

Current and Future Developments in Patents for Quantitative Trait Loci in Dairy Cattle

Joel I. Weller*

Institute of Animal Sciences, A. R. O., The Volcani Center, P. O. Box 6, Bet Dagan 50250, Israel

Received: October 5, 2006; Accepted: December 5, 2006; Revised: December 7, 2006

Abstract: Many studies have proposed that rates of genetic gain in dairy cattle can be increased by direct selection on the individual quantitative loci responsible for the genetic variation in these traits, or selection on linked genetic markers. The development of DNA-level genetic markers has made detection of QTL nearly routine in all major livestock species. The studies that attempted to detect genes affecting quantitative traits can be divided into two categories: analysis of candidate genes, and genome scans based on within-family genetic linkage. To date, 12 patent cooperative treaty (PCT) and US patents have been registered for DNA sequences claimed to be associated with effects on economic traits in dairy cattle. All claim effects on milk production, but other traits are also included in some of the claims. Most of the sequences found by the candidate gene approach are of dubious validity, and have been repeated in only very few independent studies. The two missense mutations on chromosomes 6 and 14 affecting milk concentration derived from genome scans are more solidly based, but the claims are also disputed. A few PCT in dairy cattle are commercialized as genetic tests where commercial dairy farmers are the target market.

Keywords: Quantitative trait locus, Quantitative trait nucleotide, Marker-assisted selection, DNA microsatellite, Single nucleotide polymorphism, Linkage disequilibrium, Genome scan, Genetic markers, Dairy cattle.

INTRODUCTION

Scientific breeding programs for dairy cattle began in the 1950's. These programs were based on phenotypic selection without regard to the individual genes responsible for the phenotypic variation. Many studies have proposed that rates of genetic gain can be increased by direct selection on the individual quantitative loci (QTL) responsible for the genetic variation in these traits, or selection on linked genetic markers termed marker-assisted selection (MAS) (reviewed by [1]). Until 1990 QTL detection was limited by the lack of suitable genetic markers in dairy cattle populations. Since then, the development of DNA-level genetic markers; specifically, DNA microsatellites and single nucleotide polymorphisms (SNP), have made detection of QTL nearly routine in all major livestock species. The studies that attempted to detect genes affecting quantitative traits via linkage to genetic markers can be divided into two categories: analysis of candidate genes, and genome scans based on within-family genetic linkage.

The principles of modern breeding programs for dairy cattle will be described, and their accomplishments and limitations will be reviewed. Next the basic principles and the current state of MAS in dairy cattle will be reviewed. Detection of segregating QTL via the candidate gene approach, genetic linkage, and methods to determine the actual polymorphism responsible for the observed effect will be considered in the following sections. The specific US patents and PCT in which DNA polymorphisms are claimed to affect expression of economic traits in dairy cattle then

will be analyzed, and in the last section an attempt will be made to predict future developments and several open questions with respect to QTL patents in dairy cattle will be addressed.

TRAIT-BASED SELECTION, ACCOMPLISHMENTS AND LIMITATIONS

Modern dairy cattle breeding schemes are based on the following principles:

1. Nearly all economic traits are expressed only in females.
2. Female fertility is very limited while male fertility via artificial insemination is nearly unlimited.
3. As compared to most other agricultural species, cattle breeding programs are constrained by the long generation interval and the high value of each animal.

Thus unlike the situation in poultry or most plant species, dairy cattle breeding programs are based on selection within the commercial population, rather than selection within specific populations controlled by breeding enterprises. Due to the biological limitations noted above, most modern dairy cattle programs are based on the "progeny test" scheme. Most genetic gain is obtained by selection of the bulls, even though bulls do not have production records. Genetic evaluations are derived by the Individual Animal Model, including the numerator relationship matrix. Thus the genetic evaluations of animals without records are derived from the production records of all their female relatives [1].

An example of the Israeli Holstein breeding program is given in (Fig. 1). This population consists of approximately 120,000 cows of which 90% are milk recorded. Approximately 20 bulls are used for general service. Each year about 300 elite cows are selected as bull dams. These are mated to the two to four best local bulls and an equal

*Address correspondence to this author at the Institute of Animal Sciences, A. R. O., The Volcani Center, P. O. Box 6, Bet Dagan 50250, Israel; Tel: 972-8-9484430; Fax: 972-9-9470587; E-mail: weller@agri.huji.ac.il

