use of focused SNP panels in this context has promise and could be made available at lower cost.

Fine mapping of three chromosome regions with pleiotropic effects on a number of traits, including reproduction traits, revealed several plausible candidate genes. This study was unable to definitively resolve possible causative genes in the associated regions. Several genomics datasets that are currently becoming available will aid the identification of causative genes, functional mutations and haplotypes that will result in more accurate DNA markers and genomic predictions.

This project has created a world-leading database of high-density SNP genotypes for a valuable population of tropically-adapted female cattle. The extensive phenotypic database that has been generated for the Northern Breeding Project can now be mined for additional traits (such as aspects of lifetime reproductive performance), and the SNP association to these new traits can be established without the need to acquire further data.

In addition, the project team was also able to create opportunities to extend this work to male populations. It is clear that there are strong genetic links between aspects of male and female performance. By having created large genomics datasets on male and female reproduction resource populations, the genomic and biological basis for these links can be explored. This knowledge may result in more cost-effective multi-stage selection strategies, where phenotypic selection is applied before investing in genotyping a portion of the young bulls.

The industry will soon be able to benefit through this work, via the availability of more accurate and comprehensive EBV. The industry sector that is likely to begin applying this R&D first is the seedstock sector, with benefits flowing through to breeding herds over a couple of generations. The benefits from this work will be seen in increased weaning rates, particularly from Brahman herds. This will start to happen as bulls with poor predicted daughter fertility are gradually eliminated from the herd.

There is potential for further technology developments that would give rise to lower cost and more accurate DNA-based tools which may be applied more widely and speed up genetic progress in the industry. In particular, the development of new approaches to making genomic predictions or marker panels more accurate and cost-effective would value-add to the existing industry co-investments in this area. The future integration of transcriptomics and genome sequence data sets into the analysis approaches has great potential to make better genomic predictions. Another area for co-investment is in partnerships between industry and R&D organisations to validate applications for focused SNP panels in different industry settings.

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1 Background

Reproduction rate is an economically relevant trait for beef production in tropical and subtropical environments of Australia (Burns et al., 2010). Improvement of reproduction rate in beef cattle using traditional selection practices has had limited success because female fertility traits are only expressed in cows and have low heritabilities, at least for industry measured traits, such as calving rate and days to calving (Cammack et al., 2009; Johnston and Bunter, 1996; Meyer et al., 1990).

Recently the heritability of specific components of reproduction rate has been estimated as moderate to high in tropically adapted cattle. In Brahmans, the heritability of age at puberty (AGECL-measured by the first detected corpus luteum (CL)) was estimated at 0.29 and length of postpartum anestrous interval (PPAI-measured by first detected CL after first calving) at 0.57 (Johnston et al., 2010; Johnston et al., 2009). These findings mean that genetic improvement strategies for reproduction rate have promise for effecting positive change in the industry. However, these traits are expensive to measure and accurate estimated breeding values will only become available long after young bulls or replacement heifers have been selected. The identification of genetic markers linked to these traits is therefore an important strategy for genetic improvement of cow reproductive performance.

Tools for genome-wide association studies of the bovine genome have become available in the wake of the bovine genome sequence (Elsik et al., 2009). The Illumina bovine SNP50 chip, which collects data at >50,000 single nucleotide polymorphism (SNP) marker positions simultaneously for each DNA sample analysed, was chosen to explore the SNP marker associations with reproductive traits in tropically-adapted female beef cattle. During the course of the project, a further opportunity arose to apply high-density genotyping (Illumina bovine HD SNP chip with >700,000 SNP marker assayed per sample) to Beef CRC populations. Using HD SNP chip data on close relatives, it is possible to impute high-density genotypes for individuals genotyped at a lower density. Genome-wide SNP association studies were therefore conducted on imputed high-density genotypes for 996 Brahman and 1,097 Tropical Composite cows with very detailed reproduction phenotypes.

AGECL and PPAI are complex traits influenced by growth and fatness (Johnston et al., 2009). Our hypothesis was that some of the genes responsible for fatness or growth also determine AGECL and PPAI. Therefore, in addition to reproductive traits, we analysed phenotypes for 18 other growth and body composition traits measured in the same Brahman and Tropical Composite beef heifers. The aim was to identify some of the chromosome locations responsible for the variability in AGECL or PPAI using a genome wide association study (GWAS). Although the two breeds are not genetically identical, we compared the GWAS of Brahman to Tropical Composites to determine whether any of the traits studied showed association to the same genes or genome regions in the two breeds.

A further aim of the genome-wide association studies for cattle reproductive traits was to identify potential causative genes for the traits in question and to characterise the specific mutations or haplotypes that may be linked to the phenotype of interest. The high-density genotypes generated during this project allowed us to fine-map regions harbouring SNP with large effects on the phenotype. Three chromosomal regions, on *Bos taurus* autosome (BTA) 5, BTA14, and on BTA21, were selected for more detailed investigations.

2 Project objectives

The overall objective of this project was to deliver reliable gene markers associated with female puberty and subsequent lifetime reproductive performance. To this end, the project completed a whole genome scan of the Beef CRC Northern Breeding Project resource population, to achieve the following objectives:

2.1 Objective 1

Assess the current phenotypic data in the CRC2.3 'Northern Breeding Project population', choose appropriate animals and complete a whole-genome scan of the Northern Breeding Project resource population to identify significant chromosomal regions for puberty and postpartum reconception.

2.2 Objective 2

Fine map chromosomal regions identified in the whole genome scan and find genetic markers that can be used as DNA diagnostic markers for age at puberty and postpartum re-conception.

2.3 Objective 3

Perform targeted sequencing of genes and genome regions to identify possible functional mutations and haplotypes that may more accurately define reproductive rate phenotypes.

2.4 Objective 4

Assess the effects of identified diagnostic markers for age at puberty and postpartum reconception across other non-reproductive traits.

3 Methodology

3.1 Animals and traits

Tables summarising the number of animals used and descriptive statistics for all measured traits are provided in Tables 1 and 2.

Table 1. Descriptive statistics for Brahman heifers for each of the 20 traits

Trait	Abbreviation	No. animals	Mean	sd	h^{2*}
Age at first *corpus luteum*	AGECL (days)	941	745.79	138.93	0.56
Scanned rump fat thickness at P8 position at first *corpus luteum*	P8CL (mm)	895	4.45	2.19	0.47
Live weight at first *corpus luteum*	WTCL (kg)	928	333.76	44.97	0.56
Live weight at 18 months	W1WT (kg)	946	288.84	43.83	0.36
Average daily gain at 18 months	W1ADG (g/day)	940	607.89	147.67	0.24
Scanned rump fat thickness at P8 position at 18 months	W1P8 (mm)	946	3.75	1.94	0.45
Scanned fat depth measured between the last 2 ribs at 18 months	W1RIB (mm)	946	2.01	1.04	0.48
Scanned LM area at 18 months	W1EMA (cm^2)	944	44.36	6.58	0.25
Hip height at 18 months	W1HH (cm)	573	127.47	4.84	0.60
Body condition score at 18 months	W1CSN (1-15)	887	8.32	1.35	0.38
IGF1 concentration in blood at 18 months	W1IGF (ng/ml)	857	183.23	84.13	0.41
Live weight at 24 months	D2WT (kg)	947	321.26	57.47	0.38
Average daily gain at 24 months	D2ADG (g/day)	943	138.21	233.35	0.13
Scanned rump fat thickness at P8 position at 24 months	D2P8 (mm)	946	3.22	1.76	0.37
Scanned fat depth measured between the last 2 ribs at 24 months	D2RIB (mm)	946	1.92	1.00	0.51
Scanned LM area at 24 months	D2EMA (cm^2)	944	44.17	8.66	0.44
Hip height at 24 months	D2HH (cm)	885	132.52	4.82	0.51
Body condition score at 24 months	D2CSN (1-15)	947	7.45	1.35	0.35
IGF1 concentration in blood at 24 months	D2IGF (ng/ml)	700	216.29	91.55	0.41
Length of first postpartum anestrous interval	PPAI (days)	618	180.37	109.05	0.51

sd – standard deviation; h^{2} - heritability
*heritabilities were estimated from the full population, rather than the subset of genotyped animals

Table 2. Descriptive statistics for genotyped Tropical Composite heifers for each of the 20 traits

Trait	abbreviation	No. animals	mean	sd	h^{2*}
Age at first *corpus luteum*	AGECL (days)	1005	651.45	120.28	0.49
Scanned rump fat thickness at P8 position at first *corpus luteum*	P8CL (mm)	980	2.99	1.63	0.42
Live weight at first *corpus luteum*	WTCL (kg)	995	330.87	46.00	0.46
Live weight at 18 months	W1WT (kg)	1009	316.14	40.54	0.65
Average daily gain at 18 months	W1ADG (g/day)	1002	578.24	142.57	0.36
Scanned rump fat thickness at P8 position at 18 months	W1P8 (mm)	1009	3.20	1.79	0.52
Scanned fat depth measured between the last 2 ribs at 18 months	W1RIB (mm)	1009	2.08	1.16	0.50
Scanned LM area at 18 months	W1EMA (cm^2)	1007	46.12	6.99	0.61
Hip height at 18 months	W1HH (cm)	1008	125.28	6.05	0.57
Body condition score at 18 months	W1CSN (1-15)	1009	7.48	0.90	0.34
IGF1 concentration in blood at 18 months	W1IGF (ng/ml)	757	223.50	77.91	0.36
Live weight at 24 months	D2WT (kg)	1008	356.26	38.55	0.72
Average daily gain at 24 months	D2ADG (g/day)	1007	265.90	177.96	0.18
Scanned rump fat thickness at P8 position at 24 months	D2P8 (mm)	1008	2.96	1.70	0.72
Scanned fat depth measured between the last 2 ribs at 24 months	D2RIB (mm)	1007	1.98	1.08	0.54
Scanned LM area at 24 months	D2EMA (cm^2)	1007	49.18	6.61	0.53
Hip height at 24 months	D2HH (cm)	1003	130.26	4.77	0.78
Body condition score at 24 months	D2CSN (1-15)	1008	7.06	1.10	0.37
IGF1 concentration in blood at 24 months	D2IGF (ng/ml)	748	237.07	71.94	0.21
Length of first postpartum anestrous interval	PPAI (days)	833	141.97	109.31	0.29

sd – standard deviation; h^2 - heritability
*heritabilities were estimated from the full population, rather than the subset of genotyped animals

Cattle used in this study were from the 'Northern Breeding Project' resource population bred by the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) in the tropical regions of Northern Australia (Barwick et al., 2009a; Barwick et al., 2009b; Burrow et al., 2003; Johnston et al., 2009; Prayaga et al., 2009). A total of 1,709 female cattle were genotyped from this population consisting of 843 Brahman and 866 Tropical Composites. Briefly, Tropical Composites comprised approximately 50% tropically adapted breeds and 50% non-tropically adapted *Bos taurus* breeds. The tropically adapted component was on average half derived from Brahman and the other half from tropically adapted Taurine breeds. The *Bos taurus* component of Tropical Composites consisted of various combinations of Hereford, Shorthorn, Red Angus, Red Poll and Charolais. The heifers were the progeny of 54 Brahman and 51 Tropical Composite sires.

Within this population, 19 traits were recorded during the first three years of the female's life. The reproductive traits have been previously described (Johnston et al., 2010; Johnston et al., 2009) and included (1) AGECL, age at puberty, defined as age in days at first observed corpus luteum following frequent ovarian ultrasound scans; and (2) PPAI, the postpartum anestrous interval, measured as the number of days from calving to first ovulation postpartum, detected by ultrasound scanning (first re-breeding interval in lactating cows). The other traits included live weight at AGECL (WTCL, kg), scanned rump fat thickness at P8 position at first CL (P8CL, mm), and traits measured at a constant age as described by Barwick et al. (2009). These included - live weight (WT, kg), hip height (HH, cm), serum concentration of IGF-I (IGF, ng/mL), average daily weight gain (ADG, g/day), body condition score (CSN, recoded scores to 1-15 scale), scanned LM area (EMA, cm2), scanned rump fat thickness at P8 position (P8, mm) and scanned fat depth measured between 12th and 13th ribs (RIB, mm). These traits were measured at two time points, W1 (~18 months of age near the end of the first post weaning wet season) and D2 (~24 months of age near the end of the dry season).

3.2 Genotyping

The BovineSNP50 Bead Chip (Illumina, San Diego, CA, (Matukumalli et al., 2009)) was used to genotype 866 Tropical Composite cows and 843 Brahman cows from each resource population. Family trios and repeat samples were included for quality assurance. SNP with auto-calling rates less than 85% and SNP with minor allele frequency (MAF) less than 0.01 were excluded from later analyses.

3.3 Imputation of HD genotypes

126 Brahman and 153 Tropical Composite cows were genotyped with the high-density SNP chip (~800,000 SNP; Illumina Inc., San Diego, CA, USA). Using this data, complete genotypes were imputed using the BEAGLE 3.2 program (Browning and Browning, 2010). Quality control and imputation resulted in 729,068 SNP with complete genotypes for 969 Brahman and 1,019Tropical Composite cows.

3.4 Calculation of SNP effects

Analyses were done separately for the two breeds (Brahman and Tropical Composite). The additive effect of a SNP on each phenotype was calculated by regression analysis, with values in the covariate coded as 0, 1 or 2 copies of the variant allele, and after fitting the following mixed-model:

$$y_{i,j} = X\beta + Zu + s_{j,k} + e_{i,j} \quad (1)$$

Where yi,j represents the vector of observations from the i-th cow at the j-th phenotype, X is the incidence matrix relating fixed effects in β with observation in yi,j, Z is the incidence matrix relating random additive polygenic effects in u with observation in yi,j, sj,k represents the additive association of the k-th SNP on the j-th phenotype, and ei,j is the vector of random residual effects.

Fixed effects included in β were discussed previously (Barwick et al., 2009a; Johnston et al., 2010; Johnston et al., 2009). Polygenic effects were included to reduce the effect of family structure.

Standard stochastic assumptions applied to the random effects in Model (1), which were assumed to be distributed as multivariate normal with zero mean and variance as follows:

$$\mathrm{V}\begin{bmatrix} u \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_u^2 & 0 \\ 0 & I\sigma_e^2 \end{bmatrix} \qquad (2)$$

where A is the numerator relationship matrix across all cows and derived from the pedigree structure (Wright, 1922); σ_u^2 is the additive polygenic component of variance; I is an identity matrix; and σ_e^2 is the residual component of variance.

Variance components (2) were estimated using the WOMBAT software (Meyer, 2006b). The SNP effects in Model (1) were solved using a fast strategy of the WOMBAT software (Meyer and Tier, 2012).

