Abstract


The aims of this thesis were to improve the knowledge about the genetic background of health and reproduction traits in dairy cattle. This was accomplished by performing gene mapping studies of quantitative trait loci (QTL) affecting these traits and also by exploring the genetic variability for pathogen-specific mastitis.

Ten sire families were used in a granddaughter design to map QTL. Several QTL were detected for most of the traits studied. Inclusion of cofactors in the analyses to adjust for QTL found on other chromosomes increased both the number of QTL detected and the significance levels. In total, 20 QTL were detected in analyses without cofactors whereas 41 QTL were detected when cofactors were used. Chromosome regions with several QTL were found, e.g. on chromosome 9 and 11 we observed QTL for both health and reproduction traits. A QTL for resistance to mastitis was found close to the same marker where a QTL for the fertility measure non-return rate was detected. The QTL affecting non-return rate was fine mapped to a small region on chromosome 9. Further research is required to evaluate whether this QTL could be used in breeding programs by marker assisted selection.

Resistance to mastitis is a multifactorial trait that involves a variety of pathogens and physiological defense mechanisms. To dissect the trait into sub-traits of resistance to individual pathogens, bacteriological data on mastitis pathogens were analyzed. The most common pathogen in the data set was Staphylococcus aureus. Genetic variation in resistance to individual mastitis pathogens was found. Estimated heritabilities for the sub-traits were higher compared to heritabilities for general resistance to mastitis in most other studies. Haplotypes of a QTL previously shown to affect resistance to mastitis were analyzed for effects on resistance to pathogen specific mastitis. No significant allele substitution effect of haplotype on occurrence of the various mastitis pathogens was found, but there was a significant difference between the effects of two of the haplotypes on the presence of Streptococcus dysgalactiae.

Keywords: dairy cattle, mastitis, fertility, quantitative trait loci, fine mapping, heritability, mastitis pathogen

Author’s address: Mia Holmberg, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, 750 07 UPPSALA, Sweden.
E-mail: Mia.Holmberg@hgen.slu.se
Till min underbara familj!
## Contents

**Introduction, 9**
Health and reproduction of dairy cows, 9
  *Mastitis, 9*
  *Other diseases, 10*
  *Reproduction, 10*
  *Economic aspects of health and reproduction, 10*

**Mapping of genes, 11**
Genetic improvement of low heritability traits, 12

**Aims of the thesis, 13**

**Summary of the investigations, 14**
Materials, 14
  *QTL mapping data, 14*
  *Pathogen data, 15*
  *Haplotype data, 15*
Methods, 16
Main results, 17
  *Genome scans (Paper I, II), 17*
  *Fine mapping (Paper III), 18*
  *Mastitis pathogens (Paper IV), 19*

**General discussion, 20**
Power and precision in QTL mapping, 20
  *Advantages with the GDD, 20*
  *Use of cofactors in QTL analyses, 21*
  *Fine mapping of functional traits, 21*
QTL regions affecting more than one trait, 22
Marker assisted selection (MAS), 24

**Conclusions, 25**

**Future outlook, 26**

**Populärvetenskaplig sammanfattning, 28**

**References, 31**

**Acknowledgements, 34**
Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their roman numerals:


IV. Holmberg, M., Fikse, F., Andersson-Eklund, L., Artursson, K. & Lundén, A. Genetic analyses of pathogen specific mastitis. (Manuscript)

Paper I, II and III are reproduced by permission of the journals concerned.
Abbreviations

CAL    calving performance
CAL_{dir}  CAL direct effect
CAL_{mat}  CAL maternal effect
CFI    interval from calving to first insemination
CNS    coagulase negative staphylococci
DYD    daughter yield deviation
EBV    estimated breeding value
FTR    fertility treatments
GDD    granddaughter design
HS     heat score
INS    number of inseminations
INS_{heifer}  INS in heifers
INS_{cow}  INS in 1st lactation cows
LA     linkage analysis
LD     linkage disequilibrium
MAS    marker assisted selection
NR56   non-return rate at 56 days
NR56_{heifer}  NR56 in heifers
NR56_{cow}  NR56 in 1st lactation cows
OD     other diseases
QTL    quantitative trait loci
SCC    somatic cell count
STI    stillbirth
STI_{dir}  STI direct effect
STI_{mat}  STI maternal effect
SVA    national veterinary institute
Introduction

Functional traits of dairy cattle have other values than just economic because of their direct impact on animal welfare and consumer acceptance of the products. The term “functional traits” is used for those characters of an animal that increase efficiency not by higher output of products but by reduced costs of input (Groen et al., 1997). Examples of traits belonging to this category are fertility, calving ease, resistance to mastitis and other diseases. These traits are all quantitative which means that they are influenced by several genes and also to a large degree by environmental factors. Depending on how the traits are recorded they are either characterized by a continuous variation of phenotypic values or by phenotypes expressed in distinct classes, i.e. threshold traits.

The Swedish dairy producers face new demands from consumers asking for high quality food produced according to high ethical standards to an affordable price level. Consumers concerns regarding the welfare of farm animals and the development of multi-resistant bacteria due to extensive use of antibiotics for disease treatments of animals are growing. Genetic improvement of functional traits such as resistance to diseases may reduce the use of antibiotics and thereby also reduce the risk of residues in milk and meat products. Improved fertility and less calving difficulties would make shorter calving intervals possible, reduce the need for treatments due to fertility disorders and increase the well being of the cows. Therefore it is of great importance to improve the genetic capacity for functional traits of our dairy breeds.

Health and reproduction of dairy cows

Mastitis

Mastitis is one of the most common and costly dairy cattle diseases. It is an inflammation of the mammary gland resulting from the introduction and multiplication of pathogenic microorganisms in the udder (Harmon et al., 1994). The disease is complex as it is caused by a number of different pathogens to which there are a variety of physiological responses. Clinical signs of mastitis are swelling and pain in the udder, fever, abnormal milk appearance, altered milk composition, bacteria present in the milk and substantially reduced milk yield. Subclinical mastitis does not cause visible changes in the appearance of the milk or the udder, but milk yield is reduced, bacteria are present and the composition is altered (Harmon et al., 1994).

Genetics of mastitis resistance have been studied for a long time. Heritability estimates of clinical mastitis are in general low, usually below 0.05 (Carlén et al., 2004, Lassen et al., 2003, Rupp & Boichard, 1999). However, considerable genetic variation for the trait exists; the mastitis frequency is twice as high among daughters of sires with a low udder health index compared to sires with a high index (Aamond, 2006). Thus, rather than lack of genetic variation, the low heritability is explained by a very large phenotypic variability (Rupp & Boichard,
2003), which in turn could partly be due to a too general definition of the trait without reference to the mastitis causing pathogens.

**Other diseases**

To reach the breeding goal of healthy animals, also veterinary treatments for diagnoses other than mastitis and fertility problems are included in the genetic evaluation of dairy bulls in Sweden. The incidence of each disorder is too small to be considered individually in the genetic evaluation why the disorders are pooled and treated as one trait called “other diseases” (OD). The majority of the diagnoses in OD comprise paresis, ketosis, retained placenta and hoof lesions.

**Reproduction**

The reproductive performance of a cow includes both fertility and calving traits. Fertility traits are based on a variety of different measures that reflects the cow’s ability to become pregnant, for example in a categorical manner such as percentage non-return, or the actual number of inseminations performed during one service period. There are also the interval measures such as number of days between calving and first insemination that reflects the cow’s ability to recycle after calving. Information about heat detection and treatments for reproductive disturbances are used to express the ability to show heat and to resist fertility disorders. As regards calving traits, the most commonly used in genetic evaluations are calving performance and stillbirth. The calving performance of a cow is typically scored by the farmer in predefined categories (e.g. normal or difficult calvings). Stillbirth is often defined as a dead born calf or a calf that dies within 24 hours of parturition.