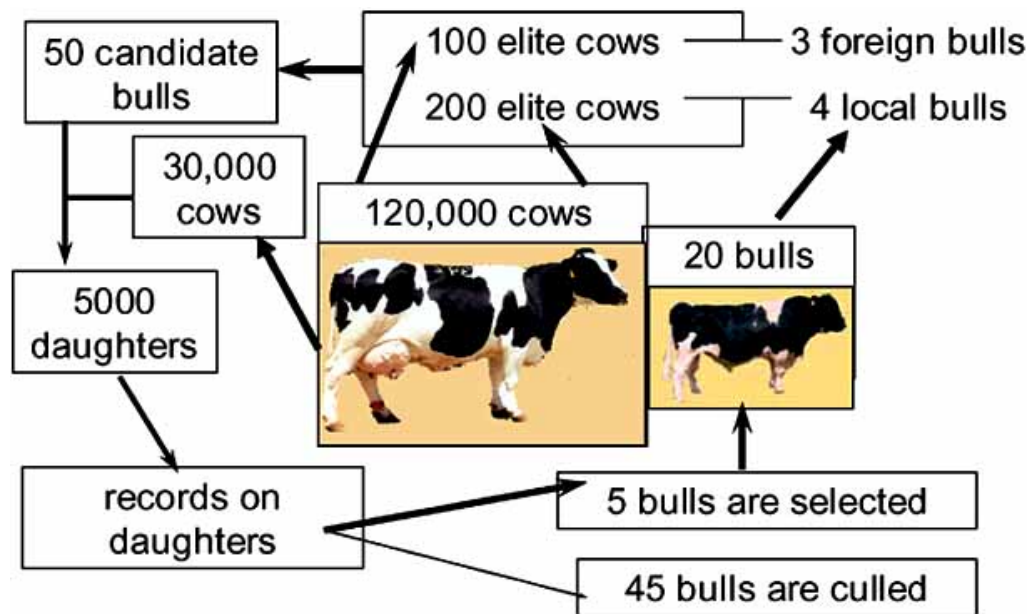


Fig. (1). The Israeli Holstein breeding programme.

number of foreign bulls to produce approximately 50 bull calves for progeny testing. At the age of one year, the bull calves reach sexual maturity, and approximately 1000 semen samples are collected from each young bull. These bulls are mated to approximately 30,000 first parity cows to produce about 5000 daughters, or 100 daughters per young bull. Gestation length for cattle is nine months. Thus the young bulls are approximately two years old when their daughters are born, and are close to four when their daughters calve and begin their first lactation. At the completion of their daughters' first lactations, most of the young bulls are culled. Only four to five are returned to general service, and a similar number of the old proven sires are culled. By this time the selected bulls are approximately five years old. Various studies have shown that rates of genetic gain by a progeny test scheme are about 0.1 to 0.2 genetic standard deviations of the selection index per year [2,3].

The progeny test scheme has several major weaknesses. First, for this system to be effective, the population must include at least several tens of thousands of animals with recording on production traits and paternity. Inaccurate recording can significantly reduce rates of genetic gain [2]. Second, generation intervals, especially along the sire-to-daughter and sire-to-son paths, are much longer than the biological requirements. The increase in generation interval reduces the rate of genetic gain per year. Since artificial insemination institutes generally pay a premium price for male calves of elite cows, these cows are often given preferential treatment in order to increase their genetic evaluations [4]. The small number of bulls actually used for general service, and the even smaller number of bulls used as bull sires tends to reduce the effective population size, which increases inbreeding and decreases genetic variance in the population. The effective population size of the US Holstein population with ten million cows has been estimated at about 100 [5]. Finally, there is virtually no selection along the

dam-to-daughter path, because 70-80% of healthy female calves produced are required as replacements of culled cows.

OVERVIEW OF MARKER-ASSISTED SELECTION

Nearly all economically important traits in domestic animal species are quantitative. That is the distribution of phenotypic values in the population has an approximately normal distribution, because these values are determined by the joint effect of many genetic and environmental factors. Sax [6] first proposed that the effects of individual genes affecting quantitative traits could be statistically isolated via linkage to genetic markers. A "genetic marker" is a polymorphism that generates an observable phenotype, and displays Mendelian inheritance.

MAS within a breed can increase the rate of genetic gain by increasing the accuracy of genetic evaluations, increasing the selection intensity, or decreasing the generation interval. Most theoretical studies have estimated the expected gains that can be obtained by MAS to be in the range of a 5 to 20% increase in the rates of genetic gain obtained by traditional selection programs [1]. Two MAS programs in dairy cattle have been initiated so far, in the German and French Holstein populations [7-9]. Both programs are based on selection of young sires prior to progeny test. These animals are selected for economically positive haplotypes of chromosomal regions in which QTL affecting economic traits have been detected.

In the French program 12 chromosomal segments, ranging in length from 5 to 30 centi-Morgans (cM) are analyzed. Regions with putative QTL affecting milk production or composition are located on *Bos taurus* chromosomes (BTA) 3, 6, 7, 14, 19, 20, and 26; segments affecting mastitis resistance are located on BTA 10, 15, and 21; and chromosomal segments affecting female fertility are located on BTA 1, 7, and 21. Each region was found to affect one to four economic traits, and on average three regions with segregating QTL were found for each trait.

Each region is monitored by two to four evenly spaced microsatellites, and each animal included in the MAS program is genotyped for at least 43 markers.

THE CANDIDATE GENE APPROACH TO QTL DETECTION

Many studies have proposed that genes with known function in metabolic pathways that determine expression of the economic traits, are prime candidates for QTL (e. g. [10]). The counter argument is that even though a specific gene may have a prime function in trait expression, the gene is not necessarily functionally polymorphic in the population of interest. In general the candidate gene approach is based on determining polymorphisms in a gene known to have an effect on the traits of interest. A sample of animals is then genotyped for these polymorphisms. A significant effect of a polymorphism on either the phenotypic or breeding values of these animals for the traits of interest is indicative that this polymorphism represents a segregating QTL.

There are three major reservations with this conclusion. First, the observed effect may be due only to linkage disequilibrium (LD) between the analyzed polymorphism and the causative DNA sequence that may reside elsewhere in the gene, or in a closely linked gene. Population-wide LD can extend over 10 cM in commercial dairy cattle populations [5]. Second, a significant effect may be due to some other factor not randomly distributed across the genotype groups for the candidate polymorphism. For example, if most of the cows are progeny of a few sires, then

the effect detected may be due to the genotypes of these sires. This problem can be solved if the relationship matrix among the animal analyzed is included in the analysis. Finally, if several polymorphisms and several economic traits are analyzed, nominal significance probability are no longer meaningful. Generally statistical significance is declared if the probability of obtaining the observed result under the null hypothesis is less than 5%. However, if one hundred statistical tests are performed, then five will display “significance” purely by chance. The multiple-comparison problem will be discussed in more detail below. Most of the PCT and patents for QTL in dairy cattle have been based on the candidate gene approach.