The false positive rate (FPR) was calculated using (Bolormaa et al., 2010):

$$FPR = \frac{P(1-S)}{P(1-P)} \qquad (3)$$

where P is the defined probability threshold and S is the proportion of SNP that are significant at that defined threshold. The expected number of true positive SNP equals (1-FPR) multiplied by the number of significant SNP at that probability threshold.

The percentage of the genetic variance accounted by the i-th SNP was computed according to the formula 4:

$$\%\mathrm{V}_i = 100 \cdot \frac{2 p_i q_i a_i^2}{\sigma_g^2} \qquad (4)$$

Where: pi and qi are the allele frequencies for the i-th SNP estimated for Tropical Composites and Brahman separately, ai is the estimated additive effect of the i-th SNP on the phenotype in question, and σ_g^2 is the REML estimate of the (poly-)genetic variance for each phenotype in question.

3.5 F_{ST} calculations

Chromosomal regions undergoing selection were identified using a calculation of fixation index (F_{ST}) according to (Weir and Cockerham, 1984). For this calculation, 565 animals were included, representing animals from each half-sib family.

3.6 Selection of top 10 SNP and estimation of effects across traits

A top 10 SNP panel was selected for AGECL and PPAI of Brahman and Tropical Composites separately. To avoid redundancy due to linkage disequilibrium, only the most significant SNP with minor allele frequency > 0.1 was selected for each chromosome. From this list, the 10 most significant SNP were retained in the SNP panel. Consequently the $-\log_{10}(p)$ cutoffs for AGECL and PPAI were 5.3 and 4.7 in Brahman, 3.9 and 3.6 in Tropical Composites, respectively. Separately for AGECL and PPAI, a multiple regression model was fitted using the top 10 SNPs to get an estimate of the total genetic variance explained by these SNP panels.

3.7 Clustering and heat maps

Hierarchical clustering of SNP association results was performed using the program PermutMatrix (Caraux and Pinloche, 2005).

4 Results and discussion

4.1 Genetic parameter estimation

AGECL and PPAI were highly heritable in Brahmans (56 and 51%, Table 1). In this breed, these traits were comparable in their heritability estimates to live weight, hip height and fatness traits. In Tropical Composites, AGECL was highly heritable at 49%, while PPAI was moderately heritable at 29% (Table 2). The production traits with the largest genetic component in these populations were adult live weight, hip height and fatness traits in Tropical Composites, while the efficiency measure ADG was the trait for which the lowest heritabilities were estimated (in both breeds, at the D2 time point).

Heritability estimates for IGF showed that this trait contained a larger heritable component at the W1 time point than at D2. Higher heritabilities were estimated for IGF in Brahmans than in Tropical Composites.

4.2 Genome-wide associations

The genome-wide association study (GWAS) for AGECL and PPAI highlighted several genome regions with important SNP associations with the two traits. As described in detail by Hawken et al ((2011), in each breed, different genome regions were found significantly associated with the same trait, and within the same breed, different genome regions were associated with each trait.

For Brahmans, the most significant associations were obtained for a region on BTA14 for AGECL, whilst for PPAI, significance values were not as high, and a region on BTA21 contained the SNP with the most significant associations (Figure 1). For Tropical Composites, BTA5 contained the most significant SNP associations with AGECL and with PPAI (Figure 2).

However, there were also some similarities between the breeds. BTA5 was also an important chromosome for AGECL and PPAI traits in Brahman. A SNP association

peak on BTA21 was seen for both traits in both Brahman and Tropical Composite breeds.

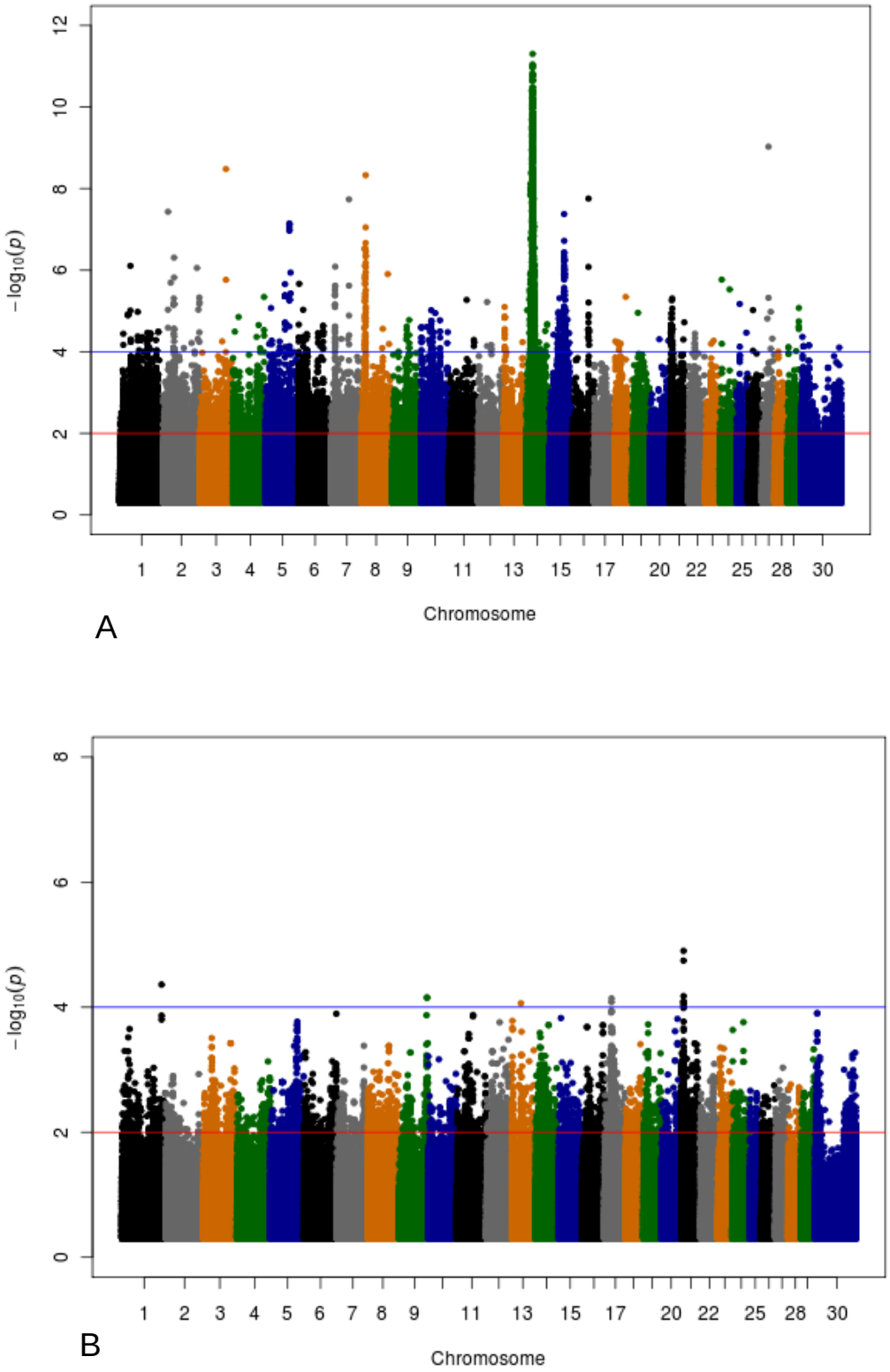
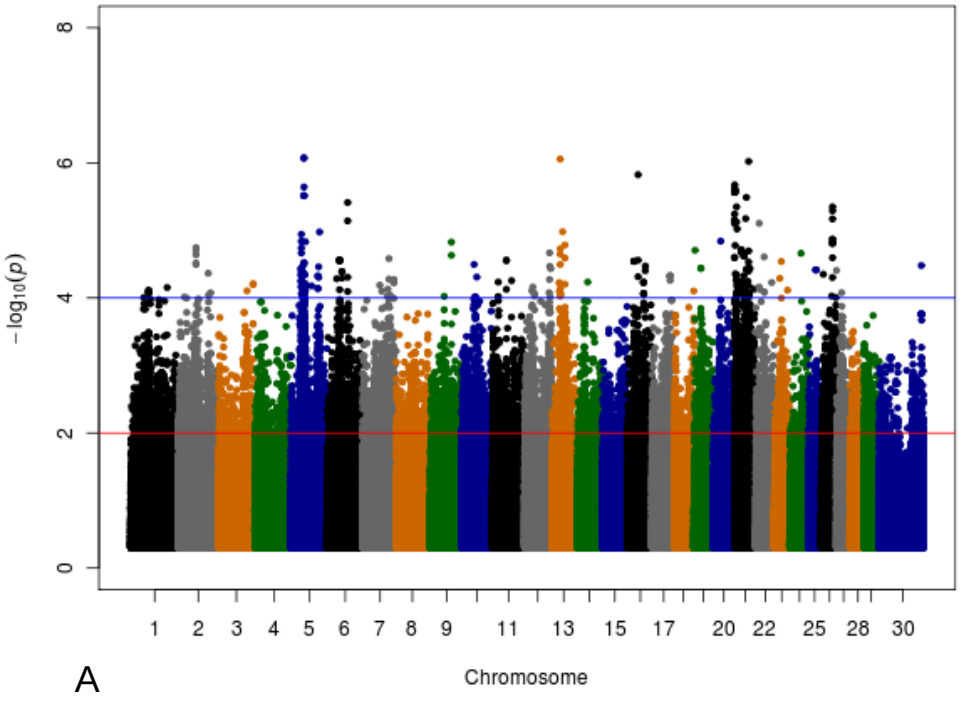


Figure 1: Manhattan Plots of SNP association in populations of Brahman heifers. A: AGECL, B: PPAI. Chromosome numbers are shown along the X-axis," 30" denotes the X-chromosome. Significance values are plotted along the Y axis. Horizontal blue and red lines indicate significance thresholds of $-\log_{10}(p) = 2$ and $-\log_{10}(p) = 4$



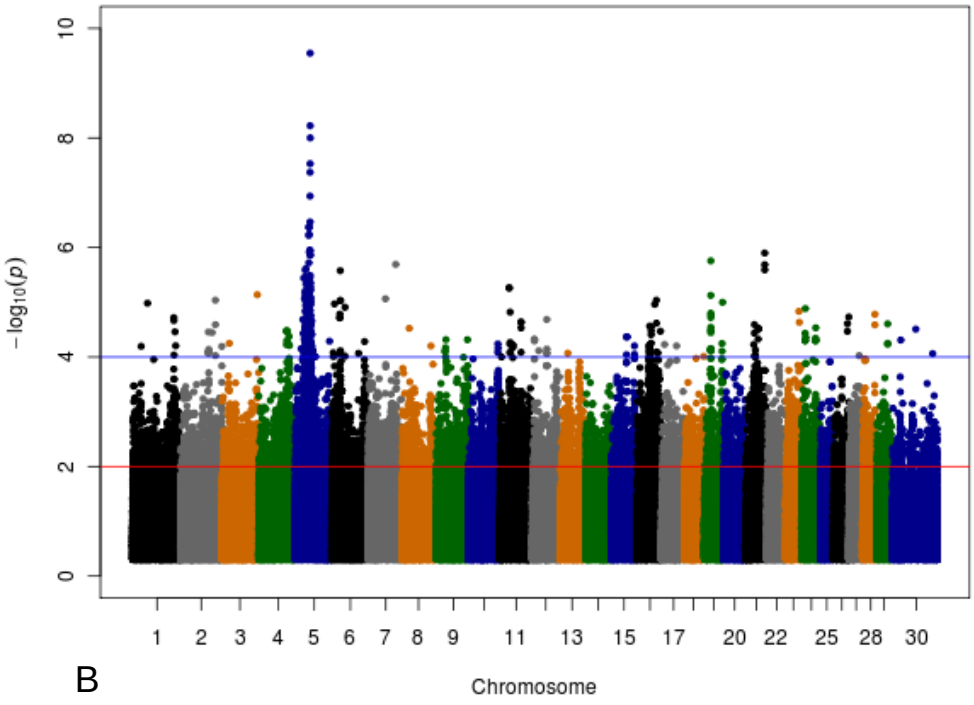


Figure 2: Manhattan Plots of SNP association in populations of Tropical Composite cows. A: AGECL, B: PPAI. Chromosome numbers are shown along the X-axis," 30" denotes the X-chromosome. Significance values are plotted along the Y axis. Horizontal blue and red lines indicate significance thresholds of $-\log_{10}(p) = 2$ and $-\log_{10}(p) = 4$

4.3 Top 10 SNP AGECL and PPAI

The top 10 SNP for each trait had $-\log_{10}(P)$ values between 3.65 and 11.33 (Tables 3 and 4). No SNP markers contributed to the set of top 10 SNP for both traits, or across the two breeds. In Brahman, SNP from chromosomes 1, 5, and 21 were included in the "top 10" list for both traits. In Tropical Composites BTA 5, 6, 11 and 13 contributed SNP to both "top 10" lists. However, different chromosomal positions were associated with each trait, with at least 10 Mb separating the markers (data not shown). The BTA5 SNP markers for PPAI and AGECL in Tropical Composites were the exception. These SNP were located only about 5 Mb apart, at 44 and 49 Mb on BTA5 respectively (data not shown).

A multiple regression model was used to obtain an estimate of the genetic variance that could be explained by the combination of the top SNP for each trait. This analysis predicts that between 23 and 78 % of the genetic variance for AGECL and PPAI is explained by the SNP panels (Tables 5 and 6).

The SNP selected from the BTA14 association peak in Brahman alone explained 7.86 % (or 33 days) of the additive genetic variance for the AGECL trait, and each individual SNP in the "top 10" list explained at least 0.03 % of the additive genetic variance. Collectively, the AGECL SNP panel in Brahmans explained 23% of the additive genetic variance. For PPAI, the SNP on BTA21 was the most significant and explained 8 % (1.1 day) of the additive genetic variance. Taken together, the "top 10" SNP for PPAI accounted for 55% of the additive genetic variance (Table 5). This figure might be an overestimate, because fewer Brahmans were phenotyped for the PPAI trait (Table 1).

The "top ten" SNP for AGECL in Tropical Composites collectively explained 44% (or 200 days) of the additive genetic variance for the trait. The SNP selected to represent the BTA5 association peak for PPAI in Tropical Composites explained 21.2 % (or 1.2 days) of the additive genetic variance for this trait. Collectively, the "top 10" SNP for PPAI in Tropical Composites appeared to explain 78 % of the additive variance for this trait. However, this is likely to be an overestimate, because the heritability for this trait was estimated at 29% (Table 2).

Overall, the estimates of the total genetic variance explained by the marker panels are likely to be overestimates, because the SNP were discovered in the same population that they were tested in. The data need to be validated either in an independent population of animals, or by splitting the populations into "discovery" and validation" subsets.

