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A genome-wide association study of tick burden and milk composition in cattle

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Abstract. To study the genetic basis of tick burden and milk production and their interrelationship, we collected a sample of 1961 cattle with multiple tick counts from northern Australia of which 973 had dairy production data in the Australian Dairy Herd Information Service database. We calculated heritabilities, genetic and phenotypic correlations for these traits and showed a negative relationship between tick counts and milk and milk component yield. Tests of polymorphisms of four genes associated with milk yield, *ABCG2*, *DGAT1*, *GHR* and *PRLR*, showed no statistically significant effect on tick burden but highly significant associations to milk component yield in these data and we confirmed separate effects for *GHR* and *PRLR* on bovine chromosome 20. To begin to identify some of the molecular genetic bases for these traits, we genotyped a sample of 189 of these cattle for 7397 single nucleotide polymorphisms in a genome-wide association study. Although the allele effects for adjusted milk fat and protein yield were highly correlated ($r = 0.66$), the correlations of allele effects of these milk component yields and tick burden were small ($|r| \leq 0.10$). These results agree in general with the phenotypic correlations between tick counts and milk component yield and suggest that selection on markers for tick burden or milk component yield may have no undesirable effect on the other trait.

Introduction

There are many studies that show that there is a physiological trade-off between production and disease or parasite resistance and this has recently been reviewed (Morris 2007). In cattle, zebu have greater growth rates and performance in the presence of parasites, in hotter climates and fed lower quality feed than taurine animals, but poorer growth rates and performance in the absence of parasites, in cooler climates and fed better quality feed (Frisch and Vercoe 1977, 1984). However, these might represent breed genetic differences because genetic correlations for growth traits and parasite resistance traits are often close to zero when analysed within a breed (Prayaga and Henshall 2005; Prayaga *et al*. 2009).

The interrelationship between genes for parasite resistance and animal productivity is not well understood at the molecular level. So far, there are no systematic studies of the interrelationships between these traits at the molecular genetic level, to determine whether some of the genes that affect host resistance to a parasite also affect animal production. In such studies we are particularly interested in the effects due to genes of moderate to large effect, partly because they simplify selection decisions and partly because selection on all the polygenic variation will reconstruct the genetic correlations seen between the traits (Meuwissen *et al*. 2001). It is possible to identify some of the genetic effects on a trait using the genome-wide association study (GWAS) methodology (Risch and Merikangas 1996; Ozaki *et al*. 2002). GWAS of traits in humans have shown that most of the associations are to quantitative trait loci (QTL) or risk alleles for complex traits (RACT) that are small to very small in size (Burton *et al*. 2007; Weedon *et al*. 2008). However, GWAS have also identified Mendelian factors causing discrete phenotypes in several species (Klein *et al*. 2005; Karlsson *et al*. 2007; Charlier *et al*. 2008), as well as either discovering, or rediscovering, QTL or RACT of moderate to large effect in humans (Todd *et al*. 1987; Ellis *et al*. 2001; Burton *et al*. 2007; Gieger *et al*. 2008; Hillmer *et al*. 2008; Pollin *et al*. 2008; Benyamin *et al*. 2009; Daly *et al*. 2009). Some of these studies found genetic effects of moderate to large size even though the sample sizes used in those studies were quite small (i.e. $100 < n < 300$), because genes of large effect can be discovered in samples that have low intrinsic power. However, small sample sizes generate estimates with large standard errors, which will cause significant results to be overestimated in size.

In this study we examined the genetic relationship between tick burden and milk production traits of dairy cattle from the tick zone in northern Australia using a bivariate analysis and performed a GWAS to identify regions of the bovine genome that influenced milk composition or tick resistance. Given previous ideas of the relationship between parasite resistance and productivity we were interested in determining whether there were any strong phenotypic or genotypic relationships between genes of large effect for milk traits and host resistance to parasites. We included four putative functional

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nucleotide polymorphisms (FNP) for dairy traits, the diacylglycerol *O*-acyltransferase homologue 1 (*DGAT1*) gene, the growth hormone receptor (*GHR*), the prolactin receptor (*PRLR*) and the ATP-binding cassette subfamily G, member 2 (*ABCG2*) that affect milk yield and milk component yield (Fries and Winter 2002; Grisart *et al*. 2002; Blott *et al*. 2003; Cohen-Zinder *et al*. 2005; Viitala *et al*. 2006). The inclusion of these four QTL allowed us to test whether any of them had direct effects on tick counts, as well as providing a way of empirically assessing the power of the GWAS for both traits.

Materials and methods

Cattle samples

We collected blood samples from 2494 dairy cattle (*Bos taurus* L.) from 16 properties across the tick zone in tropical and subtropical north-eastern Australia (Table 1), of which 1961 had duplicate tick counts. Ethics approval for the use of these animals was obtained as noted previously (Barendse *et al*. 2009*b*). The animals were selected to represent the northern Australian dairy industry, which has a broad range of breeds and farmer initiated taurine composites to deal with the hot, dry, tick-infested conditions. This allowed us to take a broad sample of breeds, breed composites and sire lines to reduce linkage disequilibrium in the combined sample.

Previous research had shown that cattle of the Jersey and Illawarra Shorthorn breeds show lower tick burdens than other taurine cattle but not enough to be called tick resistant (Utech *et al*. 1978). In the Jersey, the mechanism appears to be a form of hypersensitivity. Partly to recognise this, and partly to ensure that allele frequencies between breeds did not act to confound the analysis, the animals were divided into six breed types based on ancestry: (1) the Australian Red breed (AUR), a modern composite that includes the Illawarra Shorthorn, Red Holstein, the Scandinavian Red and the Ayrshire. An animal had to have at least two purebred grandparents of these breeds to be included in this group; (2) the Brown Swiss and its crosses (BSWX), and the same level of stringency applied here as applied for the Australian Red; (3) the Channel Isle breeds of Jersey and Guernsey and their crosses (CHA) – here the animal had to have at least three purebred grandparents of these breeds to be considered in this group, except if it was a half Holstein × Jersey or Guernsey cross-bred or composite; (4) the Holstein–Friesian and its crosses (HOLX) – here the animal had to have at least three purebred grandparents of this breed to be considered in this group; (5) mixed taurine cattle (MIXT) with no more than one known grandparent of pure breed origin; and (6) cattle with at least one grandparent of known zebu ancestry (ZEBX), usually from the Sahiwal breed through the Australian Friesian Sahiwal. Pedigree information was obtained from the farmer and from the Australian Dairy Herd Improvement Scheme (ADHIS).