A general decline in fertility of dairy cattle has been observed in many countries during the last decades (Lopez-Gatius, 2003; Lucy, 2001; Royal et al., 2000). In Sweden, impaired fertility has been the main reason for involuntary culling of dairy cows for several years (Swedish Dairy Association, 2006). As for mastitis, the heritability of fertility traits is usually below 0.05 (Roxström et al., 2001, Wall et al., 2003). The low heritability may partly be due to the large influence of management decisions (e.g. when to do the first insemination) and also that some traits are combinations of sub-traits with different physiological expressions. Owing to the complexity of the reproductive functions in a cow there is no reliable single measure that can explain variations in the entire fertility. Nevertheless, the genetic variation among available fertility measures is quite large (Philipsson & Lindhé, 2003), which enables genetic selection. There is today a widespread interest in international genetic evaluation for fertility (Jorjani, 2005).

**Economic aspects of health and reproduction**

Reproduction and health traits contribute substantially to the economy of the dairy farmer as the cow getting pregnant is a prerequisite of milk production and the health of the cow has a direct impact on production level and management costs. In addition, calving performance and calf viability are not only important traits for minimizing the suffering of cow and calf at delivery but are also economically
important as a dead born calf, an injured cow or decreased fertility due to calving difficulties causes extra costs and increased labor. Furthermore, female fertility and resistance to mastitis are unfavorably genetically correlated with milk yield (e.g. Heringstad, 2000, Roxström et al., 2001) which means that single-trait selection for increased milk production is expected to decrease the fertility and increase the mastitis incidence. Therefore it is important to consider these traits in the breeding goal of dairy cattle.

**Mapping of genes**

A quantitative trait locus (QTL) is defined as a chromosomal region that contains one or more genes that influence a quantitative trait (Andersson, 2001). Most genes affecting quantitative traits do not have a measurable effect on the traits. Therefore, the localization of genes affecting quantitative traits requires detection of co-segregation of a phenotypic measure with genetic markers on a chromosome. The development of extensive maps of genetic markers based on DNA sequence variation and the advances in statistical methodology have made it possible to map QTL affecting functional traits of interest in dairy cattle. The identification of QTL is a first step toward novel selection methods based on both phenotypic and molecular information.

A first step in identifying a QTL can be performed by conducting a genome scan. In dairy cattle the granddaughter design (GDD) (Weller et al., 1990) is commonly used to take advantage of the large pedigrees resulting from the widespread use of artificial insemination. In this design only the bulls are genotyped and the phenotypic trait values are based on the performance of many daughters of a bull, thereby obtaining reliable values (Figure 1). In a genome scan multiple markers are used to screen the whole genome (or part of a genome) to identify chromosomal regions affecting quantitative traits. As no prior knowledge of the genetic background of the trait is needed, scanning the genome provides the potential of identifying new or previously unexpected genes influencing a trait of interest. However, the mapping resolution from a genome scan is usually low. In general, QTL are mapped by linkage analysis with a confidence interval of more than 20 cM. This genetic distance may contain hundreds of genes why it is necessary to position the QTL more precisely by fine mapping methods before the QTL can be efficiently used for breeding purposes.

To fine map a QTL to a more precise location of only a few cM on a chromosome, a denser marker map is required compared with a genome scan. Methods that use linkage disequilibrium information are commonly used for fine mapping purposes. In linkage analysis only recombination events that occur between two generations are taken into account while positioning the QTL whereas in linkage disequilibrium mapping recombination events from several generations back are used which gives a higher precision.
Many QTL mapping studies have been performed worldwide and in recent years also the functional traits have been investigated for existence of QTL (review by Khatkar, 2004). Despite the low heritabilities, QTL have been found for udder health traits (Klungland et al., 2001; Schulman et al., 2004) as well as fertility and calving traits (Schrooten et al., 2000; Kühn et al., 2003).

Genetic improvement of low heritability traits

Breeding for lowly heritable traits is a challenging task with small or sometimes no progress per generation depending on the weights of the traits included in the breeding goal and the breeding plan applied. The progress, however, is heritable and therefore cumulative over generations. To increase the accuracy in genetic evaluations and to enable identification of genetic mechanisms underlying low heritability traits, it is important to have accurate trait definitions and reliable registrations of phenotypes on a large number of animals. In addition, incorporating information about identified QTL in animal breeding schemes, so called marker assisted selection (MAS), is a direct approach that may enhance the genetic progress for such low heritable traits.

Most functional traits are complex in nature, for example mastitis is a multifactorial disease involving different types of resistance mechanisms and thus many genes are likely to be involved in the regulation. To increase the heritability of resistance to mastitis, measures with a more direct association to the resistance mechanisms could be used. As mastitis is caused by a multitude of different pathogens one way to improve the heritability could be to dissect the trait into pathogen-specific resistance. De Haas et al. (2002) estimated heritabilities to between 0.02 and 0.10 for resistance to individual mastitis pathogens in 274 Dutch dairy herds, which indicates that pathogen-specific resistance could be improved by selection. By dividing mastitis into sub-traits of resistance to specific pathogens identification of QTL affecting these sub-traits would be possible.
Aims of the thesis

The overall objective of this thesis was to gain knowledge about the genetic background of health and reproduction traits in dairy cattle. This was done by performing genetic mapping of QTL affecting these traits and also to explore the genetic variability for pathogen-specific mastitis.

The more specific aims (in paper I, II, III and IV) were:

- to map QTL with an effect on mastitis resistance, resistance to other diseases, calving performance and female fertility in the Swedish dairy cattle population.
- to fine map a QTL for non-return rate on chromosome 9 by use of a combined linkage and linkage disequilibrium method
- to investigate whether there is a genetic variability regarding cow’s susceptibility to different mastitis pathogens
- to examine whether identified QTL-haplotypes with effect on general mastitis resistance has varying effects on specific mastitis pathogens
Summary of the investigations

Materials

QTL mapping data

The animal material that was used in the genome scans (I, II) consisted of ten dairy bull sire families. Nine of the families were of the Swedish Red breed and one family of the Swedish Holstein breed. The families were selected for the study as they were the largest bull sire families in Sweden at the time. The sires had on average 42 sons each and the total number of bulls was 417. In the fine mapping study (III) five of the original families were used, in total 139 bulls.

The phenotypic data used in the first three papers were field data provided by the Swedish Dairy Association in the form of estimated breeding values (EBV) from the national genetic evaluation and daughter yield deviations (DYD). The DYD are daughter group averages corrected for systematic environmental effects. The phenotypic values were based on a minimum of 50 daughters per bull. The traits studied in this thesis (defined in Table 1) were clinical mastitis (I), somatic cell score (I), other diseases (I), non-return rate (II, III), number of inseminations (II, III), fertility treatments (II), interval from calving to first insemination (II), heat intensity score (II), stillbirth (II) and calving performance (II).