Table 3: Top 10 SNP for AGECL and PPAI in Brahman (Sorted by significance within trait)

Trait	CHR	a	se	MAF	-$\log_{10}$ (p)
AGECL	14	41.25	5.98	0.46	11.30
AGECL	8	32.10	5.53	0.38	8.33
AGECL	2	-26.16	4.82	0.45	7.43
AGECL	5	-26.02	4.90	0.28	7.14
AGECL	15	43.21	8.66	0.15	6.44
AGECL	1	30.09	6.22	0.12	6.11
AGECL	7	27.44	5.68	0.25	6.08
AGECL	18	25.84	5.78	0.48	5.35
AGECL	4	24.35	5.45	0.21	5.34
AGECL	21	35.70	8.03	0.13	5.31
PPAI	21	1.31	0.25	0.44	6.86
PPAI	1	-1.88	0.40	0.14	5.76
PPAI	17	-1.11	0.25	0.37	5.29
PPAI	16	1.10	0.25	0.37	5.14
PPAI	11	1.16	0.27	0.32	5.05
PPAI	5	-1.20	0.28	0.28	4.90
PPAI	12	1.04	0.25	0.49	4.84
PPAI	19	-1.02	0.24	0.48	4.83
PPAI	X	-1.21	0.29	0.32	4.82
PPAI	13	1.10	0.27	0.32	4.73

Abbreviations: CHR –chromosome location of SNP; a - size of effect (days); se-standard error; MAF – minor allele frequency; - $\log_{10}$(p) – the logarithm of the p-value to base 10

Table 4: Top 10 SNP for AGECL and PPAI in Tropical Composites (Sorted by significance within trait)

Trait	CHR	a	se	MAF	-$\log_{10}$ (p)
AGECL	5	28.50	5.92	0.25	6.07
AGECL	13	37.31	7.76	0.12	6.05
AGECL	19	29.31	7.10	0.15	4.71
AGECL	12	-25.69	6.25	0.25	4.67
AGECL	11	-23.76	5.87	0.31	4.56
AGECL	6	-25.87	6.54	0.20	4.39
AGECL	10	-21.05	5.38	0.46	4.31
AGECL	7	-21.57	5.55	0.48	4.27
AGECL	9	-20.22	5.40	0.46	4.02
AGECL	4	24.67	6.67	0.21	3.94
PPAI	5	1.41	0.22	0.25	9.55
PPAI	28	-1.12	0.27	0.12	4.78
PPAI	6	1.04	0.25	0.15	4.72
PPAI	X	1.22	0.30	0.11	4.51
PPAI	11	1.10	0.29	0.11	4.08
PPAI	24	-1.02	0.28	0.14	3.95
PPAI	22	-1.05	0.29	0.11	3.83
PPAI	2	1.01	0.28	0.11	3.71
PPAI	7	1.02	0.29	0.13	3.69
PPAI	13	-1.01	0.29	0.11	3.65

Abbreviations: CHR –chromosome location of SNP; SNP – identity of Illumina Bovine HD Bead Chip SNP; a - size of effect (days); se- standard error; MAF – minor allele frequency; - $\log_{10}$(p) – the logarithm of the p-value to base 10

Table 5: Multiple regression results for top 10 SNP for AGECL and PPAI in Brahman (sorted by chromosome within trait)

Trait	CHR	a	se	% Va	Sum %
AGECL	1	10.90	6.14	0.37	
AGECL	2	-8.52	5.15	0.53	
AGECL	4	10.78	5.23	0.57	
AGECL	5	-2.34	5.44	0.03	
AGECL	7	10.43	5.35	0.60	
AGECL	8	19.75	5.14	2.71	
AGECL	14	32.73	5.41	7.86	
AGECL	15	33.82	7.80	4.31	
AGECL	18	19.72	5.20	2.87	
AGECL	21	32.45	7.26	3.52	23%
PPAI	1	-1.52	0.34	7.62	
PPAI	5	-1.08	0.24	6.48	
PPAI	11	0.92	0.23	5.04	
PPAI	12	0.91	0.21	5.66	
PPAI	13	0.94	0.23	5.22	
PPAI	16	0.93	0.22	5.58	
PPAI	17	-0.70	0.22	3.12	
PPAI	19	-0.80	0.21	4.35	
PPAI	21	1.09	0.21	8.00	
PPAI	X	-0.83	0.24	4.13	55%

Abbreviations: CHR –chromosome location of SNP; SNP – identity of Illumina Bovine HD Bead Chip SNP; a – size of effect; se- standard error; % Va – percent of genetic variance explained; Sum % - percent of genetic variance explained by the combination of the top ten SNP.

Table 6: Multiple regression results for Top 10 SNP for AGECL and PPAI in Tropical Composites (sorted by chromosome within trait)

Trait	CHR	a	se	% Va	Sum %
AGECL	4	30.02	6.06	6.3	
AGECL	5	21.52	5.32	3.6	
AGECL	6	-28.10	5.79	5.3	
AGECL	7	-22.46	4.96	5.3	
AGECL	9	-18.25	4.79	3.4	
AGECL	10	-20.70	4.79	4.5	
AGECL	11	-20.55	5.22	3.8	
AGECL	12	-21.77	5.60	3.5	
AGECL	13	32.22	6.98	4.6	
AGECL	19	26.30	6.37	3.7	44%
PPAI	2	0.75	0.24	4.7	
PPAI	5	1.15	0.20	21.2	
PPAI	6	0.81	0.22	7.2	
PPAI	7	0.36	0.25	1.3	
PPAI	11	0.97	0.25	7.7	
PPAI	13	-0.97	0.25	7.7	
PPAI	22	-0.84	0.24	6.0	
PPAI	24	-0.66	0.24	4.3	
PPAI	28	-0.88	0.24	6.8	
PPAI	X	1.88	0.26	11.5	78%

Abbreviations: CHR – chromosome location of SNP; a – size of effect; se - standard error; % Va – percent of genetic variance explained by SNP; Sum % - percent of genetic variance explained by the combination of the top ten SNP.

4.4 Effect of reproduction SNP on other traits

In order to test whether selection of animals with the SNP panel would affect other traits, the effects of the chosen SNP on 20 additional traits were estimated (Tables 7 to 10). To determine significant associations between PPAI and AGECL SNP and other production traits, a $-\log_{10}(P)$ cut-off of 3.65 was applied, the same as the lowest $-\log_{10}(P)$ that still resulted in the inclusion of the SNP in the panel (chromosome 13 SNP for PPAI in Tropical Composites, Table 4). In Brahman, none of the SNP for AGECL showed significant associations with PPAI and *vice versa*. In Tropical Composites, the BTA5 SNP that was selected for its significant association with AGECL also had a significant effect (in the same direction) on PPAI (Table 9).

Overall, very few significant ($-\log_{10}(P) > 3.65$) associations with non-reproduction traits were observed. This shows that a SNP marker panel may be able to be employed to make very specific selection decisions for reproduction traits. Five of the markers for AGECL in Brahmans were also associated with the trait WTCL, and this was also the case for 5 of the Tropical Composite markers for AGECL. It is not unexpected that gene marker predictions for a reduced age at puberty would also predict a lower weight at puberty, as age and live weight are closely linked.

A Brahman SNP on BTA4 that predicted an increased age at puberty also showed a significant association with a reduced IGF1 level at the W1 time point. As high IGF1 levels had been found to be significantly associated at the genetic level with reduced

age at puberty, this finding was not unexpected (Johnston et al., 2009), Corbet et al., 2011).

Hawken et al (2011) identified chromosomes 5 and 14 as having pleiotropic effects on a number of traits, not just reproduction traits, in Tropical Composites and Brahmans, respectively. This phenomenon also manifests when just one SNP per chromosome is picked to represent the effect.

The BTA5 SNP selected for its predictive value for PPAI in Tropical Composites also affects the traits W1WT, W1ADG, W1HH, WTCL, D2WT, D2HH, D2IGF. This is the only SNP marker in the Tropical Composite panels that has significant effects on other production traits (other than WTCL). If this marker alone was used to select for Tropical Composite cows with a shorter postpartum anestrous interval, the animals selected would also be expected to have a lower average daily gain (at 18 months), lower hip height (at 18 and 24 months), weigh less at puberty and at 18 months, and have significantly lower IGF1 levels at 24 months.

The SNP marker on BTA14 for AGECL on Brahmans also has significant effects on W1IGF, W1P8, W1CSN, D2IGF, D2P8, D2CSN and D2RIB. The marker on BTA14 associated with AGECL is the only Brahman SNP marker that also predicts other production traits (other than WTCL). Specifically, if only this marker was used to predict animals with reduced age at puberty on its own, they would also be predicted to have increased blood levels of IGF1 (18 and 24 months), better body condition (18 and 24 months), increased subcutaneous fat (18 and 24 months), and decreased hip height (24 months) (Table 7).

The W1ADG trait appeared to be negatively correlated with SNP predictions for an earlier age at puberty and a shorter PPAI. In some instances, for example the BTA1 marker for Brahman AGECL, our analysis would predict a large impact (15 g/day), albeit with a statistical significance of $-\log_{10}(P) = 3.07$, which is just below the significance threshold chosen here. The BTA5 SNP for PPAI in Tropical Composites is the only marker for which the predicted reduction in W1ADG (23 g/d) reached statistical significance ($-\log_{10}(P) = 4.07$). No large or statistically significant impacts of the marker panel on D2ADG were detected. Given that the negative correlation of the W1ADG trait with the reproduction traits is neither consistent (see for example BTA5 and BTA 6 markers for AGECL in Tropical Composites), or, in most cases, highly significant, it is unlikely that marker-based selection for favourable reproduction traits would result in animals with significantly slower growth.

Table 7: The effect of the 10 most significant SNP for AGECL in Brahman cows on 19 other traits

	chromosome location of SNP[2]																			
Trait[1]	BTA1		BTA2		BTA4		BTA5		BTA7		BTA8		BTA14		BTA15		BTA18		BTA21	
	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)
AGECL (d)	**30.09**	**6.11**	**-26.16**	**7.43**	**24.35**	**5.34**	**-26.02**	**7.14**	**27.44**	**6.08**	**32.10**	**8.33**	**41.25**	**11.30**	**43.21**	**6.44**	**25.84**	**5.35**	**35.70**	**5.31**
PPAI (d)	-0.13	0.45	-0.04	0.36	0.22	0.73	0.27	0.75	-0.02	0.33	0.33	1.05	0.60	2.03	0.27	0.66	0.46	1.55	0.06	0.37
WTCL (kg)	4.58	1.69	**-7.61**	**5.31**	5.86	2.87	**-7.73**	**5.20**	6.04	2.78	**9.23**	**5.73**	**15.10**	**11.43**	6.27	1.68	3.55	1.37	**11.29**	**4.33**
P8CL (mm)	0.25	1.82	-0.26	2.84	0.11	0.87	-0.26	2.74	0.06	0.53	0.26	2.25	-0.22	1.69	0.30	1.51	0.12	0.87	0.19	1.03
W1WT (kg)	-4.32	3.24	2.25	1.85	-1.90	1.31	0.51	0.51	-1.17	0.79	-0.72	0.57	2.86	1.94	-2.70	1.16	-3.33	2.48	-0.90	0.53
W1HH (cm)	0.09	0.43	0.22	0.88	-0.08	0.43	-0.10	0.51	-0.40	1.31	0.08	0.43	0.76	2.91	-0.16	0.50	-0.06	0.40	0.12	0.45
W1IGF (ng/ml)	-7.48	2.47	4.56	1.78	-8.81	3.88	4.69	1.83	-5.45	1.82	-4.46	1.46	**-23.61**	**18.00**	-2.25	0.55	-3.02	0.93	3.07	0.72
W1ADG (g/d)	-14.84	3.07	5.01	1.09	-6.20	1.19	3.84	0.83	0.83	0.37	0.61	0.36	6.26	1.10	-0.38	0.32	-4.46	0.83	-2.84	0.50
W1CSN (1-15)	-0.11	2.24	0.06	1.59	-0.07	1.65	0.01	0.46	-0.03	0.62	-0.03	0.67	**-0.19**	**5.67**	0.02	0.46	-0.05	1.05	-0.08	1.20
W1EMA (cm^2)	-0.34	0.98	0.23	0.86	-0.49	1.70	0.02	0.33	-0.06	0.40	-0.29	0.94	-0.48	1.50	-0.08	0.38	-0.62	2.20	0.33	0.76
W1P8 (mm)	-0.15	1.56	0.18	2.81	-0.20	2.66	0.20	3.21	-0.21	2.79	-0.15	1.77	**-0.65**	**11.79**	-0.08	0.62	-0.19	2.38	-0.17	1.29
W1RIB (mm)	-0.04	0.78	0.08	2.04	-0.10	2.34	0.11	3.09	-0.10	2.05	-0.07	1.35	**-0.30**	**11.70**	-0.08	0.96	**-0.16**	**4.11**	-0.02	0.47
D2WT (kg)	-3.62	2.31	2.06	1.54	-1.85	1.19	0.75	0.60	-1.69	1.03	0.30	0.40	3.12	2.01	-3.20	1.30	-3.85	2.83	0.09	0.32
D2HH (cm)	-0.36	1.37	0.14	0.73	0.07	0.46	0.06	0.44	-0.20	0.84	-0.16	0.72	**0.93**	**5.69**	-0.19	0.60	-0.21	0.87	0.06	0.38
D2IGF ng/ml	-1.09	0.43	3.25	1.01	-0.86	0.42	3.95	1.21	0.44	0.36	0.43	0.36	**-21.55**	**11.19**	5.03	0.89	-1.15	0.46	6.41	1.16
D2ADG (g/d)	3.13	0.81	-3.16	1.05	2.52	0.76	-1.61	0.60	-2.81	0.81	7.87	2.72	-1.23	0.48	-2.70	0.59	-1.78	0.59	4.77	0.95
D2CSN (1-15)	-0.05	0.76	0.07	1.39	-0.07	1.32	0.03	0.61	-0.04	0.79	-0.04	0.71	**-0.24**	**6.84**	0.10	1.13	-0.14	2.88	-0.07	0.93
D2EMA (cm^2	-0.34	1.04	0.26	1.01	-0.25	0.89	0.08	0.47	0.07	0.41	-0.10	0.48	-0.12	0.50	-0.56	1.25	-0.58	2.10	-0.06	0.37
D2P8 (mm)	-0.10	1.09	0.09	1.23	0.01	0.34	0.09	1.18	-0.11	1.34	-0.02	0.40	**-0.51**	**11.72**	0.11	0.86	-0.20	2.97	-0.12	1.02
D2RIB (mm)	-0.08	1.41	0.05	1.07	-0.05	1.07	0.09	2.18	-0.14	3.50	-0.07	1.33	**-0.33**	**11.78**	-0.06	0.78	-0.15	3.91	-0.04	0.59

