

## INDIRECT EFFECTS OF A QTL FOR HEEL DEPTH ON OTHER FEET AND LEG TRAITS IN HOLSTEIN CATTLE

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### INTRODUCTION

Feet and leg problems are of great concern to dairy producers since they are a major cause for unplanned, premature and costly culling of cows in dairy herds. Conventional selection so far in most populations has failed to improve upon this situation due to low heritabilities of the traits in question. Marker assisted selection based on the identification of QTLs should offer greater benefits. However, in a number of studies (e.g. Ashwell *et al.*, 1998a ; Ashwell *et al.*, 1998b ; Schrooten *et al.*, 2000 ; Boichard *et al.*, 2000) using grand-daughter designs and whole genome scans, a variety of QTLs have been reported on several chromosomes (9, 22, 23, 16, 24, 15) for the closely related traits foot angle, foot diagonal and heel depth. The variety of traits and chromosomes and given the fact that the Holstein sub-populations around the world should be closely related may indicate that it will be difficult to identify an important QTL based on the phenotypes used so far as these commonly are provided by national type scoring systems. In contrast to the studies mentioned, Baumgartner *et al.* (2002) reported a QTL influencing heel depth on BTA 7 based on direct measurements of the trait in bulls of age around 14 months. However, the authors were unable to verify this finding in a data set consisting of breeding values of A.I. bulls for linear type scores of their daughters. In the present study a larger data set of estimated breeding values for feet and leg traits of A.I. bulls could be used and all relevant traits were analyzed simultaneously.

### MATERIAL AND METHODS

**The QTL and its frequency.** As presented in Baumgartner *et al.* (2002), a candidate gene approach had been used for the identification of a QTL influencing heel depth. The bovine diastrophic sulphate transporter gene (BTA 7) was isolated and characterized from a genomic phage library. The gene is responsible for the sulphate uptake into cells. Within the gene, two polymorphisms could be identified of which one resulted in an amino acid exchange. Hence, two alleles were detected. A.I. bulls of which semen was available were genotyped and the results were matched with the official publication of estimated breeding values (EBV) for type traits in Germany as of August 2001. Since the data collected in this way did not stem from a planned experiment, two subsets of the entire material were created resulting in a total of three data sets. Data set I contained all bulls that had EBVs, for data set II a few old bulls born before 1987 were excluded and data set III consisted of young bulls born 1993 or later. By this stratification it was attempted to check on possible disturbing effects when including older bulls in widespread use as compared to young bulls that did not have any information from

second crop daughters in their proof. Table 1 displays the frequencies of genotypes and alleles in the three data sets.

**Table 1. Frequencies of genotypes and alleles by data set**

Data set	Absolute frequency of genotypes				Allele frequency	
	AA	AB	BB	Total	p	q
Data set I	293	439	168	900	.569	.431
Data set II	280	408	153	841	.576	.424
Data set III	144	228	88	460	.561	.439

Allele frequencies hardly differ between data sets. Furthermore, allele frequencies in the A.I. bull data set presented here do not differ greatly from those found in the young bull data set used by Baumgartner *et al.* (2002) in which p was equal to .595. From table 1, a Hardy-Weinberg equilibrium has to be assumed.

**Genetic structure of the data set.** As the data was not collected according to a planned experiment, a large number of bull sires were found in the main data set. However, this finding is typical for the German Holstein population. Only rarely bull sires with > 100 sons can be found. In our data, the largest family with 111 sons was present in all three subsets. A total of 10 sires had more than 20 sons in data set I with 111, 68, 46, 42, 39, 36, 32, 24, and 22 sons, respectively. Of the more frequently used bull sires, five sires with 32, 22, 39, 20, and 14 sons, respectively, did not have a single offspring with the genotype BB, thus suggesting that their own genotype likely was AA. In contrast, two sires with 13 and 14 sons did not have a son of genotype AA. The majority of sires had produced sons of all three possible genotypes or had too few sons to make any reliable inference on their own genotype. An overview of the genetic structure is given in table 2.

**Table 2. Genetic structure of the data sets**

Data set	Total no. of bulls	No. of sires	Average no. of sons per sire	Range of no. of sons per sire
Data set I	900	157	5.73	1 – 111
Data set II	841	138	6.09	1 – 111
Data set III	460	98	4.69	1 - 109

**Description of traits.** Estimated breeding values for type traits included the following traits related to feet and legs: Rear legs – side view (RLS), heel depth (HD), hock quality (HQ), rear legs – rear view (RLR), and feet and legs (FAL). The first four traits are scored linearly on a 1 to 9 scale. The trait FAL is an index created in the way that firstly a sub-index of RLS, HD, HQ and RLR with weights equal to 0.30, 0.30, 0.20, and 0.20, respectively, is derived and the resulting figure with a weight of 50 % is then combined with a subjective overall feet and leg score which receives another 50 % weight. This strategy has been adopted in Germany since it

was claimed that a mere index of the four linear traits would not adequately describe feet and legs in a cow.

