

DETECTION OF QUANTITATIVE TRAIT LOCI AND

MARKER-ASSISTED SELECTION

PROGRAM

COURSE NOTES AND ABSTRACTS

Sunday, August 24,
17:00 Registration
21:00 Cocktail get-together

Monday, August 25
8.30-10.15 Historical overview - J. I. Weller
10.45-12.30 Physical and genetic mapping, assumptions and scale - J. I. Weller
14.00-15.45 DNA microsatellites, the lab perspective - M. Ron
16.15-18.00 Experimental designs to detect QTL, inbred populations - J. I. Weller

Tuesday, August 26
8.30-10.15 Experimental designs to detect QTL, segregating populations - J. I. Weller
10.45-12.30 Properties of estimators and least squares parameter estimation - J. I. Weller
14.00-15.45 Magic markers and other toys for high throughput genomics - H. A. Lewin
16.15-18.00 Maximum likelihood estimation - J. I. Weller

Wednesday, August 27
8.30-10.15 Bayesian estimation of QTL parameters - J. I. Weller
10.45-12.30 Statistical power to detect QTL - J. I. Weller
14.00-18.00 Half day Tour to Jerusalem

Thursday, August 28
8.30-10.15 Optimization of experimental designs - J. I. Weller
10.45-12.30 The multiple comparison problem, multiple markers - J. I. Weller
14.00-15.45 Software packages for QTL analysis - Heike Kross
16.15-18.00 Multiple traits and multiple pedigrees - J. I. Weller

Friday-Saturday, August 29-30
Tour to the Galilee, overnight at Kibbutz Guest house in the north.

24 August - 4 September 1997

Kibbutz Mizpeh Rachel Guesthouse

JERUSALEM, ISRAEL

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14.00-18.00 Multiple trait analysis - A. B. Korol	
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14.00-18.00 Participant seminars:	
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Moving from QTL experimental results to the utilisation of QTL in current breeding programs - R. J. Spelman	
A model for population growth of laboratory animals subjected to marker assisted introgression: how many animals do we need? - O. D. Koudand	
Exploitation of Halothane gene in selection for pork quantity and quality - C. Hicks	
Towards the identification of trypanotolerance QTLs in cattle - O. Hanotte	
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10.45-12.30 Fine mapping of QTL - M. Soller	
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Forward, theory vs. results

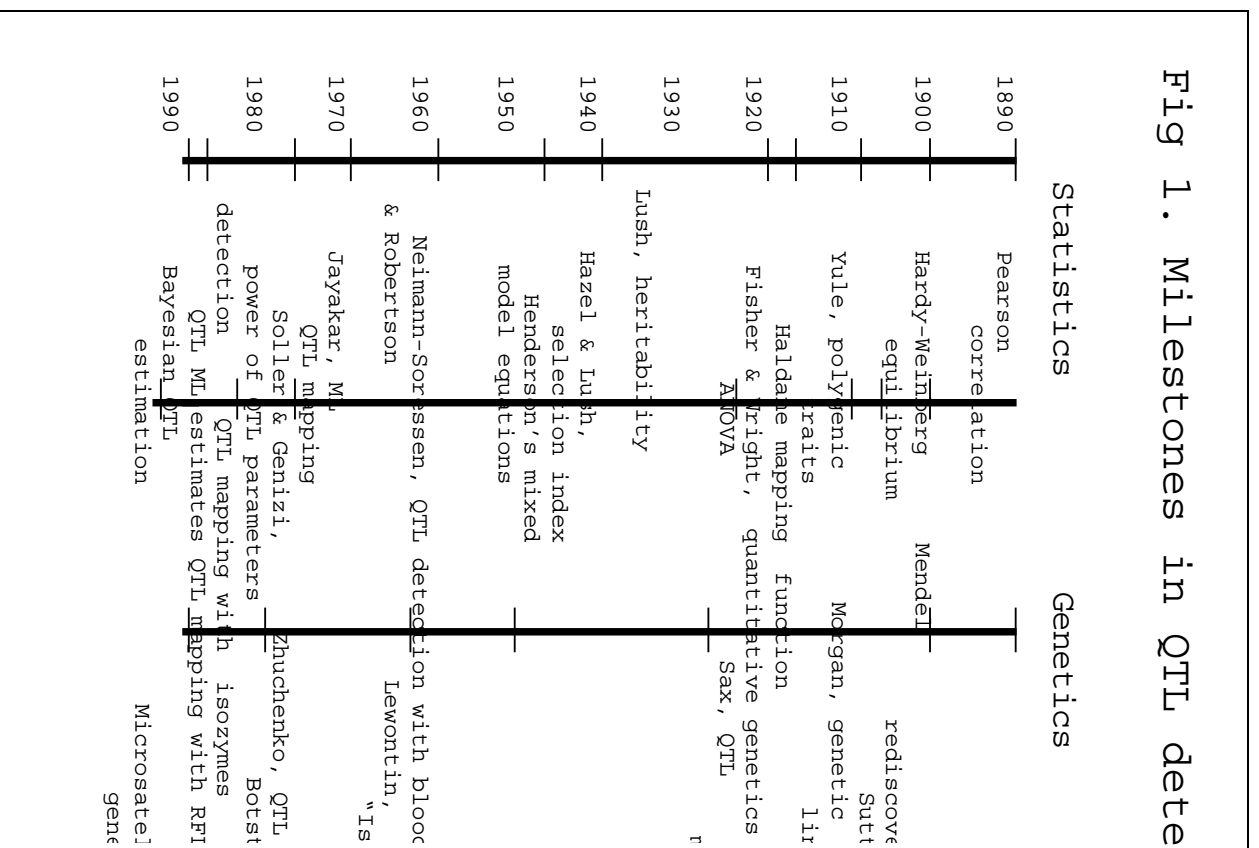
Although detection of QTL has become a "hot" topic during the last decade, the basic principles and methodology have been around since the 1920's, almost immediately after the demonstration of the chromosomal theory of inheritance, and Fisher's polygenic theory of quantitative variance. The first actual experiment was performed by Sax in 1923, and positive results were obtained. This then begs the question, "Why was this methodology more or less ignored for over 60 years?" Of course we must first answer that a number of very fine papers on QTL detection and estimation were written during this period, but no where near the explosion of literature during the past decade. Until 1980 QTL detection was definitely a scientific backwater. Most standard genetic texts written prior to 1980 do not even mention the topic.