DETECTION AND ANALYSIS OF QTL IN GENOME SCANS VIA GENETIC LINKAGE

In the genome scan approach, the objective is to detect genetic linkage between the genetic markers and the segregating QTL. This requires LD between the segregating QTL and the genetic markers. Thus instead of genotyping a random sample of individuals, the analysis is performed within families. Dairy cattle have the advantage that because of the nearly unlimited fertility of males, thousands of daughters are generated from elite sires. Furthermore, historical data on the economic trait values of these cows are available for analysis. Thus the only significant additional cost required for a QTL genome scan is genotyping. This experimental design is denoted the “daughter design”, and is illustrated in (Fig. 2). The daughters of each sire are divided

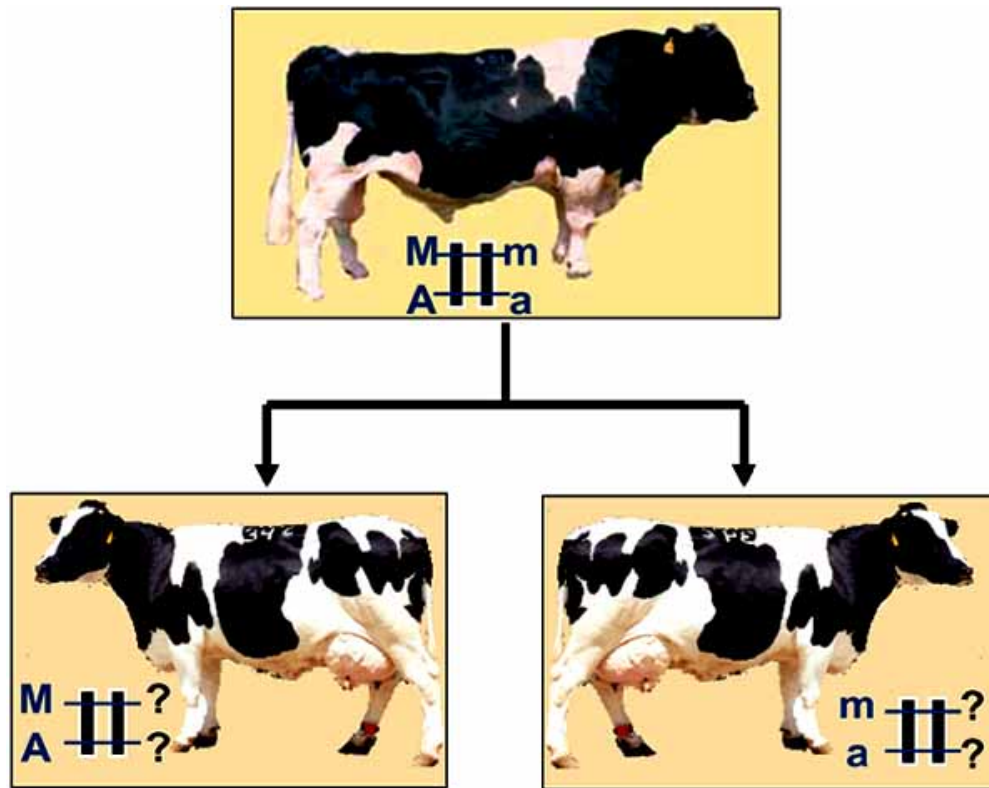


Fig. (2). The daughter design. Only a single family is shown, although in practice several families will be analyzed jointly. The sire is assumed to be heterozygous for a QTL and a linked genetic marker. The two alleles of the marker locus are denoted “M” and “m”, and the two alleles of the QTL are denoted “A” and “a”. Alleles of maternal origin are denoted by question marks.

into two groups, based on the paternal allele received. All effects other than the chromosomal segment linked to the genetic marker are assumed to be distributed randomly among the two daughter groups. Therefore, a significant effect of the economic trait associated with paternal allele for the genetic marker is indicative of a segregating QTL linked to the marker. Neimann-Sørensen and Robertson [11] were the first to apply this design using blood group polymorphisms as genetic markers.

Since linkage relationships will differ across families, effects of paternal alleles cannot be summed across families, as is done in the candidate gene approach. Furthermore, if only two functionally different QTL alleles are segregating in the population, the sires of most families will be homozygous for any specific QTL. Both of these factors reduce statistical power per individual genotyped, and thousands of daughters must be genotyped to detect QTL responsible for only a few percent of the phenotypic

variance. Statistical power per genotype can be increased if instead of genotyping daughters, sons of a specific sire, each with many daughters are genotyped. This design is denoted the “granddaughter design,” as opposed to the daughter design described above, and is diagrammed in (Fig. 3) [12].

