QUANTITATIVE TRAIT LOCI FOR MILK PRODUCTION TRAITS
IN DAIRY CATTLE

H. Bovenhuis and C. Schrooten

Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338,
6700 AH, Wageningen, The Netherlands

INTRODUCTION
Since the first complete genome scan in dairy cattle by Georges et al. (1995) many QTL mapping studies have been initiated. It is expected that information from these experiments will increase our knowledge on the genetic and biological backgrounds of milk production traits. Furthermore, information on the location and effect of these genes can be used in breeding. The aim of this paper is to summarise the main results of the QTL mapping studies that have been carried out in dairy cattle.

EXPERIMENTAL DESIGNS
Most of the QTL mapping experiments that have been carried out in dairy cattle have used a granddaughter design (Boichard, 1998). The largest granddaughter design consists of 14 grandsires and approximately 1800 sons, i.e. the first large scale QTL mapping experiment in dairy cattle, which was initiated by Georges et al. (1995) and extended in later years (Zhang et al., 1998). Experiments that have been carried out in France, Germany and The Netherlands/New Zealand consist of 1000–1500 sons, whereas the designs in Scandinavian countries have around 400 progeny tested sons (Boichard, 1998). Lipkin et al. (1998) and Mosig et al. (2001) used selective DNA pooling. The power of this design is considerably higher than the power of the granddaughter designs that are currently being carried out. However, the full power of detecting QTL only applies to one trait, i.e. protein%. Most of the studies focussed on the Holstein breed. The world-wide exchange of semen in this breed implies that most of these studies are likely to have common ancestors. In some situations the same grandsires might be included in different studies. This overlap makes it reasonable to assume that a large fraction of the segregating QTL are in fact identical.

QTL detection in a granddaughter design is limited to routinely collected traits. In all designs these comprise the milk production traits and conformation traits. Furthermore, in several countries information regarding reproduction (e.g. calving ease and non-return rate), health (somatic cell score) and workability (milking speed and temperament) is routinely collected, which enables detection of QTL for these traits. In addition, in Scandinavian countries veterinary records of individual cows are available.

Based on results from QTL mapping experiments in dairy cattle, Hayes and Goddard (2001) predicted the distribution of QTL effects for an “average” quantitative trait. Combining these results with power calculations makes it possible to construct the distribution of significantly detected QTL. Figure 1 shows that in a medium sized granddaughter design (15 grandsires with 70 sons per grandsire) around 5.4% of the QTL will be detected. For a large granddaughter design (20 grandsires with 75 sons per grandsire) this figure is approximately 7%. Hayes and Goddard (2001) concluded that, in order to explain 90% of the genetic
variance, QTL as small as 0.1σ have to be detected. At present, even the largest granddaughter designs do not meet this requirement. Using selective DNA pooling, Mosig et al. (2001) report that as much as 90% of the QTL affecting protein% have been detected.

More recently, projects using crosses between cattle breeds have been started up. At Roslin Institute (Edinburgh) a cross between the Holstein and Charolais breeds has been produced (Williams and Wooliams, 1998) while in New Zealand a cross between Jerseys and Holsteins was established (Spelman, 1998). Specifically setting up these crosses has the advantage that traits not collected routinely can be included in the experiment. On the other hand, the large generation interval and the costs of housing animals make these experiments very costly. Crosses between breeds enable the detection of QTL which explain the difference between breeds and it is therefore uncertain if QTL detected in these experiments are also segregating in purebred populations.

RESULTS OF QTL MAPPING EXPERIMENTS

Table 1 shows an overview of significant and suggestive QTL affecting milk production traits. Summarising results causes some difficulties as studies use different criteria to conclude significance. In order to make results comparable, false discovery rates used by Mosig et al. (2001) and Thomsen et al. (2001) were converted.

Table 1 shows that for protein% more significant and suggestive QTL were reported than for other production traits, even when the study by Mosig et al. (2001), reporting many QTL for protein%, is not considered. This seems surprising considering the higher heritability for protein percentage which is expected to have a negative effect on the statistical power of detecting QTL in a granddaughter design: for a medium sized granddaughter design the power of detecting a QTL with an effect of 0.4σ is 67% when the heritability is 0.3 and 31% when the heritability is 0.6. Therefore, this suggests that for protein% more QTL with large effects are segregating than for other milk production traits. Also for milk yield a considerable number of significant QTL are reported (Table 1). However, this can be attributed to the large number of QTL reported by De Koning et al. (2001). The use of significant QTL as cofactors by De
Koning et al. (2001) is expected to increase the power. However, the size of this design (~500 young bulls) suggests that the large number of QTL reported in this study might also be related to the population under study, i.e. the Finnish Ayrshire population.