The obvious answer is the lack of segregating genetic markers in species of interest. Until the last decade, the genetic markers available were morphological, blood groups, and biochemical polymorphisms. These were insufficient to provide complete genome coverage. In addition, most markers were biallelic with one allele predominating in the population, and many displayed complete dominance. These markers were not optimal for QTL detection. With the advent of DNA level genetic markers, in the early 1980's, and especially DNA microsatellites from 1990, the problem of finding suitable genetic markers can be considered solved. It is now clear that a genetic map saturated with polymorphic codominant Mendelian markers can be generated for almost any species. Nearly saturated genetic maps have already been produced for most species of economic or scientific interest.

Because of the paucity of actual results until 1980, the theory of QTL detection was ahead of experimental results. A number of theoretical papers were written under the premise: "Assuming we had segregating genetic markers in the species of interest, how should we use them?" Most of these studies, based on the current state of knowledge assumed that genetic markers would remain rare and far between. However, the recent explosion in DNA technology has turned the cart around. Experimental opportunities are now ahead of the theory and methodology necessary for analysis. The almost unlimited availability of genetic markers has created new problems not considered by the early theoretical studies.

Although one of the main objective in QTL detection in agricultural species is to incorporate this new source of information in breeding programs, much less has been written on marker-assisted selection (MAS) than on QTL detection. Furthermore, gains from MAS will be minimal. However, most studies have investigated the contribution of marker information into existing breeding programs. As with most new technologies, it will probably be necessary to modify breeding programs to fully exploit MAS. This question can no longer be put off, by saying: "First let's find the genes". Now that we are finding the genes, we must have methodology that gives reasonable answers for application of this information to improve actual breeding programs for plants and animals.

Fig 1. Milestones in QTL detection



Chapter 1. Historical overview

1.1 Introduction

Detection of QTL and parameter estimation required developments in statistics, "classical genetics" or breeding, and biochemistry. The basic theory and tools for QTL detection were all in place by 1923 when Sax did the first experiment with beans. In section 1.2 we will discuss the basic discoveries prior to 1923 that made Sax's experiment feasible. Section 1.3 is a cursory review of the major statistical advances that have had direct bearing on genetics and practical breeding, especially with respect to QTL detection. Section 1.4 considers the major theoretical advances with respect to QTL detection and parameter estimation prior to 1980. Section 1.5 considers the important advances first in biochemistry, and more recently in biotechnology that have resulted in the possibility of unlimited numbers of genetic markers for any species. Section 1.6 compares briefly the scope of the major techniques currently available for QTL localization, including genetic and physical mapping. The major advances of this century pertaining to QTL detection and analysis are summarized in Figure 1.1.