Tick counts

Tick counts of the one-host ixodid cattle tick (*Boophilus microplus* Canestrini) and DNA samples were obtained from each animal over 2–3 tick seasons. Tick numbers due to field infestations were counted in the size range 4.5–8.0 mm (Wharton *et al*. 1970) on one side of the animal by more than one counter over more than one season. A count was considered usable if the average number of counts for that day was 20 ticks per side (Table 1). Tick counts are usually log-transformed for parametric analyses (Fig. 1). The animals had to be treated for tick burden by dipping in acaricides, so tick counts were collected before dipping, at a point at which the farmer decided the cattle would be treated for the tick burden they carried.

Milk yield data

The dairy production trait values were obtained from the ADHIS database for animals in the study. We were able to obtain dairy production records for around half of the animals for which we had duplicate tick counts. Data included the national herd

Table 1. Number of tick counts per property with collection date, average number of ticks per side and the total number of cows sampled per property

Property	Collection 1	Average tick count	Collection 2	Average tick count	Collection 3	Average tick count	Collection 4	Average tick count	Collection 5	Average tick count	Total animals
301	10.x.03	13.5	25.v.04	14.6	22.x.04	37.1	26.iv.05	16.5	21.xi.05	37.7	129
302	21.xi.03	19.9	8.iv.04	45.8							210
303	2.xii.03	6.9									163
304	3.xii.03	43.5	10.ii.04	165.6	11.i.05	27.7					246
305	16.xii.03	13.3									123
406	14.i.04	9	15.iii.04	18.9	1.ii.05	12.1	27.v.05	4.8			149
407	15.i.04	8.4	9.iii.04	22.75							126
408	19.i.04	49.4									126
409	28.i.04	15.7	8.iii.05	42.1	11.v.05	46.3	17.iii.06	37.3			337
410	3.ii.04	22.2									97
411	4.iii.04	8.1									130
413	16.xii.04	190.8	21.ii.05	182.2	12.v.05	169	20.xii.05	150			232
414	17.xii.04	8.4									112
415	19.xii.04	56.8	22.ii.05	101.7	16.v.05	253	16.xii.05	71			148
516	24.i.05	15.7	19.iv.05	39.9							103
517	23.ii.05	15									63
Total											2494

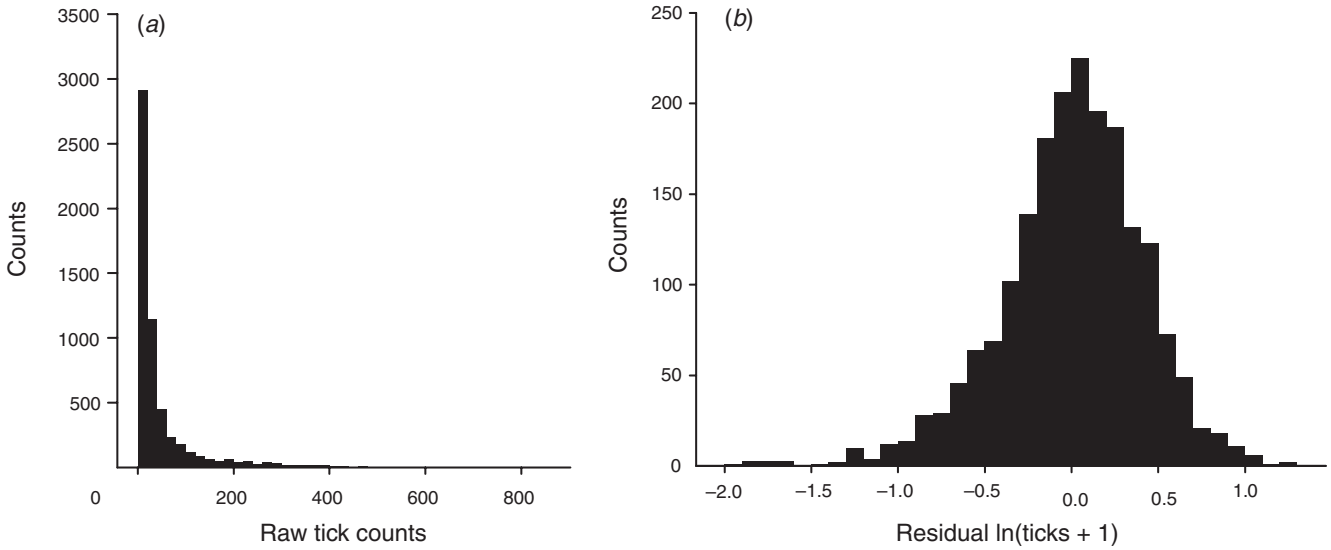

Fig. 1. (*a*) Histogram of raw tick counts. (*b*) Histogram of residual ln(ticks + 1) values for individuals.

identifier, property number, ancestry, birth date, calving date, yields of total milk, total milk protein and total milk fat, number of parities and last herd recording. We excluded data records for incomplete lactations, but could only determine that a record was incomplete in those cases were duplicate records existed. After removing duplicate or incomplete data, there were 973 of the 1961 animals that had multiple tick counts that also had milk component yield data and a DNA sample suitable for genotyping.

