Gene Mapping using Linkage Disequilibrium

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Thesis presented for the degree of Doctor of Philosophy

University of Edinburgh

2002

DECLARATION

I declare that this thesis is my own composition and is an account of analyses performed by me whilst studying for the degree of Doctor of Philosophy at the University of Edinburgh.

ACKNOWLEDGMENTS

The Pig Improvement Company International Group (PIC-Sygen) and the Biolotechnology and Biological Sciences Research Council (BBSRC) are gratefully acknowledged for their financial support. Chris Haley, Peter Visscher, Olwen Southwood and Pieter Knap thanks for your supervision, guidance and enlightenment.

I would like to thank Ricardo Pong-Wong for his invaluable help, especially for his advice on Fortran 90, Gibbs sampling and endless discussions of different (scientific and not scientific) issues. Thanks to Dave Waddington for helping me to understand complicated statistical problems. Thanks also to Luc Janss for his brilliant inspiration.

Thanks to Kwan Suk Kim and Max Rothschild for providing the MC4R dataset. Thanks to DEFRA (contract SE1744) and the EC (contract FAIR CT97 3311) for funding the BSE work, and also D. Mathews and J. Wilesmith for help obtaining the BSE samples and Daniel Pomp and Iain Maclean for technical assistance in genotyping.

Thanks to my fellow contemporary students in the Division of Genetics & Biometry, Dimitrios Vagenas, Heli Walhrus and Pau Navarro for their friendship and companionship, and to all staff (Liz, Katrin, Neil, John, Stephen, Valentin, DJ, Pam, Grant, Anthea, Caroline, Geoff) for their support.

And last but not least, I really want to thank my parents, friends and especially Laura for bearing with me all these years of hard work, frustration and, in the end, success.

'The best of science doesn't consist of mathematical models and experiments, as textbooks make it seem. Those come later. It springs fresh from a more primitive mode of thought, wherein the hunter's mind weaves ideas from old facts and fresh metaphors and the scrambled crazy images of things recently seen. To move forward is to concoct new patterns of thought, which in turn dictate the design of the models and experiments. Easy to say, difficult to achieve'

Edward O. Wilson

The diversity of life

LIST OF PUBLICATIONS

Hernández-Sánchez J, Waddington D, Wiener P, Haley CS, Williams JL (2002) Genome-wide search for markers associated with bovine spongiform encephalopathy. Mammalian Genome 13:164-168

Hernández-Sánchez J, Visscher PM, Plastow G, Haley CS (2002) Candidate gene analysis for quantitative traits using the transmission disequilibrium test: the example of the melanocortin 4-receptor in pigs. Genetics (in press)

Hernández-Sánchez J, Haley CS, Visscher PM (2002) Power of association tests to detect QTL using SNP. (submitted)

Hernández-Sánchez J, Haley CS, Woolliams JA (2002) Prediction of identity by descent based on marker information and linked gene flow theory: potential applications for fine mapping quantitative trait loci. (in preparation)

Hernández-Sánchez J, Waddington D, Wiener P, Haley CS, Williams JL (2001) Genome-wide search for markers associated with bovine spongiform encephalopathy. 7th Quantitative Trait Locus Mapping and Marker Assisted Selection Workshop, Valencia, Spain (www.ivia.es/qtlmas)

Hernández-Sánchez J, Haley CS, Woolliams JA (2002) Predicting inbreeding using markers and long-term genetic contributions. British Society of Animal Science, York, UK (www.bsas.org.uk)

Hernández-Sánchez J, Haley CS, Visscher PM (2002) Power of association and transmission disequilibrium tests. 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France (www.wcgalp.org)

ABSTRACT

Treatment of human diseases, study of evolutionary mechanisms, and artificial selection of domestic breeds will benefit from a deeper understanding of the genetic architecture of traits. The chromosomal regions harbouring gene(s) underlying continuous traits are known as quantitative trait loci (QTL). QTL have been mapped analysing marker-trait linkage within families, although with wide confidence intervals. Better QTL mapping resolution, i.e. detecting tighter linkage, is possible utilising population-wide linkage disequilibrium (LD). LD is the correlation between alleles at different linked loci. LD is influenced by evolutionary factors such as drift, selection and mutation. Population admixture and/or stratification can generate widespread spurious disequilibrium (disequilibrium without linkage) that may lead to false positive results, e.g. when comparing cases versus unrelated controls. The transmission disequilibrium test (TDT) is a LD-based method robust to the confounding effects of admixture/stratification. Originally, the TDT was designed to detect segregation distortion of alleles transmitted to affected progeny from heterozygous parents.

The power of QTL detection was studied both empirically and deterministically for several methods. TDT was more powerful than a linkage test, but less powerful than a pure association test. There were no great differences in power between TDTs. One of the TDTs was implemented in BLUP (Best Linear Unbiased Prediction) to study the effect of a candidate gene, the 4^{th} melanocortin receptor (MC4R), on growth, appetite and fatness in pigs. We found significant effects on growth and fatness but not on appetite. TDT uses within families genetic variation. A novel parameter to estimate gene effects using between families genetic variation was also included. If there is no spurious disequilibrium both estimates should be identical, otherwise only the within-families estimator is unbiased. When there was no parental information, it was more powerful to simulate missing parental genotypes with Gibbs Sampling than analysing data with sib-ship TDTs, i.e. ignoring parents. TDT was also used in a genome-wide search for markers associated with bovine spongiform encephalopathy (BSE). TDT was implemented using logistic regressions, more amenable to statistical modelling than the original form. Marker loci near the Prion Protein gene did not show any association with BSE, however, markers located on chromosomes 5, 10 and 20, did. A second study that focused on these three chromosomal regions confirmed the association for the marker on chromosome 5.

TDT has shown reasonable power and exceptional robustness when mapping QTL in structured populations. Therefore TDT should be part of the gene cartographers' continuously evolving arsenal of tools for gene mapping. However, previously published TDTs were developed for analysing human populations, whereas domestic/wild populations have different structures and histories that may require alternative statistical analyses. Linked gene flow (LGF) theory can be used for predicting identity-by-descent (IBD) probabilities between individuals. IBD probabilities are at the core of mixed model equations for mapping QTL in outbred populations via variance components estimation. In this thesis, LGF theory was used for determining inbreeding within each individual and chromosomal location using multi-marker information, hence paving the way for further developments.

CHAPTER 1

1. Literature review

1.1 INTRODUCTION

There are two Mendelian laws in genetics: 1) the gene is the unit of inheritance, and 2) genes segregate independently (Mendel 1866). Sturtevant (1913) and Payne (1918) first reported violations of the second law by demonstrating that some genes are arranged linearly in defined linkage groups. Hence, linkage could explain, for example, the association between seed size and colour in the common bean (Sax 1923), and its absence the lack of association between seed colour and shape in the common pea (Mendel 1866).

Shortly after discovering that the physical structure of genes was a long spiral molecule of deoxyribonucleic acid (DNA) (Avery et al. 1944, Heshey and Chase 1952, Watson and Crick 1953), geneticists found that most of the mammalian DNA contained no genes. The fraction of gene-free DNA was called, rather misleadingly, 'redundant' and 'junk' DNA. We know now that the whole human genetic make-up consists of approximately 31,000 genes, distributed among 23 pairs of chromosomes, constructed with only ~5% of the total 3.2 Gigabases of our genome (Baltimore 2001).

Given this scenario, the task of finding and characterising individual genes may look daunting. Nonetheless, new breakthroughs in statistics and genotyping are allowing us to unravel the puzzles that millennia of evolution have assembled in the form of complex genetic architectures, which are partially responsible for all the observed phenotypic variation.

The best approach in gene mapping consists in comparing the inheritance pattern of a trait with the inheritance pattern of chromosomal regions (Lander and Schork 1994). These chromosomal regions can be identified with DNA markers that act as point labels. Ideally, a DNA marker should be polymorphic, abundant, neutral and codominant (Falconer and Mackay 1996). Marker-based methods have largely superseded marker-free methods, e.g. complex segregation analysis (Elston 1990, Knott et al. 1991a,b), because the former are more powerful and robust than the latter.

The estimate of a gene location is usually a more or less wide chromosomal segment that is likely to contain that gene. When studying continuously distributed traits, these chromosomal segments are called quantitative trait loci (QTL). This thesis focuses on a class of statistical techniques, based on population-wide linkage disequilibrium, which have been able to provide high precision (very narrow) estimates of QTL location under certain conditions.

1.2 LINKAGE DISEQUILIBRIUM MAPPING

Linkage disequilibrium (LD) mapping is the study of marker(s)-trait association across families, as opposed to the study of such associations within families, known as linkage mapping (Hoeschele et al. 1997). In the context of human diseases, Risch and Merikangas (1996) demonstrated that LD mapping could be more powerful than linkage at finding disease genes, especially when the effects are modest, and the disease predisposing allele is at low frequency. On the other hand, Terwilliger and Weiss (1998) argued that LD mapping had worked well for rare and recessive monogenic diseases but that was not likely to work as well for complex traits.

Complex traits are those in which a clear Mendelian segregation pattern between markers and the causal mutation cannot be detected because of factors such as incomplete penetrance, phenocopies, genetic heterogeneity, pleiotropy, epistasis, polygenic inheritance and/or gene by environment interactions (Lander and Schork 1994, Ott 1996, Kruglyak 1997, Schork et al. 1998). Moreover, in addition to Mendelian inheritance, other forms of genetic inheritance may have to be considered, e.g. genetic imprinting (gene effect depends on parental origin), mitochondrial inheritance (genes inherited via maternal lineage), and anticipation (e.g. Huntington's disease becomes more severe as a pedigree develops due to a triplet codon repeat expansion) (Lander and Schork 1994).

One of the main drawbacks of some LD-based tests is that a marker-trait association can arise due to population structure rather than to linkage when using unrelated controls. The transmission-disequilibrium tests (TDTs) overcome this problem by using intra-familial controls (see Zhao 2000 for a review).

The key parameter in LD mapping is the extent of LD in the population. Although it is not necessary to estimate LD in order to map genes, it is useful to know what is LD and how can it be measured.

1.3 FACTORS AFFECTING LINKAGE DISEQUILIBRIUM

Neither the optimal marker density nor the most appropriate study design can be accurately decided without knowing the patterns of LD in the population. LD patterns vary across populations because the different forces shaping LD have probably acted with different intensities across populations. These forces are: genetic drift, population demography, admixture/migration, population structure, selection, recombination, mutation, and gene conversion (Ardlie et al. 2000). Table 1.1 summarises the most important factors determining LD patterns in populations.

Table 1.1 Main factors influencing patterns of linkage disequilibrium

Factor	Main features	Example references
Drift	Creates LD at random	Terwilliger et al. 1998 Hill and Weir 1994
Founder effect	LD is greater in small isolates than in large populations due to inbreeding.	Wright et al. 1999 Lonjou et al. 1999
Population structure	New LD is created by migrations followed by population admixture.	Cavalli-Sforza et al. 1993 Stephens et al. 1994
Selection	LD is maintained by epistasis and hitchhiking.	Zhu et al. 2001 Charlesworth et al. 1993
Recombination	Main factor eroding LD	Goldstein 2001 Johnson et al. 2001
Mutation	Creates new LD	Hill 1975
Gene conversion	Reduces LD due to chromatid exchanges	Przeworski and Wall 2001

1.3.1 Genetic drift

Genetic drift is the random change of allele frequencies over generations in a population (Falconer and Mackay 1996). Drift is more intense in small populations, and increases LD through loss of haplotype diversity. Terwilliger et al. (1998) proposed to identify genes underlying common diseases by drift mapping, i.e. mapping in old populations of small size where LD is more likely to have been produced by drift than by a founder effect. Slatkin (1994) showed that drift mapping may be practicable with the current marker map densities, i.e. extensive LD permits using sparser marker maps. On the contrary, gene mapping using LD methods will not be so effective in large, old, and stable populations where drift is negligible because most of the long-range LD will have disappeared, hence maximum likelihood surfaces for LD will be flat, i.e. uninformative (Hill and Weir 1994).

1.3.2 Population history

Population isolates (e.g. Saami, Inuit) are expected to be genetically more homogeneous than open populations (e.g. New Yorkers) (Jorde 1995, Wright et al. 1999). Genetic homogeneity implies a reduction in residual genetic variation (i.e. genetic variation not caused by the gene under study) and, as a consequence, increases the relative risk ratio or heritability of a candidate gene. The work of Hästbacka et al. (1992, 1994) exemplifies the success of gene mapping in isolated populations. However, population isolates tend to be small, and consequently, there are fewer affected cases, less opportunity for replication, and more stochastic variation than in large populations (Lonjou et al. 1999). Moreover, some studies have reported no differences in the amount of LD between isolates and cosmopolitan populations (Lonjou et al. 1999, Eaves et al. 2000, Boehnke 2000). Population isolates may not be as genetically homogeneous as previously thought with regard to common traits (Terwilliger et al. 1998), and although it can be possible to find even more extremely isolated groups, e.g. Kuusamo community in northeast Finland (Hovatta et al. 1997, Hovatta et al. 1998), their high level of relatedness can easily lead to false positive results, i.e. large chromosomal regions are shared among individuals of that community so any comparison with external controls will almost always show significant differences.

The type of mutations that can be mapped in population isolates also depends on the population history. For example, population isolates that have expanded after a founder event may be more suitable for mapping new mutations, and population isolates that have remained with a constant, and small, size may be more suitable for mapping older mutations (Laan and Pääbo 1997). One could also estimate the age of the mutation (e.g. Guo 1997). The effect of population expansion is to attenuate the effect of drift and to ensure that the pattern of LD is mainly shaped by recombination (Slatkin 1999).