For all traits, a reliability of at least 60 % was required for an estimated breeding value entering the analysis. Since the five traits have been introduced in the German type classification system at different times and hence the number of daughters scored differs even within sires from trait to trait, this lead to an irregular pattern of missing values across and within bulls.

**Statistical analysis.** Since the size of the families was too small to be suited for an analysis in the form of a grand-daughter design, a simple fixed model was applied. The only fixed effect in the model was the genotype of the bull. Contrasts between least-square means of genotypes were estimated and their significance tested using Scheffé's test under adjustment for multiple comparisons.

## RESULTS AND DISCUSSION

**Table 3. Least-square means and significance of contrasts between genotypes**

Name of trait		Least-square means			p-values of contrasts	
		AA	AB	BB	AA-AB	AA-BB
Data set I (900; 550 – 822)						
Rear legs – side view	RLS	100.2	101.0	101.0	0.72	0.84
Heel depth	HD	98.2	99.9	98.6	0.26	0.96
Hock quality	HQ	96.1	100.7	100.2	<b>0.0005</b>	<b>0.02</b>
Rear legs – rear view	RLR	99.9	101.4	101.1	0.46	0.74
Feet and legs	FAL	98.0	100.7	100.3	<b>0.02</b>	<b>0.16</b>
Data set II (841; 527 – 765)						
Rear legs – side view	RLS	100.3	100.7	100.6	0.92	0.98
Heel depth	HD	98.2	100.4	99.3	0.14	0.78
Hock quality	HQ	96.3	100.6	99.8	<b>0.001</b>	<b>0.056</b>
Rear legs – rear view	RLR	99.9	101.5	101.0	0.47	0.79
Feet and legs	FAL	98.2	101.3	101.1	<b>0.0066</b>	<b>0.075</b>
Data set III (460; 382 – 389)						
Rear legs – side view	RLS	99.4	100.3	99.6	0.79	0.99
Heel depth	HD	100.8	102.3	100.0	0.57	0.92
Hock quality	HQ	94.7	99.7	99.2	<b>0.0008</b>	<b>0.026</b>
Rear legs – rear view	RLR	100.9	102.0	101.8	0.73	0.88
Feet and legs	FAL	98.4	102.6	101.3	<b>0.011</b>	<b>0.256</b>

In Germany, estimated breeding values for all traits in the Holstein breed are expressed having means for base bulls equal to 100 and a s.d. of 12. The figures given after the names of the data sets in table 3 denote the total number of bulls with breeding values in the set and the actual range of the number of bulls included in the analysis, depending on the reliability of

their individual proof for each individual trait. For the sake of brevity, only p-values of the contrasts for the comparison of AA vs. AB and AA vs. BB are given.

The results from the main data set and its two subsets are rather similar with a tendency of larger differences between genotypes in the test bull data set III. The traits RLS and RLR are not affected by the QTL. The trait HD again is showing tendencies for a superiority of genotypes having at least one B-allele, although insignificant. In contrast to this, HQ and FAL exhibit a distinct and significant superiority of the B-allele. Analogous results, although insignificant, were found when analyzing the largest sire family separately.

In the study of Baumgartner *et al.* (2002), HD was directly measured in cm on young bulls. The three genotypes differed highly significant with the BB genotypes exhibiting the highest values. In this study, using data from the German type classification scheme, only tendencies for HD are observed while significant differences exist for HQ and FAL. Our hypothesis, supported by the fact that for HD in the German system a heritability of only around .10 has been estimated, is that HD as scored in the field may not be a suitable trait for an association study since the scoring obviously creates difficulties. However, if a cow has a sizeable heel depth and hence a more upright foot, her hocks are less susceptible to weight burdens and thus look slimmer. This in turn is responsible for higher scores for HQ and for the FAL composite.

### CONCLUSION

The availability of suitable phenotypes is a crucial factor when hunting for QTL. Phenotypic measurements first of all should bear a high repeatability. This is questionable for feet and leg traits for which only very low heritabilities have been reported. Results from an association study in this situation may then be inconclusive. However, as this study shows, indirect effects on other traits within the classification system may exist that in turn are observable. Our findings may be used as an example for a significant effect of a QTL on related traits or composite trait indices for which the interrelationships could only be unraveled jointly using data of direct measurements and scores in the field.

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