Analysis of phenotypes

Initial fixed effect models were evaluated using the R software package (R Statistical Project http://cran.r-project.org/, verified 22 March 2010). Thereafter, trait values were fitted in a mixed model using the ASReml software (Gilmour *et al.* 2002) as follows: trait ~ mean + fixed effects + animal + error, with animal and error fitted as random effects. The proportion of the residual variance (R^2) explained by a fixed effect in the model was calculated by comparing the residual sums of squares (RSS) of the model with the fixed effect (RSS_f) to the RSS of the model without the fixed effect (RSS_w), $R^2 = (RSS_w - RSS_f)/RSS_f$. Heritability estimates for the traits were obtained from the mixed model and genetic and phenotypic correlations were obtained from a bivariate analysis of these traits. In the bivariate analysis of tick counts and milk yield data, there are several tick counts per individual but only a single estimate of milk production so the mean of the ln-transformed tick counts was compared with milk production. This method of averaging tick counts was not used in the univariate analysis of tick counts due to the large variance in tick counts found between seasons, years and counters (cf. Results). In the univariate analysis, the tick counts were ln-transformed and modelled as ln(ticks + 1) ~ mean + property + season + breed type + animal + error, where the animal needed to have two tick counts out of the nine possible seasons to be included in the analysis. The model contained all available pedigree information of sire, dam and maternal grandsire identities, and season included the identity of tick counter. This model did not include effects of DNA polymorphisms. The residual tick count for each animal was extracted from the model and used as the phenotype in association analyses. The milk data were modelled as follows: milk fat or protein yield ~mean + total milk yield + birth year + property + breed type + animal + error. Total milk yield was used as a covariate for two reasons. First, as noted above, a low milk fat yield could be due purely to a low milk yield from an incomplete record. Second, we found a very strong relationship between milk yield and milk fat or protein yield (cf. Results, Fig. 2). Therefore, we fitted total milk yield to remove the possible effect of incomplete lactation records in the data and to emphasise the differences in protein or fat components, rather than analysing a proportional trait like milk fat or protein percentage. The residual protein and fat yields were extracted from the model and used as the phenotypes in association analyses. These protein and fat yields adjusted for total milk yield are called adjusted protein and fat yields below.

We evaluated associations between the trait and individual single nucleotide polymorphism (SNP) using regressions of residuals on number of copies of an allele rather than fit SNP as a covariate within the ASReml model. Both approaches have been used in the literature (Gieger *et al*. 2008; Weedon *et al*. 2008; Barendse *et al*. 2009*a*). We found that the regression of residuals on alleles gave slightly less significant results for milk trait associations than fitting the SNP as a covariate within the model (data not shown) and it is a speedier method of analysis of large datasets.

Genotyping

Animals selected for genotyping using the SNP chip were chosen in approximately equal numbers from five properties to a total of 189 plus three repeated animals, to generate two plates of 96 samples (Barendse *et al*. 2009*b*). The animals of this subset were chosen so that they were not closely related and were the offspring of 138 of the sires and 174 of the dams. They consisted of the breed types AUR ($n = 61$), BSWX ($n = 35$),

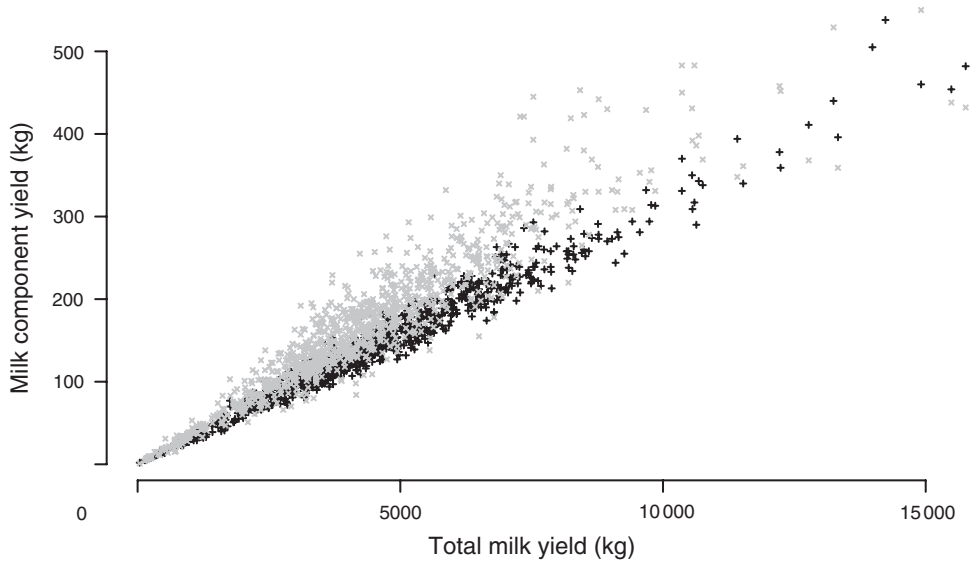

Fig. 2. Bivariate scatter plots of milk fat (grey) and protein (black) yields against total milk yield.

CHA ($n = 28$), HOLX ($n = 56$), MIXT ($n = 4$), and ZEBX ($n = 5$). The aim was to reduce the linkage disequilibrium (LD) between SNP and to reduce any bias due to large differences in numbers of animals from different sires. The animals were selected without knowing their tick burdens or milk yield data. The genotyping of the sample of 189 cattle was performed using the MegAllele Genotyping Bovine 10 K SNP Panel (Hardenbol *et al*. 2005), a fully described set of SNP, by ParAllele Inc. on an Affymetrix GeneChip Scanner 3000, yielding an average spacing of 325 kb between SNP. Further details of the SNP can be found at the link ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/snp/Btau20050310/ (verified 22 March 2010). All samples with more than 10% missing data were excluded and then all loci with more than 10% of missing data were excluded, as previously described (Barendse *et al.* 2009*b*).