Genetic epidemiological studies are benefiting from the vast amount of data generated by the human genome project, inasmuch as it is used to quantify and describe genetic variation between and within populations (Harding and Sajantila 1998). Comparative studies across populations are useful to unveil genetic heterogeneity, detect gene by environment interactions, and choosing the most homogeneous populations for each specific gene mapping study, especially when they utilise historical, ecological and genetic information (Merriman et al. 1997, Valdes et al. 1997, Stengard et al. 1998, Szabo and King 1997).

1.3.3 Population structure, admixture and migration

An extreme hypothetical case will exemplify well the effect of population structure in association studies. Let *M* and *m* be alleles at a neutral marker, and *Q* and *q* alleles at a QTL where *Q* increases the value of a trait and *q* decreases it. Assume the marker and the QTL are unlinked, and that there are two populations of equal size, one fixed for alleles *M* and *Q*, the other fixed for alleles *m* and *q*. In the absence of admixture, disregarding this population split will result in a positive association between allele *M* and allele *Q*, even though they are completely unlinked.

Migration is very common in human history (Balter 2001, Gibbons 2001), and leads to population admixture or stratification. Admixture can be observed worldwide in the form of clines, i.e. genetic variation on geographical gradients (Semino et al. 1996, Underhill et al. 1996, Jin et al. 1999). At least 5 clear genetic clines have been discovered across Europe. The strongest cline is East-West bound, probably created by the agricultural expansion from the Middle East 10,000 years ago. The second strongest cline is North-South bound, probably created by the retreat of ice sheets 12,000 years ago followed by re-colonisation (Cavalli-Sforza et al. 1993).

Admixture can generate widespread LD. In the first generation after admixture, and assuming random mating, LD is proportional to the allele frequency differences between the parental populations, and independent of genetic distance. Thereafter, LD decays at a rate of (1 - *c*) per generation in a large population, where *c* is the recombination rate between loci. Hence, spurious disequilibrium, i.e. disequilibrium between unlinked loci, is expected to halve each generation. Stephens et al. (1994) concluded that the best scenario for gene mapping in a population created by admixture 3 to 10 generations ago are: 1) markers spaced 10 to 20 cM apart, 2) minimum allele frequency difference between populations of 0.4, 3) not admixture before 3 generations ago so that the level of spurious disequilibrium is low, and 4) minimum sample size of 300 individuals. These conditions can be realistically fulfilled. For example, Wilson and Goldstein (2000) studied the Lemba from South Africa, which claim mixed Bantu and Semitic origin, and found sufficiently strong LD spanning ~20 cM. Dean et al. (1994) used a panel of 257 evenly spaced RFLPs in a comparative study between Caucasian, African American, Asian (Chinese), and American Indian (Cheyenne), and found allele frequency differences ranging from 0.15 to 0.20.

The major concern for gene cartographers that work with admixed populations is the potentially high level of spurious disequilibrium. For example, Knowler et al. (1988) found the Gm haplotype associated with non-insulin-dependent Diabetes Mellitus in a case-control study among Pima and Tohono O'odham native Indians from Arizona. Later, Hanson et al. (1995) demonstrated that Gm was actually indicating Caucasian ancestors, for which the incidence of diabetes is much lower than among native Indians.

Different risk alleles can be associated with different marker alleles across different populations. For example, D'Errico et al. (1996) found extensive evidence in the literature of ethnic differences in association between metabolic gene polymorphisms and various cancers. Finally, high level of inbreeding increases the extent of LD and, hence, makes it more difficult to map with high resolution (Nordborg et al. 2002).

1.3.4 Selection

Selection can increase LD in several ways, e.g. through epistasis, which favours particular combinations of alleles (Zhu et al. 2001), through hitchhiking effect, which sweeps up the frequency of haplotypes containing an allele that increases fitness (Parsch et al. 2001), and through selection against deleterious variants, which reduces haplotype diversity (Charlesworth et al. 1993).

1.3.5 Recombination

The rate of recombination varies across the human genome (Yu et al. 2001) as well as in other taxa (Begun and Aquadro 1992, Tanksley et al. 1992, Nachman and Churchill 1996, Copenhaver et al 1998). Although most gene mapping studies assume a ratio between physical and genetic maps of 1 Mb/cM, in reality this ratio varies from 0 to 9 across the human genome (Yu et al. 2001, Lonjou et al. 1998). Recombination events are highly localised on chromosomal hotspots, separated by regions of low recombination. This phenomenon generates a mosaic-like pattern of LD in humans (Goldstein 2001). For example, Jeffreys et al. (2001) found blocks of conserved LD spanning 60-90 kb within a 216 kb segment in the class II major histocompatibility complex (MHC), and Rioux et al. (2001) found blocks of 10-100 Kb within a region of 500 kb on chromosome 5.

On one hand, this mosaic-like pattern of LD can help association studies because most of the haplotype diversity within blocks can be explained with very few, although carefully chosen, polymorphic markers (Johnson et al. 2001, Daly et al. 2001, Patil et al. 2001). On the other,

it may hinder the localisation of causal mutations because several polymorphisms within a block share similar levels of LD (Svejgaard and Ryder 1994, Rieder et al. 1999, Farrall et al. 1999).

A common assumption is that the locations of different crossovers are independent and identically distributed. Although this assumption may be correct for long distances, chiasma interference is increasingly important in higher resolution mapping studies (Speed et al. 1992). Chiasma interference has been incorporated into mapping functions in different ways, but there is variation in intensity of interference within and between chromosomes, and between species (Crow 1990).

Noor et al. (2001) demonstrated that, in *D. melanogaster*, variation in recombination rate and non-random distribution of genes produces biased results when searching for QTLs, e.g. strong QTL effects were more likely to be detected in regions with low recombination rate and/or high gene density than in regions with low recombination and/or low gene density. Because of this bias, the authors considered that results from QTL studies should be used as hypotheses to be tested by additional genetic methods, particularly in species for which detailed genetic and physical maps are not available.

1.3.6 Mutation

Hill (1975) showed that recurrent mutation at two linked loci increases the observed level of LD when $C = 4N_e c$ is small, but have negligible effects when C is large. In general, the higher the mutation rate, the more LD will be generated per generation. However, highly mutable loci such as microsatellites and CpG dinucleotides are expected to show low levels of LD, even in the absence of historical recombinations (Ardlie et al. 2002). Mutation seems to be a less important cause of LD than gene history, which includes factors such as selection, recombination, and demographic history (Reich et al. 2002).

1.3.7 Gene conversion

Gene conversion is the transfer of very short DNA segments between sister chromatids during meisosis. It would be equivalent to a very tight double recombination event. Its high rate in humans may explain why low levels of LD are sometimes found on regions where only a few recombination events have been observed (Ardlie et al. 2001, Przeworski and Wall 2001).

1.4 MEASURES OF LINKAGE DISEQUILIBRIUM

1.4.1 Linkage disequilibrium D

Let A and a be alleles at one neutral locus, with population frequencies p and $(1-p)$, respectively, and B and b alleles at another neutral locus, with population frequencies q, and $(1-q)$, respectively. If alleles A and a segregate independently from alleles B and b, then alleles A and B will be sampled together with an expected frequency of p times q. Hence, the deviation from expectation is $D = P_{AB} - p\,q$, where P_{AB} is the observed joint frequency of alleles AB. D and other commonly used measures of LD are summarised in Table 1.2. In this thesis, linkage disequilibrium (LD) denotes non-zero D between linked loci, and spurious disequilibrium denotes non-zero D between unlinked loci (see Falconer and Mackay 1996, Lynch and Walsh 1998). LD is useful for QTL mapping purposes, whereas spurious disequilibrium may cause false positive results.

Table 1.2. Measures of linkage disequilibrium

Measure	Main features	Example references
D	Difference between observed and expected haplotype frequencies	Falconer and Mackay 1996 Weir and Cockerham 1989
D'	D as a proportion of its maximum attainable value	Lewontin 1988
r, r^2	Correlation and squared correlation between allele frequencies	Hill and Robertson 1968 Weir 1996
σ_d^2	Expected r^2	Ohta and Kimura 1969 Weir and Hill 1986
Q	Probability of IBD at one locus given IBD at another locus	Sved 1971 Sved and Feldman 1973
C	Combines population size with recombination rate	Ardlie et al. 2002
δ	Robust measure, less sensitive to allele frequencies	Kaplan and Weir 1992 Guo 1997 Morton et al. 2001

Higher-order LD measures involving alleles at three or more loci have also been developed, although their calculation becomes rapidly cumbersome (e.g. Weir 1996). One weakness of D as a measure of LD is that the values it can take depend on allele frequencies, thus D is not adequate for comparing LD between populations with different allele frequencies.

1.4.2 Standardised linkage disequilibrium D'

An alternative parameter is the standardised D (D'), which is the ratio of the observed D to its maximum possible value D_{max}, where $D_{max} = \min(p\,(1-q), (1-p)\,q)$ if $D > 0$, or $D_{max} = \min(p\,(1-p), q\,(1-q))$ if $D < 0$ (Lewontin 1988). D' ranges from -1 to 1 in all populations

regardless of allele frequencies, however two populations with identical D' may nevertheless reflect different levels of LD (N.B. D_{max} may be very different in each population). Moreover, D' estimates can be biased in small samples (Ardlie et al. 2002).

1.4.3 Linkage disequilibrium in finite populations *C*

Ardlie et al. 2002 suggested using $C = 4N_e c$ as a measure of LD because it is not based on pairwise allelic measures, thus it facilitates the comparison between chromosomal regions. However, in practice, C is difficult to measure, partly because N_e depends on certain evolutionary assumptions that are difficult or impossible to prove. Furthermore, the distribution of C is not yet fully understood.

1.4.4 Squared correlation coefficient r²

A better measure of LD is the correlation between alleles in different loci $r = D/\sqrt{p(1-p)q(1-q)}$, because it is less dependent on allele frequencies and less sensitive to small sample size (Hill 1977). The squared correlation coefficient r^2 ranges from 0 to a maximum value of $p(1-q)/(q(1-p))$, which is 1 only when $p = q$ (Weir and Cockerham 1978). Furthermore, the squared correlation r^2 is directly related to the amount of information provided by one locus about the other. For example, it is necessary to increase the sample size $\sim 1/r^2$ to have the same power to detect association at a marker locus as to detect association directly on the susceptibility locus (Ardlie et al. 2002). As a rule of thumb, $r^2 > 1/3$ has been suggested as indicating sufficient LD for gene mapping. However, two tightly linked markers may have very different r^2 values with a third one, and hence r^2 is not necessarily proportional to genetic distance between loci.

A problem with r^2 is that its distribution is not well characterised. Hudson (1985) studied the distribution of the population parameters D, D', r and r^2, and their corresponding sample statistics, via coalescent trees (Kingman 1982), and found that the approximation $E[r^2] \approx \sigma_d^2$, where $\sigma_d^2 = \frac{E[D^2]}{E[p(1-p)q(1-q)]}$, was valid conditioning on polymorphic markers. Moreover, conditioning on minimum levels of polymorphism had two other positive effects on LD measures: 1) they became independent from recurrent mutation, and 2) the likelihood profile of C became more informative. However, Hudson warned that there was not enough information in a sample of two-marker haplotypes to make inferences about C, and Hill (1977) and Hill and Weir (1988, 1994) stated that, for instance, the use of

r^2 to distinguish neutral evolution from selection (Avery and Hill 1979) is questionable due to the large variance caused by evolutionary factors.

Another approximation to E[r^2] was Q, defined as the probability of sampling identical-by-descent (IBD) alleles at one locus given that IBD alleles had been sampled at another locus (Sved 1971, Sved and Feldman 1973)

$$E[Q]=\frac{1-\left[\left(1-1/2N_e\right)(1-c)^2\right]^t}{1+(2N_e-1)c(2-c)} \sim \frac{1-exp\{-(4N_ec+1)t/2N_e\}}{4N_ec+1}$$

where t is the number of generations separating the current population from the founders. The approximation on the right hand side is valid only when c is small and N_e large. As before, letting $t \rightarrow \infty$ so that LD reaches a stable equilibrium between recombination and drift, $E[Q]=1/(1+C)$. Hill (1977) derived the following approximation

$$E[r^2] \approx \sigma_d^2 - S\sigma_d^2\left\{\frac{E[p(1-p)q(1-q)D^2]}{E[p(1-p)q(1-q)]E[D^2]} - \frac{E[p^2(1-p)^2q^2(1-q)^2]}{E^2[p(1-p)q(1-q)]}\right\}$$

which performed better than either σ_d^2 or Q (S is the probability that a population is segregating).

1.4.5 Robust linkage disequilibrium measure: δ

In the context of mapping genes for rare diseases, Devlin and Risch (1995) investigated the statistical properties of five LD measures (D', r^2, δ, Yule's coefficient and the proportional difference d (Kaplan and Weir 1992)) and concluded that the best one was $\delta = D/p_d p_n p_{2n}$, where p_d is the frequency of the disease predisposing allele, p_n the frequency of the normal allele, and p_{2n} the frequency of normal haplotypes with marker allele 2. Morton et al. (2001) reached the same conclusion: δ is the measure of LD most directly related to recombination rate and the least sensitive to variation in allele frequencies. Nevertheless, all five measures were correlated among themselves because they were all functions of δ. Guo (1997) studied the properties of all five measures in the presence of recurrent mutation and incomplete initial LD in the ancestral population. He also found that among all LD measures, δ showed the strongest robustness to changes in allele frequencies, with the proviso that mutation rates were comparatively smaller than recombination rates. However, only with complete initial LD and no mutation is δ uniquely determined by the

recombination fraction, and either high mutation rates or partial LD in the founders reduced the accuracy of prediction of LD in all measures.