1.2 From Mendel to Sax

Modern genetics is usually considered to have started with the rediscovery of Mendel's paper in 1900. However, there were major advances in both statistics and cytogenetics, prior to this watershed date, that became apparent only later. In the realm of statistics, Pearson in 1890 defined the correlation coefficient, and showed that it could be used to describe the relationship between two variables. During the last decades of the nineteenth century, important advances were also made in cytology. Chromosomes were discovered, and the stages of both meiosis and mitosis were observed and described.

The rediscovery of Mendel's laws led to a rapid first synthesis of genetics, statistics, and cytology. Sutton (1903) and Boveri (1904) first proposed the "chromosomal theory of inheritance," that the Mendelian factors were associated with the chromosomes. Using *Drosophila*, Morgan (1910) demonstrated that Mendelian genes were linked, and could be mapped into linear linkage groups with numbers equal to the haploid number of chromosomes. Harty and Weinberg in 1908 derived their famous equation to describe the distribution of genotypes in a population at equilibrium. In 1919 Haldane derived a formula to convert recombination frequencies into additive "map units" denoted "centimorgans," assuming zero interference.

Despite the early synthesis between Mendelian genetics and cytogenetics, there seemed to be no apparent connection between Mendelian genetics on the one hand, and quantitative variation and natural selection on the other. Experiments by Johansen (1903) with beans demonstrated that environmental factors are a major source of variation in quantitative traits, leading to the conclusion that the phenotype for these traits is not a reliable indicator for the genotype. Yule in 1906 first suggested that continuous variation could be explained by the

cumulative action of many Mendelian genes, each with a small effect on the trait. Fisher in 1918 demonstrated that segregation of quantitative genes in an outcrossing population would generate correlations between relatives. Thus, by 1920, the basic theory necessary to detection of individual genes affecting quantitative traits was in place.

In Sax's 1923 experiment with beans he demonstrated that the effect of an individual locus affecting a quantitative trait could be isolated through a series of crosses resulting in randomization of the genetic background with respect to all genes not linked to the genetic markers under observation. Even though all of his markers were morphological seed markers with complete dominance, he was able to show a significant effect on seed weight associated with some of his markers. This rational behind this experiment will be discussed in more detail in the next chapter.

1.3 Quantitative genetics 1920-1980, or who needs Mendel?

Since the beginning of history, plant and animal breeding was based on selecting individuals with the desired phenotype as parents for the next generation. Comparison between domestic populations and their wild progenitors demonstrate that artificial selection has been quite successful in altering phenotypes without any formal knowledge of genetics. The synthesis between Darwinism and Mendelism was completed in a series of papers by Wright, Haldane, and Fisher from 1924 through 1931 that demonstrated how natural selection could work on Mendelian factors controlling quantitative traits under selection. Fisher also demonstrated that Mendelian factors could explain the phenotypic similarity between relatives. These principles became the basis for scientific breeding of animals and plants from the 1930's onward.

Using the genetic and statistical knowledge accumulated up to 1940 Lush and Hazel developed the principles of selection index to optimize artificial selection based on known relationships among individuals and phenotypic trait information. Selection index proved a remarkable efficient and flexible methodology for practical breeding of plants and animals. Not only could selection be optimized, the expected gains from selection could also be predicted.

Selection index theory had very little connection to Mendelian genetics. The basic model assumed that each quantitative trait was controlled by an infinite number of Mendelian genes all acting in an additive manner. However, nearly identical results would be obtained if the trait was controlled by only a few loci. Only "additive" genetic variation was considered in the basic model. Dominance and epistatic interactions among loci were beyond the scope of selection index.

This biometrical methodology was advanced during the 50's, 60's, and 70's chiefly by C. M. Henderson. Using matrix notation he developed the "Mixed Model" equations combining least squares estimation with selection index in order to derive unbiased estimates of genetic values of individuals sampled in different environments, such as herds or blocks. He also devised methods to derive unbiased estimates of the genetic and environmental variance components required for solving these equations. Finally he developed a simple algorithm for inverting the "numerator relationship" matrix. This made possible the incorporation of information from all known relatives in the derivation of genetic evaluations. None of this methodology, however, required any specific information about the genetic architecture of the traits under selection.