To provide positive controls for the GWAS and to examine the effects of important milk yield QTL on tick burdens, a set of FNP with moderate to large effects on milk traits were genotyped, first on the samples used for the GWAS and then on all animals with milk component yield data. Taqman assays were designed for the following DNA variants (Table 2). These were: (1) for the *K232A* MNP (multiple nucleotide polymorphism) in the *DGAT1* gene on bovine chromosome 14 (BTA14) (Grisart *et al*. 2002; Winter *et al*. 2002); (2) for the *F289Y* SNP in the *GHR* (Blott *et al*. 2003) on BTA20; (3) for the *S18N* MNP in the *PRLR* (Viitala *et al*. 2006) on BTA20; and (4) for the *Y581S* SNP in the *ABCG2* gene on BTA6 (Cohen-Zinder *et al*. 2005; Olsen *et al*. 2007). All of these genes affect milk yield and milk component yield, and the *DGAT1* K232A MNP has an especially large effect on milk fat percentage.

Table 2. Primers and minor groove binding probes for the Taqman assays for the functional nucleotide polymorphisms associated with milk protein and fat yield

Locus	Information	DNA sequence 5′ to 3′
ABCG2-Y581S	Forward primer	GGCGTCTGGCTTCAATACTTG
ABCG2-Y581S	Reverse primer	CACGGTGACAGATAAGGAGAACAT
ABCG2-Y581S	VIC probe v1 A	TCGATACGGCTATGCGGT
ABCG2-Y581S	FAM probe m1 C	TCGATACGGCTCTGCGGT
DGAT1-K232A	Forward primer	CGCTTGCTCGTAGCTTTGG
DGAT1-K232A	Reverse primer	CGCGGTAGGTCAGGTTGTC
DGAT1-K232A	VIC probe v2 K	CGTTGGCCTTCTTACC
DGAT1-K232A	FAM probe m2 A	TTGGCCGCCTTACC
GHR-F279Y	Forward primer	TCAGATTTCCAGTTTCCATGGTTCTTAATTATT
GHR-F279Y	Reverse primer	GGTTATATCACACTTACCTTTGCTGTTTAGA
GHR-F279Y	VIC probe v1 A	AGCAGTGACATTATATTTACT
GHR-F279Y	FAM probe m1 T	TAGCAGTGACATTATTTTTACT
PRLR-S18N	Forward primer	TGCAGCATCTAGAGTGGTTTTCATT
PRLR-S18N	Reverse primer	GGGAGTGAAAAAGAACAAGACAGTCT
PRLR-S18N	VIC probe v1 N	ACTTTTTCTCAACGTCAGCC
PRLR-S18N	FAM probe m1 S	CTTTTTCTCAGTGTCAGCC

Analyses of genotypes

Genotypes were tested for Hardy–Weinberg equilibrium within breeds and genotype frequencies were compared between breeds (Weir 1996). Allelic associations were evaluated by regression of the residual phenotype on the number of copies of an allele. For association tests, the *P*-values were reported as –log*P* values to allow for uniform and consistent reporting of very small *P*-values. The R^2 was calculated from the correlation between residual phenotypes and numbers of copies of the reference allele. In the analysis of single-point associations in the GWAS, due to the small sample size, we set a threshold of a minor allele frequency (MAF) >0.05 for the analysis because a MAF <0.05 resulted in genotypic classes with one or two individuals of the rare homozygote.

The false positive rate FPR = E_p/O_p where E_p is the expected number of SNP with *P*-values below a particular significance threshold, given the number of SNP in the panel and assuming that all tests are independent, and O_p is the observed number of SNP with *P*-values below that same threshold. Standard sample size and power calculations using the critical points of the normal distribution were calculated as previously described (Snedecor and Cochran 1967).

Results

Phenotypic measures

Tick counts in the sample of cattle (n = 1961) with duplicate counts ranged from 0 to 853 (Fig. 1). The tick counts for different breeds are shown in Table 3. The heritability and standard error of ln-transformed tick counts, when analysed as multiple counts of an animal, was $h^2 = 0.37 \pm 0.02$ in these taurine dairy cattle, larger than the $h^2 = 0.23 \pm 0.15$ obtained from analysing the mean ln-transformed tick counts. The most important factor affecting ln-transformed tick counts was the combined effect of season and counter, which was highly significant ($P < 0.0001$) and accounted for $R^2 = 35.7\%$ of the residual variance in tick counts. Property, which includes the effect of management, although highly significant ($P < 0.0001$), explained a much smaller $R^2 = 2.4\%$ of the variance. Breed type was highly significant ($P < 0.001$) in these taurine cattle but explained an inconsequential amount of the variance (R^2 <0.1%) compared with the other two main effects. The amount of the variance explained by breed was consistent with the expectation that taurine dairy cattle breeds were in general similar to each other in tick burden. After accounting for property and season the MIXT, AUR and HOLX breed types all carried more ticks than the CHA breed, although the differences between these three breeds were not significant. The BSWX did not carry significantly more ticks than the CHA and the ZEBX did not carry significantly fewer ticks than the CHA.

The unadjusted milk protein, fat and total milk yields for cattle in the sample are shown in Table 4. The heritabilities for these traits are: for adjusted milk protein, $h^2 = 0.56 \pm 0.19$, for adjusted milk fat, $h^2 = 0.46 \pm 0.19$, and for total milk yield, $h^2 = 0.50 \pm 0.19$. Total milk yield was highly correlated with both milk fat yield and milk protein yield (Fig. 2), with correlations $r = 0.94$ and $r = 0.98$, $n = 973$, $P < 0.0001$. The correlation between total milk yield and milk fat yield showed visibly greater dispersion than the correlation between total milk yield and milk protein yield. Milk component yield was always adjusted for total milk yield, because nearly all of the variability in milk fat and protein yield was due to variation in total milk yield in these data. Property of origin explained $R^2 = 26$ and 24% of the residual variance respectively for adjusted milk fat and protein yield ($P < 0.0001$). Breed type explained $R^2 = 4\%$ and 5% of the residual variance respectively ($P < 0.0001$) for adjusted milk fat and protein yield. Cow birth year explained $R^2 = 3\%$ of the residual variance ($P < 0.05$) for adjusted milk fat yield but did not have a significant effect on adjusted protein yield. To determine the effect of multiple breeds on a property compared with breeds occurring on several properties, we performed an analysis of breed nested within property and of property nested within breed for adjusted milk component yield. Breed type nested within property explained $R^2 = 9\%$ and 8% of the residual variance respectively ($P < 0.0001$) for adjusted milk fat and protein yield. Property nested within breed type explained $R^2 = 32\%$ and 27% of the residual variance respectively ($P < 0.0001$) for adjusted milk fat and protein yield. Due to the known differences in yield between breeds of cattle, and due to the same considerations of data stratification,