1.4.6 Applications of some linkage disequilibrium measures

Chakravarti et al. (1984) used E[$Q(t \rightarrow \infty)$] to show that the recombination rate within the human β-globin gene cluster was not uniform, and that the gene was split in two by a recombination hotspot. A number of RFLPs within the gene were genotyped to calculate pairwise r^2 values, which, under the assumption of no mutation and constant population size, were equated to E[$Q(t \rightarrow \infty)$] to estimate C. Finally, the C estimates were regressed onto physical distance (kb) to obtain $4N_e k$, where k is the recombination rate per kb. It was on the basis of the observed variability in k that Chakravarti et al. (1984) claimed non-uniform recombination rates. However, Weir and Hill (1986) pointed out some potential flaws with the approach of equating an approximated expectation, which is only valid for populations in equilibrium without mutation, to the observed values obtained with small samples, from population that may not be in equilibrium, where mutation cannot be disregarded, and where the possibility of correlated pairwise values of r^2 is high due to the tight linkage between loci. Weir and Hill suggested that one could try to account for the variation due to small samples by adding 1/n to E[$Q(t \rightarrow \infty)$], where n is the number of chromosomes in the sample, or alternatively, using the following formula

$$E[r^2] = \frac{10+C}{22+13C+C^2}\left[1+\frac{(3+C)(12+12C+C^2)}{n(22+13C+C^2)^2}\right]$$

to which Chakravarti et al. (1986) replied that, under the conditions of their study, and especially when $C \geq 2$, the previous equation was well approximated by

$$E[r^2] \approx \frac{1}{2+C}+\frac{1}{n}.$$

Finally, Chakravarti et al. (1986) pointed out that other empirical studies agreed with their conclusions (e.g. Gerhard et al. 1984).

Hill and Weir (1994) also criticised the work of Hästbacka et al. (1992) because the latter estimated c between a marker and the DTD gene (human dwarfism) based on the expectation of LD, regardless of its distribution. Nevertheless, Hästbacka et al. (1994) cloned the DTD gene and showed that their previous theoretical estimate was only 6 kb wrong.

Finally, Kaplan et al. (1995) argued that these two studies (Chakravarti et al. 1984, Hästbacka et al. 1992) may have just been the 'lucky' ones, and many more studies could have been led astray by inaccurate theoretical approaches.

1.5 THE TRANSMISSION DISEQUILIBRIUM TEST (TDT)

1.5.1 Precursors of TDT

Association between a marker and a disease locus can be detected with a case/control study (Schork et al. 2001). Under the null hypothesis (H_0) of no association, the distribution of allele frequencies (or genotype frequencies) is expected to be the same in both groups. The main problem with this type of studies is that a significant association could be spurious if controls are not chosen from within the same genetic group as cases. One solution to this problem is to create internal controls by taking the two marker alleles transmitted to an affected offspring to form a case genotype, and the non-transmitted alleles to form a control genotype (matched tests). Table 1.3 summarises the main features of some TDT precursors.

Table 1.3. Main precursors of the Transmission-Disequilibrium Test

Test	Main features	References
MGRR	Compares transmitted and not transmitted genotypes to an individual	Rubinstein et al. 1981
GHRR	Compares genotypes between cases and controls	Falk and Rubinstein 1987
HHRR	Compares alleles between cases and controls	Terwilliger and Ott 1992 Thompson 1995
McNemar	Compares alleles transmitted and not transmitted to an individual	Terwilliger and Ott 1992

Rubinstein et al. (1981) and Falk and Rubinstein (1987) designed the matched genotype relative risk (MGRR) to test whether the frequency of a marker allele *M* differed significantly between case and control genotypes, N.B. controls are created with the two alleles no transmitted to cases, they are not real individuals. For example, if parents have the genotypes Mm and mm and the case has the genotype Mm, then the conrrol is mm. There is no distinction between a case (or a control) possessing one or two copies of M, and hence homozygote MM and heterozygote Mm cases are given equal weight (Schaid and Sommer 1994). The data for the MGRR is outlined in Table 1.4. Another possibility is to use the genotype-based haplotype relative risk (GHRR) (Table 1.5), where cases and controls are unmatched, i.e. comparing the total number of genotypes with at least one M allele among cases versus that number among controls. Note that entries in Tables 1.4 and 1.5 with the

same notation correspond to the same quantity, and that N denotes the number of nuclear families with single progeny.

$$MGRR = \frac{(B-C)^2}{B+C}$$

$$GHRR = \frac{2N(W-Y)^2}{(W+Y)(X+Z)} = \frac{(B-C)^2}{(2A+B+C)(B+C+2D)/2N}$$

Table 1.4. Table for the matched analysis of transmitted and no transmitted genotypes (MGRR)

Case	Control		Total
	M present	*M* absent	
M present	*A*	*B*	*W=A+B*
M absent	*C*	*D*	*X=C+D*
Total	*Y=A+C*	*Z=B+D*	*N*

Table 1.5. Table for the unmatched analysis of transmitted and no transmitted genotypes (GHRR)

	M present	*M* absent	Total
Case	*W*	*X*	*N*
Control	*Y*	*Z*	*N*
Total	*W+Y*	*X+Z*	*2N*

The difference between MGRR and GHRR is the estimate of the variance of *B-C*. When alleles are independent and the sample is homogeneous with regard to ethnicity, the variance estimated by the unmatched analysis is appropriate and uses more information than the matched analysis, giving a more powerful statistical test.

The problem with MGRR and GHRR is that homozygotes MM and heterozygotes Mm are grouped together, a procedure that losses information. In order to improve the analyses, Terwilliger and Ott (1992) proposed to follow the transmission of alleles, rather than genotypes, from heterozygous parents to affected offspring. Tables 1.6 and 1.7 represent counts for matched and unmatched analyses, respectively (as before, the same entry in both tables corresponds to the same quantity). For example, if a heterozygous parent Mm transmits allele M to an affected progeny then 1 is added to the b score of Table 1.6. Again, the McNemar test is used to analyse the matched data, and the haplotype-based haplotype relative risk (HHRR), also called AFBAC by Thompson (1995), for the unmatched data. The HHRR test is

$$HHRR = \frac{4n(w-y)^2}{(w+y)(x+y)} = \frac{(b-c)^2}{(2a+b+c)(b+c+2d)/4n}$$

$$McNemar = \frac{(b-c)^2}{b+c}$$

Table 1.6. Table for the matched analysis of transmitted and no transmitted alleles (McNemar)			
	Non-transmitted		
Transmitted	*M*	*m*	Total
M	*a*	*b*	*w=a+b*
m	*c*	*d*	*x=c+d*
Total	*y=a+c*	*z=b+d*	*2N*

Table 1.7. Table for the unmatched analysis of transmitted and no transmitted alleles (HHRR)			
Alleles	*M*	*m*	Total
Transmitted	*w*	*x*	*2N*
Non-transmitted	*y*	*z*	*2N*
Total	*w+y*	*x+z*	*4N*

Similarly to the MGRR and GHRR tests, the McNemar and the HHRR tests differed in the estimation of the variance of *b-c*, and the unmatched design was more powerful than the matched one for testing candidate genes or markers in the absence of spurious disequilibrium.

1.5.2 TDT for dichotomous traits

Terwilliger and Ott (1992) recommended the use of HHRR over the McNemar test because the former was theoretically more powerful than the latter. However, they failed to appreciate that the McNemar test is the only valid test in structured populations. A test is valid when it has the correct nominal significance level under H_0. Spielman et al. (1993) referred to the McNemar test as TDT, and were the first to notice its robustness. Ewens and Spielman (2001) showed that the TDT is a valid test for both linkage and association when studying independent family trios, and that it is only valid for testing linkage when studying multiplex families. This is so because even when there is not association at population level, there will be association within multiplex families when marker and disease locus are linked. Table 1.8 summarises some of the main TDTs for dichotomous traits.

Table 1.8. Transmission-Disequilibrium Tests for dichotomous traits		
Test	Main features	References
TDT	McNemar test in Table 1.3	Spielman et al. 1993
TDT_c	Testing the symmetry of cell counts in a multiallelic version of Table 1.5	Bickeboller and Clerget-Darpoux 1995 Sham and Curtis 1995
T_{mhet}	Testing the symmetry of marginal subtotals in a multiallelic version of Table 1.5	Spielman and Ewans 1996
T_s	Improved version of T_{mhet}	Sham 1997
Log-tests	Miscellaneous: allelic risk, maximum likelihood, log-linear models	Clayton and Jones 1999 Zhao 2000 Schaid and Rowland 2000
S	Rao's efficient score statistic	Schaid 1996
$_{max}TDT$	Similar to T_s and T_{mhet} with unknown MOI	Morris et al. 1997a, b
Z_1, Z_{max}	TDT for sibship data and two or more alleles, respectively	Ewens and Spielman 1998 Laird et al. 1998
RC-TDT	TDT for sibship data and reconstructed parental genotypes	Knapp 1999
SDT	Sign test TDT for sibship data and multiallelic markers	Horvath and Laird 1998 Curtis et al. 1999
Z_C	Maximum discordant sib pair TDT	Curtis 1997
AC_2	Sibship TDT	Boenhke and Langefeld 1998
T_{MSTDT}	Uses unaffected sibs as surrogates for missing parental data	Monks et al. 1998
PDT	TDT for extended pedigrees	Martin et al. 2000

Originally, TDT was developed for testing linkage in cases where disease association had already been found. The TDT compares the number of times a marker allele is transmitted from a heterozygous parent to her/his affected child versus the number of times it is not transmitted. Under the H_0 of no linkage $E[b] = E[c]$ (Table 1.6), and TDT is distributed as a χ^2 with 1 degree of freedom. Zhao (1999) re-interpreted the TDT in terms of allele risk ratios, when considering a single parent, or in terms of genotype (multiplicative) penetrance ratios, considering both parents.

Several extensions of the TDT have been proposed for analysing multi-allelic markers. Sethuraman (1997) derived the probability for each cell in a multi-allelic version of Table 1.6 as

$$p = \left\{ \begin{array}{l} (p_1\pi_{11} + p_2\pi_{12})[m_j(m_i p_1 + D_i) - c(m_j D_i - m_i D_j)] + \\ (p_1\pi_{12} + p_2\pi_{22})[m_j(m_i p_2 + D_i) - c(m_j D_i - m_i D_j)] \end{array} \right\} \Big/ K$$

where p_1, p_2, m_i are the frequencies of alleles Q and q at the disease locus, and allele i at the marker locus, respectively; π_{11}, π_{12}, π_{22} are the penetrances of disease genotypes QQ, Qq and qq, respectively; $K = p_1^2\pi_{11} + 2p_1p_2\pi_{12} + p_2^2\pi_{22}$ is the prevalence of the disease in the

population; D_i is the linkage disequilibrium between marker allele i and disease allele Q. The same expressions were used by Sham and Curtis (1995) in their logistic model, and by Morris et al. (1997 b) in their likelihood ratio tests.

Spielman and Ewens (1996) proposed to test for marginal homogeneity with the statistic

$$T_{mhet} = \frac{m-1}{m} \sum_{i=1}^{m} \frac{(n_{i\cdot} - n_{\cdot i})^2}{n_{i\cdot} + n_{\cdot i}}$$

where $n_{i\cdot} = \sum_{j=1}^{m} n_{ij}$, and $n_{\cdot j} = \sum_{i=1}^{m} n_{ij}$, are marginal subtotals in a multiallelic extension of Table 1.6 where the diagonal has been set to zero. Under H_0 of no linkage T_{mhet} should be distributed as a χ^2 with m-1 degrees of freedom, where m is the number of marker alleles. However, Sham (1997) showed that T_{mhet} does not always follow the reference χ^2 distribution by deriving its asymptotic variance and demonstrating that it exceeds $2(m\text{-}1)$ when the frequencies of different heterozygous genotypes in the parents are not the same (N.B. if $u \sim \chi^2_v$ then $\sigma^2_u = 2v$). Instead of T_{mhet}, which can be anticonservative, Sham (1997) proposed to use Stuart's score test T_s (Stuart 1955). The Stuart's score test is $T_s = d'V^{-1}d$, where $d' = [d_1 \ldots d_{m-1}]$, and $d_i = n_{i\cdot} - n_{\cdot i}$, excluding one allele to avoid aliasing. The co-variance structure of T_s is V, with diagonal elements $v_{ii} = n_{i\cdot} + n_{\cdot i} - 2n_{ii}$, and off-diagonal elements $v_{ij} = -n_{ij}$.

Bickeböller and Clerget-Darpoux (1995) tested also the symmetry of the transmission/non-transmission table with the following statistic

$$TDT_c = \sum_{i<j} \frac{(n_{ij} - n_{ji})^2}{n_{ij} - n_{ji}}$$

which, under H_0, follows a χ^2 with $m(m\text{-}1)/2$ degrees of freedom.

Sham and Curtis (1995) expressed both T_s and TDT_c with logistic regressions, which allow more flexibility in statistical modelling than the original tests. However, Miller (1997) found that the distribution of p-values was not uniform under H_0, and proposed a Monte Carlo method to evaluate significant levels. Nonetheless, the flexibility of logistic regressions has appealed to other researchers as well (Jin et al. 1994, Harley et al. 1995, Rice et al. 1995, Waldman et al. 1999).