With a battery of 50 to 100 well-spaced genetic markers spanning the genome it is possible to assay the entire genome for QTL by “interval mapping” [1]. Unlike the candidate gene approach, *a priori* no assumptions are made with respect to the effects of these markers on the traits of interest. Whole genome scans for segregating QTL have been completed for most major dairy cattle populations. The traits that have been considered in most studies are milk, milk-fat, and milk-protein production, fat and protein concentration, and somatic cell score (SCS). A number of studies have also considered cow survival, female and male fertility, persistency of milk production, calving traits, and a large number of cow conformation traits. Results have been

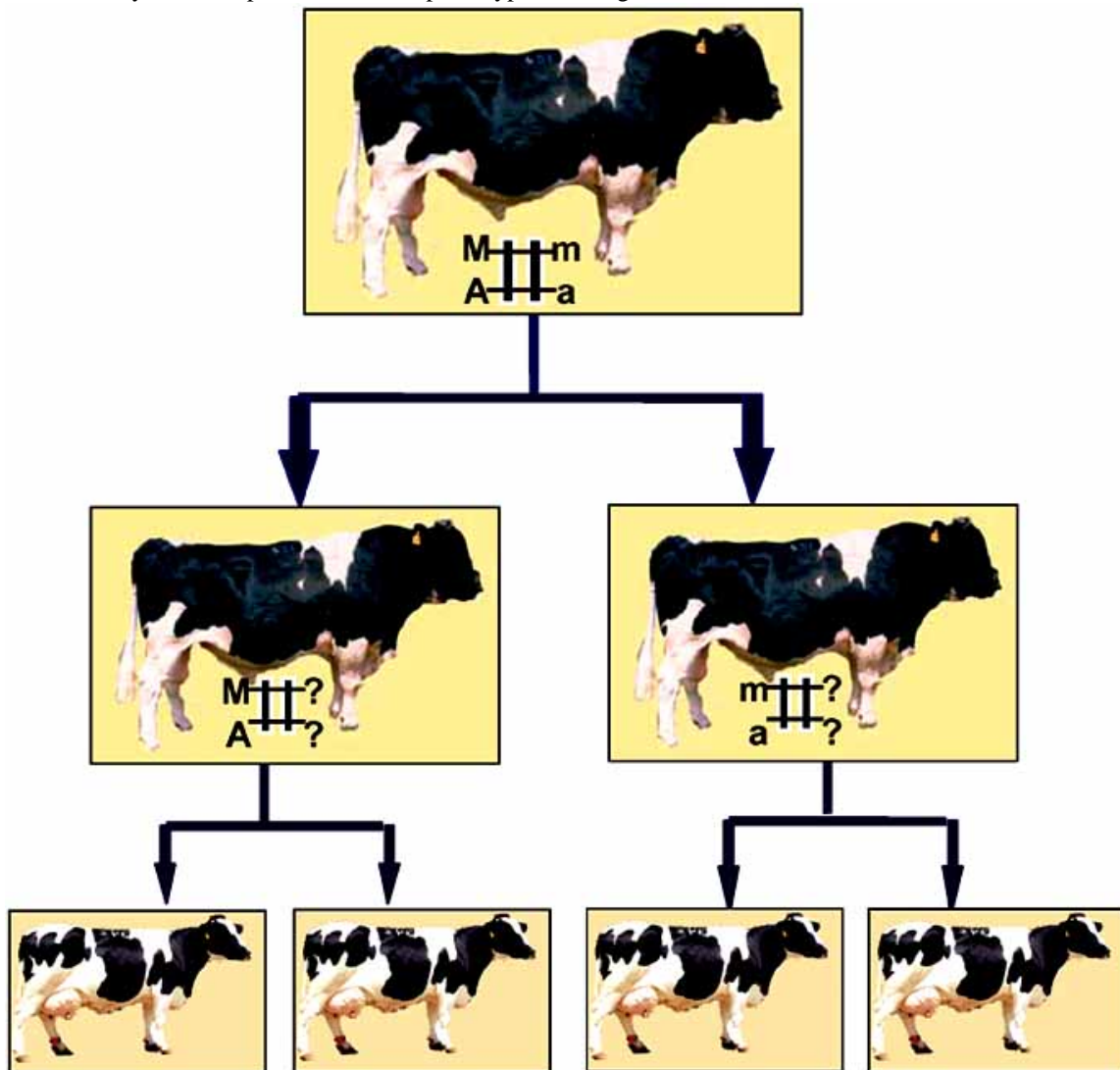


Fig. (3). The granddaughter design. The grandsire is assumed to be heterozygous for a QTL and a linked genetic marker. As in Figure 2, only a single family is shown. The two alleles of the marker locus are denoted “M” and “m”, and the two alleles of the QTL are denoted “A” and “a”. Alleles of maternal origin are denoted by question marks. Genotypes are not listed for the granddaughters because they were not genotyped.

summarized at: http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/, <http://bovineqtl.tamu.edu/index.html>, and <http://www.animalgenome.org/QTLdb/cattle.html>.

The confidence interval for QTL location from the results of a dairy cattle genome scan will still span 10 to 50 cM. Increasing marker density will only have a marginal effect [13]. Furthermore, for genome scans the multiple-comparison problem is even more acute than for the candidate gene approach. First, multiple families and traits are generally analyzed. Second, each chromosomal “site” can be considered a statistical test. Thus if a 5 or 1% type I error is declared, several chromosomal regions will show nominal point-wise “significance” purely by chance.

The classical solution to the multiple-comparison problem is to reduce the nominal type I error value to the level required so that the probability that any of the tests will display significance by chance is below a specified threshold. However, considering the large number of comparisons, reduction of the type I error to this level would mean that only the very largest segregating QTL could be detected. Although there is no complete solution to the multiple-comparison problem in genome scans, several solutions have been proposed (reviewed by [1]). In general a QTL can only be considered confirmed if it has been detected in multiple populations.