Table 1. Main results from QTL mapping studies in dairy cattle

<table>
<thead>
<tr>
<th>BTA</th>
<th>Milk Yield</th>
<th>Fat Yield</th>
<th>Protein Yield</th>
<th>Fat %</th>
<th>Protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>***(J)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>***E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>***J)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>***D)</td>
<td>***J)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>***G)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>***K)</td>
<td>***K)</td>
<td>***K)</td>
<td>***G)</td>
<td>***C)</td>
</tr>
<tr>
<td>15</td>
<td>***K)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td>***I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**A** = Spelman et al. (1996); **B** = Arranz et al. (1998); **C** = Coppieters et al. (1998); **D** = Zhang et al. (1998); **E** = Heyen et al. (1999); **F** = Velmala et al. (1999); **G** = Boichard et al. (2000); **H** = Ashwell et al. (2001); **I** = Mosig et al. (2001); **J** = De Koning et al. (2001); **K** = Thomsen et al. (2001).

Table 1 shows that especially for BTA14, 6, 20 and 3 significant QTL effects have been reported. The significant results in Table 1 do not necessarily represent the same QTL and therefore results for those 4 chromosomes will be presented in more detail (Figure 2).
Distances between genetic markers in Figure 2 were based on the MARC map. Not all of the results presented in Figure 2 reached genomewide or suggestive significance levels.

**BTA14.** Coppieters et al. (1998) report experimentwise evidence for the presence of a QTL affecting fat%, protein% and milk yield on the centromeric end of BTA14, i.e. at marker CSSM66. At the same location Boichard et al. (2000) found significant evidence for effects on milk yield, fat yield, fat% and protein%. Heyen et al. (1999) found effects on fat% and fat yield in the same chromosomal region but closer to marker ILSTS039. In the region bracketed by ILST039 and CSSM66, Looft et al. (2001) detected significant effects on milk, fat and protein yield and found substantial linkage disequilibrium between marker KIEL_E8 and milk production traits. Ashwell et al. (2001) report a QTL affecting fat% and fat yield at marker BMS1678. At the other end of BTA14, close to marker BM6425, Mosig et al. (2001) report a QTL affecting protein%. This is likely to be a different QTL from the ones reported in other studies.

In a follow-up of the study by Coppieters et al. (1998), Riquet et al. (1999) describe the fine mapping of a QTL on BTA14 by developing a high density marker map and searching for identity-by-descent regions. Recently, Grisart et al. (2002) reported the positional cloning of this QTL. This is the first QTL in dairy cattle that has been successfully identified. The proposed candidate gene is *DGAT1* and is located close to ILSTS039. The gene catalyses the final step in triglyceride synthesis and as 98% of the milk fat consists of triglycerides this is a likely candidate. Grisart et al. (2002) hypothesise that the functionality of the enzyme has changed due to the identified mutation which would explain the effect on fat%. In the Dutch population 51% of the variation in fat% could be explained by the mutation indicating that this is a gene with a major effect. For the New Zealand population 31% of the variation in fat% could be explained by the mutation. Allele frequencies were 0.63 in the Dutch population and 0.30 in the New Zealand population. The reason a QTL with such large effects is still segregating in both populations is probably that the gene has a negligible effect on the net-merit index used in both countries.

In addition to the QTL affecting milk production traits a number of studies reported QTL with effects on non-production traits. The effects on somatic cell score by Zhang et al. (1998) and front teat placement and fore udder attachment by Ashwell et al. (2001) might be the result of pleiotropic effects of one gene.

**BTA6.** The QTL detected by Georges et al. (1995) on BTA6 in combination with the presence of the casein gene cluster encouraged many researchers to study this chromosome. Figure 2 shows the location of reported QTL. There is a remarkable consistency between different studies on the location of a QTL affecting protein%. Most of the studies locate the QTL at or close to marker BM143 and Ron et al. (2001) report a 95% confidence interval of 4 cM around this marker. In addition to the effect on protein%, Zhang et al. (1998) and Ron et al. (2001) also report a QTL affecting fat% in the same region. The estimated locations suggest that this is the same gene. Note that Klungland et al. (2001) found significant evidence for a QTL affecting clinical mastitis close to marker BM143. Boichard et al. (2000) and Mosig et al. (2001) report a QTL affecting protein% that is located closer to the casein gene cluster. In the same region several studies reported a QTL with an effect on milk yield.
Spelman et al. (1996), Zhang et al. (1998) and Velmala et al. (1999) fitted a 2-QTL model to BTA6. Velmala et al. (1999) found evidence for the presence of 2 QTL affecting protein% milk yield and fat yield. Their analyses suggest the presence of one QTL close to BM143 and another QTL located around the casein cluster. Zhang et al. (1998) found significant evidence for 2 QTL affecting fat%. These two loci were only 12 cM apart: one locus was located close to BM1329 and the other at TGLA37. Zhang et al. (1998) indicated that in all the cases where there was evidence in favour of a 2 QTL model, the two linked loci were in repulsion phase in those families where both of them were segregating. Selection might have induced negative covariances between loci. Closely linked QTL in repulsion phase are expected to remain undetected in single QTL analyses, as was illustrated by Velmala (1999) for the QTL affecting fat yield on BTA6.