Schaid (1996) used a conditional likelihood to model offspring genotype as a function of parental genotypes and offspring disease status as follows:

$$P(g_c|g_m,g_f,D)=\frac{P(D|g_c,g_m,g_f)P(g_c|g_m,g_f)P(g_m,g_f)}{\sum_{g^*\in G}P(D|g^*,g_m,g_f)P(g^*|g_m,g_f)P(g_m,g_f)}$$

where D denotes disease status, g_c, g_m, and g_f are the marker genotypes of the affected offspring, mother and father, respectively, and g^* is one of the four possible genotypes G of the child conditional on parental genotypes. Given $P(D|g_c,g_f,g_m)=P(D|g_c)$, then the above equation reduces to

$$P(g_c|g_m,g_f,D)=\frac{r(g_c)}{\sum_{g^*\in G}r(g^*)}$$

where $r(g)$ is the relative risk of disease for genotype g. If g consists of two haplotypes i and j, and assuming a multiplicative model, then $log\ r(g)=log\ r(i,j)=\beta_i+\beta_j$ (Zhao 2000), and, more generally $h[r(i,j)]=\beta_i+\beta_j=\frac{1}{2}\{h[r(i,i)]+h[r(i,i)]\}$ (Clayton and Jones 1999), where h is an unspecified monotone increasing function.

Schaid (1996) proposed the following model

$$log\ r(g)=X'\beta$$

where X is the coded vector for the observed genotype g. The H_0 of no association, i.e. $\beta=0$, can be tested with Rao's efficient score statistic

$$S=U'V^{-1}U$$

where $U=\frac{\partial ln\,L}{\partial\beta}|_{\beta=0}$ and $V_{ij}=-E\left\{\frac{\partial^2 ln\,L}{\partial\beta_i\partial\beta_j}|_{\beta=0}\right\}$.

When the mode of inheritance is unknown, two statistics, the T_s discussed above and the $_{max}$TDT statistic defined as

$$_{max}TDT=\max_i\left\{\frac{(n_{i\cdot}-n_{\cdot i})^2}{n_{i\cdot}+n_{\cdot i}-2n_{ii}},\ i=1,\ldots,k\right\}$$

were powerful alternatives specified by the relative risks of marker genotypes (Morris et al. 1997 a).

All previous TDTs require parental genotype information. The following TDTs were designed to analyse diseases for which parental genotypes may not be available, e.g. late-onset diseases such as Alzheimer's or Parkinson's. The minimum unit of information per family is a pair of discordant sibs with different marker genotypes.

Ewens and Spielman (1998) developed a sib-TDT that was as powerful as the original TDT when there were equal numbers of affected and unaffected progeny per sibship, and less powerful otherwise. For a biallelic marker, this TDT was named Z_1, and for multiallelic markers Z_{max} = max $|Z_j|$, where $j = 1...k$, and k is the total number of marker alleles. The statistic Z_j, for marker allele j, can be found as follows. Let N be the number of sibships, and within each sibship let r be the number of marker genotypes jj, s the number of marker genotypes ij ($i \neq j$), a the number of affected progeny, u the number of unaffected progeny, and t the size of the sibship ($t = a + u$). Hence, using a continuity correction, the Z_j for allele j can be written as

$$Z_j = \frac{|Y_j - A_j| - \frac{1}{2}}{\sqrt{V_j}}$$

where Y_j is the total number of marker alleles j in the data set, $A_j = \sum_{i=1}^{f} [(2r+s)a/t]_i$, and $V_j = \sum_{i=1}^{f} [au(4r(t-r-s)+s(t-s))/(t^2(t-1))]_i$, where f is the number of sibships in the sample. Asymptotically, Z_1 is normally distributed. Significant thresholds can be obtained with permutations for both Z_1 and Z_{max}.

A sample is likely to consist of different type of families, some with and some without parental information. Spielman and Ewens (1998) showed how to combine the original TDT and Z_1 in a unique statistic, although not for Z_{max}. Finally, Spielman and Ewens (1998) and Laird et al. (1998) discussed the similarities and differences of this combination of statistics with the Mantel-Haenszel test, which is commonly used in the joined analysis of several χ^2 contingency tables.

Another way of analysing data without parental genotypes is reconstructing them from genotypes of their progeny. However, this procedure may introduce an error (Curtis 1997, Spielman and Ewens 1998, Knapp 1999). Knapp (1999) developed the reconstruction

combined TDT (RC-TDT), providing necessary and sufficient conditions for the observed marker genotypes in the offspring to allow reconstruction of parental genotypes, and showed that RC-TDT was more powerful than Z_1 and Z_{max}. Parental genotypes could also be reconstructed stochastically via Gibbs Sampling.

Bias can also be introduced when one parental genotype is missing and ambiguous families are discarded (e.g. when the parent and the child have the same *Mm* genotype). Under this circumstance, Curtis and Sham (1995) proposed discarding progeny with genotypes *MM*, *Mm* and *mm*, when the only known parental genotype is *Mm*. Sun et al. (1998, 1999) proposed two new tests to analyse families with one missing parental genotype. The power of these tests was roughly equal to Z_1 and Z_{max} when one affected and one unaffected sibs were sampled, and both needed approximately twice as much records as TDT (Wang and Sun 2000).

All methods described so far are based on comparing affected and unaffected sibs. Teng and Risch (1999) suggested that there is additional information available in the sample from the relative frequency of the different sibship genotype constellations. They showed that two unaffected sibs without parents requires approximately 50% more families than when parents are available, however their strategy of grouping records by combinations of progeny genotypes may be sub-optimum (Zhao 2000).

Horvath and Laird (1998) developed the SDT (sibship disequilibrium test) to analyse families without parental information. Let us assume two alleles, M and m, and let m_A and m_U denote the average number of M alleles in affected and unaffected progeny, respectively. For biallelic markers, the SDT is the following nonparametric sign test on differences d, where $d = m_A - m_U$,

$$SDT = \frac{(b-c)^2}{b+c}$$

where b is the number of sibships for which $d > 0$, and c is the number of sibships for which $d < 0$. For a marker with $k > 2$ alleles, the sign test is

$$T = S'W^{-1}S$$

where $S' = [s^1 \ldots s^{k-1}]$, and $s^j = \sum_{i=1}^{n} sgn\left(d_i^j\right)$ summing across all n sibships, the sign being 1, 0 or -1 when $d > 0$, d = 0 or d < 0, respectively, and W is a matrix with elements

$w_{jl} = \sum_{i=1}^{n} sgn(d_i^j)\ sgn(d_i^l)$. Under H_0 the expectation of S is 0 and T is asymptotically distributed as a χ^2 with k-1 degrees of freedom. The SDT can be combined with the TDT when the data consist of a mixture of families with and without parental information, both for biallelic markers (Horvath and Laird 1998) and for multiallelic markers (Curtis et al. 1999).

Curtis (1997) proposed to choose one affected progeny at random, and then select one unaffected offspring whose marker genotype maximally differs from the affected one. Each marker allele in the affected child is compared against his/her sibs, and if both alleles are the same then they are ignored, but if they are different then ½ is added to T_{ij}, where i denotes the allele from the affected child and j the allele from the unaffected one. For biallelic markers, the test is

$$Z_C = \frac{T_{12} - (N_2 + N_1/2)}{\sqrt{N_2 + N_1/4}}$$

where N_i is the number of sibships that increase the test statistic of either T_{12} or T_{21} by i. For multiallelic markers Curtis used a likelihood model such as in Sham and Curtis (1995), however Monks et al. (1998) found a poor approximation of the likelihood ratio test to the χ^2 distribution.

For markers with $k > 2$ alleles, Boenhke and Langefeld (1998) constructed 2 x k contingency tables where the rows represented disease status. The most powerful way of analysing these tables was ignoring alleles shared between affected and unaffected sibs and only focusing in those alleles from which sibs differed using the following statistic:

$$AC_2 = \sum_{j=1}^{k} \frac{(n_{1j} - n_{2j})^2}{n_{1j} + n_{2j}}$$

where n_{1j} and n_{2j} are counts of marker allele j in affected and unaffected sibs, respectively. Significant thresholds were obtained permuting affection status among sibs.

Monks et al. (1998) proposed the T_{MSTDT} and compared it with Z_c (Curtis 1997), Z_l and Z_{max} (Spielman and Ewens 1998) and AC_2 (Boehnke and Langefeld 1998). They found that, for biallelic markers, these TDTs had similar power, however differences arose when testing multiallelic markers. In general, AC_2 and T_{MSTDT} had similar power in all scenarios; Z_{max} was more powerful than the previous two TDTs when one of the marker alleles was more

strongly associated with the disease allele than the rest of the alleles, and was less powerful when all marker alleles were equally associated with the disease allele; and finally, Z_c was the least powerful test in all situations.

Martin et al. (2000) developed the pedigree disequilibrium test (PDT) as a valid test for linkage disequilibrium when the sample consists of multiple related nuclear families. PDT is based on the average measure of LD calculated across all triads and discordant sib pairs. PDT was more powerful than Z_1 and Z_{max} (Spielman and Ewens 1998) and SDT (Horvath and Laird 1998). PDT gives larger weight to larger sibships and nuclear families within a pedigree, but equal weights to all pedigrees. It may be better to give more weight to more informative pedigrees over less informative ones. PDT also gives equal weight to triad and discordant sib-pairs, however if unaffected sibs may have been misclassified then it would be a better approach to give higher weight to triad than sib-pairs.

Schaid and Rowland (2000) developed a general method for simultaneously estimating linkage and LD based on logistic regressions in multiplex families. This method can detect linkage without LD, but the presence of LD increases the power of detecting linkage because it contains information on parental phases, i.e. haplotypes. The probability of transmitting allele 1 in phase with the disease allele conditional on the phenotype of y of the progeny, e.g. affected, is

$$a(\alpha|y) = \frac{e^{\alpha_0+\alpha_1 y}}{1+e^{\alpha_0+\alpha_1 y}}$$

where α is a vector with linkage parameters α_0 and α_1, which are measures of log-odds of transmission to unaffected and affected progeny, respectively. Under H_0, no linkage, $a(\alpha \mid y)$ = ½, otherwise $a(\alpha \mid y) > ½$. The logit function $log\left[\frac{a}{1-a}\right] = log\, it = \alpha_0 + \alpha_1 y$ can include additional regressors to model age of onset and other covariates. This method can be used also to analyse quantitative and categorical traits. A likelihood function for detecting linkage in a multiplex family can be constructed for each allele of a heterozygous parent. These likelihoods are then weighed by the probability of being in phase with the disease allele (using LD information) and a composite likelihood can be constructed by multiplying all marginal likelihoods across all heterozygous parents. Schaid and Rowland (2000) warned that when allele action is not multiplicative, a residual correlation may arise between the paternal and maternal alleles transmitted to affected progeny, in which case, a correction based on robust covariance matrix estimation is necessary.

1.5.3 Summary of TDT for dichotomous traits

TDT analyses the association between disease occurrence in progeny and transmission of a particular marker allele from parents. Further extensions of the TDT have allowed the analysis of multiallelic markers and/or missing parental genotypes (i.e. sib-TDTs). For biallelic markers, a TDT using parental information can be combined with a sib-TDT. Extended pedigrees can also be analysed after decomposing them into nuclear family units. Although several TDTs have been developed as contingency tables, alternative parameterisations based on linear and log-linear models offer greater flexibility (e.g. correcting simultaneously for fixed effects). Finally, some tests allow for the joint estimation of association and linkage.

1.5.4 TDT for quantitative traits

Many human diseases, e.g. obesity, osteoporosis, and most agricultural traits of interest, e.g. growth, fatness, are continuously distributed. In order to analyse these traits several quantitative TDTs have been developed. Table 1.9 summarises the main TDTs in this section.

Table 1.9. Transmission-Disequilibrium Tests for quantitative traits

Test	Main features	References
$TDT_{Q1\text{-}Q5}$	Tests with and without prior phenotypic ascertainment.	Allison 1997
Multiple regression	Miscellaneous tests involving multiple linear regressions.	George et al. 1999 Xiong et al. 1998 Yang et al. 2000
TDT_R	Correlation between allele transmission and trait	Rabinowitz 1997
T_{QP}, T_{QS}, T_{QPS}	TDT, sib-TDT and TDT that uses sib and parent information, respectively	Monks and Kaplan 2000
TDT_G	Contrast between group means regarding transmitted allele	Xiong et al. 1998 Szyda et al. 1998
S	Permutation-based TDT	Allison et al. 1999
Variance-component TDT	Estimates between and within QTL variances	Fulker et al. 1999 Sham et al. 2000 Abecasis et al. 2000
b_{TD}, b_{PD}	Estimation of between and within (i.e. TDT) QTL fixed effects	Janss (pers. comm.) Hernández-Sánchez et al. 2002

Allison (1997) developed five different quantitative TDTs to analyse samples of family trios. Tests TDT_{Q1} to TDT_{Q4} required trios with a single heterozygous parent, and whereas TDT_{Q1} and TDT_{Q3} assumed random sampling of trios regarding the trait value, TDT_{Q2} and TDT_{Q4}

were analysed extreme phenotypic samples. The preferred test was TDT_{Q5} because: 1) it had a consistently high power under all modes of inheritance, 2) it analysed together families with either one or two heterozygous parents, and 3) statistical modelling was facilitated by the multiple linear regression approach of TDT_{Q5}.

In the TDT_{Q5}, the quantitative trait was first regressed to a dummy variable indicating the type of informative parental genotype combination (i.e. *MM* x *Mm*, *Mm* x *Mm*, or *mm* x *Mm*), and secondly, regressed to the same explanatory variable plus two additional variables encoding allele transmission (one for modelling additive effects, the other dominant effects). TDT_{Q5} is an F-test for comparing how much phenotypic variation is explained with the extended model over the reduced one.