FROM QTL TO QTN

Once a QTL has been detected and confirmed by additional studies in a specific chromosomal region the objective is to determine the actual gene, and the specific DNA polymorphism within this gene responsible for the observed effect. This is denoted the “quantitative trait nucleotide” (QTN). Similar to other mammals, the bovine genome includes 3×10^9 base pairs (bp), and the map length is approximately 3000 cM. The human genome is estimated to encode 20,000 – 25,000 protein-coding genes [14], and it can be assumed that the number of genes in other mammals, including cattle, should be quite similar. Thus a single map unit, on the average, includes approximately 8 genes and one million bp. Except for the largest QTL, confidence intervals determined by a daughter or granddaughter design will generally include several tens of cM [13]. Considering that each cattle cM includes ~8 genes and one million bp, detection of the QTN appears at first glance to be a daunting task.

Meuwissen and Goddard [15] proposed that confidence intervals for QTL location could be reduced to individual cM by application of LD mapping. If a QTL polymorphism is due to a relatively recent mutation or to a relatively recent introduction from another population, then it should be possible to detect population-wide LD between the QTL and closely linked genetic markers. The closer the marker to the QTL, the greater will be the extent of LD. They developed a method to estimate QTL location and confidence intervals based on LD between a QTL and a series of closely linked markers.

In order to determine the actual gene responsible for the QTL, most studies have used a modification of the candidate gene approach, described above. Likely candidates for the QTL among those genes within the QTL confidence interval

are determined based on the gene function. The candidate genes are then sequenced in order to detect polymorphisms, and the effects of the polymorphism on the trait of interest are estimated. However, even if a polymorphism is detected in the candidate gene, and the polymorphism is associated with a major effect on the QTL, how does one prove that this polymorphism is not merely in LD with the actual QTN? Mackay [16] proposed two alternatives for proof positive that a candidate polymorphism is in fact the QTN, namely, co-segregation of intragenic recombinant genotypes in a candidate gene with the QTL phenotype, and functional complementation where the trait phenotype is “rescued” in a transgenic organism. Neither of these is applicable to QTL in dairy cattle. In this case, Mackay [16] postulated that the only option to achieve the standard of rigorous proof for identification of a gene underlying a QTL in commercial animal populations is to collect “multiple pieces of evidence, no single one of which is convincing, but which together consistently point to a candidate gene”. Evidence can be provided by quantitative differences of gene expression in physiologically relevant tissues, SNP capable of encoding a non-conservative amino acid change, protein differences in cows with contrasting genotypes for the QTN, orthologous QTL in other species (genes that are derived from a common ancestral gene), and alteration of gene protein in bovine cell lines by “short interfering RNA” (siRNA) technology. (The siRNA molecules bind with proteins to form a unit called the “RNA-induced silencing complex” that suppresses the expression of the gene to which it corresponds in the viral genome, silencing the gene from which the siRNA is derived.)

For dairy cattle, so far, the most compelling evidence is “concordance” i.e. that the deduced QTL genotypes of a sample of individuals correspond completely to their genotypes for the putative QTN. All individuals heterozygous for the QTL should be heterozygous for the putative QTN, with the same QTN allele associated with the same QTL allele in all individuals, and all individuals homozygous for the QTL should also be homozygous for the QTN. Theoretically, the sample of individuals analyzed should be large enough to reject statistically the hypothesis that concordance was obtained by chance. However, in dairy cattle, the only individuals for which QTL genotype can be derived with any level of reliability are sires that have been analyzed by either a daughter or granddaughter design, and the number of these individuals will always be limited. Furthermore, there is at present no accepted theory to compute concordance probabilities by chance, considering that any polymorphism very close to the QTN will display significant LD. Two studies have addressed the problem [17, 18]. The case for identification of the QTN is clearly more compelling if concordance is obtained in several different populations.

In the candidate gene approach, it is usually assumed that if a significant effect on an economic trait is associated with a specific polymorphism in an analysis of a random sample of animals then this polymorphism is in fact the QTN. In the genome scan approach this is not the case, because *a priori* there is no reason to assume that an anonymous marker should directly affect the trait of interest. Starting with QTL detected by anonymous markers there are only two claims in

dairy cattle of QTN determination. Both are disputed and will be considered in the following section.

PATENTS AND PCT FOR DNA SEQUENCES AFFECTING MILK PRODUCTION TRAITS

A list of US patents and PCT for DNA sequences that include the claim the detect polymorphism affects at least one milk production trait is given in Table 1. Of these, three were detected first by genome scans, but two [19,20] refer to the same polymorphism in the gene *DGATI* on BTA14. This gene was considered a prime candidate for the QTL, due to its role in fat metabolism, and because mice with a knock out mutation for this gene do not lactate [21, 22]. Both groups claimed complete concordance between a missense mutation in this gene and the QTL status of sires as determined by granddaughter designs. However, other studies claim that the *DGATI* missense mutation described by both PCT does not explain the entire effect associated with this QTL [23,24]. Some individuals homozygous for this mutation were nevertheless heterozygous for the QTL. This PCT is licensed to Merial, an animal health company, under the trade name "IGENITY OptiYIELD" (http://us.igenity.com/dairy/igenity_dairy_opti.html). Merial will genotype animals for this polymorphism for a fee, and the target market is commercial dairy farmers.