Table 1. Abbreviations and trait definitions

<table>
<thead>
<tr>
<th>Trait/abbreviation</th>
<th>Trait definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical mastitis</td>
<td>Incidence of clinical mastitis, based on veterinary records and culling reports</td>
</tr>
<tr>
<td>SCC</td>
<td>Somatic cell count, the lactation mean of log-10 transformed SCC values (in 10,000/mL)</td>
</tr>
<tr>
<td>OD</td>
<td>Incidence of other diseases, veterinary treatments for diseases other than mastitis and fertility problems (mainly paresis, ketosis, retained placenta and hoof lesions)</td>
</tr>
<tr>
<td>NR56&lt;sub&gt;heifer&lt;/sub&gt;</td>
<td>Non-return rate at 56 days after the first insemination in heifers</td>
</tr>
<tr>
<td>NR56&lt;sub&gt;cow&lt;/sub&gt;</td>
<td>Non-return rate at 56 days after the first insemination in 1&lt;sup&gt;st&lt;/sup&gt; lactation cows</td>
</tr>
<tr>
<td>INS&lt;sub&gt;heifer&lt;/sub&gt;</td>
<td>Number of inseminations per service period in heifers</td>
</tr>
<tr>
<td>INS&lt;sub&gt;cow&lt;/sub&gt;</td>
<td>Number of inseminations per service period in 1&lt;sup&gt;st&lt;/sup&gt; lactation cows</td>
</tr>
<tr>
<td>FTR</td>
<td>Incidence of fertility treatments, veterinary treatments for fertility disturbances</td>
</tr>
<tr>
<td>CFI</td>
<td>Interval from calving to first insemination (days)</td>
</tr>
<tr>
<td>HS</td>
<td>Heat intensity score, scored by farmer in predefined categories</td>
</tr>
<tr>
<td>STI&lt;sub&gt;dir&lt;/sub&gt;</td>
<td>Incidence of stillbirth, calves dead at birth or within 24 h, direct effect (the evaluated bull is father of the calf)</td>
</tr>
<tr>
<td>STI&lt;sub&gt;mat&lt;/sub&gt;</td>
<td>Incidence of stillbirth, calves dead at birth or within 24 h, maternal effect (the evaluated bull is maternal grandfather of the calf)</td>
</tr>
<tr>
<td>CAL&lt;sub&gt;dir&lt;/sub&gt;</td>
<td>Calving performance, scored by farmer in predefined categories, direct effect (the evaluated bull is father of the calf)</td>
</tr>
<tr>
<td>CAL&lt;sub&gt;mat&lt;/sub&gt;</td>
<td>Calving performance, scored by farmer in predefined categories, maternal effect (the evaluated bull is maternal grandfather of the calf)</td>
</tr>
</tbody>
</table>

<sup>1</sup>All traits are based on records from 1<sup>st</sup> lactation cows except the heifer specific traits
DNA from each bull was extracted from frozen semen samples obtained from the Swedish breeding company Svensk Avel. Microsatellite markers were genotyped on a total of 20 bovine chromosomes. In paper I, 17 chromosomes were covered with 116 markers, in paper II 20 chromosomes were analyzed with 145 markers and in paper III a part of chromosome 9 was fine mapped with 25 markers. The average marker interval was around 20 cM in the genome scans and 2.8 cM in the fine mapping study.

Pathogen data

Each year milk samples from approximately 9000 cows are sent to the National Veterinary Institute (SVA) in Sweden for bacteriological analyses. Samples are collected both from cows with suspected subclinical mastitis and clinical mastitis. Information on outcome of the bacteriological culturing has been stored in a database since 1993.

Data on mastitis pathogens (IV) were obtained from SVA. Data collection period was from 1993 until 2004. The total number of cows tested in the original data was 110,881. After editing the data such that cows without bacterial growth in the milk samples, cows without known pedigree and cows by sires with less than 20 daughters in the dataset were deleted, the number of cows in the final dataset was 21,834. The pathogen data were scored as a categorical trait with 1 (present) or 0 (absent) for each pathogen at each observation. Each infected quarter of a cow was treated as a separate observation. The total number of observations used in the analyses was 38,607. Pathogens studied were *Staphylococcus aureus* (*S. aureus*), coagulase negative staphylococci (CNS), *Escherichia coli* (*E. coli*), *Streptococcus dysgalactiae* (*Str. dysgalactiae*), *Streptococcus uberis* (*Str. uberis*), and streptococci other than *Str. dysgalactiae*, *Str. uberis* and *Str. agalactiae* (*Str. species*).

Haplotype data

A QTL affecting resistance to clinical mastitis was identified and mapped to an interval of less than 1 cM on bovine chromosome 9 by Sahana et al. (submitted). Haplotypes with varying effects on clinical mastitis resistance were identified. The study was based on three Nordic red breeds in which 14 sire families of the Swedish red breed were included. To investigate whether the QTL haplotypes give resistance to specific mastitis pathogens rather than a general effect on the resistance to clinical mastitis we extracted pathogen data from daughters of bulls with known haplotypes. Haplotype information was available on 114 of the sires that had at least five daughters with bacteriological data. The number of identified QTL haplotypes among these bulls was 20. We analyzed the seven most frequent haplotypes plus a residual group of haplotypes with frequencies of 3% or below. The less frequent haplotypes accounted for 15% of all haplotypes.
Methods

The granddaughter design was used in the genome scans (paper I and II), and the same statistical method was used in both studies. Each trait was analysed separately with a multimarker regression approach (Knott et al., 1996, Vilki et al., 1997). The phenotypes were regressed on the probability of inheriting the first or second paternal QTL allele for each cM on the chromosome. The analysis was nested within families to allow for different linkage phases between marker and QTL alleles between grandsires. The following regression model was applied:

\[ Y_{ijk} = \mu + g_{si} + b_i X_{ijk} + e_{ijk} \]

where \( Y_{ijk} \) is the EBV or DYD of son \( k \), of grandsire \( i \), with marker genotype \( j \); \( \mu \) is the overall mean; \( g_{si} \) is the effect of grandsire \( i \); \( b_i \) is the regression coefficient within grandsire \( i \); \( X_{ijk} \) is the probability of QTL allele 1 being inherited by son \( k \) from grandsire \( i \), given the pair of flanking markers \( j \) of son \( k \), and \( e_{ijk} \) is the residual effect. Initially, the chromosomes were analyzed individually to identify candidate regions for QTL. The analyses were performed both across and within grandsire families. To increase the power of the analysis, putative QTL that were found in the across family analysis were included as cofactors in the further analysis.

Two significance levels were used in the genome scans, i.e. chromosome-wise and genome-wise significance. Tests performed within a chromosome can not be considered independent because loci on the same chromosome are linked, whereas tests on different chromosomes are independent of each other. To correct for multiple tests performed on each chromosome, significance levels were set by a randomization procedure (permutation test) (Churchill & Doerge, 1994). The permutation was repeated 10,000 times for each chromosome and trait separately. Genome-wise significance thresholds were calculated from the chromosome-wise thresholds by the Bonferroni correction, to account for multiple testing on several chromosomes.

Marker order for each chromosome and map distances were estimated (paper I, II and III) using CRIMAP version 2.4 software (Green et al., 1990) and the Haldane map function.

In the fine mapping study (paper III) both the regression approach and a variance component linkage analysis (LA) was applied to the data before linkage disequilibrium (LD) and the combined LDLA methods (Lund et al., 2003) were used. Linkage disequilibrium based methods are commonly used for fine mapping of QTL. The LD methods give a more precise location of the QTL as they allow for the utilization of all recombination events since the mutation occurred (Meuwissen & Goddard, 2000). The combined approach requires evidence for LD at the QTL position as well as indication of a QTL from LA, thereby reducing the risk of finding false positive QTL (Goddard & Meuwissen, 2005).
The incidence of infection by a mastitis pathogen (paper IV) was recorded as a binomial trait why a threshold sire model was used to estimate variance components. The analyses were based on Markov Chain Monte Carlo methodology, using a Gibbs sampling algorithm implemented in the Maggi program package (Janss, 1998). The threshold model assumes an underlying non-observable continuous variable called liability. Each pathogen was analyzed separately and heritabilities on the liability scale were calculated from the estimated variance components by multiplying the sire variance by four and dividing by the sum of the sire and residual variances.