Multiple regression techniques for studying marker-trait associations have also been applied by Xiong et al. (1998), George et al. (1999), Yang et al. (2000), and Zhu and Elston (2001). George et al. (1999) developed a regression model that analysed linkage and association between a marker and a trait using arbitrary family structures. All TDT methods test for linkage in the presence of association, therefore George et al. (1999) suggested testing for linkage only after a significant association had been detected. This method was more powerful than any TDT proposed by Allison (1997). The correlation structure among pedigree members was accounted for by assuming that the residual random effect is composed by two additive and independent random components: a familial effect and an individual-specific residual effect. The familial effect was assumed to lead to a correlation structure such as would be expected, under random mating, from polygenic inheritance. Thus, the residual correlation between a pair of j^{th}-degree relatives is taken to be of the form $f^2/2^j$, where $f^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$, and σ_g^2 is the common sibship variance due to polygenes, and σ_e^2 is the individual residual variance. Zhu and Elston (2001) proposed an alternative regression model, and compared the power of these two regression approaches using simulations.

Yang et al. (2000) estimate the association between a candidate gene and a quantitative trait using linear regression models without adjusting for confounding effects due to population stratification/admixture, and augmented the model with additional regressors that accounted for such confounding effects when estimating the form and strength of the association.

Rabinowitz (1997) developed a TDT based on the correlation between a quantitative trait and the transmission of a particular allele from heterozygous parents to progeny. For a biallelic marker this TDT was T/σ_T , where $T = \sum_{i,j=1}^{n_{ij}} (Y_{ij} - k)(X_{ij}^{f} + X_{ij}^{m})$, and where Y_{ij} is the phenotype of the jth sib in the ith family, X_{ij}^{m} (X_{ij}^{f}) a variable encoding for the transmission of alleles from the mother (father) to the jth sib in the ith family, and k a constant (e.g. population or sibship mean). The denominator is the standard deviation of T. Under the H_0 of no linkage and/or no association, this statistic has a t-distribution with $\sum_{i=1} n_{i\cdot} - 1$ degrees of freedom, where $n_{i\cdot}$ denotes the number of sibs in the ith family. Rabinowitz also considered multiplex families and multiple alleles per marker. This method was generalised to include families with missing parental information (Sun et al. 2000).

When testing a marker with multiple alleles, a researcher could use the maximal TDT to compare the effect of each allele against the overall effect of all others, and focus on the highest score statistic. However, this approach suffers from two drawbacks. First, the number of false positives may increase due to multiple testing. Second, some significant alleles may be missed due to a 'swamping' effect, when they are compared against the 'all others' category that includes both high and low risk alleles (Schaid 1996). Moreover, the maximal TDT has an unknown distribution under the H_0 of no linkage and/or association. Nevertheless, Betensky and Rabinowitz (2000) developed a deterministic method for calculating the upper bound for type I error rates and p-values for the maximal TDT, which was less conservative than the Bonferroni's correction.

Schaid and Rowland (1999) reformulated Rabinowitz's TDT using linear regression and extended it to simultaneously analyse families with and without parental genotype information. L. Janss (pers. comm.) used Rabinowitz's TDT to estimate allele effects within and between families using linear regression. The former estimate was robust to spurious association, and the latter was sensitive to spurious association, hence providing the basis for a test of spurious disequilibrium (Hernández-Sánchez et al. 2002).

In order to use all available information, Monks and Kaplan (2000) proposed to calculate three statistical tests: T_{QP}, T_{QS}, and T_{QPS}. The T_{QP} uses parental genotype information and is identical to the test proposed by Rabinowitz (1997). When no parental information is available, the T_{QS} is calculated using families with at least two sibs having different genotypes. The third statistic, T_{QPS}, combines both T_{QP} and T_{QS}. When different family

structures provided unequal amount of information to these statistics, a permutation procedure was suggested for obtaining p-values. For multiallelic markers, Monks and Kaplan followed the approach of Rabinowitz (1997), and suggested to calculate a maximal statistical test.

Xiong et al. (1998) and Szyda et al. (1998) developed independently the same TDT, called TDT_G by the former authors TDT_G is an extension of the TDT_{QI} (Allison 1997) for multiallelic markers, being as follows:

$$TDT_G = \frac{m-1}{m}\sum_{i=1}^{m}\frac{\left(\overline{Y}_{i\cdot} - \overline{Y}_{\cdot i}\right)^2}{\sigma_i^2}$$

where m is the number of alleles, $\overline{Y}_{i\cdot}$ ($\overline{Y}_{\cdot i}$) is the mean phenotype of the progeny receiving (not receiving) allele i from a heterozygous parent, and σ_i^2 is the variance of the i^{th} difference in the numerator. Deng et al. (2001) used simulations to conclude that polygenes can increase the power of TDT_G. However, this result was an artefact of their simulations as they fixed the total phenotypic variance (the sum of residual, polygenic and QTL variances) so that when the polygenic variance was increased, and assuming a constant QTL variance, the residual variance decreased. Instead, if only the residual and QTL variances are assumed constant, increasing the polygenic variance may reduce the power.

Allison et al. (1999) proposed two statistics for testing the H_0 of no linkage using sibship data. The first statistic was based on the following mixed linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where Y_{ijk} denotes the phenotype of the k^{th} sib with the j^{th} allele in the i^{th} sibship, μ is the overall mean, α_i is a random effect corresponding to the i^{th} sibship, β_j is the fixed effect corresponding to the j^{th} allele, and the interaction $(\alpha\beta)_{ij}$ is also modelled as random. The second statistic was based on permutations and is

$$S = \frac{k-1}{k}\sum_{j=1}^{k}\frac{\left[\sum_{i=1}^{n}\left(\sum_{l=1}^{n_i} Y_{il} - \mu_{ij}\right)\right]^2}{\sum_{i=1}^{n} V_{ij}}$$

where μ_{ij} and V_{ij} are the mean and variance, from the i^{th} sibship and j^{th} allele, respectively. The permutation-based statistic showed, in general, more power than the statistic based on a mixed-linear model, and has the extra advantage of being distribution-free.

Fulker et al. (1999) used variance component methods to test for both linkage and association of markers and QTL in sibships. They partitioned the sibship variation in between and within sib-pair components, and constructed a robust test with the latter component of variation. Sham et al. (2000) concluded that the power of detecting association with this method was proportional to the QTL heritability and the square of LD, and the power of detecting linkage was proportional to the square of the QTL heritability. Therefore, confirming the idea that, when LD is strong, testing for association is more powerful than testing for linkage. Abecasis et al. (2000) extended this method to accommodate any number of sibs, with or without parental genotypes.

1.5.5 Summary of TDT for continuous traits

Continuously distributed traits can be analysed with TDT. Multiple regression techniques have been widely used for this purpose. This statistical framework allows studying general pedigrees (e.g. accounting for the correlation structure among sibs), simultaneous estimation of association and linkage, sibship data, and multiple hypotheses testing. Moreover, TDTs formulated more rigidly (e.g. Rabinowitz 1997) have been re-formulated with multiple regressions (e.g. Schaid and Rowland 1999, L Janss (pers. comm.)). As in the case of dichotomous traits, TDTs that use parental information or just sib information can be combined together in complex pedigrees with several nuclear family structures. Finally, comparing the power across TDTs has become crucial in order to discriminate among such a prolific bibliography.

1.6 LINKAGE DISEQUILIBRIUM MAPPING VIA MODELLING POPULATION HISTORY

Methods of analysis that do not take into account evolutionary variances and covariances, particularly when studying large and heterogeneous populations, may be overoptimistic. For example, Terwilliger (1995) developed a maximum likelihood (ML) method to obtain recombination rate estimates between a marker locus and the hypothetical location of a disease gene by measuring the excess frequency of a marker allele in a sample of affected individuals with respect to the frequency in the whole population. Moreover, this ML

combined information from multiple, and multiallelic, markers per chromosomal region. However, Devlin and Risch (1995) pointed out that, notwithstanding the similarities with the approach of Kaplan et al. (1995), this ML did not explicitly model the evolutionary variance in the population and, as a result, the likelihood profile was too sharp and the confidence intervals for gene location too narrow.

Using historical information in gene mapping studies has been crucial in some cases. For example, the most striking result of gene mapping by LD methods has been the mapping of the Distrophy Dysplasia gene (DTD) (Hästbacka et al. 1992). DTD is a rare and recessive condition characterised by dwarfism, which has a relatively high prevalence in Finland. A previous linkage analysis had located the DTD gene within a broad region of chromosome 5. A finer location of the DTD gene was not possible via linkage analysis because the sample size could not be easily increased. Instead, estimates of recombination rates between markers and the potential DTD locus were obtained by modelling the Finnish demographic history. They assumed the current population had been steadily growing from a small group of individuals that arrived in Finland 100 generations ago. A single copy of the DTD predisposing allele was probably introduced then, and, therefore most of the DTD alleles must be its direct descendents. Affected individuals not only inherited two copies of the same DTD allele, but also common background haplotypes. The Finnish history was modelled using the approach of Luria and Delbruck (1943), which was originally designed to study the exponential growth phase of bacteria. The results were encouraging: the DTD gene was predicted to lie within 70 Kb from the CSF1R marker. Finally, the DTD gene was physically mapped at 64 kb from CSF1R (Hästbacka et al. 1994).

Despite this success, Hill and Weir (1994), Kaplan et al. (1995), and Kaplan and Weir (1995) argued that Hästbacka and co-workers underestimated the upper confidence intervals for the location of DTD. Instead, they modelled the evolution of the haplotype containing the DTD allele as a Poisson branching process, assuming the frequency of normal haplotypes remained constant over generations. The maximum likelihood (ML) estimator of the upper confidence interval was twice as large as that obtained by Hästbacka et al. (1992). Although ML estimators are preferable to point estimators, the Poisson branching model was still based on population assumptions that are difficult to verify. Moreover, explicit derivations of these likelihood functions are very difficult, and the authors used computationally demanding Monte Carlo simulations.

Xiong and Guo (1997) suggested the following approximated likelihood for estimating the recombination rate between a marker and a disease locus, c, assuming the frequency of normal haplotypes (p_n) constant over generations, and modelling the variation in disease haplotypes (p_d),

$$L(p_n, c) \approx L(p_n, \pi) + \frac{1}{2} Tr[L''(p_n, \pi) Var(p_d)]$$

where $E[p_d] = \pi$, and $L''(p_d, \pi)$ is the matrix of second derivatives. Xiong and Guo (1997) compared this approximated likelihood with a first order approximation, consisting of the first term alone, which ignores stochastic changes in the frequency of disease haplotypes over time. The first order approximation coincided with other deterministic methods (Hästbacka et al. 1992, Terwilliger 1995). Moreover, Xiong and Guo (1997) showed how to use information from several linked markers, multiple alleles per marker, and mutations at both markers and disease loci.

Devlin et al. (1996) developed a composite maximum likelihood method (CML) based on maximising the likelihood of –log(δ), where $\delta = \frac{\pi_{11}\pi_{22} - \pi_{12}\pi_{21}}{\pi_{+1}\pi_{22}}$ and π_{ij} is the frequency of haplotypes with marker allele i and disease allele j (j = 1 is the disease-predisposing allele), for each marker, given the recombination rate between marker and disease locus, and multiplying across all markers. Although Devlin and colleagues were able to re-map accurately genes underlying simple monogenic diseases, e.g. cystic fibrosis, DTD, on the basis of published data, there were two main problems with their approach. First, all markers were assumed independent, and second, they assumed a Gamma distribution for –log(δ), which will produce a consistent estimator of variance only if the shape of the distribution is correctly specified (Clayton 2000).

Collins and Morton (1998) and Lonjou et al. (1998) applied the Malecot model, originally derived to describe kinship as a function of distance between populations, to gene mapping, where distance is between marker and disease locus. The decay of association (ρ_i) between marker i and disease locus over generations is predicted with the Malecot model as $\rho_i = (1-L)Me^{-\varepsilon d_i} + L$, where ρ_i = E[r_i], L denotes the level of spurious association, M denotes the probability of monophyletic origin of the disease gene, d_i is the assumed genetic distance between marker i and the disease locus, and $\varepsilon d_i = tc_i$ where t is the number of generations since the disease locus entered the population, and c_i and d_i are the

recombination rate and the physical distance between marker i and the disease locus, respectively. Information from multiple linked markers was combined in a composite likelihood that was a function of the joint distribution of ρ_i (assuming values for L, M, ε), and the allele frequencies at the marker and disease locus. The Malecot model re-mapped accurately major genes, e.g. hemochromatosis, Huntington disease and cystic fibrosis, although it was not tested with complex traits.

The coalescent process (Kingman 1982) has also been used to model the history of a sample of haplotypes either in the context of diseases (Rannala and Slatkin 1998, Thompson and Neel 1997, Graham and Thompson 1998) or quantitative traits (Zhang and Zhao 2001). In the coalescent, a sample of haplotypes is brought backwards in time, allowing the histories of pairs of haplotypes to meet (a coalescent event), and eventually reaches the single common haplotype from which all the current ones descended. Each coalescence event represents either a recombination or a mutation event.

1.7 HAPLOTYPE ANALYSIS AND GENE CLONING

Positional cloning has been defined as the isolation of a gene solely on the basis of its chromosomal location (Lander and Schork 1994). The observed mosaic-like pattern of LD may render association studies powerless for mapping the causal mutation, because long stretches of conserved LD means that several polymorphisms can be strongly associated with a trait. For example, the most studied polymorphism in the gene encoding angiotensin converting enzyme (ACE), which hypothetically contributes to cardiovascular disease (CVD) by controlling blood pressure, has been a 287 bp intronic insertion/deletion called Alu. However, the effects of Alu on CVD have been contradictory (Schmidt et al. 1993, Barley et al. 1996). There is absolute LD between Alu and 17 polymorphic SNPs in a region covering exons 13 to 18 of the gene (Rieder et al. 1999). If a causal mutation were in that area then an association study would find it very difficult to pinpoint it. Farrall et al. (1999) discarded exons 1 to 5 from harbouring the causal mutation by demonstrating that from the 3 monophyletic Alu-deletion haplotypes, 2 were associated with similar ACE level in plasma, and hence, the causal mutation was unlikely to be in the region differing between these two clades. This region was studied by Zhu et al. (2001) and found that a SNP located upstream from the 5' end of the ACE encoding gene explained 6% of the variance of ACE concentration in plasma. In the same study, a second SNP located within exon 17, very close to the Alu site, explained 19% of the variance of ACE concentration. The best model for analysing marker-trait linkage included these two loci and an additive x additive interaction

between them. These two loci were significantly associated with blood pressure, although no evidence for linkage was found. This study highlighted two things, 1) association studies can be more powerful than linkage in regions of high LD, although mapping the causal mutation may still be very difficult, and 2) that interactions (e.g. epistasis, genetic x environment) may play an important role in mapping genes underlying complex traits such as hypertension.