The remaining PCT that refers to a QTN first detected by a genome scan claims that the causative mutation for a QTL chiefly affecting milk-protein concentration on BTA6 is variation in a poly-A sequence in the promoter of the *OPN* gene [25]. However, Cohen-Zinder *et al.* [17] claim that a missense mutation in the gene *ABCG2* is the causative mutation for the observed effects on milk, and fat and protein concentration. The latter group filed a PCT in June 2006. All of the remaining PCT and patents can be considered effects detected by the candidate gene approach.

Most of these are of dubious validity, and have been repeated in only very few independent studies. It should be noted that the literature is skewed towards reports of significant effects, because non-significant effects are generally not published. Over 1500 studies have shown that the injection of the growth hormone, also denoted somatotropin, into lactating cows increases milk production (reviewed by Cunningham, [26]). Thus the growth hormone receptor (*GHR*) gene is a prime candidate for a QTL affecting milk production. Nevertheless, Dybus [27] reviewed the literature on the *GHR* gene and concluded that "it is difficult to accept the bovine growth-hormone gene as a major gene for quantitative traits of cattle."

Three patents describe missense mutations in this gene. Collier *et al.* [28] describe a leucine-to-valine missense mutation at position 126, while two PCT both refer to a phenylalanine-to-tyrosine substitution at position 279 [29, 30]. The fact that significant effects were detected in two different populations reduces the probability that this is a "false positive". Blott *et al.* [31] provide the most recent scientific study of the effect of a polymorphism in this gene. Although two sires heterozygous for the QTL were also heterozygous for this polymorphism, they did not report the *GHR* genotypes for any of the sires that were homozygous for the QTL. Furthermore, two sires with significant effects

on milk production traits on this chromosome were homozygous for this polymorphism. The PCT of Blott *et al.* is also licensed to Merial under the trade name "IGENITY ComponentMAKER" (http://us.igenity.com/dairy/igenity_dairy_component.html).

The effect associated with prolactin found by Cowan *et al.* [32] was only repeated in one other independent study [33], but the polymorphism analyzed was different. Similarly, only one other study in addition to Renaville *et al.* [34] found an effect associated with *PIT-1* on milk production [35]. Two US patents were issued for polymorphisms in this gene affecting milk production traits [36, 37]. Several studies have found effects on milk production associated with polymorphisms in the leptin gene, but the polymorphisms considered were not the same in all studies [38-41]. No studies other than Khatib *et al.* [42,43] reported on an effect of polymorphisms at the protease inhibitor gene on milk production traits, but at least two genome scans found QTL affecting milk production and health traits at position 56 cM on BTA21 [44]. Two patents [45,46] detected by the candidate gene approach that have not been verified by other studies.

The QTL on BTA6 and 14 explain 50% of the phenotypic variance for protein and fat percentages, respectively [17,47,48]. However, in both cases, the polymorphism included in the PCT apparently do not account for the entire effect observed in these chromosomal regions [17, 23, 24]. The effect associated with the missense mutation in *ABCG2* explains the entire effect observed on milk yield and fat and protein concentration, but does not explain the effects associated with fat and protein yield. Both of these QTL have disadvantages with respect to application in MAS. The allele of *DGATI* that increases fat production also decreases water content in the milk, which are both desirable. Furthermore, this allele is at moderate to low allelic frequencies in most dairy cattle populations [49]. Thus there is significant scope for selection for this allele. However, this allele also slightly decreases protein yield, which is the most important trait in most current breeding indices [50]. The allele of *ABCG2* which decreases milk production and increases protein percentage is clearly the favorable allele in nearly all current selection indices, but this allele is already at a very high frequency in all major dairy cattle populations [51]. Thus there is very little scope for further selection.

CURRENT & FUTURE DEVELOPMENTS

Only a few of the patents so far in dairy cattle are commercialized, and only at the level of providing genotypes to commercial farmers. The two ongoing MAS programs have not incorporated any of these patents in their programs. It is likely that in the near future additional QTN will be resolved. Discovery of QTN for secondary traits may be more economically valuable than those for the primary milk production traits. Secondary traits generally have lower heritability, and have been under less selection than milk production. Thus despite low to moderate heritability it is still likely that relatively large QTL for these traits are still segregating in commercial populations. Khatkar *et al.* [44] found significant effects on BTA 1, 3, 9, and 10 by a meta-analysis of multiple studies; in addition to the effects described on BTA 6, 14, and 20. However, as can be seen

Table 1. Patents and PCT for DNA Sequences Affecting Milk Production Traits

Patent type	Number and reference	Gene	Polymorphism type	Economic traits affected	Chromosome	Filing date
US	5,041,371 [32]	Prolactin	Restriction enzyme site	Milk	23	20.8.1991
	5,374,523 [28]	GHR	Missense	Milk	20	20.12.1994
	5,614,364 [36]	PIT-1	Restriction enzyme site	Milk	1	25.3.1997
	6,383,751 [45]	Thyroglobulin, RARG, RDH5	Not determined	Milk-fat, and meat marbling	14, 5, 10	7.5.2002
	6,492,142 [37]	PIT-1	Restriction enzyme site	Milk	1	10.12.2002
PCT	NZ2001000245 [20]	DGAT1	Missense	Milk, fat, protein and concentration	14	31.10.2001
	EP2002007520 [19]	DGAT1	Missense	Milk-fat, and meat marbling	14	5.7.2002
	NZ2002000157 [29]	GHR	Missense	Milk, fat, protein and concentration	20	16.8.2002
	EP2003014837 [30]	GHR and k-casein	Missense	Milk, protein and meat production	20	26.11.2003
	CA2004000405 [46]	Leptin	Missense	Milk	4	18.3.2004
	US2004040675 [43]	PI	SNP	Milk, health, reproduction	21	3.12.2004
	US2006001207 [25]	OPN	Indel in promoter	Milk, fat and protein concentration	6	12.1.2006

from the examples given, it is a long way from discovery of a significant QTL to an economically viable patent.