[1] refer to Table 1 for trait definitions, [2] refer to Table 3 for identity of SNP; SNP effects with (–log 10(P) > 3.65) shown in **bold** type and in grey cells

Table 8: The effect of the 10 most significant SNP for PPAI in Brahman cows on 19 other traits

	chromosome location of SNP[2]																			
Trait[1]	BTA1		BTA5		BTA11		BTA12		BTA13		BTA16		BTA17		BTA19		BTA21		BTA30	
	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)
PPAI (d)	**-1.88**	**5.76**	**-1.20**	**4.90**	**1.16**	**5.05**	**1.04**	**4.84**	**1.10**	**4.73**	**1.10**	**5.14**	**-1.11**	**5.29**	**-1.02**	**4.83**	**1.31**	**6.86**	**-1.21**	**4.82**
AGECL (d)	-19.28	1.69	-7.40	0.85	10.95	1.34	-9.21	1.23	5.66	0.76	7.05	0.92	7.05	0.91	-6.14	0.84	-4.29	0.63	13.92	1.62
WTCL (kg)	-0.23	0.33	-2.68	0.86	1.39	0.56	-0.22	0.34	1.52	0.62	-0.79	0.45	2.08	0.77	-0.43	0.38	-3.51	1.29	4.11	1.28
P8CL (mm)	-0.27	1.22	0.00	0.31	0.09	0.66	0.09	0.67	0.11	0.78	0.13	0.95	-0.09	0.66	-0.04	0.47	-0.20	1.45	0.28	1.81
W1WT (kg)	4.03	1.67	-1.01	0.62	-0.01	0.30	-0.85	0.61	0.06	0.32	-2.09	1.29	1.64	1.00	1.48	0.96	-1.19	0.76	0.12	0.33
W1HH (cm)	0.73	1.58	-0.13	0.49	-0.24	0.74	-0.11	0.49	0.23	0.75	-0.19	0.66	0.35	1.10	0.16	0.59	-0.38	1.19	0.07	0.39
W1IGF (ng/ml)	6.22	1.18	2.09	0.61	-4.21	1.16	0.71	0.41	-2.11	0.67	0.54	0.38	-1.63	0.56	1.42	0.54	-1.52	0.55	-1.08	0.44
W1ADG (g/d)	8.83	0.99	-3.86	0.65	7.06	1.14	-5.40	0.96	-4.62	0.82	-6.02	1.03	2.26	0.51	2.46	0.55	-2.96	0.60	9.14	1.44
W1CSN (1-15)	0.10	1.23	0.05	0.86	0.06	1.11	-0.03	0.70	0.05	1.04	-0.03	0.57	-0.02	0.56	0.06	1.28	-0.08	1.61	0.09	1.44
W1EMA (cm^2)	-0.11	0.41	-0.46	1.21	-0.22	0.66	0.11	0.47	0.10	0.46	-0.65	2.15	0.15	0.55	0.61	2.14	-0.27	0.84	0.30	0.80
W1P8 (mm)	0.08	0.63	0.09	0.86	-0.08	0.79	-0.09	0.93	0.05	0.59	0.02	0.43	-0.13	1.32	0.08	0.84	-0.20	2.45	0.19	1.72
W1RIB (mm)	0.10	1.12	0.03	0.57	-0.05	0.82	-0.01	0.42	0.05	0.86	0.02	0.51	-0.01	0.31	0.07	1.43	-0.06	1.14	0.09	1.36
D2WT (kg)	4.12	1.59	-0.92	0.56	-0.58	0.46	-1.51	0.90	0.49	0.45	-2.62	1.58	2.03	1.17	1.96	1.19	-1.77	1.04	-0.29	0.37
D2HH (cm)	0.66	1.75	-0.25	0.85	0.10	0.50	0.09	0.49	-0.30	1.19	-0.45	1.90	0.22	0.85	0.24	0.97	-0.09	0.50	0.13	0.53
D2IGF ng/ml	1.82	0.45	1.20	0.43	-2.60	0.66	-2.68	0.70	-4.03	0.99	0.17	0.32	1.05	0.43	4.61	1.20	2.16	0.61	0.21	0.32
D2ADG (g/d)	-0.40	0.33	0.54	0.36	-3.96	0.99	-4.89	1.36	1.07	0.45	-2.89	0.79	8.10	2.52	3.45	0.96	-2.25	0.67	-1.41	0.48
D2CSN (1-15)	-0.10	1.02	0.02	0.46	-0.05	0.78	-0.01	0.38	0.04	0.65	-0.01	0.37	0.04	0.64	0.08	1.39	-0.11	2.01	0.08	1.10
D2EMA (cm^2	-0.07	0.36	-0.44	1.22	-0.02	0.33	-0.27	0.87	0.03	0.34	-0.57	1.97	-0.05	0.38	0.42	1.43	0.09	0.45	-0.12	0.47
D2P8 (mm)	-0.02	0.37	-0.04	0.51	-0.03	0.44	-0.08	0.93	-0.04	0.57	0.05	0.61	-0.09	0.99	0.04	0.58	-0.07	0.78	0.08	0.83
D2RIB (mm)	0.05	0.64	0.01	0.38	-0.02	0.50	-0.03	0.56	-0.03	0.66	0.04	0.70	-0.04	0.68	0.07	1.31	-0.06	1.07	0.04	0.72

[1] refer to Table 1 for trait definitions, [2] refer to Table 3 for identity of SNP; SNP effects with (–log 10(P) > 3.65) shown in **bold** type and in grey cells

Table 9: The effect of the 10 most significant SNP for AGECL in Tropical Composite cows on 19 other traits

	10 most significant SNP for AGECL in Tropical Composite cows: chromosome location[2]																			
Trait[1]	BTA4		BTA5		BTA6		BTA7		BTA9		BTA10		BTA11		BTA12		BTA13		BTA19	
	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)
AGECL (d)	**24.67**	**3.94**	**28.50**	**6.07**	**-25.87**	**4.39**	**-21.57**	**4.27**	**-20.22**	**4.02**	**-21.05**	**4.31**	**-23.76**	**4.56**	**-25.69**	**4.67**	**37.31**	**6.05**	**29.31**	**4.70**
PPAI (d)	0.28	0.94	0.81	4.38	-0.38	1.30	-0.27	1.13	-0.19	0.80	-0.03	0.37	0.16	0.67	-0.17	0.67	0.46	1.39	0.47	1.53
WTCL (kg)	4.12	1.25	**10.37**	**5.46**	**-12.14**	**5.94**	**-7.79**	**3.82**	-2.27	0.86	-4.51	1.81	-3.41	1.18	**-9.89**	**4.60**	6.12	1.68	**9.98**	**3.83**
P8CL (mm)	0.06	0.57	0.12	1.09	-0.07	0.66	0.02	0.41	-0.06	0.65	0.04	0.55	0.04	0.48	-0.01	0.33	0.26	2.04	0.21	1.80
W1WT (kg)	-2.34	0.97	1.58	0.77	-1.48	0.68	0.58	0.45	2.53	1.33	3.08	1.68	4.32	2.36	-2.15	0.96	-2.41	0.88	-2.43	0.95
W1HH (cm)	-0.47	1.41	0.70	2.79	-0.54	1.67	0.10	0.49	0.07	0.44	0.17	0.66	0.19	0.67	-0.09	0.44	-0.14	0.50	0.12	0.47
W1IGF (ng/ml)	-5.40	1.07	-10.11	2.60	8.11	1.74	5.27	1.25	1.42	0.48	2.43	0.65	1.37	0.46	4.30	0.90	1.71	0.45	-6.35	1.17
W1ADG (g/d)	0.17	0.31	12.73	1.94	-10.75	1.37	-0.48	0.33	0.11	0.31	7.28	1.12	1.40	0.40	-3.65	0.57	-1.22	0.36	-16.18	2.07
W1CSN (1-15)	-0.13	2.28	-0.09	1.61	0.05	0.84	0.03	0.62	0.02	0.57	0.02	0.49	0.03	0.65	-0.02	0.52	-0.01	0.39	-0.04	0.69
W1EMA (cm^2)	-0.40	0.91	-0.51	1.30	0.28	0.69	0.05	0.37	0.60	1.84	0.41	1.17	0.71	2.03	-0.28	0.72	-0.96	2.05	-0.36	0.78
W1P8 (mm)	-0.07	0.72	0.01	0.38	0.13	1.27	0.13	1.44	0.09	1.03	0.22	3.14	0.20	2.39	0.17	1.77	-0.07	0.61	-0.03	0.46
W1RIB (mm)	-0.08	1.17	-0.09	1.57	0.10	1.44	0.10	1.90	0.05	0.86	0.12	2.46	0.07	1.14	0.07	1.13	0.04	0.54	-0.04	0.65
D2WT (kg)	-3.64	1.51	1.03	0.56	-2.24	0.91	-0.72	0.48	1.49	0.76	2.85	1.44	3.49	1.67	-3.89	1.75	-2.32	0.81	-2.00	0.77
D2HH (cm)	-0.47	1.39	0.83	3.51	-0.66	2.18	-0.15	0.61	0.22	0.79	0.20	0.75	0.29	0.97	-0.47	1.50	-0.25	0.68	0.23	0.67
D2IGF ng/ml	-1.76	0.47	-7.53	1.72	3.88	0.77	-0.93	0.41	2.19	0.60	2.37	0.64	1.54	0.48	2.51	0.59	0.41	0.33	1.08	0.40
D2ADG (g/d)	-1.26	0.42	2.44	0.59	-3.05	0.64	-2.81	0.69	-1.01	0.42	-1.64	0.50	-6.16	1.33	-9.85	2.20	2.19	0.48	-0.28	0.32
D2CSN (1-15)	-0.06	0.88	-0.10	1.87	0.10	1.64	-0.02	0.56	0.03	0.73	0.00	0.32	0.05	0.87	-0.02	0.48	-0.02	0.47	-0.08	1.19
D2EMA (cm^2	-0.22	0.60	-0.53	1.44	0.54	1.30	0.43	1.24	0.14	0.52	0.48	1.41	0.88	2.87	-0.53	1.33	-0.57	1.15	-0.38	0.85
D2P8	-0.04	0.52	-0.12	1.25	0.21	2.22	0.11	1.32	0.08	0.88	0.16	2.09	0.19	2.35	0.14	1.39	0.00	0.32	-0.03	0.43
D2RIB	-0.06	0.80	-0.18	3.57	0.05	0.71	0.02	0.44	0.04	0.69	0.14	2.72	0.16	3.06	0.10	1.47	0.01	0.35	-0.02	0.45

[1] refer to Table 2 for trait definitions, [2] refer to Table 4 for identity of SNP ; SNP effects with (–log 10(P) > 3.65) shown in **bold** type and in grey cells

Table 10: The effect of the 10 most significant SNP for PPAI in Tropical Composite cows on 19 other traits

	10 most significant SNP for PPAI in Tropical Composite cows: chromosome location[2]																			
Trait[1]	BTA2		BTA5		BTA6		BTA7		BTA11		BTA13		BTA22		BTA24		BTA28		BTA30	
	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)	Size of effect	-log (*P*)
PPAI (d)	**1.01**	**3.71**	**1.41**	**9.55**	**1.04**	**4.72**	**1.02**	**3.69**	**1.10**	**4.08**	**-1.01**	**3.65**	**-1.05**	**3.83**	**-1.02**	**3.95**	**-1.12**	**4.78**	**1.22**	**4.51**
AGECL (d)	9.72	0.92	18.99	2.70	10.44	1.10	-16.15	1.61	1.11	0.35	-18.71	1.89	-24.30	2.63	-12.14	1.17	2.86	0.44	-3.17	0.44
WTCL (kg)	3.57	0.87	**13.82**	**7.57**	3.06	0.85	-3.34	0.85	-0.09	0.31	-5.55	1.37	-9.34	2.63	-4.36	1.08	0.77	0.40	1.05	0.42
P8CL (mm)	0.04	0.43	0.14	1.19	0.09	0.71	-0.02	0.37	-0.15	0.97	-0.06	0.50	-0.25	1.76	-0.02	0.35	-0.03	0.40	0.03	0.39
W1WT (kg)	-2.01	0.71	**7.73**	**4.86**	0.88	0.47	3.24	1.12	-1.56	0.59	-0.82	0.44	0.37	0.36	-0.62	0.41	-2.33	0.83	0.11	0.32
W1HH (cm)	0.05	0.35	**1.67**	**9.95**	0.03	0.34	0.34	0.85	-0.03	0.33	0.02	0.32	-0.30	0.72	-0.19	0.56	-0.29	0.74	-0.31	0.70
W1IGF (ng/ml)	-7.13	1.13	-8.66	1.83	4.62	0.83	2.60	0.54	-3.27	0.59	-0.64	0.35	-0.58	0.34	-2.72	0.54	-1.64	0.44	0.67	0.34
W1ADG (g/d)	-8.84	0.88	**23.24**	**4.07**	-4.28	0.57	2.69	0.44	-0.15	0.31	-3.77	0.50	2.73	0.43	2.41	0.42	-9.30	0.97	-1.48	0.37
W1CSN (1-15)	-0.07	0.90	-0.13	2.47	0.06	0.84	0.10	1.29	0.02	0.40	0.02	0.40	0.03	0.49	0.01	0.37	-0.03	0.55	0.01	0.36
W1EMA (cm^2)	-1.16	2.46	-0.55	1.29	0.26	0.60	0.52	0.97	-0.52	0.91	0.43	0.79	0.00	0.30	0.03	0.32	0.21	0.52	-0.02	0.31
W1P8 (mm)	-0.14	1.05	0.05	0.54	0.15	1.24	0.06	0.55	-0.01	0.34	0.03	0.42	0.00	0.31	0.09	0.75	0.00	0.31	-0.12	0.83
W1RIB (mm)	-0.01	0.33	-0.05	0.78	0.11	1.50	-0.02	0.40	-0.07	0.80	-0.04	0.52	-0.07	0.82	0.08	0.98	0.02	0.42	0.04	0.50
D2WT (kg)	-0.69	0.41	**9.52**	**6.38**	0.23	0.34	2.79	0.91	-0.05	0.31	-0.89	0.44	-0.42	0.36	-1.89	0.67	-1.91	0.69	1.72	0.58
D2HH (cm)	-0.18	0.52	**1.93**	**12.00**	0.32	0.85	0.25	0.67	0.44	0.96	0.35	0.82	-0.23	0.60	-0.12	0.45	-0.20	0.57	-0.33	0.73
D2IGF ng/ml	3.07	0.57	**-15.91**	**4.66**	-1.29	0.41	-5.39	0.85	2.35	0.48	7.77	1.18	4.97	0.77	0.41	0.33	4.32	0.74	6.17	0.91
D2ADG (g/d)	8.76	1.33	11.80	2.72	2.71	0.56	-11.98	2.04	1.68	0.42	-4.50	0.70	0.10	0.31	-4.38	0.73	1.51	0.42	6.27	0.90
D2CSN (1-15)	-0.09	1.18	-0.13	2.43	0.06	0.93	-0.01	0.34	-0.10	1.25	-0.06	0.77	-0.06	0.78	0.08	1.01	-0.03	0.52	0.08	0.99
D2EMA (cm^2	-0.23	0.54	-0.61	1.53	0.04	0.34	0.46	0.89	0.12	0.41	0.17	0.47	0.14	0.43	0.02	0.32	0.39	0.80	0.14	0.42
D2P8 (mm)	-0.03	0.42	0.01	0.37	0.05	0.55	0.06	0.59	-0.17	1.24	0.10	0.78	-0.04	0.47	0.16	1.24	-0.07	0.60	0.06	0.51
D2RIB (mm)	0.01	0.36	-0.15	2.43	0.06	0.72	0.06	0.73	-0.16	1.79	-0.04	0.50	0.01	0.34	0.13	1.55	0.01	0.38	0.02	0.42

[1] refer to Table 2 for trait definitions, [2] refer to Table 4 for identity of SNP; SNP effects with (–log 10(P) > 3.65) shown in **bold** type and in grey cells

4.5 Clustering analysis revealing correlations and trade-offs between traits based on SNP effects

The associations between SNP markers and the 20 traits studied were subjected to hierarchical clustering and shown as “heat maps” where the most significant associations are represented as brighter tones of green or red colours. Negative associations are shown as red, while positive associations are coloured green. The output from the clustering program groups traits with correlated SNP associations.