The haplotype data set (paper IV) was used to study the effect of QTL haplotype on the risk of being infected by a given mastitis pathogen. An allele substitution model was used where the effect of each haplotype was expressed as a deviation from the mean.

Main results

Genome scans (Paper I, II)

Two genome scans were performed involving the same ten grandsire families. Associations between markers and traits were tested on a total of 20 chromosomes and altogether 14 traits were analyzed. In the across-family analysis without cofactors a total of 20 significant QTL ($P < 0.05$) were found on 10 chromosomes, and QTL were detected for all traits except FTR, CAI_dir and CFI. When cofactors were added the number of QTL increased to 41, and the significance levels were higher for most QTL (Table 2). In addition to the results of the across-family analysis several QTL were found to be segregating only in individual families. The number of families segregating ($P_{\text{chromosome}} < 0.10$) for each QTL varied between one and six out of the ten analyzed families. Two different significance levels were used, at the genome level we used a more stringent threshold. Two of the QTL were significant at the genome level in the analyses without cofactors, on chromosome 9 (NR56_heifer) and 20 (NR56_cow). Eighteen QTL were significant at the genome level in analyses with cofactors.

Chromosome regions with effect on more than one trait were found. Six QTL with effect on both health traits and reproduction traits were found each on chromosomes 9 and 11 (with cofactors). On chromosome 9, four out of the six detected QTL were found close to marker TGLA73. The most promising result with highest significance level was the QTL affecting NR56_heifer on chromosome 9. Five families segregated for this QTL and four of these had the highest test statistics in the same region, toward the distal end of chromosome 9.
Table 2. Significant QTL found in the genome scans performed in paper I and II. Results are given per chromosome.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Trait</th>
<th>Marker or marker bracket</th>
<th>Cof.</th>
<th>No cof.</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NR56_cow</td>
<td>ILSTS104-BM6506</td>
<td>*</td>
<td>NS</td>
<td>4.9</td>
</tr>
<tr>
<td>1</td>
<td>FTR</td>
<td>TGLA49</td>
<td>**</td>
<td>NS</td>
<td>2.57</td>
</tr>
<tr>
<td>2</td>
<td>INS_cow</td>
<td>INRA197</td>
<td>*</td>
<td>NS</td>
<td>2.10</td>
</tr>
<tr>
<td>2</td>
<td>FTR</td>
<td>BM2924</td>
<td>**</td>
<td>NS</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>HS</td>
<td>MGT405</td>
<td>*</td>
<td>NS</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>STI_mat</td>
<td>BMS1237-HJ673</td>
<td>***</td>
<td>NS</td>
<td>1.38</td>
</tr>
<tr>
<td>4</td>
<td>SCC</td>
<td>BM6026</td>
<td>*</td>
<td>NS</td>
<td>2.79</td>
</tr>
<tr>
<td>5</td>
<td>CAL_heifer</td>
<td>BM143</td>
<td>***</td>
<td>**</td>
<td>5.710</td>
</tr>
<tr>
<td>6</td>
<td>CAL_heifer</td>
<td>BM1329</td>
<td>***</td>
<td>**</td>
<td>2.35</td>
</tr>
<tr>
<td>7</td>
<td>HS</td>
<td>BMS522</td>
<td>***</td>
<td>NS</td>
<td>2.6</td>
</tr>
<tr>
<td>7</td>
<td>STI_mat</td>
<td>BM6105</td>
<td>***</td>
<td>*</td>
<td>1.48</td>
</tr>
<tr>
<td>9</td>
<td>Clinical mastitis</td>
<td>TGLA73</td>
<td>**</td>
<td>*</td>
<td>2.679</td>
</tr>
<tr>
<td>9</td>
<td>SCC</td>
<td>CSSM56</td>
<td>**</td>
<td>NS</td>
<td>6.710</td>
</tr>
<tr>
<td>9</td>
<td>OD</td>
<td>UWC9-TGLA73</td>
<td>**</td>
<td>*</td>
<td>1.55</td>
</tr>
<tr>
<td>9</td>
<td>HS</td>
<td>BMS817</td>
<td>***</td>
<td>NS</td>
<td>2.69</td>
</tr>
<tr>
<td>9</td>
<td>INS_heifer</td>
<td>TGLA73</td>
<td>*</td>
<td>*</td>
<td>1.8</td>
</tr>
<tr>
<td>9</td>
<td>NR56_heifer</td>
<td>TGLA73</td>
<td>***</td>
<td>***</td>
<td>1.258,9</td>
</tr>
<tr>
<td>11</td>
<td>Clinical mastitis</td>
<td>INRA177</td>
<td>*</td>
<td>NS</td>
<td>5.9</td>
</tr>
<tr>
<td>11</td>
<td>SCC</td>
<td>BMS7169</td>
<td>***</td>
<td>**</td>
<td>2.7910</td>
</tr>
<tr>
<td>11</td>
<td>OD</td>
<td>INRA177</td>
<td>***</td>
<td>*</td>
<td>2.348</td>
</tr>
<tr>
<td>11</td>
<td>INS_cow</td>
<td>ILST8036</td>
<td>***</td>
<td>*</td>
<td>3.5678</td>
</tr>
<tr>
<td>11</td>
<td>NR56_cow</td>
<td>INRA177</td>
<td>***</td>
<td>NS</td>
<td>1.2410</td>
</tr>
<tr>
<td>11</td>
<td>STI_mat</td>
<td>BMS7169</td>
<td>***</td>
<td>*</td>
<td>3.589</td>
</tr>
<tr>
<td>13</td>
<td>HS</td>
<td>AGLA232</td>
<td>**</td>
<td>*</td>
<td>5.67</td>
</tr>
<tr>
<td>13</td>
<td>CAL_mat</td>
<td>BM1352</td>
<td>***</td>
<td>*</td>
<td>2.36910</td>
</tr>
<tr>
<td>15</td>
<td>OD</td>
<td>RM4</td>
<td>**</td>
<td>*</td>
<td>1.24510</td>
</tr>
<tr>
<td>15</td>
<td>INS_cow</td>
<td>NCAM</td>
<td>*</td>
<td>NS</td>
<td>3.5</td>
</tr>
<tr>
<td>15</td>
<td>NR56_cow</td>
<td>NCAM</td>
<td>***</td>
<td>**</td>
<td>1.347</td>
</tr>
<tr>
<td>15</td>
<td>CAL_mat</td>
<td>NCAM</td>
<td>**</td>
<td>*</td>
<td>2.79</td>
</tr>
<tr>
<td>18</td>
<td>NR56_cow</td>
<td>BMS2639</td>
<td>***</td>
<td>NS</td>
<td>2.347</td>
</tr>
<tr>
<td>18</td>
<td>STI_mat</td>
<td>BMS2785</td>
<td>***</td>
<td>NS</td>
<td>3.10</td>
</tr>
<tr>
<td>19</td>
<td>NR56_cow</td>
<td>BM1C013</td>
<td>***</td>
<td>NS</td>
<td>5.9</td>
</tr>
<tr>
<td>20</td>
<td>NR56_cow</td>
<td>BMS1282</td>
<td>***</td>
<td>***</td>
<td>1.4567</td>
</tr>
<tr>
<td>22</td>
<td>FTR</td>
<td>BM4102</td>
<td>*</td>
<td>NS</td>
<td>3.78</td>
</tr>
<tr>
<td>23</td>
<td>SCC</td>
<td>BM1443</td>
<td>*</td>
<td>*</td>
<td>4.10</td>
</tr>
<tr>
<td>25</td>
<td>Clinical mastitis</td>
<td>ILST102-RM404</td>
<td>*</td>
<td>NS</td>
<td>2.36</td>
</tr>
<tr>
<td>25</td>
<td>OD</td>
<td>ILST102</td>
<td>***</td>
<td>*</td>
<td>2.3610</td>
</tr>
<tr>
<td>25</td>
<td>HS</td>
<td>RM404</td>
<td>**</td>
<td>NS</td>
<td>1.67</td>
</tr>
<tr>
<td>29</td>
<td>INS_cow</td>
<td>BMS1244-BMC8012</td>
<td>**</td>
<td>*</td>
<td>1.26</td>
</tr>
<tr>
<td>29</td>
<td>NR56_cow</td>
<td>BMS1600-ILST81</td>
<td>***</td>
<td>NS</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Note: Results are presented with significance levels both from the analyses with cofactors and without cofactors.