A final point to consider relates to the scope of the claims of all the patents listed in Table 1. A specific polymorphic DNA sequence is described in each patent. As noted previously, population-wide LD can extend over rather large chromosomal segments in dairy cattle. This then raises the question as to whether the claims of these patents also cover additional polymorphism in strong LD to those specifically described in the patent. The French MAS program includes chromosomal segments with effects on milk production traits on BTA6, 14, and 20. Patents or PCT within all of these chromosomal segments are listed in Table 1. Yet selection in the French program is apparently based on markers other than the specific polymorphisms listed in these patents [52].

ACKNOWLEDGMENTS

This research was supported by grants from the Israel milk marketing board and the European Sixth Research and Technological Development Framework Programme, Proposal No. 016250-2 SABRE.

REFERENCES

[1] Weller JI. Quantitative Trait Loci Analysis in Animals. CABI Publishing. London. 2001. 287 pp.
 [2] Israel C, Weller JI. Effect of misidentification on genetic gain and estimation of breeding value in dairy cattle populations. J Dairy Sci 2000; 83: 181-187.
 [3] Nicholas FW, Smith C. Increased rates of genetic change in dairy cattle by embryo transfer and splitting. Anim Prod 1983; 36: 341-353.

[4] Powell RL, Norman HD. Accuracy of cow indexes according to repeatability, evaluation, herd yield, and registry status. J Dairy Sci 1988; 71: 2232-2240.
 [5] Farnir F, Coppieters W, Arranz JJ, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Mni M, Nezer C, Simon P, Vanmanshoven P, Wagenaar D, Georges M. Extensive genome-wide linkage disequilibrium in cattle. Genome Res 2000; 10: 220-227.
 [6] Sax K. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 1923; 8: 552-560.
 [7] Bennewitz J, Reinsch N, Thomsen H, Szyda J, Reinhart F, Kuhn C, Schwerin M, Erhardt G, Weimann C, Kalm E. Marker assisted selection in German Holstein dairy cattle breeding: outline of the program and marker assisted breeding value estimation. Ann Meet Europ Ass Anim Prod. 54th Session; Rome, Italy (2003) G1.9.
 [8] Boichard D, Fritz S, Rossignol MN, Boscher MY, Malafosse A, Colleau JJ. Implementation of marker-assisted selection in French dairy cattle. Proc 7th World Cong Genet Appl Livest Prod. Montpellier, France (2002) 33: 19-22.
 [9] Boichard D, Fritz S, Rossignol MN, Guillaume F, Colleau JJ, Druet T. Implementation of marker-assisted selection: practical lessons from dairy cattle. Proc 8th World Cong Genet Appl Livest Prod., Belo Horizonte, MG, Brazil (2006) 22: 11.
 [10] Parmentier I, Portetelle D, Gengler N, Prandi A, Bertozzi C, Vleurick L, Gilson R, Renaville R. Candidate gene markers associated with somatotrophic axis and milk selection. Domest Anim Endocrin 1999; 17: 139-148.
 [11] Neimann-Sørensen A, Robertson A. The association between blood groups and several production characters in three Danish cattle breeds. Acta Agr Scand 1961; 11: 163-196.
 [12] Weller JI, Kashi Y, Soller M. Power of "daughter" and "granddaughter" designs for genetic mapping of quantitative traits in dairy cattle using genetic markers. J Dairy Sci 1990; 73: 2525-2537.
 [13] Weller JI, Soller M. An analytical formula to estimate confidence interval of QTL location with a saturated genetic map as a