Table 3. Number of records, mean, and standard deviation (s.d.) of tick counts and ln-transformed tick counts in different breed types

AUR, Australian Red breed; BSWX, Brown Swiss and its crosses; CHA, Channel Isle breeds of Jersey and Guernsey and their crosses; HOLX, Holstein–Friesian and its crosses; MIXT, mixed taurine cattle; ZEBX, cattle with at least one grandparent of known zebu ancestry. *$P < 0.05$, **$P < 0.01$

Type	AUR	BSWX	CHA	HOLX	MIXT	ZEBX	Total
No. of animals with multiple tick count records	332	193	266	509	650	11	1961
No. of tick count records	1004	639	733	1355	1844	36	5611
			Tick count				
Mean	39.6	88.4	30.0	41.0	46.6	124.8	47.1
s.d.	76.9	114.7	46.5	71.8	75.4	134.0	79.7
			ln(ticks + 1)				
Mean	2.79	3.66	2.82	2.99	3.03	3.88	3.03
s.d.	1.30	1.40	1.12	1.17	1.31	1.73	1.29
l.s.e.[A]	0.21*	–0.03	–0.29	0.22**	0.19*	–0.31	–
s.e.	0.10	0.09	–	0.07	0.07	0.23	–

[A]Least-square estimates of fixed effects with CHA as the reference breed. Note ASReml estimates n-1 levels and the last level is the negative sum of the others.

Table 4. Number of records, mean, and standard deviation (s.d.) for milk yield, milk fat yield and milk protein yield in different breed types
AUR, Australian Red breed; BSWX, Brown Swiss and its crosses; CHA, Channel Isle breeds of Jersey and Guernsey and their crosses; HOLX, Holstein–Friesian and its crosses; MIXT, mixed taurine cattle; ZEBX, cattle with at least one grandparent of known zebu ancestry

Type	AUR	BSWX	CHA	HOLX	MIXT	ZEBX
n	204	84	136	388	153	8
			Yield (kg)			
Mean	3890	4553	3421	4917	4146	3439
s.d.	1536	2149	1782	2594	1973	1505
			Fat (kg)			
Mean	144.7	182.1	141.8	187.7	169.2	142.9
s.d.	63.5	85.4	74.1	103.0	89.8	58.9
			Protein (kg)			
Mean	126.2	145.0	115.6	151.7	135.6	109.1
s.d.	52.6	69.0	61.5	82.5	65.2	50.5

breed type, property and birth year of the cow were fitted as effects in the association analyses.

The correlations between mean ln-transformed tick counts and milk and milk component yield varied substantially. The phenotypic correlation and standard error between adjusted milk fat and milk protein yields was $r_P = 0.53 \pm 0.02$ and the genetic correlation and standard error was $r_g = 0.73 \pm 0.15$. Correlations between adjusted milk component yield and mean ln-transformed tick counts could not be performed due to a lack of residual variance when total milk yield was fitted as a covariate. When total milk yield was removed from the model, the correlations between milk fat yield and mean ln-transformed tick counts were $r_P = -0.01 \pm 0.03$ and $r_g = -0.80 \pm 0.40$, and for milk protein yield and mean ln-transformed tick counts were $r_P = -0.01 \pm 0.03$ and $r_g = -0.73 \pm 0.41$. The correlations between total milk yield and ln-transformed tick counts were $r_P = -0.01 \pm 0.03$ and $r_g = -0.63 \pm 0.44$. These large negative genetic correlations between tick burden and milk production have large standard errors and so should not be over-interpreted, but they are in the direction of lower tick burdens with higher milk component yields.

Effect of the milk FNP on traits

The allele substitution effect of the *DGAT1* K232A MNP was significantly ($P = 0.0008$, i.e. $-\log P = 3.05$) associated with adjusted fat yield, it was one of the most significant associations for milk composition in the GWAS subsample and explained 6.4% of the residual variance in the GWAS sample (Table 5). The allele substitution effect of the *GHR* F289Y SNP was significantly ($P < 0.05$) associated with both adjusted fat and protein yield, explaining 2.7 and 3.0% of the R^2, respectively, with the same favourable homozygote for both traits. The *ABCG2* Y581S SNP and the *PRLR* S18N MNP were not significantly associated with either adjusted protein or fat yield. The sample of 189 animals had a power of detecting an effect of 6.9% of the variance at a 5% significance threshold with 90% power.

The genotyping of the four FNP on all available animals ($n = 973$) with adjusted fat and protein yields showed a significant effect of all these FNP (Table 6). The effect of the FNP at *DGAT1* was very highly significant ($-\log P > 15$) and was five times larger than those of the other three FNP, having an effect of 10.3% of the R^2 of adjusted fat yield. It had also a moderate effect on adjusted protein yield, with the same favourable homozygote for both traits. The other three FNP had effect sizes of between 1.1 and 2.1% of the R^2 of adjusted milk protein yield. The *GHR* FNP had an effect on adjusted milk fat yield with the same favourable homozygote, but neither the *ABCG2* nor the *PRLR* FNP had significant effects at the 5% threshold on adjusted fat yield in these data.

