Genetics of Pig Health Symposium 2003

Genetic Approaches to Improving Health and Reproduction in Cattle

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Animal breeders have used selection strategies to increase production in livestock species since the late eighteenth century. These selection strategies, including progeny testing, are time consuming and expensive, but have contributed to enormous production gains in the major livestock species. For example, in the period from 1991-2000, the amount of milk produced per cow in the United States increased 21% (http://www.usda.gov/nass/aggraphs/milk1.htm, Figure 1). However, this gain in milk production has come at a cost, with increased incidence of disease and infertility. It is estimated that mastitis costs dairy producers $2 billion, milk fever $216 million, and ketosis $48 million each year. Infertility is the primary cause of involuntary culling in dairy herds today (Bascom and Young, 1998) and reports indicate that disease incidence and reduced fertility are becoming more and more common as milk production continues to increase.

Figure 1. Milk per cow from 1991 to 2000 (USDA-NASS, 2/16/01).

Traditional selection methods have been effective in improving milk production in dairy cattle without DNA marker information, but the same is not true for lowly heritable traits, including health and reproduction. In the late 1980s and early 1990s, several groups (i.e., Oddgeirsson et al., 1988; Weigel et al., 1990) selected genes encoding the bovine major histocompatibility complex (MHC) as candidate genes affecting mastitis, due to the MHC’s role in immune response. A large number of studies were conducted, looking at Class I and II alleles and their associations with different mastitis traits, including incidence of clinical mastitis, somatic cell counts, and antibody response to experimental challenges. Review of all these studies (Rupp and Boichard, 2003) found that many alleles were associated with either susceptibility or resistance to mastitis but many reports were inconsistent. The inconsistencies could arise for several reasons; different pathogens may have been present during the different studies, or, more likely,
the polymorphisms were not the causal mutations but were linked to other genes involved in resistance, giving different results in each family (Rupp and Boichard, 2003).

**Figure 2.** Selection for milk yield over time (C. VanTassell, USDA-ARS, AIPL).

During the mid 1990s, cattle linkage maps were developed (Bishop et al., 1994; Barendse et al., 1994; Ma et al., 1996; Barendse et al., 1997; Kappes et al., 1997), providing large numbers of DNA markers to the research community. Once the maps were developed, the bovine genome could be scanned in search of chromosomal regions that affected traits of economic interest. These regions are referred to as quantitative trait loci, or QTL.

The first genome scans conducted in either beef (Keele et al., 1999) or dairy (Georges et al., 1995) cattle focused on production traits, because phenotypic data was readily available for these quantitative traits. For dairy cattle in the U.S., little reproductive or disease data is routinely collected and made available to the USDA’s Animal Improvement Programs Laboratory (AIPL), which calculates most of the genetic evaluations for the dairy industry. In many European countries, reproduction and disease phenotypic data are available, making studies to identify QTL affecting these traits possible (Schrooten et al., 2000; Klungland et al., 2001; Larsson and Andersson-Eklund, 2002).

In 1994, AIPL began providing evaluations for somatic cell score (SCS), which was the first disease-related trait reported by the group. Although it is an indirect measure, the number of somatic cells in milk is correlated with the incidence of mastitis (Coffey et al., 1986). Researchers from the Gene Evaluation and Mapping Laboratory (GEML) at USDA’s Agricultural Research Service started a search for QTL affecting mastitis using these new SCS evaluations. For this study, they selected seven Holstein grandsire families from the Dairy Bull DNA Repository (DBDR; Da et al., 1994). This collection, initiated by Harris Lewin (University of Illinois) in collaboration with many U.S. artificial insemination companies, was a resource
population that could be used for QTL mapping studies. Initially, bovine chromosome 23 (BTA23) was targeted because it contains the MHC loci. Selected individuals from the seven families were genotyped at many DNA markers on this chromosome in order to identify QTL affecting mastitis, as measured by somatic cell score. Results of this initial study found only one DNA marker on BTA23 that was potentially associated with SCS (Ashwell et al., 1996).

![Graph showing selection for somatic cell score over time.](image)

**Figure 3.** Selection for somatic cell score over time.

However, with the advent of high-throughput instruments, including automated DNA sequencers and high-capacity PCR thermal cyclers, the project was expanded to include the study of eight DBDR families at DNA markers located on all bovine chromosomes. The GEML group selected approximately 150 DNA markers that were genotyped in the eight families. At the same time, the group at the University of Illinois was evaluating some of the same families at approximately 170 different DNA markers. Each group identified QTL affecting milk production, somatic cell score and productive life traits and reported their results independently (Heyen et al., 1999; Ashwell et al., 2001). Last year these two genotypic datasets were merged and a more comprehensive analysis was completed. Seven chromosomes (BTA5, 7, 15, 20, 23, 26 and 29) showed evidence of putative QTL affecting somatic cell score in these Holstein families (Ashwell et al., in press). Further study to refine the location of these QTL will be needed before the information can be incorporated into a selection program.