1.4 QTL detection 1930-1980, theory and experiments

During these 50 years, there were relatively few successful detections of marker-QTL linkage in plant and animal populations, and of these even fewer were repeated. A major problem that continues up to today is the relatively small size of most experiments. In most cases in which QTL effects were not found, power was too low to find segregating QTL of a reasonable magnitude (Soller, Brody and Genizi, 1976). During the period 1960-1980 there were important methodological advances in QTL detection and parameter estimation, even though the lack of segregating markers was beyond doubt the main limiting factor for this technology.

In 1961 Neimann-Soressen and Robertson proposed a half-sib design for QTL detection in commercial dairy cattle populations. Although the actual results were disappointing, this was the first attempt to detect QTL in an existing segregating population. All previous studies were based on experimental populations produced specifically for QTL detection. This study was also ground breaking in other aspects. It was the first study to use blood groups rather than morphological markers, and the proposed statistical analyses, a chi-squared test, based on a squared sum of normal distributions, and ANOVA were also unique. This was the first study that attempted to estimate the power to detect QTL, and to consider the problem of multiple comparisons. Law (1965) completed the first successful QTL mapping experiment in an agricultural species. He localized a QTL in wheat using substitution lines.

Jayakar (1970) proposed that maximum likelihood could be used to map QTL. Two years later, Haseman and Elston (1972) proposed a sib pair analysis method for QTL detection in human populations. They also presented a likelihood function to estimate recombination frequency and QTL parameters. Soller, Genizi, and Brody (1976) and Soller and Genizi (1978) estimated power for crosses between inbred lines and segregating populations. For segregating populations they considered large half-sib and full-sib families. Their studies clearly showed that very large samples, generally more than 1000 individuals, were required to obtain reasonable power to detect a QTL explaining one percent of the phenotypic variance.

1.5 From biochemistry to biotechnology, or more markers than we will ever need.

Marker-QTL linkage studies in crosses between inbred lines require markers that distinguish between the lines. In *Drosophila*, strains carrying multiple mutants served this purpose very effectively. However, this is not the case for agricultural species. In plants the only markers initially available were morphological differences. Clearly, these were insufficient to cover the genome. Furthermore, the direct effect on the phenotype of most of these markers was quite dramatic. Thus, even if an effect was found on the trait of interest associated with the marker, it was very likely that this effect was a pleiotropic effect of the marker. In animals marker-QTL linkage studies are generally carried out within populations, and require as markers loci that are polymorphic within the population of interest. Prior to 1980, the only

suitable Mendelian loci were blood groups, which were naturally prevalent in all populations, often multiallelic, and had no visible effect on the phenotype for any traits of interest. However, it eventually became clear that the total number of polymorphic blood loci was quite limited. Thus, blood groups were clearly not a solution for QTL detection in animal populations.

The first biochemical polymorphism was detected for sickle cell anemia by Pauling in 1949. Lewontin and Hubby showed in 1966 that electrophoresis could be used to uncover large quantities of naturally occurring enzyme polymorphisms in *Drosophila*. Almost all enzymes checked showed some polymorphism that could be detected by migration in an electric field. This large quantity of naturally occurring polymorphism created quite a shock, and there seemed to be no obvious explanation. However, later studies with domestic plant and animal species found that electrophoretic polymorphism was much less common. During the 1980's there were a number of QTL detection studies in agricultural plants based on isozymes using crosses between different strains or even species in order to generate sufficient electrophoretic polymorphism (Edwards et al., 1987; Kahler and Wihethahn, 1986; Tanksley et al., 1982; Weller et al., 1988). It was becoming clear though that large quantities of naturally occurring polymorphism could only be detected at the DNA level.

The first detected DNA-level polymorphisms were restriction fragment length polymorphisms (RFLP). Grodzicker et al. (1974) first showed that restriction fragment band patterns could be used to detect genetic differences in virus. Kan and Dozy (1978) used methods developed by Southern (1975) to detect polymorphism near the human hemoglobin gene. The next year Solomon and Bodmer (1979) and Bolstein et al. (1980) proposed RFLP as a general source of polymorphism that could be used for genetic mapping. Although RFLP are diallelic, initial theoretical studies demonstrated that they should be prevalent throughout the genome. Beekmann and Soller (1982) proposed using RFLP for detection and mapping of QTL. The first genome wide scan for QTL using RFLP was performed on tomatoes by Paterson et al. (1988). Since then, many additional QTL mapping studies based on RFLP have been carried out successfully in plant species. In animal species, however, RFLP markers, because of their diallelic nature, have a rather low polymorphism information content, and hence have not been as useful for QTL mapping as was initially anticipated.