1. Results are presented with significance levels both from the analyses with cofactors and without cofactors.
2. Cof. number of segregating ($P < 0.10$) families from the cofactor analysis.

**Fine mapping (Paper III)**

The fine mapping study was performed to confirm the QTL for NR56\_heifer detected in paper II and to narrow down the QTL region. Five families that segregated for
the QTL (in paper II) were genotyped for markers covering a region of 67 cM of chromosome 9. Results from the regression analysis and the variance component LA supported the previously detected QTL for NR56\textsubscript{heifer}. However, the peak obtained from the variance component method was broad despite the denser marker map (Figure 2). The same was true also for the regression analysis.

The combined LDLA analysis resulted in a sharp peak with a well defined maximum at the same position as where the highest peak from the LD analysis was found (Figure 2). The highest likelihood ratio test statistics was found in the marker interval BMS1724-BM7209, where the distance between the markers was 2 cM. No significant results were obtained in the combined LDLA analyses of the correlated trait INS.

![Fig. 2. QTL profiles on chromosome 9 for variance component linkage analysis (□), linkage disequilibrium (■) and combined linkage/linkage disequilibrium analysis (♦) of non-return rate. Triangles on the x-axis indicate relative positions of markers. The horizontal line is the 5% significance threshold (from paper III).](image)

**Mastitis pathogens (Paper IV)**

The six most frequently isolated groups of pathogens were analyzed, and the frequencies when all presumed pathogens were included in the edited data were for *S. aureus* 35%, CNS 29%, *Str. uberis* 13%, *Str. dysgalactiae* 11%, *E. coli* 4% and *Str. species* 2%. Less frequent pathogens comprised 6%. Estimated heritabilities for acquired infections with the different mastitis pathogens were in the range from 0.03 (*E. coli*) to 0.18 (CNS). However, the sire effect was not significant for *E. coli* and *Str. species*. The highest herd variance was found for *S. aureus*.

No significant haplotype substitution effect on the variation in occurrence of pathogen specific mastitis was found. However, when comparing the effects of different haplotypes within pathogen we found that the effect of haplotype 3 was significantly different from the effect of haplotype 6 regarding the presence of *Str. dysgalactiae*. Haplotype 6 was associated with a higher risk (approximately four times) of an infection of *Str. dysgalactiae* compared to haplotype 3.
General discussion

Identification of QTL is a first step towards the identification of the genes involved in the regulation of a quantitative trait. However, even if the actual genes are not known it is possible to enhance the selection efficiency by use of genetic markers that are closely linked to a QTL of interest. This could be particularly useful for low heritability traits that are difficult to improve efficiently by traditional selection methods, and especially in dairy cattle where many traits of economic interest are sex-limited and thus cannot be measured directly on the bulls.

Power and precision in QTL mapping

Advantages with the GDD

The GDD is suitable to use in commercial dairy cattle populations where large half-sib families exist due to extensive use of a limited number of AI-bulls, and where DNA is available only on the bulls in the population. The phenotypic trait values (e.g. estimated breeding values) in the analyses are based on recordings on a large number of daughters, which gives smaller standard errors of the estimates. The appropriate population structure and the phenotypic data already exists in dairy cattle populations for selection purposes, and DNA can be extracted from frozen semen samples that are available even on bulls that are no longer active.

The advantage of the GDD over the daughter design, where both genotypes and phenotypes are obtained on daughters of a common sire (half-sibs), is most pronounced for low heritability traits. The detection power of GDD increases when the heritability decreases, provided the number of granddaughters per son is high (Weller et al., 1990). Small effects are, however, difficult to detect and would require many informative families (i.e. grandsires heterozygous for both marker alleles and QTL alleles). Power in the GDD increases with number of grandsires, sons per grandsire, daughters per son and size of gene effect. Doubling the number of granddaughters per son had nearly the same effect as doubling the number of sons per grandsire. For low heritability traits it is therefore advantageous to base the analysis on grandsires with many granddaughters per son (Weller et al., 1990).

We found QTL for almost all the 14 examined traits, QTL for eleven traits were detected without cofactors in the analyses and twelve with cofactors. Other studies that used the GDD also found a large number of QTL for functional traits (e.g. Schrooten et al., 2000; Kühn et al., 2003). One reason for the many QTL found in our genome scans is the relatively high reliability of the phenotypic values that are based on a large number of daughters per bull. Furthermore, the largest sire families available in the Swedish dairy cattle population were chosen for this study to obtain as high power as possible.

In spite of these considerations, there are limitations in the material we used. The families were relatively small (21 to 58 sons per grandsire) for gene mapping purposes and number of markers used could have been larger. The average marker
interval in the genome scans was around 20 cM, but on some chromosomes the average interval was over 30 cM. However, because this was a population study the aim was to explore whether we could detect QTL for low heritability traits of interest in the Swedish dairy population. We concluded that it was possible. This shows the potential for dairy cattle populations where reproductive performance and health status are recorded on a routine basis.

Use of cofactors in QTL analyses

In paper I only results from the cofactor analysis were presented. However, in the initial analysis without cofactors the QTL on chromosome 11 and 25 for clinical mastitis, and the QTL on chromosome 5 and 9 for SCC did not reach the significance threshold. For OD the same QTL were found in both analyses, but reached a higher significance level when cofactors were used. In paper II, results were presented from both analyses and by including cofactors the number of QTL detected increased from 13 to 30.

In cofactor analysis, for each chromosome putative QTL found on other chromosomes are taken into account by fitting them as cofactors in the following analyses. A higher power for detection of QTL is expected due to a reduced residual variance when correcting for variance explained by the cofactors (de Koning et al., 2001). However, there is a high risk that additional QTL detected in cofactor analysis are false positives. In a small family there is a higher probability of observing a random association between marker alleles and phenotypes just by chance. According to Sahana et al. (2006) an increase in type I error is a general phenomenon in cofactor analysis and a liberal threshold for inclusion of cofactors increases the number of false positive QTL. The recommendation from Sahana et al. (2006) was to use cofactors only in experiments with very high power to reduce the false positives and not use cofactors to increase the power. This is in contradiction to de Koning et al. (2001) who suggested a liberal threshold for inclusion of cofactors to increase the power, and a more stringent to declare a QTL significant. However, a genome scan is the first step in the identification of a QTL and should not be too conservative to avoid premature exclusion of true QTL. The QTL that are found should be confirmed by other methods before they are used.

Fine mapping of functional traits

A low precision is obtained by QTL mapping based on LA because the method only utilizes recombination events that occur between two generations. The low number of recombination events between closely linked markers does not enable localization of the QTL to segments smaller than approximately 10-20 cM even if the study has a dense marker map and high power (Darvasi et al., 1993). A more precise estimation of the QTL position can be obtained by combining LA and LD mapping. The advantage with this method is that it combines information on historic recombination events with recent recombinations observed in the family structure under study, thereby reducing the risk of finding false positive QTL (Goddard & Meuwissen, 2005). The combined LDLA method has proven to result
in a mapping resolution accurate enough to narrow down the QTL confidence interval to a few cM (Meuwissen et al., 2002; Olsen et al., 2005).