The same set of data was used to detect QTL affecting conformation traits and daughter pregnancy rate. Identification of QTL for conformation traits was initiated because these traits are genetically correlated with milk production, resistance to disease and fertility (Rogers et al., 1999). Using the same merged genotypic dataset and phenotypic data provided by the Holstein Association USA (Brattleboro, VT), fifteen chromosomes were identified as carrying putative
QTL affecting the linear conformation traits, including feet and leg score, several udder traits, and dairy form (unpublished data).

Dairy form is a conformation trait based on the “dairyness” of a cow, including evaluation of the animal’s body condition. It has a moderate heritability and is correlated with milk production (Misztal et al., 1995). Therefore, as milk production continues to increase the cows appear to have less fat on their bodies. Rogers et al. (1999) reported that higher phenotypic values for the dairy form trait were genetically correlated with increased incidence of metabolic diseases. Therefore, identification of DNA markers that could be used to select for lower phenotypic values for dairy form should lead to dairy cattle that are more metabolically balanced, reducing the incidence of many diseases, including ketosis and milk fever.

**Figure 4.** Maps of chromosomes 23 and 27 (Source: ARKdb http://www.thearkdb.org).

GEML researchers began a project to fine-map putative QTL affecting dairy form on BTA27. This project has involved evaluation of additional DNA markers in the DBDR families found to be segregating for the QTL (Van Tassell et al., in press). This work required expanding the pedigrees to include more recent generations of the original DBDR families. Inclusion of a more complex pedigree structure provides greater power to detect and pinpoint the location of the gene...
or genes responsible for the observed effects. Several positional candidate genes have been identified using comparative mapping data from the human genome sequence. These candidate genes are currently being evaluated for polymorphisms in the coding sequences that may explain the observed differences.

As mentioned, the same data were analyzed to identify putative QTL affecting fertility, defined by AIPL researchers as the daughter pregnancy rate (DPR; VanRaden and Tooker, 2003; VanRaden et al., 2003). This trait is defined as the percentage of non-pregnant cows that become pregnant during each 21-day period. The DPR is related to days open, with a 1% increase in DPR equaling four fewer days open (http://www.aipl.arsusda.gov/reference/fertility/dpr.htm). Six chromosomes (BTA6, 14, 16, 18, 27, 28) were found to carry DPR QTL (Ashwell et al., in press). Four chromosomes are already known to harbor QTL affecting non-return rate (Kühn et al., 2003), milk fat content (Grisart et al., 2002) and dairy form (Ashwell et al., 2001).

One of these chromosomes is BTA27, which carries QTL affecting the dairy form conformation trait. The same region of BTA27 seems to contain QTL affecting dairy form (Van Tassell et al., in press), marbling in beef cattle (Casas et al., 2002) and fertility (Ashwell et al., in press). Therefore, results suggest at least one gene affecting fat metabolism is located on this chromosome that may also affect fertility. Rogers et al. (1999) studied the genetic correlations between type traits and three groups of diseases: reproductive, foot and leg, and metabolic and digestive diseases. Results from this study showed that the genetic correlation between dairy form and the three disease categories were negative and moderate in magnitude. Metabolic disorders (such as milk fever and ketosis) are indicators of negative energy balance (Rogers et al., 1999), which is known to affect reproductive performance (de Vries and Veerkamp, 2000). Therefore, selection for increased dairy form may lead to cows that are more prone to reproductive and metabolic diseases (Rogers et al., 1999).

One can speculate that identification of the genes underlying the pregnancy rate QTL may actually be genes affecting body condition and the metabolic state of dairy animals. In several cases there are effects on milk production traits in regions where putative pregnancy rate and disease resistance QTL have been detected, so it may be difficult to improve these traits without sacrificing some milk production. However, it may be more economically advantageous to sacrifice some gains in milk production in order to be able to breed healthier cows in a shorter period of time.

Identification of broad QTL locations is just the first step in understanding the biology behind these economically important traits. Identification of the genes responsible for the effects will involve comparisons across different populations, genotyping of complex, multi-generation families and more sophisticated analysis methods. Application of functional genomics and proteomics methods can also be used to identify the genes and gene products responsible for the observed effects. Such studies can be long and expensive, but they are needed so that dairy producers can overcome the financial impact of disease and infertility, improve animal well-being and compete in today’s global marketplace.

REFERENCES


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Progress due to traditional selection

Costs to dairy producers

Milk production

Cost of diseases:
- Mastitis: $2 billion
- Milk fever: $216 million
- Ketosis: $48 million

Infertility:
- More days open
- More inseminations
- Involuntary culling

Intense selection for milk yield

MHC Class I alleles

Weigel et al., 1990:
- A1: Clinical mastitis
- A11: Resistance

Mejdell et al., 1994:
- A2: Clinical mastitis
- A7: Clinical mastitis
- A26: Somatic cell count

Aarestrup et al., 1995:
- A11: Somatic cell count
- A26: Susceptibility

Summary:
- Many alleles associated with susceptibility or resistance to mastitis
- Based on at least 2 independent studies:
  - A11: Resistance
  - A26: Susceptibility
**MHC Class II DRB3 alleles**