A rare and recessive form of progressive epilepsy may be caused by a single mutation in the ausosomal gene EPM1 (epilepsy mioclonus) (Lehesjoki et al. 1993). EPM1 was first located on chromosome 21q22.3 with linkage analysis. Subsequently, more data were collected and the region potentially locating EPM1 was narrowed down to 7 cM. Further resolution was achieved by studying LD. A haplotype that included alleles from 4 tightly linked marker loci, spanning ~3 Kb, was found in 60% of the cases and only 1% of the controls. That region was embedded within a slightly larger region of conserved LD. Although LD was helpful for narrowing down the resolution of location estimates, physical cloning of EPM1 will need a more direct approach, e.g. sequencing all bases along the 3 kb region.

Even though several other studies (e.g. Copeman et al. 1995, Watkins et al. 1994, Snarey et al. 1994, Yu et al. 1994) used LD for gene mapping in a similar way as shown in the aforementioned studies (e.g. EPM1 and ACE), the physical localisation of causal mutations is still a major undertaking (e.g. Kerem et al. 1989, MacDonald et al. 1991, HDCRG 1993).

Nevertheless, the study of haplotypes, as opposed to single markers, may help refining the location of a causative SNP (Lynch and Walsh 1998). Templeton (1995) suggested creating cladograms, or evolutionary tree, with the observed haplotypes. The rationale is that the closer two haplotypes are, the higher the chance of them sharing the same QTL and, hence, of having similar phenotypic effects. Meuwissen and Goddard (2000) compared the expected covariances between haplotype effects given a postulated QTL position to the covariances found in the data. These expected covariances were proportional to the probability that the QTL is IBD given the marker haplotype information, and were calculated stochastically (i.e. genedropping). This stochastic method was subsequently substituted by deterministic theory based on Sved (1971) (Meuwissen and Goddard 2001). In one application of this method, a QTL for twinning rate in Norwegian cattle was mapped within a 1.3 cM region on chromosome 15, which is substantially narrower than the usual results reported from standard linkage analysis.

Haplotypes are more informative than single markers, for example in obtaining IBD estimates at specific point locations. However, the analysis of haplotypes brings new

problems, e.g. phase construction, high diversity, and optimal haplotype length. Ultimately, the aim of LD mapping must be to estimate as far as possible the ancestry of chromosomes carrying the gene of interest in the current sample, and to place marker mutations and recombinations on this (Clayton 2000).

The total number of SNPs in the human genome exceeds 1.69 million (Chakravarti 1998, Miller and Kwok 2001, The international SNP map working group 2001), accessible at NCBI (www.ncbi.nlm.nih.gov/SNP) and ENSEMBL (www.ensembl.org). Most of these SNPs may be redundant for characterising blocks of conserved LD. Patil et al. (2001) developed a strategy for selecting a minimum number of informative SNPs that could explain most of the haplotype diversity on chromosome 21. They found that from a total of 35,989 SNPs identified in a sample of 20 chromosomes, 2793 SNPs sufficed to identify common blocks, which covered 81% of the 32.4 Mb of chromosome 21. Concentrating in a much smaller region of 135 Kb, but with a much larger sample size than the previous study, and focusing on known genes rather than anonymous regions, Johnson et al. (2001), managed to explain most of the haplotype variation observed with a total of 122 SNPs with just 34 of them. In the future, optimisation algorithms may be developed to facilitate researchers choosing among available SNPs a minimum number that contains most (e.g. 95%) of the LD information, hence reducing statistical thresholds due to multiple testing, diminishing the level of complexity in haplotype analyses, and facilitating the interpretation of results in association studies.

1.8 EXPERIMENTAL DESIGN

There has been a lot of debate about what marker densities, what family structures, what traits, and what populations are ideal for LD mapping in humans. These issues have not been explored yet in an agricultural context.

Based on simulations and assuming a constant demographic expansion since humans left Africa 100,000 years ago, Kruglyak (1999) predicted that LD would be rare beyond ~3 kb. If this was confirmed, at least 500,000 SNPs would be needed in genome-wide scans. On the other hand, Ott (2000) criticised Kruglyak's assumptions, predicting LD over longer distances, and reducing to 30,000 the minimum number of SNPs needed in genome-wide scans. Finally, Jeffreys et al. (2001) speculated that if the pattern of LD on the MHC region (i.e. LD blocks flanked by recombination hotspots) was the norm, then the human genome could be a mosaic of about 40,000 recombinationally suppressed segments of DNA. In this

case, genome-wide association scans would be feasible with 80,000-200,000 SNPs, or an average of 2 to 5 per LD domain.

Brown (1975) showed that relatively large samples were needed to detect LD, however König et al. (2001) suggested that it was possible to reduce sample sizes by up to 20% using optimised group sequential study designs, leading to considerable reductions in cost and time. There are two ways in which a sequential study can be practised: 1) a genome-wide analysis is performed with sparse marker maps, and only those promising regions are followed up by adding new markers, and 2) the sample size is increased sequentially, starting off with a few probands or cases, and increasing the sample only if results are not entirely conclusive in either rejecting or accepting H_0.

Olson and Wijsman (1994) and Chapman and Wijsman (1998) showed that the best scenario for detecting an association between marker and disease loci was the following: 1) perfect haplotype data, 2) 6-8 alleles per marker, especially for weak associations, 3) equal frequency for all alleles at a marker, 4) equal number of cases and controls in case-control studies, 5) rare, recessive, and monophyletic Mendelian diseases, 6) selected samples within genetically homogeneous populations, and 7) young mutations, e.g. not older than 20 generations.

Goldgar and Easton (1997) studied the power for detecting association between a marker and a disease locus *A*, under a two disease loci model (*A* and *B*). They considered different nuclear family structures, and levels of information with respect to locus *B*, taking into account all experimental costs in each scenario. Their conclusions were: 1) family trios (both parents and a single child) were the best family structures to sample, 2) knowing the causal polymorphism at locus *B* enhanced the chances of detecting locus *A*, and 3) recessive loci were easier to map than dominant loci.

Finally, the power of QTL detection depends on the values for the QTL, polygenic and residual variances, for instance polygenic variation can increase the power of association studies when multiple sibs are sampled (Deng et al. 2001). Selection can maintain different patterns of LD, which will impact upon the observed levels of polygenic variance of a trait (Lynch and Walsh 1998). For instance, polygenic variation can be partially hidden, compared to the level expected under random segregation of alleles, when selection favours repulsive disequilibria, i.e. positive and negative alleles alternate on haplotypes (Bulmer 1971, 1976), or it can be increased when selection favours coupling disequilibria, i.e. haplotypes contain either positive or negative alleles (Lynch and Deng 1994).

1.9 QUANTITATIVE TRAIT LOCI MAPPING IN LIVESTOCK USING LINKAGE DISEQUILIBRIUM

The animal breeding industry is being revolutionised by the new advances in genomics, as it has already happened in medicine and plant breeding. The advanced stage of research in model organisms (e.g. *Saccaromices cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus* and *Arabidopsis thaliana*) is now benefiting genomic studies in the main livestock species (e.g. pig, cattle, sheep and poultry) for example in terms of high throughput genotyping technology and comparative mapping (Georges 2001).

The list of monogenic traits being characterised is continuously increasing (www.angis.org.au/Databases/BIRX/omia or http://probe.nalusda.gov:8300/animal/omia.html) but the real challenge lies in characterising complex traits affected by numerous genes interacting with each other as well as with the environment. Although linkage analysis is well established among gene cartographers interested in livestock populations (Haley 1995, Bovenhuis et al. 1997), this approach has rendered very poor resolution (Baret and Hill 1997). On the other hand, LD mapping has already been successful in high-resolution mapping of QTL, albeit sporadically (Farnir et al. 2000, Bink et al. 2000, Kim et al. 2002).

We agree with Baret and Hill (1997) in that 'the transposition of (LD) methods developed in human genetics to livestock is dependent on the choice of studied populations and on knowledge of their genetic history' but disagree in that 'potential applications in livestock are limited to discrete traits in specific populations'. First, nowadays any trait can be studied from a LD perspective. Second, most modern livestock populations originated from a past hybridisation event between very distinct (pure?) breeds, potentially creating extensive LD. Since then, animal breeders have kept some of these populations rather isolated (i.e. genetically homogeneous) and moderately inbred (i.e. low N_e). Recombination, drift, and selection, the latter in a more localised fashion, have shaped current LD patterns. Theoretically, this population history can maintain levels of LD so that medium density marker maps allow genome-wide scans (e.g. at least one order of magnitude less number of markers would be required as in comparable human studies). Moreover, general guidelines (Stephens et al. 1994) and notation (Ewens and Spielman 1995) have been developed in the context of admixture-based gene mapping.

Finally, the rate of genetic progress that can be achieved through selection is determined by four factors: genetic variation, selection accuracy, selection intensity, and generation interval (Falconer and Mackay 1996). Molecular knowledge of QTL can favourably affect each of these factors through MAI (marker assisted introgression; van Heelsum 1997a,b), MAS (marker assisted selection; Hospital et al. 1992, Whittaker et al. 1995), and GAS (gene assisted selection; Pong-Wong and Woolliams 1999).

In conclusion, we believe that LD mapping is a necessary tool for fine mapping QTL in livestock populations that will improve current selection programs. However, the applications of LD mapping have been limited to few theoretical (Du et al. 2002, Grapes et al. 2002, Nsengimana and Baret 2002) and even fewer practical studies (Meuwissen and Goddard 2002, Kim et al. 2002, Hernández-Sánchez et al. 2002) in animal breeding. Nevertheless, we forecast a brighter future for LD mapping in livestock: LD mapping is here to stay.

1.10 OBJECTIVES OF THE THESIS

This thesis focuses on the study and development of statistical tools for high resolution mapping of QTL and disease genes. Linkage disequilibrium (LD) underlies population-wide marker-trait association that can be utilised for fine mapping. The transmission-disequilibrium test (TDT) is a single-marker test of population association that is robust to spurious association (i.e. without linkage). Chapter two compares the power and robustness of three TDTs and two ANOVAs for testing marker-trait association at population level. Power was obtained by means of deterministic formulae and validated with stochastic simulations. Chapter three explores how to implement a TDT within the BLUP-REML framework, which is a more appropriate way of analysing livestock data. Moreover, in Chapter three, allele substitution effects are estimated using within family variation, i.e. with TDT, and between family variation, and shows how statistical differences between these two estimators provide evidence of spurious association. Chapter four uses TDTs in a genome-wide search for genes related to susceptibility to BSE in Holstein-Friesian cattle. Chapter five sets the basis of a novel approach for high-resolution QTL mapping that combines Linked Gene Flow (LGF) theory with IBD-based variance component methods. The LGF theory provides a framework from which to obtain linkage disequilibrium information under the assumption of a common history of the population.

CHAPTER 2

Power of association tests to detect Quantitative Trait Loci using Single Nucleotide Polymorphisms

2.1 INTRODUCTION

Geneticists have been successful in mapping genes underlying rare, monogenic disorders showing a clear pattern of Mendelian inheritance (e.g. Kerem et al. 1989, Hastbäcka et al. 1992, 1994). However, mapping genes underlying complex traits, such as common multifactorial diseases, has been more difficult (Terwilliger and Weiss 1998, Schork et al. 1998). Two broad strategies are currently employed in gene mapping: linkage and association. Although both strategies exploit the cosegregation of markers and phenotypes, there are some striking differences between them. For example, scanning the human genome searching for significant associations could require between 30,000 to 500,000 single nucleotide polymorphisms (SNPs) (Kruglyak 1999, Ott 2000), whereas significant linkage may be detected with just 200 to 400 microsatellites (Neale et al. 1999). The main disadvantage regarding linkage analysis is that the confidence intervals on location estimates of a gene are usually wide (Boehnke 1994), whereas the main disadvantage regarding association analysis is that some tests are not robust to genetic heterogeneity (Wright et al. 1999). Moreover, theoretical work suggested that a genome wide association scan employing every polymorphic marker in the human genome may have greater power to detect complex disease causing polymorphisms than a genome wide linkage scan, even after compensating for the increased number of false positives expected from testing such a large number of markers (Risch and Merikangas 1996). In deriving this conclusion, the authors assumed that the causative polymorphism (or a marker in complete association with it) was one of the markers tested.

Despite being less powerful, family-based association tests, such as the transmission/disequilibrium test (TDT) (Spielman et al. 1993), have been favoured, over non-family-based association tests, e.g. case-control (Clayton 2001, Schork et al. 2000), because the former are robust to spurious disequilibrium generated by population

stratification, or recent admixture. TDT gives the researcher confidence that an observed SNP/phenotype association is not simply a population artefact and, therefore, it would be prudent to confirm any population-level association with a TDT-type statistical test (Long and Langley 1999).