Clusters of associated traits in Brahmans and Tropical Composites show clear “condition” and “stature” groupings (Figure 3 and 4). This indicates that SNP-based selection for traits will tend to result in correlated responses in traits that reflect altered growth and metabolism. For example, SNP-based selection for AGECL and PPAI will tend to select for animals with a genetic predisposition to be fatter and have better body condition (Figure 3 and 4).

However, Figure 3 also shows that the significant associations of the BTA14 SNP with hip height in Brahmans, and the trend for body weight are in an opposite direction to the trend displayed by the remaining SNP in the panel. In other words, the remainder of the marker panel would predict animals with a reduced age at puberty that would also tend to have higher body weights and hip height at the 18 month and 24 month time points (–log 10(P) < 3.24), while the BTA14 SNP would predict that those animals would be shorter and tend to weigh less.

The BTA14 “PLAG1” region is already well documented for its pleiotropic effects (Karim et al., 2011). Genetic variation in this region may affect the animals’ set point for “mature height”, therefore permitting animals to reach puberty at a lower live weight.

The separate SNP on BTA5 selected to represent AGECL and PPAI in Tropical Composites are located in the same broad region (see section 4.7) and show the same overall trend in their pleiotropic effects on the condition and stature traits in Tropical Composites.

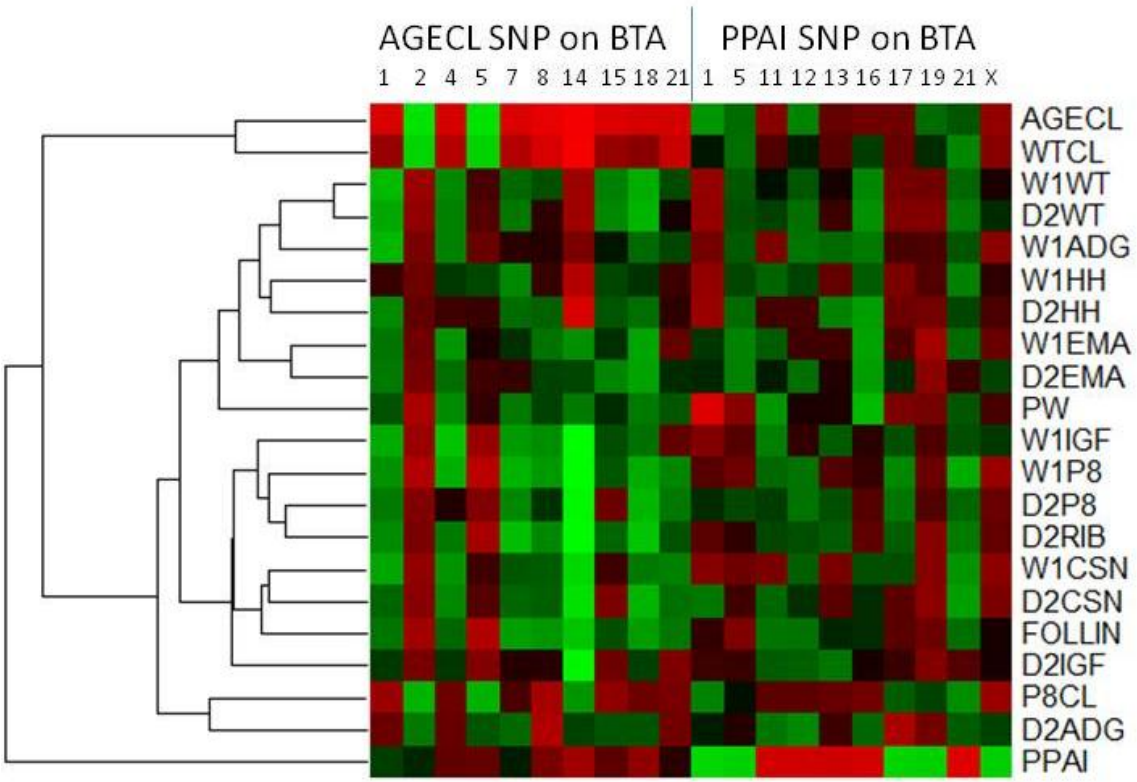


Figure 3: Heatmap of the association (effect/se) of SNP for AGECL and PPAI in Brahman with all traits. Intensity of colour corresponds to strength of statistical association, green or red corresponds to negative or positive values (for numerical values, see Tables 7 and 8)

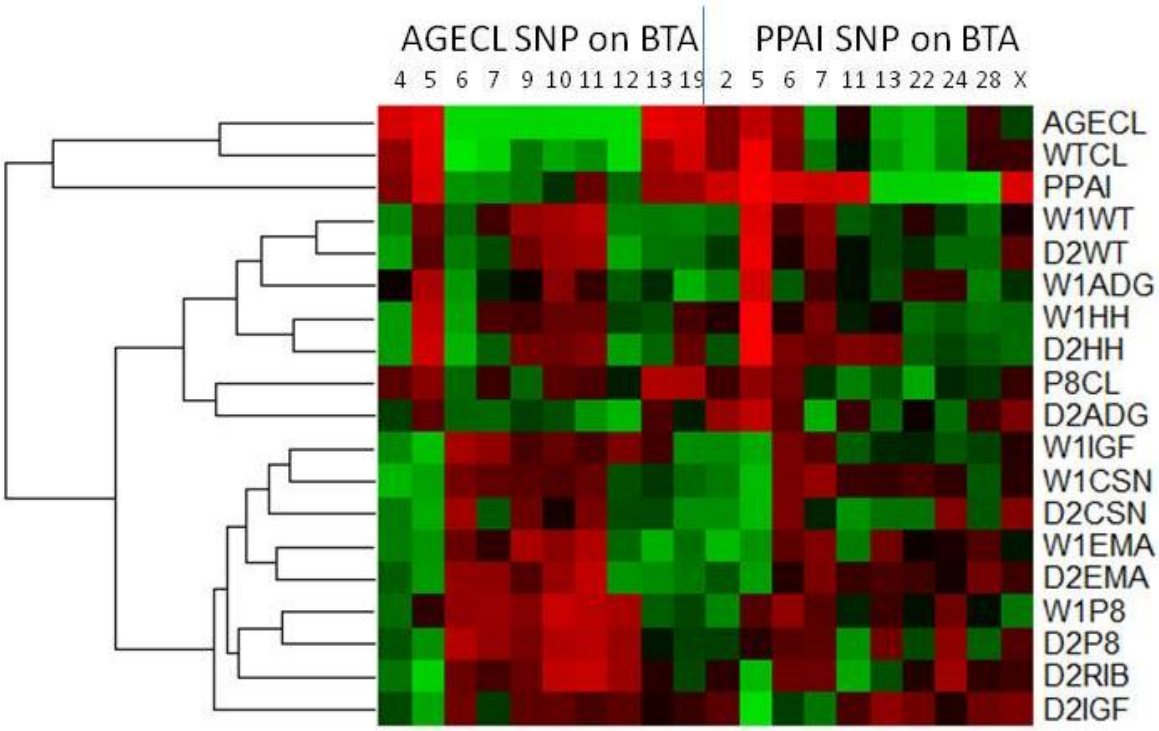


Figure 4: Heatmap of the association (effect/se) of SNP for AGECL and PPAI in Tropical Composites for all traits. Intensity of colour corresponds to strength of statistical association, green or red corresponds to negative or positive values (for numerical values, see Tables 9 and 10).

4.6 Fine mapping of BTA14

This report has identified a region in the *B. taurus* autosome (BTA) 14, between 20 and 30 Mb UMD3 genome assembly), that harbours SNPs associated with age at puberty in males and females, blood levels of insulin-like growth hormone I (IGF-I), weight, hip height and fat deposition in *B. indicus* influenced cattle, Brahman and Tropical Composites (Fortes et al., 2012b; Hawken et al., 2011; Karim et al., 2011). This BTA14 region was also associated with fat deposition phenotypes (post slaughter) in a study that included *B. taurus*, *B. indicus*, Tropical Composites and crossbred cattle (Bolormaa et al., 2011; Karim et al., 2011). This evidence suggests that mutations located between 20 and 30 Mb of BTA14 might underpin the genetic correlation between weight, height, fat deposition and reproduction.

Other groups also found this same region of BTA14 to be associated with stature (height and weight, or frame size) in a population of *B. taurus* dairy cattle (Karim et al., 2011) and with feed intake in crossbred beef cattle (Karim et al., 2011; Lindholm-Perry et al., 2012). The study with dairy cattle was critical as it proposed a causative mutation, a SNP in the 3' UTR of the *PLAG1* gene identified as rs109231213 that could be responsible for the differences in stature observed in their population. It is not known whether this same mutation is also responsible for the SNP associations reported for the region of BTA14 with differences in age at puberty, IGF-I, fat deposition or feed intake, as observed in previous studies.

Motivated by these findings, the objective of the present study was to further characterise this region in terms of its pleiotropic effects and to test whether *PLAG1* could be confirmed as the candidate causative gene in this region in Brahman cattle. We tested the association of rs109231213 in addition to all the 23,495 SNP of the HD Illumina chip that were located at BTA14 across a range of growth, fat deposition, feed intake and reproduction phenotypes. In order to maximise our power to detect and fine-map associations between individual SNP and relevant traits, we used the combined population of Brahman bulls (n=1,014; described in the Final Report for B.NBP.604) and cows (n=996) for this study.

A number of bovine genes map to the 25 Mb region on BTA14 region. The UMD 3.0 version of the bovine genome browser shows annotations for *XKR4, TMEM68, TGS1, LYN, RPS20, MOS, PLAG1, CHCD7, SDR16C5, SDR16C6, PENK, IMPAD1, FAM110B*, between 24 and 26 Mb. Figure 5 shows that the *PLAG1* SNP rs109231213 can substitute for all the SNP associations observed over the 20 to 30 Mb BTA14 region.

Table 11 shows that the *PLAG1* SNP rs109231213 did not emerge as the top marker for all the traits that show significant associations to this region. It was the most significant association for IGF in bulls, and showed very highly significant associations to AGECL and hip height and body condition traits in cows, but it was not the only gene that could be linked to these traits.

It is possible that more than one type of variation, affecting more than one gene, is responsible for the pleiotropic effects of the *PLAG1* region in cattle. It is equally possible that the number of genotypes examined here is not sufficient to resolve the region in enough detail to confirm the *PLAG1* rs109231213 SNP as the causative mutation.

Future R&D in this area may be able to resolve the question of whether specific gene markers can be identified for the different production traits with significant SNP associations to the region. This detailed knowledge would benefit industry by providing clear information about trade-offs (hip height, body condition) that may or may not have to be made to achieve an earlier age at puberty in Brahman cows.

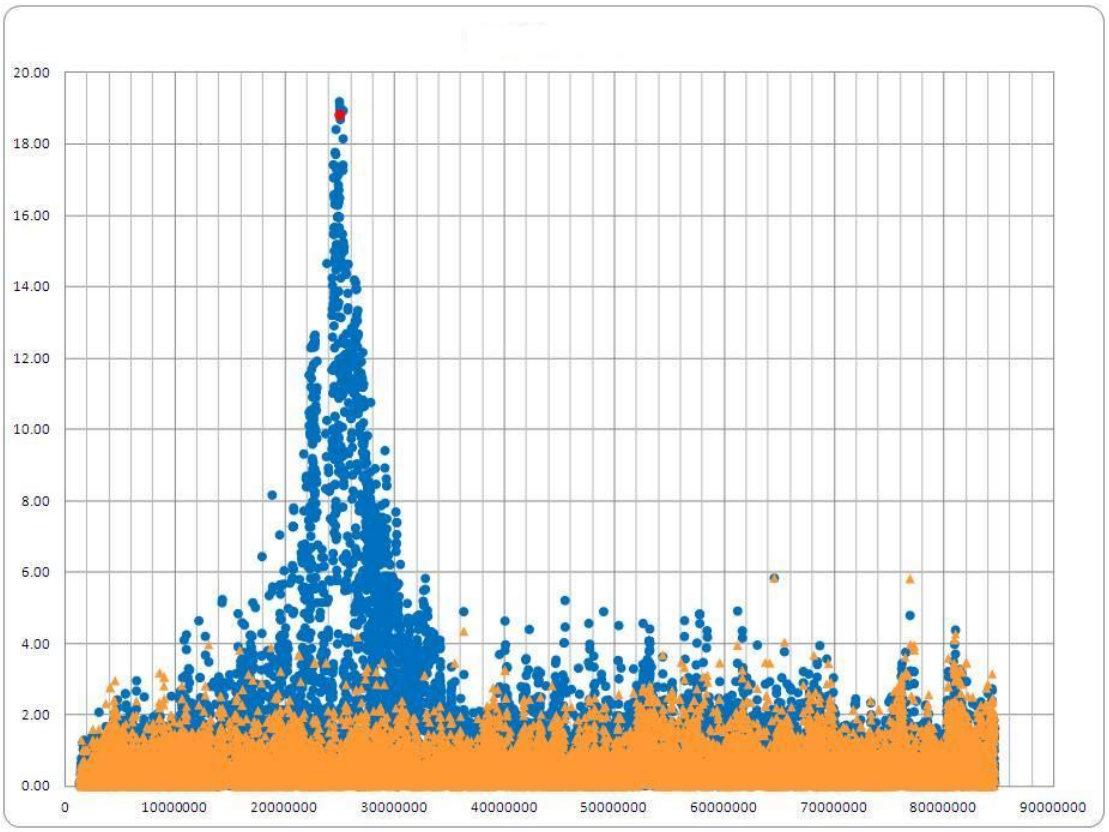


Figure 5. Significance of SNP located in BTA 14 before (in blue) and after (in orange) fitting the rs109231213 SNP as a fixed effect in the association analysis for IGF1 hormone levels in *B. indicus* (Brahman) cattle. The significance of the rs109231213 SNP fitted in isolation is represented by the red dot.