GHR and *PRLR* were on the same chromosome so the significant association of *PRLR* with adjusted protein yield may be due to LD between *GHR* and *PRLR*, although these genes are 7 Mb apart. We found that when *GHR* and *PRLR* were fitted as main effects in a mixed model, with *PRLR* fitted after *GHR*, the effect of *PRLR* on adjusted milk protein yield was still statistically significant ($-\log P = 2.4$). Moreover, *PRLR* was also statistically significant ($-\log P = 1.63$) when nested within

Table 5. Association between four causative genes and adjusted milk fat and protein yield in the genome-wide association study sample
p, allele frequency; R^2, proportion of the residual variance; α, allele substitution effect; s.e., standard error of the allele substitution effect

Single nucleotide polymorphism	Chromosome	Position (Mb)	*p*	R^2	α	s.e.	$-\log P$
			Adjusted fat yield				
ABCG2Y581S	6	37.4	0.97	0.0006	–2.802	9.055	0.12
DGAT1K232A	14	0.4	0.77	0.0639	11.330	3.345	3.05
GHRF279Y	20	33.9	0.18	0.0268	8.180	3.761	1.51
PRLRS18N	20	41.4	0.51	0.0050	2.883	3.085	0.45
			Adjusted protein yield				
ABCG2Y581S	6	37.4	0.97	0.0065	–4.095	3.848	0.54
DGAT1K232A	14	0.4	0.77	0.0070	1.567	1.445	0.55
GHRF279Y	20	33.9	0.18	0.0303	3.708	1.599	1.67
PRLRS18N	20	41.4	0.51	0.0107	1.791	1.311	0.76

Table 6. Significant associations between four causative genes and adjusted milk fat and protein yield in the full sample

p, allele frequency; R^2, proportion of the residual variance; α, allele substitution effect; s.e., standard error of the allele substitution effect

Single nucleotide polymorphism	*n*	*p*	R^2	α	s.e.	–log*P*
			Adjusted fat yield			
DGAT1K232A	951	0.70	0.1026	13.86	1.34	15.35
GHRF279Y	938	0.20	0.0172	5.52	1.36	4.27
			Adjusted protein yield			
ABCG2Y581S	952	0.97	0.0143	–5.42	1.47	3.62
DGAT1K232A	951	0.70	0.0212	2.61	0.58	5.16
GHRF279Y	938	0.20	0.0210	2.52	0.56	5.10
PRLRS18N	951	0.55	0.0111	1.72	0.53	2.95

GHR genotypes. There was no statistically significant interaction between *GHR* and *PRLR*, although such an analysis is not a formal test of epistasis.

None of these four FNP for milk fat and protein had significant associations to tick burden either when analysed on the GWAS sample or when tested on the full sample of animals with milk component yield data (data not tabulated). A sample of 973 animals should detect an effect of R^2 = 1.1% with 90% power at the 5% significance threshold.

GWAS results for milk composition

Of the SNP in the 10 K Affymetrix panel, there were 7397 that passed quality control and were used for association mapping and of these, there were 6532 that had MAF >0.05 in the combined GWAS sample (*n* = 189). There are 6.5 SNP expected to be significant at the 0.1% threshold by chance if one assumed that the SNP tests were independent.

At the same threshold as that for *DGAT1* in the GWAS sample (i.e. –log*P* = 3), there were six SNP with MAF >0.05 associated with adjusted milk fat yield (Table 7). The FPR was therefore 100% for milk component traits at the 0.1% significance threshold. Of these SNP, the one located immediately 3′ to the *GPR126* gene on BTA9 was interesting because the same favourable homozygote had an effect on milk protein yield (–log*P* = 2.76, α = –4.158, s.e. = 1.307) on a chromosome with known QTL effects for milk fat and protein yield (Georges *et al*. 1995). A second SNP was in the *NCOA2* gene, which is a co-activator of steroid hormone receptors including steroid, thyroid, retinoic acid and vitamin D receptors, including the oestrogen receptor (Hong *et al*. 1997). *DGAT1* is some 30 Mb away from *NCOA2* so this association is unlikely to be due to LD to *DGAT1*, and the variation in milk fat and protein yields on BTA14 was not fully explained by *DGAT1* variation (Kaupe *et al*. 2007). At the same threshold there was one SNP with MAF >0.05 associated with adjusted protein yield (Table 6), which is much fewer than expected by chance. None of the other genes was a potential candidate gene.

GWAS results for tick burden

For tick burden, at a threshold of –log*P* = 3.0, there were 27 SNP with MAF >0.05 of which 25 could be located to the bovine genome (Table 8). The FPR = 24% for tick burden associations at the 0.1% significance threshold. The –log*P* values were plotted against genome location in Fig. 3. Several of these SNP were either in an exon or intron of a gene that had a known role in the adaptive immune system of mammals, or was within 50 kb of such a coding sequence. *TNFSF8* (*CD30*) is a cytokine belonging to the tumour necrosis factor ligand family and has been shown to be upregulated in cutaneous inflammation and mediates degranulation-independent chemokine secretion (Fischer *et al*. 2006). *SIRPA* is a member of the immunoglobulin superfamily involved in the differentiation of monocytes to dendritic cells (Brooke *et al*. 1998), the most potent antigen presenting cell, and has a known role in binding to *CD47* (Oldenborg *et al*. 2000). Of the others with a potential link to the immune system, *SATB2* binds to the matrix attachment region of the immunoglobulin micro locus and enhances expression in pre-B-cells (Dobreva *et al*. 2003). *MAN2A1* mutations are involved in systemic autoimmune disease (Chui *et al*. 2001), and the expression of *ABCA9* was affected by cholesterol levels in blood and it belongs to a family of genes that are induced during monocyte differentiation into macrophages (Piehler *et al*. 2002). None of the genes listed in Table 8 was identified by gene expression profiling in cattle following artificial challenge using *B. microplus* larvae (Wang *et al*. 2007).