- **DRB3.2*24:**
  - Intra mammary infections
  - Clinical mastitis
  - Somatic cell count
  - Summary: Allele 24 consistently associated with susceptibility to mastitis

- **DRB3.2*16:**
  - Somatic cell count

- **DRB3.2*8:**
  - Clinical mastitis
  - All other alleles—contradictory findings across 5 studies

**Development of linkage maps**

- As of 2001—2725 loci in database
- More than 1500 were microsatellite markers

**What is a QTL??**

- Quantitative trait locus (loci):
  - A chromosomal location carrying a gene or genes that varies among animals
  - Those variations cause differences in economically important traits, e.g., disease resistance, reproduction

**How do you find QTLs?**

- Select “obvious” candidate genes—look for variation and association with trait in a specific population
- Scan the genome—look for associations between anonymous markers and traits in a specific population

**Granddaughter Design**

- DNA → Bull → Sons → Granddaughters

**Scanning the bovine genome**

- Ten large US Holstein families—granddaughter design
- 367 DNA markers located throughout genome
- Identified significant marker-trait associations (putative QTL)
**Phenotypic traits**

- Milk production
- Milk, fat, protein yields
- Fat, protein %
- Somatic cell score
- Productive life
- Calving ease

**Conformation traits:**

- Feet and leg
- Body size
- Daughter pregnancy rate

**Putative QTL affecting SCS**

BTA5: 54 cM

BTA7: 61 cM

BTA26: 0 cM, family 3

BTA26: 0 cM, family 7

BTA23: 50 cM

BTA29: 50 cM

**Putative QTL affecting type traits**

Why?—correlated with milk production, disease resistance and fertility

<table>
<thead>
<tr>
<th>BTA</th>
<th>Traits</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>Rear udder height, dairy form, feet/leg score</td>
</tr>
<tr>
<td>4</td>
<td>Strength</td>
</tr>
<tr>
<td>5</td>
<td>Fore udder attachment, rumen angle, front udder placement</td>
</tr>
<tr>
<td>7</td>
<td>Udder cleft, body depth, feet/leg score</td>
</tr>
<tr>
<td>8</td>
<td>Test length, rumen angle, rear udder attachment</td>
</tr>
<tr>
<td>9</td>
<td>Rumen angle, dairy form</td>
</tr>
<tr>
<td>13</td>
<td>Dairy form</td>
</tr>
<tr>
<td>15</td>
<td>Stature, rear udder attachment, udder depth</td>
</tr>
<tr>
<td>16</td>
<td>Udder depth</td>
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<tr>
<td>18</td>
<td>Rear udder height</td>
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<td>22</td>
<td>Front udder placement</td>
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<tr>
<td>24</td>
<td>Body depth, strength</td>
</tr>
<tr>
<td>26</td>
<td>Udder cleft, udder depth, rear udder attachment</td>
</tr>
<tr>
<td>27</td>
<td>Dairy form</td>
</tr>
<tr>
<td>28</td>
<td>Udder depth</td>
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</tbody>
</table>

**Dairy Form**

Extremely Coarse | Extremely Dairy

**Dairy form QTL on BTA27**

Family 8

- Building human/bovine comparative maps
- Identifying positional candidate genes

Family 2

- Sequencing to identify SNPs

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Complex pedigree for fine-mapping

-six generation pedigree used to fine-map BTA27 QTL

Phenotypic Trend for Days Open

Daughter pregnancy rate

- DPR: percentage of non-pregnant cows that become pregnant during each 21-day cycle
- ↑1% DPR = ↓Days open by 4 days

Chromosomes carrying putative DPR QTL:
6, 14, 16, 18, 27, 28

Bovine chromosome 27 QTL

-Marbling QTL in beef cattle
-Casas et al., 2000
-Is there a “fat” gene affecting fertility??

Findings

-Rogers et al. (1999) calculated genetic correlations between type traits and 3 groups of diseases:
  - Reproductive
  - Foot and leg
  - Metabolic and digestive diseases

-Correlations negative and moderate in magnitude

Conclusions

-Several chromosomes with pregnancy rate and SCS QTL also have production QTL

-Genes underlying these important traits may affect body condition and metabolic state of dairy animals

-May be difficult to improve these traits without sacrificing some production

-May be advantageous to sacrifice some production to breed healthier cows in shorter time
Dairy Form

Days open
Disease incidence

Extremely Coarse  Extremely Dairy

Acknowledgements

USDA-ARS:
- Erin Connor
- Tad Sonstegard
- Curt Van Tassell
- Becky Deluca
- Tina Sphon
- Larry Shade
- Marsha Atkins

AIPL--
- Paul VanRaden
- George Wiggans
- Bob Miller

Univ. of IL/ARO:
- Wayne Heyen
- Yang Da
- Harris Lewin
- Micha Ron
- Joel Weller

Bull semen contributors:
- Accelerated Genetics
- ABS Global
- AltaGenetics
- Genex
- Select Sire Power
- Semex Alliance

Conformation trait data:
- Tom Lawlor
- Holstein
- Bert Klei

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