Table 11: SNP associations in the *PLAG1* region of BTA14

Trait	*PLAG1* SNP		SNP with best $-\log_{10}(P)$		
	$-\log_{10}(P)$	Rank	position (Mbp)	gene	$-\log_{10}(P)$
Cows					
AGECL	9.72	55	25.2	*PENK*	11.22
W1IGF	18.82	5	25.0	*MOS*	19.19
W1WT	10.12	47	24.5	*XKR4*	11.63
W1HH	6.25	27	29.3	*NKAIN3*	8.16
W1CSN	4.59	269	26.5	*NSMAF*	6.70
D2P8	12.21	162	25.1	*LOC526726*	14.88
D2RIB	1.06	3591	83.4	*ENPP2*	5.29
					Bulls
Age at puberty	5.44	26	24.1	*RP1*	9.05
IGF1	22.46	2	25.0	*PLAG1*	22.63
Live weight	2.45	390	84.4	*SNTB1*	5.80
Hip height	5.58	8	24.3	*XKR4*	6.06
Body Condition	2.16	200	45.4	*MRPS28*	3.56

4.7 BTA5

In the current study, BTA5 featured as the chromosome containing the most significant markers for age at puberty, postpartum anestrous interval and many weight and fat traits in the Tropical Composite data set (Table 10, Figure 6). This chromosome has been identified in a large number of studies investigating quantitative trait loci (QTL) for reproduction, including ovulation and twinning rate (Kappes et al., 2000; Schrooten et al., 2000; Cruickshank et al., 2004; Daetwyler et al., 2008; Schulman et al., 2008; Allan et al., 2009; Kim et al., 2009).

The 45-50Mb region of BTA5 shows significant associations with reproductive phenotypes in two studies, one investigating age at first service (Daetwyler et al., 2008) and one with twinning (Cruickshank et al., 2004). The two BTA5 SNP included in the Top 10 SNP for AGECL and PPAI in Tropical Composites map at 44 and 49 Mb, respectively. Interestingly, 5 of the 9 significant ($P < 0.001$) markers for both PW and PPAI in Tropical Composites in this chromosome region on BTA5, also have an F_{ST} value greater than 0.484 (1% genome wide significance level) indicating that this region could be under selection. This signature of selection on BTA5 was also identified in dairy and beef cattle breeds (Barendse et al., 2009; Chan et al., 2010).

The High Mobility Group AT-Hook 2 gene (*HMGA2*) maps to the 48 Mb position on BTA5. This gene has been identified and validated in multiple studies as associated with stature in humans (Lettre, 2009; Meyer, 2006a). One very significant SNP in our cattle study was rs29016809, which is within an intron of the *HMGA2* gene in cattle. This SNP is significant in Tropical Composites at $P < 0.001$ for many traits including both reproduction and growth and weight traits (PPAI, PW, W1SEMA, D2IGF, W1WT, D2WT, WTCL, D2HH, and W1HH) but also significant at $P < 0.01$ for

AGECL, D2SRIB, W1ADG. At a reduced level of significance (P < 0.01), this SNP is also significant for D2CS, D2SEMA, D2HH, D2SP8 in BRM, although not for reproduction traits. Furthermore, it has an extreme F_{ST} value (0.64) between our Brahman and Tropical Composite populations.

A second gene of potential interest for reproduction traits in this region is interferon gamma (*IFNG*). It has been shown that IFNG acts synergistically with tumour necrosis factor alpha to induce necrosis in endothelial cells in the bovine corpus luteum. This process is essential for luteolysis, and therefore for re-setting the ovarian cycle so the animal can ovulate again (Hojo et al., 2010).

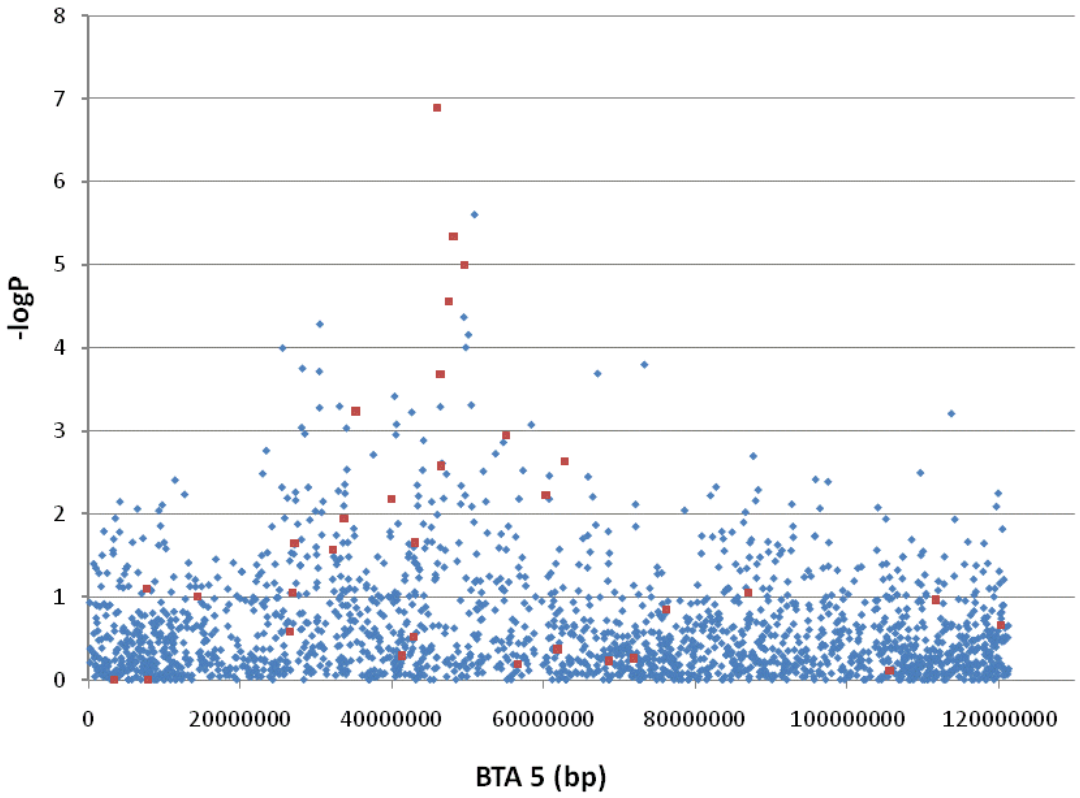


Figure 6: Association peaks for PPAI SNP on chromosome 5 in Tropical Composites. Red squares indicate those SNPs with an FST value >0.484.

4.8 BTA21

The GWAS for female reproductive traits identified an association peak on BTA21, particularly for AGECL in Brahmans (Hansen et al., 1967; Hawken et al., 2011). On closer examination, we found SNP located in and around *IGF1R* that presented associations with AGECL in both Brahmans and Tropical Composites. In the Brahman GWAS, three SNPs in *IGF1R* were associated with AGECL (one with P = 0.00009; two with P < 0.05). The SNP in *IGF1R* with the highest association had an estimated effect of 49 days (SE = 12.17 days) and explained 2.04% of the total variance (Table 12). A single SNP explaining 2% of the total variance of a complex trait should be of interest given similar results found for functional polymorphisms

(Hansen et al., 1967; Turner et al., 2010). Given this evidence and the known involvement of this receptor in the maturation of the gonadotropin-releasing hormone axis during sexual maturation, *IGF1R* can be considered a candidate gene associated with the peak found in BTA21. We have selected additional SNP in the *IGF1R* region and genotyped the population, discovering haplotypes of *IGF1R* that were associated with AGECL in both Brahman and Tropical Composite cattle. The detailed results have been published (Fortes et al., 2012a).

Table 12: Single-nucleotide polymorphisms (SNPs) from the *IGF1R* region on BTA21: significance of the association with AGECL, SNP effect and minor allele frequencies.

		Tropical Composite				***Brahman***			
SNP ID	***BTA21 position***	***P***	***Effect***	***R^2***	***MAF (866)***	***P***	***Effect***	***R^2***	***MAF (843)***
ARS-BFGL-NGS-115411	***6535070***	***0.925***	***1.3***	***0***	***0.009***	***0.018***	***85***	***0.55***	***0.005***
ARS-BFGL-NGS-86900	***6633472***	***0.043***	***12.2***	***0.64***	***0.389***	***0.03***	***-21.5***	***0.53***	***0.081***
ARS-BFGL-BAC-34544	***6661835***	***0.41***	***1.5***	***0.01***	***0.42***	***0.064***	***10.9***	***0.44***	***0.409***
ARS-BFGL-NGS-111433	***6696772***	***0.043***	***-11.2***	***0.57***	***0.458***	***0.527***	***6.7***	***0.16***	***0.375***
ARS-BFGL-NGS-16316	***6730037***	***0.817***	***-1.8***	***0.01***	***0.45***	***0.888***	***-4.1***	***0.03***	***0.113***
ARS-BFGL-NGS-15702	***6762467***	***0.14***	***7.5***	***0.18***	***0.234***	***0.063***	***-22.9***	***0.55***	***0.074***
Hapmap35256-BES3_Contig435_690	***6806087***	***0.343***	***-8.1***	***0.21***	***0.22***	***0.00009***	***49***	***2.04***	***0.059***
ARS-BFGL-NGS-23160	***8337533***	***0.23***	***9.5***	***0.24***	***0.18***	***0.512***	***-8.5***	***0.09***	***0.094***
ARS-BFGL-NGS-24429	***8361477***	***0.21***	***9.0***	***0.26***	***0.229***	***0.578***	***-8.1***	***0.08***	***0.093***

SNP ID: polymorphism ID according to Illumina codes or dbSNP access number. *P*, *P* value of each SNP association with AGECL (age of puberty defined by first observed corpus luteum); Effect, additive effect on AGECL in days calculated for each SNP; R^2, percentage of the variation that is explained by the SNP; MAF (n), minor allele frequency (number of animals genotyped).

5 Success in achieving objectives

5.1 Overall success

The success of this project exceeded the original objectives. Due to technology advances that occurred during the lifetime of the Beef CRC, the project was able to achieve far more comprehensive genome scans than originally planned.

In response to the technology developments, significant restructuring and re-negotiation of the Beef CRC's goals occurred. As a result of this, a major output of this project has not been the release of genetic marker panels and the identification of causative genes, but the first genomic prediction equations for reproductive traits

in Australian beef breeds, and their validation. These important project outcomes are detailed in the Beef CRC's final report (Program 7), and will be incorporated into Breedplan as genomically-enhanced EBV (gEBV).

In addition, the success of the genome scans performed in this project formed the basis for analysing populations of Brahman and Tropical Composite beef bulls in the same way. MLA projects B.NBP.604 and B.NBP.723 have generated whole genome SNP data for around 1,000 bulls from each breed with reproduction phenotypes. These data have provided important information about the genomic links between male and female reproduction traits and will form the basis for future improvements to genomic predictions.

Eleven peer-reviewed publications have resulted from this project so far. The database resulting from this research is likely to result in additional publications over the coming years, as additional phenotypes are tested for associations to SNP.

5.2 Objective 1

Assess the current phenotypic data in the CRC2.3 'fertility population', choose appropriate animals and complete a whole-genome scan of the fertility resource population to identify significant chromosomal regions for puberty and postpartum reconception.

This objective was achieved as described in section 4.2. A peer reviewed publication (Hawken et al., 2011) resulted from this work, and the genome-wide association data were used as the basis for achieving Objective 2.

5.3 Objective 2

Fine map chromosomal regions identified in the whole genome scan and find genetic markers that can be used as DNA diagnostic markers for age at puberty and postpartum re-conception.

This objective was achieved by imputing high-density genotypes across the Brahman and Tropical Composite cows used in this study. The outcomes from this work are detailed in section 4.3 of this report.

5.4 Objective 3

Perform targeted sequencing of genes and genome regions to identify possible functional mutations and haplotypes that may more accurately define reproductive rate phenotypes.

Section 4.6 – 4.8 describe the activities that took place in fulfilment of this objective.

During the lifetime of the Beef CRC, technology became available to perform far more dense genotyping (50 K SNP coverage instead of 10K), and during the final year of the CRC, all 50K genotypes were imputed to high density (800 K SNP) coverage. To a large extent, these technology advances have made fine mapping of targeted genome regions obsolete.

However, targeted sequencing of key genome regions (sequence capture technology) was attempted in 2009, but proved unsuccessful at the time. This was due to the less advanced state of this technology in the context of the bovine genome at the time. This objective was then given lower priority, as resources were allocated to whole genome sequencing of individual animals from the Beef CRC population.

The whole genome sequencing data is only now becoming available, and will be fully analysed for the identification of functional mutations and accurate haplotypes over the coming years.

5.5 Objective 4

Assess the effect of identified diagnostic markers for age at puberty and postpartum reconception across other non-reproductive traits

This objective has been achieved and the results are detailed in section 4.4 and 4.5 of this report.

6 Impact on meat and livestock industry – Now and in five years' time

6.1 The role of genetic markers in genetic improvement strategies

The rate of genetic gain that can be achieved by selective breeding strategies depends on a number of factors. These factors are included in the following equation used to calculate the expected rate of genetic gain per year (ΔG):

$$\Delta G=(A\sigma_g i)/I$$

where A is the accuracy of selection, σ_g is the standard deviation of the additive genetic variation in the population, i is the selection intensity (proportion selected for further breeding), and I is the generation interval (age of breeding). The σ_g factor cannot be easily changed within a breed, while the other quantities in the equation can be changed.

The use of genetic markers and genomic prediction technology, by enabling more accurate selection decisions at an earlier age, impacts on the A and I factors in this equation. In some cases, genetic markers and genomic prediction technology will also improve the selection intensity.

For example, for a sex-limited trait such as cow reproductive performance, bulls will not acquire an accurate EBV for daughter reproductive performance until their female offspring has reached reproductive age. By using DNA-based technology, bulls could acquire a genomic prediction for their daughters' reproductive performance at birth. This would allow the selection intensity (i) for this trait to increase, as it would allow stud breeders to focus resources on young bulls with high predicted genetic merit. In other words, the use of DNA-based predictions may mean that it becomes cost-effective to use a broader base from which to recruit bulls into a selective breeding program.