**BTA20.** On BTA20 effects on protein% were reported by Arranz et al. (1998), Zhang et al. (1998), Boichard et al. (2000), Ashwell et al. (2001), and Mosig et al. (2001). All these effects were located in a chromosomal region of approximately 40 cM and therefore might be due to a single QTL. In the same chromosomal region, Zhang et al. (1998), also showed significant evidence for a QTL affecting fat%. Boichard et al. (2000) and Ashwell (2001) reported effects on udder characteristics at the same chromosomal region as where effects on protein% were found.

**BTA3.** On BTA3 QTL for protein% were reported by and Zhang et al. (1998), Heyen et al. (1999), Boichard et al. (2000) and Ashwell et al. (2001). These effects were located in an area of about 40 cM. In addition, in the same region significant effects were found for protein yield...
DISCUSSION
It was concluded that even the largest QTL mapping studies carried out to date are expected to detect only a limited fraction of the QTL underlying milk production traits. More QTL can be detected by increasing the size of the design or by using alternative designs, e.g. selective DNA pooling. However, in most studies the information present has not been fully exploited. Most of the studies used regression methods for QTL detection. Regression methods have been proven to be robust, relatively simple to apply and computationally not very demanding which allows the use of permutation tests for calculation of significance thresholds. However, regression methods assume unrelated families and can handle only two generations of genotyped individuals. In dairy cattle pedigrees, usually additional relations exist, e.g. due to relationships between grandsires or due to dams with multiple genotyped sons. Bink and Van Arendonk (1999) and Bolard and Boichard (2001) showed that including additional relationships increases the power of the experiment. These methods have not been fully exploited in most experiments. An alternative strategy for increasing the power of existing designs was demonstrated by De Koning et al. (2001). By fitting cofactors De Koning et al. (2001) significantly detected 8 QTL affecting milk yield. Without the use of cofactors 5 suggestive QTL were detected.

Genes influencing more than one trait are believed to be the main source for genetic correlations between traits. Little attention has been paid to potential pleiotropic effects of chromosomal regions. However, before MAS for certain chromosomal regions can be implemented, effects of chromosomal regions on multiple traits need to be studied. Figure 2 demonstrates that chromosomal regions affecting milk production QTL also have effects on other traits. So far attention has focussed on single trait analyses. Multiple trait analyses could be used to determine whether pleiotropic gene effects play a role.

While results from QTL mapping experiments accumulate, focus will turn towards fine mapping, cloning genes and utilising this information in breeding. So far, the first gene affecting milk production has been identified on BTA14 (Grisart et al., 2002). Linkage has been confirmed on three other chromosomes; BTA3, BTA6 and BTA20 where BTA6 is likely to contain more than one QTL. It is expected that in the coming years more chromosomal regions affecting milk production traits will be detected. However, these QTL are likely to have smaller effects, which will complicate positional cloning.

REFERENCES
Coppieters, W., Riquet, J., Arranz, J.-J., Berzi, P., Cambisano, N., Grisart, B., Karim, L.,
Marcq, F., Moreau, L., Nezer, C., Simon, P., Vanmanshoven, P., Wagenaar, D. and
Georges, M., Nielsen, D., Mackinnon, M., Mishra, A., Okimoto, R., Pasquino, A.T., Sargeant,
Genetics 139 : 907-920.
Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Cambisano, N., Mni, M., Reid, S.,
Heyen, D.W., Weller, J.I., Ron, M., Band, M., Beever, J.E., Feldmesser, E., Da, Y., Wiggans,
Plante, Y., Gibson, J.P., Nadesalingam, J., Mehrabani-Yeganeh, H., Lefebvre, S., Vandervoort,
Riquet, J., Coppieters, W., Cambisano, N., Arranz, J.-J., Berzi, P., Davis, S.K., Grisart, B.,
Farnir, F., Karim, L., Mni, M., Simon, P., Taylor, J.F., Vanmanshoven, P., Wagenaar,
727-735.
Schmutz, S.M., Stooky, J.M., Winkelman-Sim, D.C., Waltz, C.S., Plante, Y. and Buchanan,
795–806.
Genetics 144 : 1799-1808.
Spelman, R.J., Huisman, A.E., Singireddy, S.R., Coppieters, W., Arranz, J., Georges, M. and
Thomsen, H., Reinsch, N., Xu, N., Looff, C., Grupe, S., Kuhn, C., Brockmann, G.A.,
Schwerin, M., Leyhe-Horn, B., Hiendleder, S., Erhardt, G., Medjugorac, I., Russ, I.,