Global association between tick burden and milk composition

In a genomic selection framework, all of the SNP in the 10-K SNP panel may be used to predict the breeding values for a trait, so to

Table 7. Locations of significant associations to adjusted milk and protein yield in the genome-wide association study

p, allele frequency; R^2, proportion of the residual variance; α, allele substitution effect; s.e., standard error of the allele substitution effect

Chromosome	Position (Mb)	*p*	R^2	α	s.e.	–log*P*	Gene symbol
			Adjusted fat yield				
3	65	0.19	0.06	12.61	3.73	3.05	Near LOC781323
9	83	0.62	0.06	–10.47	3.05	3.12	3′ to GPR126
14	34	0.16	0.06	14.27	4.23	3.03	NCOA2
24	30	0.89	0.06	16.98	4.98	3.09	Not near gene
24	30	0.89	0.06	16.98	4.98	3.09	Not near gene
26	40	0.63	0.06	10.90	3.21	3.06	PRDX3
			Adjusted protein yield				
25	30	0.35	0.07	–4.66	1.33	3.25	GBAS

Table 8. Locations of significant associations to tick burden in the genome-wide association study sample
p, allele frequency; R^2, proportion of the residual variance; α, allele substitution effect; s.e., standard error of the allele substitution effect

Chromosome	Position (Mb)	*p*	R^2	α	s.e.	–log*P*	Gene
1	95	0.35	0.09	0.43	0.10	4.48	Near NAALADL2
2	92	0.69	0.06	–0.38	0.11	3.00	3′ to SATB2
2	92	0.44	0.11	–0.45	0.09	5.55	3′ to SATB2
4	16	0.43	0.07	0.36	0.09	3.70	GLCCI1
6	23	0.53	0.06	0.33	0.10	3.13	LOC518821
6	23	0.47	0.06	–0.33	0.10	3.13	LOC518821
6	23	0.53	0.06	0.32	0.10	3.06	LOC518821
7	111	0.37	0.06	–0.37	0.10	3.35	Near MAN2A1
8	109	0.80	0.07	–0.43	0.12	3.34	Near TNFSF8
10	69	0.86	0.08	0.56	0.14	3.92	SAMD4A
11	45	0.10	0.06	–0.54	0.16	3.01	Not near gene
13	10	0.87	0.07	–0.60	0.16	3.73	Near FLRT3
13	10	0.17	0.06	0.49	0.14	3.26	Near FLRT3
13	10	0.13	0.07	0.59	0.16	3.52	Near FLRT3
13	54	0.30	0.06	–0.38	0.11	3.18	Near SIRPA
13	59	0.19	0.06	–0.45	0.13	3.22	Near VAPB
14	77	0.37	0.06	0.35	0.10	3.08	RALYL
19	28	0.88	0.08	–0.68	0.17	4.17	LOC512248
19	43	0.92	0.06	–0.56	0.17	3.10	CNP
19	63	0.38	0.06	–0.36	0.10	3.37	Near ABCA9
19	63	0.37	0.06	–0.35	0.10	3.27	Near ABCA9
20	32	0.94	0.07	–0.75	0.19	3.78	Near MRSP30
20	32	0.94	0.07	–0.80	0.21	3.76	Near MRSP30
20	45	0.30	0.07	–0.40	0.11	3.63	Not near gene
26	30	0.35	0.06	–0.36	0.10	3.30	3′ to PARP8

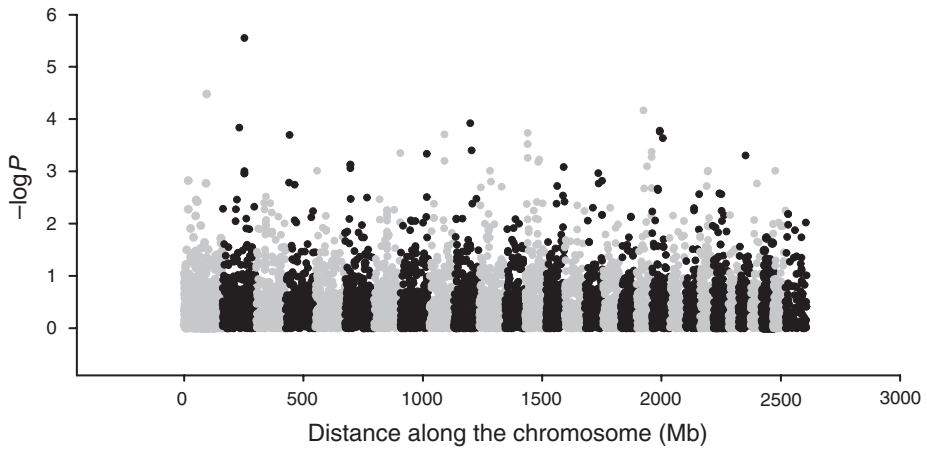

Fig. 3. Manhattan plot of –log*P* of allele effects for tick burden against chromosome location: odd numbered chromosomes grey, even numbered chromosomes black.

estimate the relative effects of these SNP on several traits, correlations were calculated between the allele substitution effects of all three traits. The allele substitution effects for adjusted milk fat and adjusted milk protein yield were correlated with $r = 0.66$, those for adjusted milk fat yield and tick burden were slightly positively correlated with $r = 0.10$, and those for adjusted milk protein yield and tick burden were effectively uncorrelated with $r = –0.03$. These correlations are in general agreement with the phenotypic correlations for these traits (cf. above).

Discussion

In this study we calculated heritabilities and correlations between tick counts and milk production traits, examined the effects on parasite resistance of genes with known effects on milk yield and milk component yield, performed a low resolution GWAS to identify genomic regions associated with tick counts or milk component yield and compared the allele substitution effects of these traits across the genome to determine whether the effects for tick counts were correlated with those for milk component yield.