- function of experimental design. *Theor Appl Genet* 2004; 109: 1224-1229.
- [14] Collins FS, Lander ES, Rogers J, Waterston RH. Finishing the euchromatic sequence of the human genome. *Nature* 2004; 431: 931-945.
- [15] Meuwissen THE, Goddard ME. Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. *Genetics* 2000; 155: 421-430.
- [16] Mackay TF. The genetic architecture of quantitative traits. *Ann Review Genet* 2001; 35: 303-339.
- [17] Cohen-Zinder M, Seroussi E, Larkin D M, Looor JJ, Everts-van der Wind A, Lee JH, Drackley JK, Band MR, Hernandez AG, Shani M, Lewin HA, Weller JI, Ron M. Identification of a missense mutation in the bovine *ABCG2* gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. *Genome Res* 2005; 15: 936-944.
- [18] Schnabel RD, Kim JJ, Ashwell MS, Sonstegard TS, Van Tassell CP, Connor EE, Taylor JF. Fine-mapping milk production quantitative trait loci on BTA6: Analysis of the bovine osteopontin gene. *Proc Nat Acad Sci USA* 2005; 102, 6896-6901.
- *[19] Fries, H-R., Winter, A.: WO03004630 (2003).
- *[20] Georges, M.A.J., Coppieters, W.H.R., Grisart, B.M-J.J., Snell, R.G., Reid, S.J., Ford, C.A., Spelman, R.J.: PCT/NZ2001/000245 (2001).
- [21] Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E, Novak S, Collins C, Welch CB, Lusis AJ, Erickson SK, Farese RV Jr. Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc Nat Acad Sci USA* 1998; 95: 13018-13023.
- [22] Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, Farese RV Jr. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat Genet* 2000; 25: 87-90.
- [23] Bennewitz J, Reinsch N, Paul S, Looft C, Kaupe B, Weimann C, Erhardt G, Thaller G, Kuhn C, Schwerin M, Thomsen H, Reinhardt F, Reents R, Kalm E. The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. *J Dairy Sci* 2004; 87, 431-442.
- [24] Kuhn C., Thaller G., Winter A., Bininda-Emonds O.R.P., Kaupe B., Erhardt G., Bennewitz J., Schwerin M. & Fries R. (2004) Evidence for multiple alleles at the DGAT1 locus better explains a quantitative tip trait locus with major effect on milk fat content in cattle. *Genetics* 167, 1873-1881.
- *[25] Schnabel, R.D., Sonstegard, T.S., Van Tassell, C.P., Ashwell, M.S., Taylor, J.F.: WO06076563 (2006).
- [26] Cunningham EP. The use of bovine somatotropin in milk-production - a review. *Irish Vet J* 1994; 47: 207-210.
- [27] Dybus A. Bovine growth-hormone gene. *Medycyna Weterynaryjna* 2002; 58: 942-945.
- *[28] Collier, R.J., Hauser, S.D., Krivi, G.G., Lucy, M.C.: US5374523 (1994).
- *[29] Blott, S., Kim, J.J., Schmidt-Kuntzel, A., Cornet, A., Berzi, P., Cambisano, N., Grisart B., Karim, L., Simon, P., Georges, M., Farnir, F., Coppieters W. Moio, S., Vilkki J., Johnson, D., Spelman R., Ford, C., Snell R.: WO03104492 (2003).
- [30] Renaville, R., Parmentier, I.: WO04048609 (2004).
- [31] Blott S, Kim J J, Moio S, Schmidt-Kuntzel A, Cornet A, Berzi P, et al. Molecular dissection of a quantitative trait locus: A phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics* 2003; 163: 253-266.
- [32] Cowan, C.M., Dentine, M.R., Ax, R.L., Schuler, L.A.: US5041371 (1991).
- [33] He F, Sun DX, Yu Y, Wang YC, Zhang Y. Association between snps within prolactin gene and milk performance traits in Holstein dairy cattle. *Asian-Australasian J Anim Sci* 2006; 19: 1384-1389.
- [34] Renaville R, Gengler N, Vrech E, et al. Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. *J Dairy Sci* 1997; 80: 3431-3438.
- [35] de Mattos KK, Del Lama SN, Martinez ML, Freitas AF. Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls. *Pesquisa Agropecuaria Brasileira* 2004; 39: 147-150.
- [36] Tuggle, C.K., Freeman, A.E.: US5614364 (1997).
- [37] Renaville, R., Portetelle, D.: US20026492142 (2002).
- [38] Buchanan FC, Van Kessel AG, Waldner C, Christensen DA, Laarveld B, Schmutz SM. Hot topic: An association between a leptin single nucleotide polymorphism and milk and protein yield. *J Dairy Sci* 2003; 86: 3164-3166.
- [39] Komisarek J, Szyda J, Michalak A, Dorynek Z. Impact of leptin gene polymorphisms on breeding value for milk production traits in cattle. *J Anim Feed Sci* 14; 2005: 491-500.
- [40] Liefers SC, te Pas MFW, Veerkamp RF, van der Lende T. Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. *J Dairy Sci* 2002; 85: 1633-1638.
- [41] Moussavi AH, Ahouei M, Nassiry MR, Javadmanesh A. Association of leptin polymorphism with production, reproduction and plasma glucose level in Iranian Holstein cows. *Asian-Australasian J Anim Sci* 2006; 19: 627-631.
- [42] Khatib H, Heifetz E, Dekkers JCM. Association of the protease inhibitor gene with production traits in Holstein dairy cattle. *J Dairy Sci* 2005; 88: 1208-1213.
- [43] Khatib, H. Method and compositions for genetically detecting improved milk production.: WO05056758 (2005).
- [44] Khatkar MS, Thomson PC, Tammen I, Raadsma HW. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genet Sel Evol* 2004; 36: 163-190.
- [45] Barendse, W.J.: US20026383751 (2002).
- [46] Marquess, F.L.S., Laarveld, B., Van Kessel, A.G., Schmutz, S.M., Wladner, C., Christensen D.: WO04083456 (2004).
- [47] Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, et al. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res* 2002; 12: 222-231.
- [48] Winter A, Kramer W, Werner FAO, Kollers S, Kata S, Durstewitz G, Buitkamp J, Womack JE, Thaller G, Fries R. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. *Proc Nat Acad Sci USA* 2002; 9: 9300-9305.
- [49] Kaupe B, Winter A, Fries R, Erhardt G. DGAT1 polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. *J Dairy Res* 2004; 7: 182-187.
- [50] Weller JI, Golik M, Seroussi E, Ezra E, Ron M. Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. *J Dairy Sci* 2003; 86: 2219-2227.
- [51] Ron M, Cohen-Zinder M, Peter C, Weller JI, Erhardt G. *ABCG2* polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. *J Dairy Sci* 2006; 89: 4921-4923.
- [52] Druet T, Fritz S, Boichard D, Colleau JJ. Estimation of genetic parameters for quantitative trait loci for dairy traits in the French Holstein population. *J Dairy Sci* 2006; 89: 4070-4076.