The addition of DNA-based information to pedigree-based predictions invariably increases the accuracy of EBV estimates at an early age, thereby impacting the "A" factor in the above equation. One of the largest impacts of DNA-based predictions on a sex-limited trait is the dramatic reduction in generation interval. By adding DNA-based predictions to pedigree-based estimates, it will be possible to confidently use bulls before their own offspring has been evaluated.

DNA-based estimation of breeding value is having a dramatic impact in the dairy industry, where genomic predictions on a similarly sex-limited trait (milk production) are applied to young dairy bulls. While the beef industry has not yet achieved the

same prediction accuracies that now prevail in the Holstein-based global dairy industry, even at lower accuracies the impacts of DNA-based technology on the rate of genetic gain in beef selective breeding programs will become evident in the near future.

6.2 Impact of genetic markers on industry – now and in five years' time

By including DNA-based predictions in existing delivery mechanisms such as genomically enhanced EBV through Breedplan, this technology will impact on bull selection in the stud breeding sector in the foreseeable future. By eliminating, for example, the worst performers for predicted daughter fertility from the Brahman stud sector, the impact of these bulls on overall herd performance will gradually decrease. This will start showing up as an increase in the weaning rates of Brahman breeder herds, once the daughters of bulls genomically-selected for improved female reproductive performance reach reproductive age themselves.

Improved accuracies and cheaper genotyping will eventually lead to DNA-based predictions extending from the stud level to the commercial herds. For example, multiplier herds may not only use the bulls with the highest genetic merit but also be able to rank prospective herd bulls based on their DNA predictions. DNA technology may be extended to the selection of females at the stud level and of replacement females in breeder herds. With continued investment, it is conceivable that the Australian industry will be applying genetic markers for female reproductive performance at different levels of the industry within five years.

The impact on the industry will be felt in a permanent and cumulative improvement in weaning rates from extensive breeding herds in northern Australia, particularly Brahman herds.

7 Conclusions and recommendations

7.1 Prospects for gene marker technology in improving beef cow fertility

As a result of the outputs from this work, the Australian Beef CRC was able to publish the world's first study of genome-wide SNP associations with reproductive traits measured in tropically-adapted beef cows. One of the clear results from this study is that the genomic determinants of reproductive traits between the two traits and two breeds studied differ profoundly. Therefore, separate gene marker tools and genomic predictions need to be developed for Brahmans and Tropical Composites, and for the other major tropical breeds in northern Australia such as Santa Gertudis and Droughtmaster.

The extensive data generated as part of this research also show that a small set of gene markers can potentially predict a significant proportion of the genetic component of the traits, age at puberty and length of postpartum anestrus. There was no overlap between the gene markers that might be used to predict the two traits or between the two breeds. The focussed marker panels that were assembled for assessment in this study could potentially be run at a very low cost compared to SNP chips.

The work presented here lays the foundations for identifying functional mutations or highly accurate SNP haplotypes linked to reproductive traits. More closely linked or causative gene markers will lead to higher accuracy and lower cost of genotypic

predictions, as well as being more likely to applicable across the entire industry and across different cattle breeds. In addition, low cost tests that use a small number of highly predictive markers could be used where DNA quantity is limiting and fast turnaround is crucial, for example in screening embryos before transfer to recipients – thereby shortening the generation interval.

The R&D necessary to achieve these goals will be aided by the large data sets currently becoming available. For example, the NextGen sequencing technology has made it possible to sequence and assemble the genomes of several individuals from the Beef CRC populations, and in addition, transcriptomics data sets (RNASeq) based on reproduction experiments in *Bos indicus*- influenced cattle are becoming available at the same time.

7.2 Recommendations for future R&D investment

7.2.1 Gene markers for reproduction traits

The focused SNP marker panels assembled and examined in this report will need to be validated in industry settings, before commercialisation can take place. It is likely that this validation will be carried out in collaboration with a company or companies that are interested in incorporating SNP marker panels into their array of services offered to beef producers. The publication of the identity and performance of the" top 10" SNP panels will be delayed until we have explored the possibility of engaging with commercial partners on validation activities.

Testing the SNP marker panel might employ the following steps:

1. Divide the available heifer data into two, use one data subset as "discovery" and the other for "validation".
2. Identify independent genotyped validation populations of the two breeds that carry EBV or have phenotypic measures and conduct *in silico* testing of the markers.
3. Recruit additional numbers of genotyped animals into the "discovery" population and optimise the marker panels.
4. Optimise marker panels by covering known candidate or causative mutations.
5. Identify commercial populations of animals with good phenotypic or EBV data, and perform DNA tests to evaluate marker performance.
6. Estimate accuracy of marker panel and calculate likely impact of markers on genetic improvement strategies, using simple modelling approaches.

In the future, SNP marker panels could find a market as a lower-cost tool for ranking animals in situations where the investment of a SNP chip assay to generate genomically-enhanced EBV is not a cost-effective option.

7.2.2 Continued investment in R&D

A vast amount of *Bos indicus* whole genome sequence and RNASeq data relevant for the breeds and traits studied in this project is only now becoming available. Two further whole genome association studies in Brahman and Tropical Composite bulls (B.NBP.604 and B.NBP.723), to mirror the studies reported here, have just been concluded. The methods required to integrate and fully exploit these data are still under development. It is therefore important for MLA to continue investing in

collaborative teams to work on beef cow reproduction. The work that needs to be done in the coming years includes:

- The identification of additional SNP markers for utility specifically in *Bos indicus*- influenced breeds,
- The identification of causative mutations or "perfect" markers for traits of interest,
- The development and validation of genomics-based tools tailored to different industry sectors,
- The development of SNP chips optimised for genomic predictions
- The development of computational methods for more efficient genomic predictions from genotyping data
- Generation of knowledge on the biological basis of reproductive traits and their links to other traits (in both bulls and cows)
- Industry-based proof-of-concept herds that will serve as development and extension resources for genomics approaches

Continued investment in this area will produce the following important outcomes for the northern beef industry:

1. Lower cost genotyping tools specifically aimed at *Bos indicus*-influenced cattle,
2. Lower cost validated prediction tools for reproductive traits that can be used to rank commercial bulls and/or replacement females,
3. More accurate genomic predictions based on SNP chips.

Overall, if the opportunities that currently present themselves can be exploited by Australian researchers, these developments will result in DNA-based technology contributing to genetic progress on reproductive performance in tropically-adapted beef cattle. gEBV or other molecular predictions will extend the reach of science-based genetic selection to sectors of the industry where genetic evaluations are not currently used, such as non-BREEDPLAN herds or commercial animals in extensive production situations. By widening the impact of genetic technology in Northern Australia in particular, these developments will have a large impact on the competitiveness and sustainability of the Australian beef industry.

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9 Bibliography

Allan, M. F., Kuehn, L. A., Cushman, R. A., Snelling, W. M., Echternkamp, S. E., Thallman, R. M., 2009. Confirmation of quantitative trait loci using a low-density single nucleotide polymorphism map for twinning and ovulation rate on bovine chromosome 5. Journal of Animal Science. 87**,** 46-56.

Barendse, W., Harrison, B. E., Bunch, R. J., Thomas, M. B., Turner, L. B., 2009. Genome wide signatures of positive selection: the comparison of independent samples and the identification of regions associated to traits. BMC Genomics. 10**,** 178.

Barwick, S. A., Johnston, D. J., Burrow, H. M., Holroyd, R. G., Fordyce, G., Wolcott, M. L., Sim, W. D., Sullivan, M. T., 2009a. Genetics of heifer performance in 'wet' and 'dry' seasons and their relationships with steer performance in two tropical beef genotypes. Animal Production Science. 49**,** 367-382.

Barwick, S. A., Wolcott, M. L., Johnston, D. J., Burrow, H. M., Sullivan, M. T., 2009b. Genetics of steer daily and residual feed intake in two tropical beef genotypes, and relationships among intake, body composition, growth and other post-weaning measures. Animal Production Science. 49**,** 351-366.

Bolormaa, S., Neto, L. R. P., Zhang, Y. D., Bunch, R. J., Harrison, B. E., Goddard, M. E., Barendse, W., 2011. A genome-wide association study of meat and carcass traits in Australian cattle. Journal of Animal Science. 89**,** 2297-2309.

Bolormaa, S., Pryce, J. E., Hayes, B. J., Goddard, M. E., 2010. Multivariate analysis of a genome-wide association study in dairy cattle. J Dairy Sci. 93**,** 3818-33.

Browning, S. R., Browning, B. L., 2010. High-Resolution Detection of Identity by Descent in Unrelated Individuals. American Journal of Human Genetics. 86**,** 526-539.

Burns, B. M., Fordyce, G., Holroyd, R. G., 2010. A review of factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf-Implications for reproductive efficiency in northern Australia. Animal Reproduction Science. 122**,** 1-22.

Burrow, H. M., Johnston, D. J., Barwick, S. A., Holroyd, R. G., Barendse, W., Thompson, J. M., Griffith, G. R., Sullivan, M., 2003. Relationships between carcass and beef quality and components of herd profitability in Northern Australia. 50 years of DNA: Proceedings of the Fifteenth Conference, Association for the Advancement of Animal Breeding and Genetics, Melbourne, Australia, 7-11 July 2003. 359-362.

Cammack, K. M., Thomas, M. G., Enns, R. M., 2009. Reproductive traits and their heritabilities in beef cattle. Professional Animal Scientist. 25**,** 517-528.

Caraux, G., Pinloche, S., 2005. PermutMatrix: a graphical environment to arrange gene expression profiles in optimal linear order. Bioinformatics. 21**,** 1280-1.

Chan, E. K., Nagaraj, S. H., Reverter, A., 2010. The evolution of tropical adaptation: comparing taurine and zebu cattle. Anim Genet. 41**,** 467-77.

Corbet, N. J., Burns, B., Corbet, D. H., Crisp, J. M., Johnston, D. J., McGowan, M. R., Venus, B. K., Holroyd, R. G., 2011. Bull traits measured early in life as

indicators of herd fertility. Proceedings of the Association for the Advancement of Animal Breeding and Genetics. 19**,** 55-58.

Cruickshank, J., Dentine, M. R., Berger, P. J., Kirkpatrick, B. W., 2004. Evidence for quantitative trait loci affecting twinning rate in North American Holstein cattle. Anim Genet. 35**,** 206-12.

Daetwyler, H. D., Schenkel, F. S., Sargolzaei, M., Robinson, J. A., 2008. A genome scan to detect quantitative trait loci for economically important traits in Holstein cattle using two methods and a dense single nucleotide polymorphism map. J Dairy Sci. 91**,** 3225-36.

Elsik, C. G., Tellam, R. L., Worley, K. C., Gibbs, R. A., Muzny, D. M., Weinstock, G. M., Adelson, D. L., Eichler, E. E., Elnitski, L., Guigo, R., Hamernik, D. L., Kappes, S. M., Lewin, H. A., Lynn, D. J., Nicholas, F. W., Reymond, A., Rijnkels, M., Skow, L. C., Zdobnov, E. M., Schook, L., Womack, J., Alioto, T., Antonarakis, S. E., Astashyn, A., Chapple, C. E., Chen, H. C., Chrast, J., Camara, F., Ermolaeva, O., Henrichsen, C. N., Hlavina, W., Kapustin, Y., Kiryutin, B., Kitts, P., Kokocinski, F., Landrum, M., Maglott, D., Pruitt, K., Sapojnikov, V., Searle, S. M., Solovyev, V., Souvorov, A., Ucla, C., Wyss, C., Anzola, J. M., Gerlach, D., Elhaik, E., Graur, D., Reese, J. T., Edgar, R. C., McEwan, J. C., Payne, G. M., Raison, J. M., Junier, T., Kriventseva, E. V., Eyras, E., Plass, M., Donthu, R., Larkin, D. M., Reecy, J., Yang, M. Q., Chen, L., Cheng, Z., Chitko-McKown, C. G., Liu, G. E., Matukumalli, L. K., Song, J., Zhu, B., Bradley, D. G., Brinkman, F. S., Lau, L. P., Whiteside, M. D., Walker, A., Wheeler, T. T., Casey, T., German, J. B., Lemay, D. G., Maqbool, N. J., Molenaar, A. J., Seo, S., Stothard, P., Baldwin, C. L., Baxter, R., Brinkmeyer-Langford, C. L., Brown, W. C., Childers, C. P., Connelley, T., Ellis, S. A., Fritz, K., Glass, E. J., Herzig, C. T., Iivanainen, A., Lahmers, K. K., Bennett, A. K., Dickens, C. M., Gilbert, J. G., Hagen, D. E., Salih, H., Aerts, J., Caetano, A. R., Dalrymple, B., Garcia, J. F., Gill, C. A., Hiendleder, S. G., Memili, E., Spurlock, D., Williams, J. L., Alexander, L., Brownstein, M. J., Guan, L., Holt, R. A., Jones, S. J., Marra, M. A., Moore, R., Moore, S. S., Roberts, A., Taniguchi, M., Waterman, R. C., Chacko, J., Chandrabose, M. M., Cree, A., Dao, M. D., Dinh, H. H., Gabisi, R. A., Hines, S., Hume, J., Jhangiani, S. N., Joshi, V., Kovar, C. L., Lewis, L. R., Liu, Y. S., Lopez, J., Morgan, M. B., Nguyen, N. B., Okwuonu, G. O., Ruiz, S. J., Santibanez, J., Wright, R. A., Buhay, C., Ding, Y., Dugan-Rocha, S., Herdandez, J., Holder, M., Sabo, A., Egan, A., Goodell, J., Wilczek-Boney, K., Fowler, G. R., Hitchens, M. E., Lozado, R. J., Moen, C., Steffen, D., Warren, J. T., Zhang, J., Chiu, R., Schein, J. E., Durbin, K. J., Havlak, P., Jiang, H., Liu, Y., Qin, X., Ren, Y., Shen, Y., Song, H., Bell, S. N., Davis, C., Johnson, A. J., Lee, S., Nazareth, L. V., Patel, B. M., Pu, L. L., Vattathil, S., Williams, R. L., Jr., Curry, S., Hamilton, C., Sodergren, E., Wheeler, D. A., Barris, W., Bennett, G. L., Eggen, A., Green, R. D., Harhay, G. P., Hobbs, M., Jann, O., Keele, J. W., Kent, M. P., Lien, S., McKay, S. D., McWilliam, S., Ratnakumar, A., Schnabel, R. D., Smith, T., Snelling, W. M., Sonstegard, T. S., Stone, R. T., Sugimoto, Y., Takasuga, A., Taylor, J. F., Van Tassell, C. P., Macneil, M. D., Abatepaulo, A. R., Abbey, C. A., Ahola, V., Almeida, I. G., Amadio, A. F., Anatriello, E., Bahadue, S. M., Biase, F. H., Boldt, C. R., Carroll, J. A., Carvalho, W. A., Cervelatti, E. P., Chacko, E., Chapin, J. E., Cheng, Y., Choi, J., Colley, A. J., de Campos, T. A., De Donato, M., Santos, I. K., de Oliveira, C. J., Deobald, H., Devinoy, E., Donohue, K. E., Dovc, P., Eberlein, A., Fitzsimmons, C. J., Franzin, A. M., Garcia, G. R., Genini, S., Gladney, C. J., Grant, J. R., Greaser, M. L., Green, J. A., Hadsell, D. L., Hakimov, H. A., Halgren, R., Harrow, J. L., Hart, E. A., Hastings, N., Hernandez, M., Hu, Z. L., Ingham, A., Iso-Touru, T., Jamis, C., Jensen, K., Kapetis, D., Kerr, T., Khalil, S. S., Khatib, H., Kolbehdari, D., Kumar, C. G., Kumar, D., Leach, R., Lee, J.