Not many QTL for functional traits have been fine mapped in dairy cattle. The QTL for NR56_{heifer} was fine mapped in paper III by using the combined LDLA method giving a considerably sharper peak compared to the regression analysis. This QTL was supported by Shrooten et al. (2000) who detected a QTL for NR in the same region. One other study has fine mapped a QTL for fertility; Meuwissen et al. (2002) mapped a QTL affecting twinning rate to chromosome 5.

In a fine mapping study involving three Nordic dairy breeds, Sahana et al. (submitted) mapped a QTL affecting resistance to clinical mastitis to an interval of less than 1 cM on chromosome 9. They were able to identify QTL haplotypes that had similar effect on general mastitis resistance across populations. We were not able to confirm the effect of individual QTL haplotypes on resistance to pathogen specific mastitis (paper IV). No significant effects were found except when comparing effects between haplotypes within pathogen. However, due to limitations in the material, the power of the haplotype analyses was low. We analyzed the seven most frequent haplotypes among the bulls in our sample and it is possible that the haplotypes with largest effect on general mastitis resistance were not among these. Further studies are needed to investigate the effect of the QTL haplotypes on resistance to individual pathogens.

QTL regions affecting more than one trait

Given that SCC and clinical mastitis are correlated traits it is interesting to compare the QTL reported for these traits. Quite a few studies have found QTL for SCC (e.g. Ashwell et al., 2004, Kühn et al., 2003, Schrooten et al., 2000) whereas only few have found QTL for clinical mastitis (Klungland et al., 2001, Schulman et al., 2004). Several chromosomal regions seem to have effect on mastitis resistance, although, no clear correspondence has been found between the QTL for SCC and clinical mastitis (Khatkar et al., 2004). On chromosome 9 and 11 we found QTL for both traits, whereas on chromosome 5 and 23 only SCC QTL were detected, and on chromosome 25 we detected a QTL for clinical mastitis but not for SCC. Estimates of the genetic correlation between SCC and clinical mastitis are usually around 0.7 (Rupp & Boichard, 2003), which means that a QTL for SCC not necessarily affects resistance to clinical mastitis. Considering the variety of physiological responses to different mastitis causing pathogens it is likely that partly different genes are involved in the resistance to clinical versus subclinical mastitis infections.

There is a high genetic correlation (0.94) between the traits NR56 and INS (Wall et al., 2003); both traits describe the heifer’s ability to become pregnant after insemination. Non-return rate is a categorical trait where the cow either succeeds or fails to maintain a pregnancy during the first 56 days of gestation. Compared to NR, INS is based on more information as all inseminations that were done during one service period is included. Thus, with INS it is possible to discriminate
between cows receiving two or four inseminations. In the cofactor analysis QTL were detected for both traits on chromosome 9, 11, 15, and 29. In addition, there were four QTL found with effect on only one of the traits. However, some of these QTL may be false positives. No QTL have previously been reported for INS, however, NR is more widely used as a fertility measure compared to INS (Jorjani, 2005). For example, in Norway NR is the only fertility trait included in the genetic evaluation (Ranberg et al., 2003).

From our results it seems likely that several different QTL, or alternatively a few pleiotropic QTL, are located on chromosome 9 and 11. On chromosome 9 we found significant QTL for both clinical mastitis (paper I) and NR56 in heifer (paper II) close to the same marker (TGLA73). The two QTL profiles are shown together in Figure 3, although, the marker maps differed slightly between the two studies as we had one additional marker in paper II. Two families segregated for both QTL. The coinciding QTL locations suggest either a QTL with pleiotropic effects on both traits or two QTL in the same region with effects on the different traits. In a study of Norwegian red cows, Heringstad et al. (2006) found no correlation between clinical mastitis and NR56 in first lactation cows. However, Kadarmideen et al. (2000) found a moderately high correlation of 0.41 between clinical mastitis and number of services per conception in Holstein Friesian cows, suggesting that selection for reduced mastitis incidence would result in a reduced number of inseminations per conception.

In our studies QTL were mapped for individual traits by single trait analyses. When two traits are genetically correlated it is because of pleiotropic effects of QTL or a result of linkage disequilibrium between two or more QTL, each affecting one trait only (Falconer & Mackay, 1996). It is possible to distinguish between the two types of effects by performing a multi-trait fine mapping QTL analysis (Lund et al., 2003). Furthermore, a higher power of detection and a higher precision of QTL positions can be achieved by performing a multi-trait analysis (Sørensen et al., 2003). In paper III, a multi-trait analysis was performed on INS

---

**Fig. 3.** QTL profiles of chromosome 9 from the genome scans (paper I and II) of clinical mastitis and non-return rate. The marker map is slightly modified from paper II.
and NR, however, due to convergence problems the analysis did not refine the position of the QTL.

A prerequisite for detecting QTL is that the families under study segregate for both QTL alleles and marker alleles. Thus, it is more likely to find several QTL in a chromosome region where many families segregate for the markers used in the study. This could be one explanation for the many QTL found on some of the chromosomes. The number of segregating families per QTL varied between one and six in the cofactor analysis and without cofactors the maximum was five families. In general, a QTL effect in a single family is not sufficient to make the QTL significant in the across-family analysis. However, analyzing individual families can reveal QTL that are not detected in a joint analysis of all families due to a low degree of heterozygosity at the QTL in the population.

**Marker assisted selection (MAS)**

In recent years identification of QTL has received increasing attention. As multiple ovulation and embryo transfer allows for a larger number of full-sibs in dairy cattle, MAS will enable pre-selection among these before progeny testing, thus increasing selection differentials, shortening generation interval and increasing genetic gain (Mackinnon & Georges, 1998). Results from theoretical and simulation studies show that application of MAS has the potential to enhance the response to selection, especially if traditional selection is less effective (e.g. low heritability traits) (Abdel-Azim & Freeman, 2002).

To be able to use a QTL for selection across families or populations, or to identify the functional genes behind the QTL effects, a high level of resolution is required in the mapping. QTL affecting clinical mastitis resistance or fertility may be used also in populations where records on clinical mastitis and fertility are not available, assuming they segregate for the same QTL. Furthermore, knowledge about QTL may increase the genetic potential for traits with an unfavorable genetic correlation to production by direct selection on loci with positive effect on these traits.

To be effective, MAS requires a very detailed information of the identified QTL in terms of number of segregating alleles and their respective effects on the traits under selection. It is advisable to investigate the effect of a QTL on all traits included in a breeding goal before applying MAS in practical breeding. For example, a QTL with effect on general mastitis resistance may have different effects regarding the resistance to the specific pathogens depending on QTL allele. An allele with a large positive effect on one pathogen may have an adverse effect on another pathogen. As higher heritabilities were found for resistance to specific mastitis pathogens compared to estimates of the general resistance (paper IV), a more accurate and efficient selection response could be achieved by MAS using a QTL with pathogen specific effect. For best results, it would be desirable to utilize all available QTL affecting a trait in MAS.
Individual QTL only account for part of the phenotypic variance, the rest being due to environmental factors as well as other QTL. Quantitative traits are affected by many genes and the effect of each individual gene is generally small to moderate even though there may be few loci with large effects. Consequently, the benefit from MAS is limited by the proportion of the genetic variance explained by known QTL.

Conclusions

- It was possible to map several QTL for health and reproduction traits despite the low heritabilities. Significant QTL ($P < 0.05$) were found in the genome scans for almost all the studied traits.