Many epidemiological investigations of the common chronic diseases have focused on continuously distributed risk factors of disease (e.g. ACE concentration levels and blood pressure (Zhu et al. 2001)). Power studies of association tests for quantitative traits will aid to elucidate statistical properties of these tests, to set appropriate experimental designs, and to choose the most powerful, given the conditions of the experiment, among available tests.

Allison (1997) proposed five different TDTs for analysing quantitative traits under different ascertainment conditions. We included the most powerful of these tests, named TDT_{Q5}, in this study, and show how its power can be increased with respect to the original implementation. Long and Langley (1999) studied the power of five non-family based association tests and the TDT_{Q5} proposed in Allison (1997). They showed that TDT_{Q5} was always less powerful than non-family based tests, and that, among the latter tests, it was more powerful to analyse genotypes than either alleles or haplotypes. Nevertheless, these authors also acknowledged that under genetically heterogeneous conditions, such as when population stratification is present, the type-I error rate of non-family based tests can rise above that nominally set for the experiment. Xiong et al. (1998) extended TDT_{Q1} (see Allison 1997), to accommodate any number of sibs per family, any number of heterozygous parents, and any number of alleles at the marker locus, naming it TDT_G (see Szyda et al. 1998 for an application of TDT_G in a cattle population). TDT_G was always more powerful, sometimes substantially so, than TDT_{Q1}, the Haseman-Elston linkage test (Haseman and Elston 1972), and an extreme discordant sib pair test. However, TDT_{Q1} is less powerful than TDT_{Q5} (Allison 1997), which is the test chosen to be studied here. Sham et al (2000) developed approximations for the non-centrality parameters of linkage and association tests in the context of variance-components analysis when data consist of large sib-ships, randomly sampled with regards to a normally distributed trait. They concluded that, for a given experimental design, there could be more power to detect associations within sib-ships than to detect linkage when the effect of the quantitative trait locus (QTL) is small. (N.B. It is also possible to estimate associations between sib-ships, but they are not robust to spurious associations due to population stratification and/or admixture). Page and Amos (1999) compared different TDTs in terms of power via simulations. They showed that, in a population where as much disequilibrium is being created by drift as it is being lost by

recombination, sampling from both extremes of the trait distribution greatly increases the power of another test, the *truncated measured allele* (TMA). However, when corrected for admixture, the TMA was less powerful than TDT_{Q3} (see Allison 1997).

Finally, TDT is valid for testing jointly linkage and association (i.e. linkage-disequilibrium) when analysing independent family trios, where 'valid' denotes that the statistical distribution of the test under H_0 is known. However, when multiplex families are analysed, TDT is not a valid test of association because of the sibs' lack of independence (Ewens and Spielman 2001). Martin et al. (1997) considered the set of transmissions to affected sibs in the whole family, rather than the transmissions to each child separately, thus retaining the necessary independence property. Other studies have also investigated the properties of more sophisticated TDTs when sampling multiplex families (e.g. Monks and Kaplan 2000), or extended pedigrees (e.g. George et al. 1999, Martin et al. 2000). Nevertheless, simpler TDTs may still prove valid for testing association if permutation testing is used, instead of standard statistical distributions, for setting significant thresholds (e.g. Doerge and Churchill 1996).

In summary, we have developed a deterministic approximation to predict power of several association tests, and simulations have been used to validate its accuracy. This methodology is sufficiently general to be used in predicting power of other association tests. In this study, several tests of association, and for some also of linkage, (Table 2.1) have been compared in terms of power, both empirically and deterministically.

Table 2.1. Main features of tests compared in this study

Abbreviation	Testing	[a] H_0	Reference
One-way	Genotype effects	Association	Sokal and Rohlf (1995)
TDT_{Q5}	Genotype effects after correcting for family effects	Linkage and association	Allison (1997)
TDT_R	Allele-phenotype correlation	Linkage and association	Rabinowitz (1997)
TDT_G	Allele means difference	Linkage and association	Xiong et al. (1998), Szyda et al. (1998)
Nested	Genotype effects within family type	Association within family type	Sokal and Rohlf (1995)

[a] The null hypothesis (H_0) for TDT is linkage and association when testing simplex families, but only linkage when testing multiples families (Ewens and Spielman 2001).

2.2 MATERIAL AND METHODS

2.2.1 Tests

The power of five tests to detect both linkage and population-wide association between a marker locus and a QTL was studied empirically (via simulations) and deterministically. These tests were the one-way analysis of variance (one-way), the nested analysis of variance (nested), the TDT_{Q5} of Allison (1997), the TDT_R of Rabinowitz (1997), and the TDT_G proposed independently by Xiong et al. (1998) and Szyda et al. (1998) (Table 2.1). A general deterministic method for predicting power at a linked marker is proposed in this study, and implementation examples are given for one-way, TDT_R and TDT_{Q5}.

One-way ANOVA. The one-way contrasts marker genotype means among all progeny. This is the simplest and most powerful test of association (Long and Langley 1999), although it is prone to high rates of false positive results (type-I errors) in the presence of spurious association, viz. disequilibrium without linkage. This is so because the null hypothesis (H_0) being tested by one-way is *no association, independently of linkage*. Therefore H_0 could still be rejected when testing unlinked marker loci ($c = ½$) if there was a sufficiently strong population wide (spurious) association ($D \neq 0$). This lack of robustness is a drawback common to tests that do not use intra-familial controls, e.g. case-control studies (Shork et al. 2000) although some theoretical solutions to this problem have been proposed (Clayton 2001).

Nested ANOVA. A way of overcoming this problem with one-way is contrasting marker genotype means among progeny within mating types, thus using a nested design. Mating type represents the particular combination of parental marker genotypes within a family. Thus, the H_0 being tested by nested is *no association within mating types*. Nested is expected to be robust to spurious associations created between families. There are six possible mating types regarding a biallelic marker locus (Table 2.2). Nested uses only those families with at least one heterozygous parent (referred to as informative families in what follows) because there must be at least two progeny with different marker genotypes within each mating type for there to be a contrast. This family ascertainment applies to all TDTs as well. However, this type of ascertainment will reduce the number of residual degrees of freedom (residual df) available for testing H_0, and, consequently, a loss of power is expected compared to one-way.

TDT_{Q5}. The original statistic for TDT_{Q5} is $\frac{(SS_F - SS_R)/2}{(1-SS_F)/(n-5)}$ (Allison 1997), where SS_R is the sum of squares explained by a reduced model fitting an overall mean and mating type as fixed factors, hence, at most, estimating three parameters (e.g. the overall mean and two mating type means), and SS_F is the sum of squares explained by a full model fitting the reduced model plus two more factors, one to estimate additive gene effects, and the other to estimate dominant gene effects (hence, at most, estimating five parameters). The total number of informative families is n. Hence, TDT_{Q5} is testing whether a significant amount of phenotypic variation can be explained by marker genotypes in the progeny, over and above the variation already explained by mating type. When the residuals are normally distributed and H_0 is true, TDT_{Q5} follows a $F_{2,n-5}$ distribution. The TDT_{Q5} is equivalent to a two-way cross-classified design analysis of variance (two-way) where the factors are mating type and progeny marker genotype (Appendix c).

TDT_R. The TDT_R is calculated as T/σ_T when the marker is biallelic, where T measures the strength of the covariance between the transmission of an arbitrarily chosen allele from heterozygous parents to progeny and the phenotype of these progeny, and σ_T is the standard deviation of T. We will next describe TDT_R in detail, as this information will be needed later for further statistical developments. The numerator, T, is $\sum_i^n (y_i - \bar{y})w_i$, where y_i is the phenotype of the i^{th} child, $\bar{y}$ is a constant (usually the overall mean, or the mean among informative families), and w_i are weights for each trio given in Table 2.2. The sum is over all n informative trios, assuming them unrelated and having been drawn at random with respect to the phenotype from a wider population. The variance of T is $\sigma_T^2 = \frac{1}{4}\sum_i^n (y_i - \bar{y})^2 H_i$, where H_i is the number of heterozygous parents in a family (Table 2.2). When H_0 is true, TDT_R follows a t-distribution with n-1 degrees of freedom (Rabinowitz 1997).

TDT_G. The last test being considered is TDT_G, that for a biallelic marker is $\frac{(\bar{Y}_M - \bar{Y}_m)^2}{\left(\frac{1}{n_M} + \frac{1}{n_m}\right)S^2}$, where $\bar{Y}_M$ and $\bar{Y}_m$ are the means among progeny having inherited allele M or m from heterozygous parents, respectively, n_M and n_m are the number of times such

parents transmits allele M or m, respectively, and $\left(\frac{1}{n_M}+\frac{1}{n_m}\right)S^2$ is the variance of $\overline{Y}_M - \overline{Y}_m$. Assuming the trait is normally distributed, and the sample size is large, TDT_G follows a χ^2 with 1 df (Xiong et al. 1998, Szyda et al. 1998).

2.2.2 Empirical power

Power was empirically calculated as the proportion of significant results out of 1000 analyses of independent data sets generated under specific combinations of parameter values. Genotypes were generated for all individuals, and phenotypes only in the progeny. We considered a single QTL with alleles Q and q, and frequencies p_Q and p_q. The difference between both homozygous genotype means was 2a, and there was no dominance effect. The residual variance was set to unity, and the polygenic variance to zero (no other QTLs were simulated). A single biallelic marker with alleles M and m, and frequencies p_M and p_m, was simulated linked to the QTL with a recombination rate c. The level of association (correlation) between alleles Q and M was given by the standardised linkage disequilibrium parameter D' (Lewontin 1988). The total number of phenotypic records was n. The specific set of parameter values used will be given in the Results section for each simulation.

Family trios (i.e. father, mother, child), also known as simplex families, are classified with respect to particular combinations of marker genotypes in all three family members. Although the basic unit of information was a family trio, we also investigated the effect on power of including more progeny per family, and varying the number of families. All informative families are treated as independent. Even if multiplex families are decomposed into several simplex families, TDTs are still valid as tests for linkage, although not for association (see Introduction).

2.2.3 Deterministic power

We have developed a compound method with two parts for predicting power of association tests deterministically. The first part consisted in calculating the expected 'apparent' effect of marker genotypes as functions of underlying QTL genotypes, conditional on population parameters and family type. Family type was defined as each particular combination of marker genotypes within family trios. The second part consisted in calculating the non-centrality parameters (λ) as functions of specific genotypic contrasts in each test. The first part of the method can be used to predict power in other association tests, in addition to the

ones in this study. The second part of the method is test-specific, hence needs being calculated in each test.

2.2.4 Expected marker effects

Every family trio can be classified regarding the marker genotypes of its members. For example, there are 10 different types of trios at a biallelic marker (Table 2.2). Let X_j be a vector with the marker genotypes of child, father, and mother in the j^{th} trio, e.g. X_1=[MM, MM, MM] (see Table 2.2). Let G_i denote the i^{th} QTL genotype of the child, i.e. G_1 = QQ, G_2 = Qq, and G_3 = qq. Assuming a biallelic QTL with an additive effect of allele substitution equal to α, and no dominance, the expected phenotype (Y) of a child given the i^{th} trio is

$$E[Y \mid X_i] = \alpha[P(G_1 \mid X_i) - P(G_3 \mid X_i)] \qquad [1].$$

The conditional probabilities $P(G_1|X_i)$ and $P(G_3|X_i)$ can be calculated using Tables A1-A4 in Appendix A (Jayakar 1970, Hill 1975). For example, the probability of QTL genotype QQ given X_1 is

$$P(G_1 \mid X_1) = \frac{P(G_1 \cap X_1)}{P(X_1)} = \frac{h_1^2 p_M^2}{p_M^4} = \frac{h_1^2}{p_M^2}$$

where $P(G_1 \cap X_1)$ is the joint probability of QTL genotype QQ in the child, and marker genotype MM in all members of the family, $P(X_1)$ is the probability of trio type 1, and h_1 is the probability of drawing haplotype QM from the population, which assuming random mating and no segregation distortion, is $h_1 = h_{QM} = p_Q p_M + D_{QM}$ (N.B. $D_{QM} = D' \cdot D_{max}$, and if $D' > 0$ then $D_{max} = \min\{p_q p_M, p_Q p_m\}$ (Weir 1996)). The joint probability $P(G_1 \cap X_1)$ can be obtained from Table 2.A4 by multiplying the third and the sixth columns and adding all up. All the conditional probabilities $P[G_i|X_j]$, for i=1, 2, 3 and j =1...10, are summarised in Table 2.3.

Table 2.2. Variables and probabilities used in TDT_R, and expected marker genotype effects within each type of trio.

Parents	Child	Probability	t	w	het	Effect
MM x MM	MM	p_M^4	*	0	0	b_1
MM x Mm	MM	$2p_M^3p_m$	1	½	1	b_2
	Mm	$2p_M^3p_m$	0	-½	1	b_3
MM x mm	Mm	$2p_M^2p_m^2$	*	0	0	b_4
Mm x Mm	MM	$p_M^2p_m^2$	1	1	2	b_5
	Mm	$2p_M^2p_m^2$	*	0	2	b_6
	mm	$p_M^2p_m^2$	0	-1	2	b_7
Mm x mm	Mm	$2p_Mp_m^3$	1	½	1	b_8
	mm	$2p_Mp_m^3$	0	-½	1	b_9
mm x mm	mm	p_m^4	*	0	0	b_{10}

p_M, p_m: Frequencies of marker alleles M and m, respectively.
t: Transmission indicator (1 if a parent Mm transmits M, 0 otherwise)
w: Weight per trio, $w = \sum het \cdot (t - 1/2)$, summing over both parents.
het: Heterozygosity indicator (1 if a parent is Mm, 0 otherwise)
Effect: Expected marker genotype effects (b) of progeny within trios

Table 2.3. Conditional QTL genotype probabilities in a child, given marker genotypes in the trio, and population parameters D, c, p_M, p_m, p_Q, and p_q.