The genetic correlation between tick and milk traits was negative with large standard errors, while the heritabilities of these traits were similar to previous estimates. Compared with previous estimates the heritability for tick burden in this study was larger than the value of $h^2 = 0.15$ found in zebu and zebu composite Brahman and Tropical Composite cattle for tick scores (Prayaga *et al*. 2009), but similar to the value of $h^2 = 0.41 \pm 0.08$ calculated for taurine cattle using multiple tick counts (Henshall 2004) and within the range of $h^2 = 0.34$–0.49 previously calculated for cattle in Queensland (Wharton *et al*. 1970; Mackinnon *et al*. 1991). Heritabilities for the milk yields were similar to previously estimated values for these traits (Pander *et al*. 1992) but higher than previous estimates restricted to Australian Holstein and Jersey (Visscher and Goddard 1995). Phenotypic and genetic correlations for the milk traits are similar to values found in larger datasets (Visscher and Goddard 1995). We were not able to find estimates of tick burden and milk or milk component yield in the literature. The phenotypic correlations between milk and milk component yield and tick counts were close to zero but the genetic correlations were strongly negative. These latter estimates have large standard errors and so should not be over-interpreted, because the effects could be much weaker and are not >2 s.e. below zero, but they are in the direction of lower tick burdens correlated with higher milk and milk component yields. Selection for milk and milk component yield should therefore not increase tick burdens and may improve tick burdens if the genetic correlations are to be believed. The similarity of the correlations and heritabilities of other traits in this study with estimates from the literature suggests that the sample and analyses were not unusual, giving some credence to the correlations between tick counts and milk production traits.

The four previously identified FNP of moderate to large effect for milk and milk component yield, in the genes *ABCG2*, *DGAT1*, *GHR* and *PRLR*, all showed associations to milk compositional traits, including that the effect of *DGAT1* on a phenotype was much greater than the effect of the other genes. Furthermore, we found that although *GHR* and *PRLR* were on the same chromosome, we were able to identify separate effects of these genes on milk component traits. This analysis agreed with Viitala *et al*. (2006) who had found separate effects for both *GHR* and *PRLR* with milk production traits in Finnish Ayrshire cattle whereas Blott *et al*. (2003) had not observed separate effects for *PRLR* in an earlier study primarily of Holstein cattle. These four FNP did not show a statistically significant association to tick counts in this sample – this sample cannot rule out very small effects, but if those small effects exist they are substantially smaller than the effects of these genes on milk traits. Their use to improve cattle production should have no negative effect on the host resistance of these cattle to ticks. Although the milk yield traits used in this study are for adjusted milk fat and protein yield, these four gene tests also have known direct effects on milk yield.

The results from the GWAS showed several promising results but these need to be kept in perspective. The sample of 189 animals had a power of detecting an effect of 6.9% of the variance at a 5% significance threshold with 90% power, which will result in many false negatives and false positives. False negatives would be examples of associations such as *ABCG2* and *PRLR* to milk protein yield that are not significant at the 5% threshold in the GWAS sample – we know that they have an effect in these data because they showed statistically significant results in the full dataset. To identify false positives, additional research will be needed to confirm the associations of SNP to traits identified by the GWAS. Although many of these are highly significant ($P < 0.001$), they need to be confirmed in independent samples of cattle before they could be used for genetic testing. Many of these are likely to be false positives, and diagnostic tests need to be based on results from thousands of cattle (Barendse 2005). Nevertheless, we found little relationship between the allele effects for adjusted milk protein or fat yield and tick burden, consistent with the phenotypic correlations between these traits in this dataset. These results are in agreement that selection for milk component yield should have no or little effect on parasite loads in these cattle, whether this is based on phenotype or based on gene tests.

The FPR in the GWAS showed 100% for milk composition associations but only 24% for tick burden associations. There are at least three possible explanations for this difference. First, this could be a chance event, partly due to the small sample size used in the GWAS, although it is not clear how one would evaluate the likelihood of such a chance event – the sampling distribution of number of successful hits in a GWAS is not well understood when analysed over a range of traits in the same experiment. Second, the SNP in this panel are not uniformly distributed across the genome and show large gaps of more than 1 Mb between adjacent SNP in some areas (Barendse *et al*. 2009*b*). If the QTL for milk composition were located where SNP are less dense then fewer milk composition QTL would be found. There is some evidence to support this – there are no SNP within 3 Mb of *DGAT1* and the other FNP were also flanked by a sparse number of SNP. Moreover, it is likely that other SNP near *DGAT1* would also show LD to milk fat concentration, potentially increasing the number of SNP with very small *P*-values. However, arguing against this evidence, the *P*-value for *DGAT1* was only slightly less than the 0.1% threshold in the GWAS sample, so it is not certain that other SNP in strong LD to *DGAT1* would have been equally significant at that threshold. The third explanation is that there are more SNP of large effect associated with tick burden than for milk component yield segregating in the population. This would be consistent with strong selection for milk production traits and weak selection for host resistance to ticks, because persistent selection on a trait will cause genes of larger effect to become fixed sooner in a population (Kimura 1982). Selection for milk production is strong because it occurs on bulls in a national system of evaluation where many bulls are progeny tested, most outside the tick zone. Selection for host resistance to ticks is likely to be relatively weak because it occurs largely through culling of cows that carry extremely large tick burdens. Large numbers of ticks are not seen every year (Sutherst *et al*. 1979), and regular treatment with acaricides would also reduce the opportunity of seeing cows with large tick burdens, blunting opportunities for genetic selection.

To help identify genes that might have large effects on host resistance to parasites, we analysed the gene content near the most significant SNP and then determined whether the region

had previously been identified to contain a signature of selection. It is unlikely that a QTL that makes a small contribution to the phenotypic variance will have a population genetic signature of selection (Kim and Stephan 2002). Locations on chromosome BTA2 showed an intersection of all three criteria: (1) significant allele effects associated with tick burden in a GWAS; (2) appropriate positional candidate genes; and (3) a signature of selection (Gibbs *et al*. 2009). Further research that may identify FNP for these QTL in the future will be able to determine whether the FNP were responsible for the selection signatures or whether some other trait, or combination of traits, is responsible for the selection signature.

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