A major breakthrough came at the end of the decade with the discovery of DNA-microsatellites. Mullis et al. (1986) proposed the "Polymerase Chain Reaction" (PCR) to specifically amplify any particular short DNA sequence. Using the PCR, large enough quantities of DNA could be generated so that standard analytical methods could be applied to detect polymorphisms consisting of only a single nucleotide. In 1989 three laboratories independently found that short sequences of repetitive DNA were highly polymorphic with respect to the number of repeats of the core element (Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989). The most common of these repeat sequences were poly(TG), which was found to be very prevalent in all higher species. These sequences were denoted "simple sequence repeats" (SSR) or "DNA-microsatellites". Finally the ultimate genetic marker was at hand. Microsatellites were prevalent throughout all genomes of interest. Nearly all poly(TG) sites were polymorphic even within commercial animal populations. These markers were by definition codominant, and were nearly always polyallelic. Thus, most

individuals were also heterozygous. In short, "Just what the doctor ordered!"

1.6 Physical and genetic mapping, questions of scale

The genome can be considered on various levels, and various techniques have been developed for gross and fine mapping of specific sites. The basic units used to measure the genome are DNA base pairs (bp), genes, recombination frequencies or centimorgans (cM), and chromosomes. Although the scales of these units are quite different, the relationship among them are more than a simple question of scale, such as converting meters to inches. The number of chromosomes is known without error, while genome lengths in base pairs and centimorgans can now be determined quite accurately. However, the total number of genes is still just an educated guess. For example, the bovine genome consists of 29 autosomes, about 3000 cM, and 3.75×10^9 bp. The total number of genes has been estimated as 60,000. Thus, the average bovine autosome has about 100 cM. Likewise on the average, a 10 cM chromosomal segment will have about 200 genes, and 1.25×10^7 bp. However there is significant variation in the correspondence between the physical and genetic maps. Furthermore the genetic map also varies among individuals and between sexes.

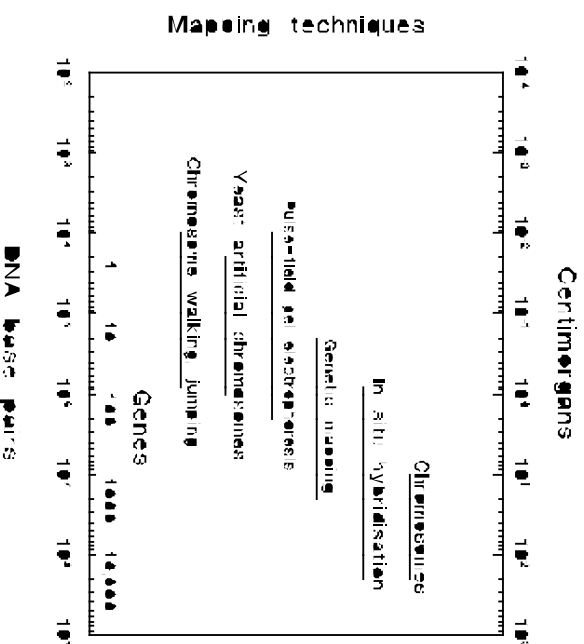


Figure 1.2 Comparison of the bovine physical and genetic maps

The physical genome in bp and the genetic map in cM are compared in Figure 2.1 on a log scale. The effective ranges for genetic mapping and the various techniques that can be used for physical gene mapping are also indicated. Genetic mapping which is the focus of this course occupies the middle ground of the log scale, from about 0.1 to 20 cM, or from 10^5 to 10^7 bp. Genetic mapping cannot be used to identify individual loci, but other techniques are available to accomplish this objective.

1.7 Summary

In this chapter we reviewed the history of QTL detection from the three aspects of statistics, formal genetics and biochemistry. By 1923 genetic theory had developed to the point that it was understood that traits with continuous distributions were controlled by individual Mendelian genes, and that the effects of these genes could be detected with the aid of genetic markers and an appropriately designed experiment. Statistical methods to accurately estimate QTL parameters were only developed fifty years later. Even though all the necessary theory was in place by 1980, QTL detection only became a major field of scientific research towards the end of the 1980's with the discovery of prevalent DNA-level polymorphism. The objective of this course is to describe the statistical methods useful for QTL detection and analysis, thus we will not consider genotyping techniques, or modern methods of physical mapping in detail. We will, however, consider many of the statistical methodologies mentioned above in more detail.

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