- Inclusion of cofactors in the analyses (to adjust for QTL found on other chromosomes) increased both the number of QTL that were found and the significance level. The total number of QTL increased from 20 to 41 with inclusion of cofactors in the analyses. However, some of the results from the cofactor analysis may be false positives and require further validation.

- The most interesting chromosome regions with QTL for several traits were found on chromosome 9 and 11. QTL with effects on six different traits were found on each of these chromosomes.

- Fine mapping of the QTL affecting NR56 heifer on chromosome 9 confirmed the results from our previous study and refined the position of the QTL. By using the combined LDLA approach the QTL was mapped to an interval of 2 cM in between markers BMS1724 and BM7209.

- Genetic variation in resistance to specific mastitis pathogens was found. Estimated heritabilities for resistance to specific mastitis pathogens were higher compared to estimates of general clinical mastitis resistance in most other studies.

- The most common mastitis pathogen was *S. aureus*. The highest heritability was estimated for CNS (0.18) whereas the highest herd variance was found for *S. aureus*. However, the pathogen data could not be considered to constitute a random sample of the population as the bacteriological data were based on milk samples from cows that were tested for a reason (e.g. high cell count).

- There was a significant difference between the effects of two QTL haplotypes previously shown to affect general mastitis resistance on the presence of *Str. dysgalactiae*. Hence, it is possible that the investigated haplotypes have varying effects on the resistance to specific mastitis pathogens even though no significant allele substitution effect of haplotype on resistance to pathogen-specific mastitis was found. The
The power of the analyses performed on the haplotype data was low as the number of genotyped bulls was limited and also because of few daughters with bacteriological observations per bull.

**Future outlook**

An important goal in dairy cattle production is to improve the health and reproductive functions of our dairy cows and thereby increase cow productivity and reduce the need for disease and fertility treatments. One way to achieve this is by selection. To increase the genetic progress, information about QTL affecting important functional traits could be incorporated in the breeding schemes by means of MAS. To enable this, results from fine mapping studies with more specific information about QTL haplotypes is desirable. Furthermore, for complex traits such as mastitis, measures with a more direct association to the resistance to disease should be used in genetic evaluations. As mastitis is caused by a variety of different pathogens one way to improve the selection response could be to use pathogen-specific information. Examples of specific projects that could be performed to upgrade the breeding for important low heritability traits are:

- Additional fine mapping studies could be performed on some of the detected QTL affecting health (e.g. OD on chr 11 and 25), fertility (e.g. NR56cow on chr 20), and calving (e.g. CALmat on chr 6 and 13) to confirm and refine the results of our studies.

- New phenotypic measures that more directly reflect the physiologic background of the traits could be used in QTL mapping studies to find more specific QTL. Progesterone level, for example, could be used as a fertility measure. Furthermore, the main health disorders included in OD could be examined separately to find disease specific QTL.

- Further research regarding the QTL for NR56heifer is required to evaluate whether this QTL could be used in breeding by MAS. The marker density in the QTL region should be increased by use of single nucleotide polymorphism (SNP) markers. More individuals in additional sire families should be genotyped to confirm the QTL and to enable the identification of haplotypes with effect on NR56heifer.

- The bacteriological data that was used in the analyses could not be considered a random sample of the population. A national routine registration of bacteriological data would enable genetic evaluation for resistance to specific mastitis pathogens and QTL studies on specific pathogens would be possible. A mastitis index including information on both general and pathogen specific mastitis and genotype information regarding pathogen specific QTL could be developed for breeding purposes.
• For future research on bacteriological data it would be desirable to include the unique id number of the cow for each record to be able to identify all repeated records of a cow and also to obtain the pedigree information needed for genetic studies. Additional information such as calving date, lactation number and reason for testing are also desirable.

• The power of the analyses performed on the haplotype data was low. To enlarge the haplotype dataset additional bulls that have many daughters with bacteriological data could be genotyped for the haplotypes with effect on general mastitis resistance. For future research, milk samples that are sent in for bacteriological analyses could be stored to enable genotyping of individual cows for a more accurate estimation of the effects of QTL haplotypes on pathogen specific mastitis.
**Populärvetenskaplig sammanfattning**


Egenskaper som påverkar djurs hälsa och reproduktionsförmåga är så kallade kvantitativa egenskaper vilka styrs av ett stort antal gener och dessutom i hög grad påverkas av miljön. Många viktiga egenskaper som påverkar reproduktionsförmåga och sjukdomsresistens har låg arvbarhet vilket försvårar genetiska framsteg genom traditionellt avelsarbete eftersom egenskaperna till stor del påverkas av miljömässiga faktorer. Även om arvbarheten är låg uppvisar dessa egenskaper genetisk variation. Kunskapen om hur många och vilka gener som påverkar dessa egenskaper är emellertid mycket begränsad.

Trots att hälsoegenskaper har funnits med i avelsmålet för mjölkor i Sverige i årtionden har mjölkbönderna stora problem med juver inflammationer (mastit) i besättningarna. En av anledningarna är att det finns ett ogynnsamt genetiskt samband mellan mjölkmängd och mastitfrekvens vilket innebär att avel för en hög mjölkproduktion ökar benägenheten för mastit. Frukt samhet har också en ogynnsam genetisk korrelation med mjölkproduktionen och sjukdomsresistens. Andra egenskaper som är viktiga att inkludera i avelsprogram ur såväl etisk som ekonomisk synpunkt är kalvningssvårigheter och dödfödslar.

Ett kromosomsegment som innehåller en eller flera gener med effekt på en kvantitativ egenskap kallas för ett QTL, från engelskans "quantitative trait loci". Förekomsten av QTL kan påvisas genom att inom tjurfamiljer studera sambandet mellan variation i genetiska markörer och variation i avelsvärde för en egenskap. En genetisk markör är ett variabelt DNA-segment som nedärvs från förälder till avkomma. Markörerna används för att avgöra vilken variant av kromosomsegmentet en avelstjur är avtravat av sin far. Man jämför sedan avelsvärden för de egenskaper man är intresserad av mellan grupper av söner som ärtravat endera kromosomsegment. En signifikant skillnad i avelsvärden mellan söngrupper som ärtravat olika kromosomsegment indikerar på förekomst av QTL. För att kunna kartlägga QTL för en viss egenskap måste ett stort antal genetiska markörer (t.ex. mikrosatelliter) genotypbestämmas. Studier av QTL på mjölkor genomförs ofta i en så kallad ”granddaughter design” där enbart tjurarna genotypas medan fenotypvärdena utgörs av tjurarnas avelsvärden vilka baseras på egenskapsregistreringar av tjurarnas döttrar.

Avelsvärden för de analyserade egenskaperna i den här avhandlingen kom från Svensk Mjölnks avelsvärdering medan DNA från tjurarna renframställdes från frusen sperma som erhållits från Svensk Avel. Totalt analyserades 14 egenskaper och genotyper bestämdes för markörer på sammanlagt 20 kromosomer. Tio
tjurfamiljer (nio SRB och en SLB) med i medeltal 42 söner användes i analyserna. Flera QTL med signifikant effekt påvisades för de flesta av de analyserade egenskaperna. En del kromosomregioner visade sig innehålla QTL för både hälso- och fruktanhetsegenskaper, t.ex. på kromosom 9 och 11 påvisades sex QTL vardera för olika egenskaper. Ett QTL för mastitisresistens förefaller sammanfalla med ett QTL för fertilitetsegenskapen 56 dagars non-return (andelen kor som inte löper om inom 56 dagar efter första inseminering) på kromosom 9. Eftersom precisionen i kartläggningen av QTL var låg är de lokализerade kromosomregionerna omfattande och innehåller troligtvis ett hundratal gener.