C., Li, C., Logan, K. M., Malinverni, R., Marques, E., Martin, W. F., Martins, N. F., Maruyama, S. R., Mazza, R., McLean, K. L., Medrano, J. F., Moreno, B. T., More, D. D., Muntean, C. T., Nandakumar, H. P., Nogueira, M. F., Olsaker, I., Pant, S. D., Panzitta, F., Pastor, R. C., Poli, M. A., Poslusny, N., Rachagani, S., Ranganathan, S., Razpet, A., Riggs, P. K., Rincon, G., Rodriguez-Osorio, N., Rodriguez-Zas, S. L., Romero, N. E., Rosenwald, A., Sando, L., Schmutz, S. M., Shen, L., Sherman, L., Southey, B. R., Lutzow, Y. S., Sweedler, J. V., Tammen, I., Telugu, B. P., Urbanski, J. M., Utsunomiya, Y. T., Verschoor, C. P., Waardenberg, A. J., Wang, Z., Ward, R., Weikard, R., Welsh, T. H., Jr., White, S. N., Wilming, L. G., Wunderlich, K. R., Yang, J., Zhao, F. Q., 2009. The genome sequence of taurine cattle: a window to ruminant biology and evolution. Science. 324**,** 522-8.

Fortes, M. R., Li, Y., Collis, E., Zhang, Y., Hawken, R. J., 2012a. The IGF1 pathway genes and their association with age of puberty in cattle. Anim Genet.

Fortes, M. R. S., Lehnert, S. A., Bolormaa, S., Reich, C., Fordyce, G., Corbet, N. J., Whan, V., Hawken, R. J., Reverter, A., 2012b. Finding genes for economically important traits: Brahman cattle puberty. Animal Production Science. 52**,** 143-150.

Hansen, M. P., Van Zandt, J. N., Law, G. R. J., 1967. Differences in susceptibility to Marek's disease in chickens carrying two different B locus blood group alleles. Poultry Science. 46**,** 1.

Hawken, R. J., Zhang, Y. D., Fortes, M. R. S., Collis, E., Barris, W. C., Corbet, N. J., Williams, P. J., Fordyce, G., Holroyd, R. G., Walkley, J. R. W., Barendse, W., Johnston, D. J., Prayaga, K. C., Tier, B., Reverter, A., Lehnert, S. A., 2011. Genome-wide association studies of female reproduction in tropically adapted beef cattle. Journal of Animal Science.

Hojo, T., Oda, A., Lee, S. H., Acosta, T. J., Okuda, K., 2010. Effects of tumor necrosis factor alpha and Interferon gamma on the viability and mRNA expression of TNF receptor type I in endothelial cells from the bovine corpus luteum. J Reprod Dev. 56**,** 515-9.

Johnston, D., Barwick, S., Fordyce, G., Holroyd, R., Understanding the Genetics of Lactation Anoestrus in Brahman Beef Cattle to Enhance Genetic Evaluation of Female Reproductive Traits. 9th World Congress on Genetics Applied to Livestock Production., Leipzig, Germany, 2010.

Johnston, D. J., Barwick, S. A., Corbet, N. J., Fordyce, G., Holroyd, R. G., Williams, P. J., Burrow, H. M., 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Animal Production Science. 49**,** 399-412.

Johnston, D. J., Bunter, K. L., 1996. Days to calving in Angus cattle: Genetic and environmental effects, and covariances with other traits. Livestock Production Science. 45**,** 13-22.

Kappes, S. M., Bennett, G. L., Keele, J. W., Echternkamp, S. E., Gregory, K. E., Thallman, R. M., 2000. Initial results of genomic scans for ovulation rate in a cattle population selected for increased twinning rate. Journal of Animal Science. 78**,** 3053-9.

Karim, L., Takeda, H., Lin, L., Druet, T., Arias, J. A. C., Baurain, D., Cambisano, N., Davis, S. R., Farnir, F., Grisart, B., Harris, B. L., Keehan, M. D., Littlejohn, M. D., Spelman, R. J., Georges, M., Coppieters, W., 2011. Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. Nature Genetics. 43**,** 405-413.

Kim, E. S., Berger, P. J., Kirkpatrick, B. W., 2009. Genome-wide scan for bovine twinning rate QTL using linkage disequilibrium. Anim Genet. 40**,** 300-7.

Lettre, G., 2009. Genetic regulation of adult stature. Curr Opin Pediatr. 21**,** 515-22.

Lindholm-Perry, A. K., Kuehn, L. A., Smith, T. P. L., Ferrell, C. L., Jenkins, T. G., Freetly, H. C., Snelling, W. M., 2012. A region on BTA14 that includes the

positional candidate genes LYPLA1, XKR4 and TMEM68 is associated with feed intake and growth phenotypes in cattle(1). Animal genetics. 43**,** 216-9.

Matukumalli, L. K., Lawley, C. T., Schnabel, R. D., Taylor, J. F., Allan, M. F., Heaton, M. P., O'Connell, J., Moore, S. S., Smith, T. P., Sonstegard, T. S., Van Tassell, C. P., 2009. Development and characterization of a high density SNP genotyping assay for cattle. PLoS One. 4**,** e5350.

Meyer, K., 2006a. Left ventricular dysfunction and chronic heart failure: should aqua therapy and swimming be allowed? Br J Sports Med. 40**,** 817-8.

Meyer, K., "WOMBAT" - digging deep for quantitative genetic analyses by restricted maximum likelihood. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Minas Gerais, Brazil, 13-18 August, 2006. Instituto Prociencia, Minas Gerais, 2006b, pp. 27-14.

Meyer, K., Hammond, K., Parnell, P. F., Mackinnon, M. J., Sivarajasingam, S., 1990. Estimates of heritability and repeatability for reproductive traits in Australian beef-cattle. Livestock Production Science. 25**,** 15-30.

Meyer, K., Tier, B., 2012. "SNP Snappy": a strategy for fast genome-wide association studies fitting a full mixed model. Genetics. 190**,** 275-7.

Prayaga, K. C., Corbet, N. J., Johnston, D. J., Wolcott, M. L., Fordyce, G., Burrow, H. M., 2009. Genetics of adaptive traits in heifers and their relationship to growth, pubertal and carcass traits in two tropical beef cattle genotypes. Animal Production Science. 49**,** 413-425.

Schrooten, C., Bovenhuis, H., Coppieters, W., Van Arendonk, J. A. M., 2000. Whole Genome Scan to Detect Quantitative Trait Loci for Conformation and Functional Traits in Dairy Cattle. Journal of Dairy Science. 83**,** 795-806.

Schulman, N. F., Sahana, G., Lund, M. S., Viitala, S. M., Vilkki, J. H., 2008. Quantitative trait loci for fertility traits in Finnish Ayrshire cattle. Genet Sel Evol. 40**,** 195-214.

Turner, L. B., Harrison, B. E., Bunch, R. J., Neto, L. R. P., Li, Y. T., Barendse, W., 2010. A genome-wide association study of tick burden and milk composition in cattle. Animal Production Science. 50**,** 235-245.

Weir, B. S., Cockerham, C. C., 1984. ESTIMATING F-STATISTICS FOR THE ANALYSIS OF POPULATION-STRUCTURE. Evolution. 38**,** 1358-1370.

Wright, S., 1922. Coefficients of inbreeding and relationship. American Naturalist. 56**,** 330-338.

10 Publications

10.1 Publications arising from this project

10.1.1 Refereed journal articles and book chapters:

1. Fortes MRS, Sasazaki S, Kemper K, Reverter A, Pryce JE, Barendse W, Bunch R, McCulloch R, Harrison B, Bolormaa S, Zhang Y, Hawken RJ, Goddard ME, Lehnert SA (in preparation) Evidence for pleiotropism and recent selection in the *PLAG1* region in Australian Beef cattle.

2. Reverter A and Fortes MR (in press) Association Weight Matrix: A network-based approach towards functional genome-wide association studies. In: “Genome-Wide Association Studies, Humana Press, C Gondro, J van der Werf and B Hayes, eds.

3. Fortes MR, Reverter A, Hawken RJ, Bolormaa S, Lehnert SA (in press) Candidate Genes Associated with Hormone Levels of Inhibin, Luteinising

Hormone, and Insulin-like Growth Factor 1, Testicular Development and Sperm Quality in Brahman Bulls. *Biology of Reproduction*

4. Fortes MR, Li Y, Collis E, Zhang Y, Hawken RJ. (2012) The IGF1 pathway genes and their association with age of puberty in cattle. *Anim Genet.* 2012 May 4 . [Epub ahead of print]

5. Fortes MR, Lehnert SA, Bolormaa S, Reich C, Fordyce G, Corbet NJ, Whan V, Hawken RJ and Reverter A (2012) Finding genes for economically important traits: Brahman cattle puberty. *Animal Production Science.* **52**: 143–150

6. Johnston D J, Tier B., Graser H -U (2012) Beef cattle breeding in Australia with genomics: opportunities and needs. Anim. Prod. Sci. 52: 100-106

7. Snelling WM, Cushman RA, Fortes MR, Reverter A, Bennett GL, Keele JW, Kuehn LA, McDaneld TG, Thallman RM, Thomas MG. (2012) Physiology and Endocrinology Symposium: How single nucleotide polymorphism chips will advance our knowledge of factors controlling puberty and aid in selecting replacement beef females. *J Anim Sci.* **90**:1152-1165

8. Hawken RJ, Zhang YD, Fortes MR, Collis E, Barris WC, Corbet NJ, Williams PJ, Fordyce G, Holroyd RG, Walkley JR, Barendse W, Johnston DJ, Prayaga KC, Tier B, Reverter A, Lehnert SA. (2012) Genome-wide association studies of female reproduction in tropically adapted beef cattle. *J Anim Sci.* **90**:1398-1410

9. Collis E, Fortes MR, Zhang Y, Tier B, Schutt K, Barendse W, Hawken R. (2011) Genetic variants affecting meat and milk production traits appear to have effects on reproduction traits in cattle. *Anim Genet.* 2011 Oct 18. [Epub ahead of print]

10. Meyer K, Tier, B (2011) "SNP Snappy": A Strategy for Fast Genome-Wide Association Studies Fitting a Full Mixed Model. *Genetics* 190: 275-277

11. Fortes MR, Reverter A, Nagaraj SH, Zhang Y, Jonsson NN, Barris W, Lehnert S, Boe-Hansen GB, Hawken RJ (2011) A single nucleotide polymorphism-derived regulatory gene network underlying puberty in 2 tropical breeds of beef cattle. *J Anim Sci.* **89**:1669-1683.

12. Fortes M R S, Reverter A, Zhang Y, Collis E, Nagaraj S H, Jonsson N N, Prayaga K C, Barris W, Hawken R J. (2010) An Association Weight Matrix for the Genetic Dissection of Puberty in Beef Cattle. *Proceedings of the National Academy of Sciences, USA* **107**, 13642-13647

10.1.2 Conference abstracts:

1. Fortes MR, Sazasaki S, Kemper K, Reverter A, Pryce J, Barendse W, Bunch R, Zhang Y, Hawken RJ, Goddard ME, Lehnert SA (2012) Mutations in the *PLAG1* region affect height, weight, puberty, IGF1 levels and fat deposition in beef cattle. Oral presentation at International Society for Animal Genetics Conference, Cairns, Australia July 2012

2. Reverter A, FortesMR, Bolormaa S, Zhang Y and Lehnert SA (2012) Accuracy of Genomic Selection for Fertility Traits in Australian Brahman Cattle. 4th ICQG conference Edinburgh, UK, June 2012

3. Fortes MR, Reverter A, Zhang Y, Snelling WM, Thomas MG, Hawken RJ, Lehnert SA (2012) Finding markers associated with reproductive performance in beef cattle. Plant and Animal Genome conference, San Diego, US, January 2012

4. Hawken R J, Zhang Y, Fortes M R S, Collis E, Reverter A, Barris W, Johnston D, Fordyce G, Holroyd R, Tier B, Burrow H, and Lehnert SA (2011) Dissecting the genetics underlying reproduction rate in tropically adapted beef cattle. Applied Genomics for Sustainable Livestock Breeding, Melbourne, Australia, May 2011

5. Fortes M R S, Bolormaa S, Porto Neto L R, Holroyd R G, Reverter A. (2011) Principal Component Analysis in a Population of Brahman bulls genotyped with 50K SNP chip revealed a genetic structure. AAABG, Perth, Australia, July 2011

6. Zhang Y, Tier B and Hawken R (2011) Genetic Evaluation of Post-weaning Anoestrous Status in Northern Beef. AAABG, Perth, Australia, July 2011

7. Fortes M R S, Li Y, Collis E, Zhang Y, Hawken R J. IGF1R: a Candidate Gene for Cattle Puberty. 32nd Conference for the International Society for Animal Genetics (ISAG). Edinburgh, UK, July 2010

8. Fortes M R S, Reverter A, Zhang Y, Collis E, Nagaraj S. H, Jonsson NN, Barris, Hawken RJ A new method for exploring genome-wide associations applied to cattle puberty. 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 2010

9. Zhang Y D, Tier B and Hawken R. (2010) Whole Genome Analysis of Heifer Puberty in Brahman Cattle. 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 2010

10. Johnston D., Barwick S., Fordyce G., Holroyd R. (2010) Understanding the Genetics of Lactation Anoestrus in Brahman Beef Cattle to Enhance Genetic Evaluation of Female Reproductive Traits . 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 2010

10.1.3 PhD thesis

Marina Rufino Salinas Fortes: “Genes and Genetic Markers Associated with Puberty in Beef Cattle” The University of Queensland, School of Veterinary Science, May 2012

10.1.4 Industry communication:

MLA Feedback magazine June 2012 issue: “Genomics boost Brahman fertility”

Beef Bulletin Quarter 1 2012: “Tackling delayed puberty in Brahmans”

11 Appendix

11.1 gEBV for Reproductive Performance in Tropically-Adapted Beef Cattle

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