För att förbättra precisionen i kartläggningen av QTL gjordes en finmappningsstudie. Genotyper bestämdes för ytterligare ett antal markörer i den region på kromosom 9 som påvisat effekt på egenskapen 56 dagars non-return. Med en tätare markörkarta och nya statistiska metoder identifierades en mindre region (ca 2 cM) på kromosom 9 med signifikant effekt på egenskapen. Resultaten behöver emellertid bekräftas i ett större material och precisionen förbättras ytterligare innan genotypinformation om detta QTL kan användas i avelsarbetet för selektion av avelsdjur.

Förbättrad kunskap om egenskapernas nedärvning kan på sikt leda till ett effektivare avelsarbete. Störst nytta av information om QTL har man troligtvis vid urval inom familjer exempelvis vid selektion av tjurfalvar till avkommerövning bland en grupp hel- eller halvsyster. En sådan förselektion kan leda till ett snabbare genetiskt framsteg genom ökad precision i avelsurvalet och kortare generationsintervall eftersom man tidigt kan få uppgift om en tjurs nedärvningsförmåga bl.a. för egenskaper som endast uttrycks hos kor, t.ex. mastitisresistens.

Mastit orsakas av många olika typer av patogener i kombination med faktorer i miljön och betraktas därför som en komplex sjukdom. Olika mastit-patogener ger upphov till mastit med olika svårighetsgrad. *Echerichia Coli* förekommer exempelvis främst hos kor med kliniska symptom på mastit (t.ex. flockor i mjölen samt svullet och ömt juver) medan coagulasnegativa staphylococci är vanligt förekommande hos kor med subklinisk mastit (utan kliniska symptom). Med tanke på mängden olika resistensmekanismer (fysiska barrer samt immunologiska) som påverkar motståndskraften mot mastit är det sannolikt att egenskapen påverkas av många olika gener.

Bakteriologiska data avseende mastit-patogener i mjölkprover analyserade vid Statens Veterinärmedicinska Anstalt (SVA) användes för att undersöka om det finns en genetisk variation för resistens mot specifika mastit-patogener. Analyserna baserades på data från perioden 1993 till 2004 och bestod i huvudsak av mjölkprover från kor med subklinisk mastit men även kliniska mastiter ingick. Eftersom data från materialet så att endast kor med bakterieväxt i mjölen, känt härstamning och en far med minst 20 döttrar i materialet sparades återstod totalt 38,607 mjölkprover från 21,834 kor.
Ett mindre dataset plockades fram ur det redigerade materialet från SVA för att undersöka om ett sedan tidigare identifierat QTL med påvisad effekt på mastitresistens även har effekt på förekomst av specifika patogener. Genotyper för detta QTL fanns tillgängligt för ett antal tjurar av SRB-ras. Av dessa hade 114 tjurar minst fem döttrar var med bakteriologisk information.


Sammanfattningsvis var det möjligt att identifiera ett flertal kromosomregioner med effekt på hälsa och reproduktionsförmåga. De QTL som identifierats omfattar en relativt stor del av respektive kromosom men kan användas som utgångspunkt för vidare genetiska studier. Ett QTL för fertilitet påvisades på kromosom 9 med större precision i kartläggningen, resultaten behöver dock bekräftas i ett större djurtmaterial och precisionen förbättras ytterligare innan information om detta QTL kan implementeras i aveln. Genetisk variation för resistens mot specifika mastit-patogener kunde påvisas och en högre arvbarhet skattades för resistens mot individuella patogener än för generell resistens. Även om mer forskning behövs visas resultaten på en möjlighet att förbättra det genetiska framsteget avseende motståndskraft mot mastit genom att utnyttja information om resistens mot specifika patogener i avelsarbetet.
References


Green, E., Falls K., and Crooks S. 1990. Documentation of CRI-MAP, Version 2.4. Washington University School of Medicine, St Louis, MO.


Acknowledgements

The studies presented in this thesis were carried out at the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences. The work was partly funded by Swedish Farmer’s Foundation for Agricultural Research, The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas), and the European Commission: Quality of Life and Management of Living Resources.

I would like to thank all my past and present colleagues at the Department of Animal Breeding and Genetics for providing a stimulating work environment and for interesting discussions regarding everything from skiing to dogs, babies and statistics... It has been fun working with you and I will miss you all!

Lena Andersson-Eklund, min huvudhandledare, tack för att du gav mig chansen att förkorea mig i husdjursgenetikens mysterier. För din konstruktiva kritik och din positiva inställning till projektet även när jag tvivlade på att det skulle gå vägen. Tack för allt stöd jag har fått under åren och för att du även har lätit mig arbeta mycket självständigt.

Anne Lundén, min handledare, tack för ditt stora engagemang i alla texter som du har lusläst under åren, det har varit till en stor hjälp. Tack också för att du har tagit dig tid och ställt upp framförallt under det sista årets hektiska period.

Freddy Fikse, min räddare i nöden, utan dig hade vi inte hunnit färdigt i tid. Tack för att du engagerade dig i projektet och för att du delar med dig av din kunskap.

Goutam Sahana, my statistical expert, thank you for collaborating with me and for answering all my questions regarding QTL analyses.

Ana Fernandez, my lab supervisor, thank you for excellent supervision during my short but intense period in the lab.

Anki Roth, Svensk Mjölk, tack för allt jobb med att plocka fram ”DYDar” till mina analyser och tack även Kjell Johansson för att du hjälpte till att få ordning på dessa.

Karin Artursson, SVA, tack för ett gott samarbete och för dina expertkommentarer.

Mogens Lund, thank you for taking care of me during my visit to Foulum.

Erling Strandberg, avdelningschef, tack för att du alltid ställer upp och svarar på frågor samt för att du är en riktigt bra avdelningschef.

Britt-Marie, Monica, Siw, Dan och Jörgen, tack för att ni håller så bra ordning på institutionen och hjälper till närhelst man behöver.
Alla nuvarande och före detta doktorander på institutionen och på renheden, tack
för att spåder glad och gör att det känns roligt att komma till jobbet varje dag.
Jag kommer att sakna er. Ett speciellt tack till Emma Carlén för att du varit en så
bra ”nästarumskompis”, tur att vi aldrig delade rum då hade det nog inte blivit
många avhandlingar skrivna. Tack också Emma Thorén, min förste bekantskap på
Ultuna, för alla trevliga fikaraster och andra viktiga avbrott i en doktorands
arbetsdag.

Tjejgången, Maria, Christina och Madde, tack för att ni alltid finns där även om jag
ibland inte är så bra på att höra av mig…

Mina syskon, Charlotte och Björn, tack för att ni alltid finns där för mig och ställer
upp när det behövs och för allt roligt vi har ihop.

Mamma och pappa, tack för att ni alltid har uppmuntrat mig till att göra det jag vill.
Tack för allt stöd jag får oavsett vad det gäller och för att ni alltid tror att jag ska
klara av det jag gör.

Wilma, tack för alla mysiga lunchpromenader och för att du alltid är så glad. Tack
också till övriga hundar och hundägare på institutionen för alla trevliga
promenader och för att ni skapar en avslappnad atmosfär.

Min egen underbara familj, tack för att ni har stått ut med mig under de stressade
perioderna av avhandlingsarbetet. Björn, tack för allt, för att du aldrig tvivlar på
min förmåga, för att det vi har tillsammans överträffar allt, du och jag är miraklet,
 jag älskar dig! Ålskade Felix, vår lilla goding, världens finaste kille, tack för att du
har visat hur liten betydelse en disputation har, egentligen…