Father	Mother	Child	QTL gentoype in child		
			QQ	Qq	qq
MM	MM	MM	h_1^2/p_M^2	$2\,h_1h_3/p_M^2$	h_3^2/p_M^2
MM	Mm	MM	$h_1(h_1p_m\text{-}CD)/p_M^2p_m$	$[2h_1h_3p_m+(h_1\text{-}h_3)CD]/p_M^2p_m$	$h_3(h_3p_m+CD)/p_M^2p_m$
		Mm	$h_1\,(h_2p_M+CD)/p_M^2p_m$	$[p_M\,(h_1h_4+h_2h_3)+CD(h_3\text{-}h_1)]/\ p_M^2p_m$	$h_3(h_4p_M\text{–}CD)/p_M^2p_m$
MM	mm	Mm	h_1h_2/p_Mp_m	$(h_1h_4+h_2h_3)/p_Mp_m$	h_3h_4/p_Mp_m
Mm	Mm	MM	$[(h_1p_m\text{–}CD)/(p_Mp_m)]^2$	$2[h_1h_3p_m^2+CD(h_1\text{-}h_3)p_m\text{–}C^2D^2]/\ (p_Mp_m)^2$	$[(h_3p_m+CD)/(p_Mp_m)]^2$
		Mm	$[h_1h_2p_Mp_m+c(1\text{-}c)D^2]/\ (p_Mp_m)^2$	$[(h_1h_4+h_2h_3)p_Mp_m\text{–}2C(1\text{-}c)D^2]/\ (p_Mp_m)^2$	$[h_3h_4p_Mp_m+c(1\text{-}c)D^2]/\ (p_Mp_m)^2$
		mm	$[(h_2p_M+CD)/(p_Mp_m)]^2$	$2[h_2h_4p_M^2+CD(h_4\text{–}h_2)p_M\text{–}C^2D^2]/\ (p_Mp_m)^2$	$(h_4p_M\text{–}CD)^2/(p_Mp_m)^2$
Mm	mm	Mm	$h_2(h_1p_m\text{-}CD)/(p_Mp_m^2)$	$[(h_1h_4+h_2h_3)p_m+CD(h_2\text{-}h_4)]/\ (p_Mp_m^2)$	$h_4(h_3p_m+CD)/(p_Mp_m^2)$
		mm	$h_2(h_2p_M+CD)/(p_Mp_m^2)$	$[2p_Mh_2h_4\text{-}(h_2\text{–}h_4)CD]/\ (p_Mp_m^2)$	$h_4(h_4p_M\text{ -}CD)/(p_Mp_m^2)$
mm	mm	mm	h_2^2/p_m^2	$2h_2h_4/p_m^2$	h_4^2/p_m^2

D: Linkage disequilibrium
c: Recombination rate
p_M, p_m: Frequencies of marker alleles M and m, respectively
p_Q, p_q: Frequencies of QTL alleles Q and q, respectively
h_1, h_2, h_3, h_4: Frequencies of haplotypes QM, Qm, qM, and qm, respectively, where
$h_1=p_Qp_M+D$; $h_2=p_Qp_m\text{-}D$; $h_3=p_qp_M\text{-}D$; $h_4=p_qp_m+D$
Note that, for example, to calculate the second row of probabilities we divide by $p_M^2p_m$ instead of by $2p_M^2p_m$ (see Table 2.2) as we consider one mating type, MM x Mm, but not the reciprocal, Mm x MM (the first genotype corresponds to the father and the second to the mother).

2.2.5 Non-centrality parameters (λ)

The non-centrality parameter for the one-way ANOVA (λ_O) can be obtained applying formula (81) in Searle (1971, p101),

$$\lambda_O \sim \frac{B'X'XB}{\sigma_e^2} = \frac{\sum \mu_i^2 n_i}{\sigma_e^2} \qquad [2].$$

The sum in equation [2] is over all 3 genotype classes MM, Mm and mm, the vector B' contains the three marker genotype means $[\mu_{MM}, \mu_{Mm}, \mu_{mm}]$, and X'X is a matrix with diagonal elements $[n_{MM}, n_{Mm}, n_{mm}]$ and zeroes elsewhere, where n_i is the sample size

corresponding to marker genotype i. Equation [2] represents the sum of squares due to both the marker locus and the sample mean. The appropriate λ_O can be obtained after correcting [2] for the sum of squares due to the sample mean (μ), i.e. $N\mu^2$, where $N = n_{MM} + n_{Mm} + n_{mm}$. In doing so, we are assessing how much variation is explained by a model fiting an overall mean and genotypes as fixed factors over and above a model fiting only the overall mean. When testing the QTL (i.e. conditioning on c = 0, D' = 1, and $p_Q = p_M$), and assuming no dominance, equation [2] simplifies to

$$\lambda_O = N\frac{\sigma^2_{QTL}}{\sigma^2_e} \qquad [3]$$

where $\sigma^2_{QTL} = 2p_Qp_qa^2$ (Falconer and Mackay 1996).

In Appendix C, we have shown that TDT_{Q5} is equivalent to a two-way ANOVA analysis, where data are modelled fitting mating type and genotype as fixed factors, in addition to μ. Taking this equivalence into account, the non-centrality parameter λ_{Q5}, derived in Appendix B, is

$$\lambda_{Q5} = \frac{\sum_{i=1}^{10} b_i^2 n_i I_i - \sum_{j=1}^{6} F_j^2 f_j I_j}{\sigma^2_e} \qquad [4]$$

where b_i is the marker genotype effect in the progeny of class i trios (see Table 2.2), n_i is the number of class i trios, $I_{i(j)}$ is an indicator variable that takes the value 1 when the trio is informative (viz. at least one heterozygous parent), and 0 otherwise, F_j is the mean value of the j^{th} family class, and f_j the number of these families (see Appendix B). Equation [4] measures, in σ^2_e units, the amount of total sum of squares explained by the marker, after subtracting the family effect. When testing the QTL, equation [4] reduces to

$$\lambda_{Q5} = N\frac{\sigma^2_{QTL}}{2\sigma^2_e} = \frac{\lambda_O}{2} \qquad [5].$$

The non-centrality parameter for TDT_R (λ_R) is approximately

$$\lambda_R \approx \left[p_M^2(b_2 - b_3) + p_Mp_m(b_5 - b_7) + p_m^2(b_8 - b_9)\right]\sqrt{\frac{Np_Mp_m}{\sigma^2_e + \sigma^2_{QTL}}} \qquad [6]$$

(Appendix D). When testing the QTL, equation [6] simplifies to

$$\lambda_R \approx a\sqrt{\frac{Np_Qp_q}{\sigma^2_e + \sigma^2_{QTL}}} = \sqrt{\frac{N}{2 + 2\sigma^2_e/\sigma^2_{QTL}}} \qquad [7].$$

Finally, the non-centrality parameter for TDT_G (λ_G) is

$$\lambda_G \approx N\frac{[(1-2C)Da]^2}{p_M p_m\left(\sigma_e^2+\sigma_{QTL}^2\right)} \qquad [8]$$

where D is the usual measure of linkage disequilibrium (Xiong et al. 1998). When testing the QTL in family trios, the appropriate non-centrality parameter is

$$\lambda_G = \frac{N}{2}\frac{\sigma_{QTL}^2}{\sigma_e^2+\sigma_{QTL}^2/2} = \frac{N}{1+\left(2\sigma_e^2/\sigma_{QTL}^2\right)} \qquad [9]$$

(equation 10 in Xiong et al. 1998).

2.3 RESULTS

2.3.1 Empirical power

Empirical power was studied using combinations of three parameter values: allele frequencies at the marker (p_M) and the QTL (p_Q) {$p = p_Q = p_M$ 0.5, 0.3, 0.1}, standardised linkage disequilibrium (D') {1, 0.5, 0}, and a range of recombination rates (c) from 0 to 0.5, in steps of 0.05. Each parameter combination was analysed 1000 times in each test. Two hundred unrelated family trios were randomly sampled with respect to phenotypes and genotypes. The difference between QTL homozygotes was 2a, and there was no dominance.

Table 2.4a shows power of the tests for a given c, whilst averaging across values of D' and p. The most powerful test was one-way ANOVA, followed by TDT_{Q5} (N.B. Employing all family trios), TDT_G, TDT_R, and nested ANOVA. The last row in Table 2.4a (where marker and QTL were unlinked, i.e. c = ½) corresponds to the empirical proportion of false positive results, or type-I error, for each test. The empirical error was expected to be ~5% in all tests. The one-way ANOVA was the only test for which the empirical error exceeded expectations (e.g. ~20%, averaged across D' and p), a fact that has also been documented elsewhere (e.g. Long and Langley 1999). On the basis of this result, one-way ANOVA is not a valid test of association when spurious association (D'≠ 0 and c = ½) is present in the population. As expected, power declined steadily as c increased, because the amount of σ_{QTL}^2 explained a marker decreases as inter-loci distance increases. The ranking of tests regarding power was maintained across all c values.

Table 2.4b shows power given D', whilst averaging across values of p and c. When D' = 1, the power of one-way ANOVA reached ~72% compared to just ~39% for the second most

powerful test (TDT_{Q5}). Undoubtedly, if spurious association is not an issue, significant extra power can be obtained through testing genotype differences directly, as opposed to using robust tests. In the absence of disequilibrium (D' = 0, last row in Table 2.4b), all tests, including one-way ANOVA, showed ~5% false positive results.

Finally, Table 2.4c shows power given p, whilst averaging across values of c and D'. Power decays as allele frequency becomes more extreme because 1) the number of informative families diminishes, and 2) from these informative families, the proportion with one heterozygous parent increases, whilst the proportion with two decreases, and the within family variation, which is the variation exploited by TDT, is expected to be lower in the former than in the latter type of family, these factors mean that, for a fixed QTL effect, σ^2_{QTL} decreases (the trait becomes less genetic). Allison (1997) showed that, for dominant and additive modes of inheritance, power increases as p_M decreases. Note that Allison kept σ^2_{QTL} constant, so when $p_M \rightarrow 1$ (or $p_M \rightarrow 0$) the additive genetic effect (a) of the QTL must increase, rendering greater mean differences between marker genotypes, and hence, more powerful contrasts.

Table 2.4. Empirical power (%) of tests per single parameter.

2.4a. Averaging across D' and p

c	Oneway	TDT_{Q5}	TDT_G	TDT_R	Nested
0	46.5	39.2	38.4	39.7	28.5
0.05	44.6	35.1	33.8	32.5	24
0.1	42.8	31.4	29.3	28.4	20.7
0.15	10.4	26.4	24.7	23.3	16.8
0.2	37.6	22.4	19.2	19.6	13.5
0.25	35.6	17.6	16.1	14.8	10
0.3	33.3	13.5	11.8	10.9	8.4
0.35	30.2	10.4	9.1	8.4	6.4
0.4	26.7	7.8	6.8	6.4	6.1
0.45	23.5	5.8	6	4.8	5.4
0.5	20.5	5.5	5.4	4.8	4.9

2.4b. Averaging across c and p

D'	Oneway	TDT_{Q5}	TDT_G	TDT_R	Nested
1	71.9	38.6	35.5	34.1	25
0.5	27.2	15	14	13.1	9.2
0	5	5.1	5.2	4.7	5.2

2.4c. Averaging across D' and c

p	Oneway	TDT_{Q5}	TDT_G	TDT_R	Nested
0.5	41.4	23.6	21.9	21.4	15.8
0.3	38.6	21.7	19.9	19.5	14.1
0.1	24.1	13.3	12.9	11.1	9.6

c: Recombination rate
D': Standardised Linkage Disequilibrium
p: Frequency of alleles Q and M (assumed equal)
Oneway: Oneway ANOVA
TDT_{Q5}: TDT in Allison (1997); analysis across all trios.
TDT_G: TDT in Xiong et al. (1998)
TDT_R: TDT in Rabinowitz (1997)
Nested: Nested ANOVA

The set of parameters given in Allison (1997) was also used to compare the power of these tests when analysing a QTL. Table 2.5 shows simulation results across σ^2_{QTL} (as a proportion of the total phenotypic variance), p_Q (frequency of the positive effect allele), and N (number of informative trios).

Table 2.5. Power when analysing the QTL, using parameters for which TDT_{Q5} was reported to achieve 80% power (Allison 1997).									
	5% [a]			10% [a]			15% [a]		
p_Q	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5
N	114	219	247	56	105	118	36	67	75
One-way	85.5	91.7	89.9	86.2	88.3	89.9	85.4	89.1	89.9
TDT_R	85.8	85.7	84.5	83.6	84.8	87.2	79.8	83.4	85.9
TDT_{Q5}	79	85.2	79.5	75	76.6	77.4	75.9	77.1	78.8
TDT_G	80.5	81	70	79.2	72	71.4	75.8	71	69.5
Nested	70.8	77	67.6	67.1	64.1	68	68.7	66.2	67.5
[a] Percentage of total variation explained by the QTL p_Q: Frequency of QTL allele Q N: Number of informative trios									

In Table 2.5, one can observe that one-way ANOVA and TDT_R have similar power, likewise TDT_{Q5} and TDT_G. The nested ANOVA was always the least powerful test. In our analysis, TDT_{Q5} was slightly less powerful than predicted by Allison (1997), who obtained 80% power for the same parameters used in Table 2.5.

2.3.2 Empirical versus deterministic power

We developed formulae to obtain the non-centrality parameters of one-way ANOVA (λ_O), TDT_{Q5} (λ_{Q5}) when using only informative trios, and TDT_R (λ_R). Once these λ's are obtained, power can be calculated from the appropriate non-central distributions. The equation for calculating the non-centrality parameter of TDT_G (λ_G) can be found in Xiong et al. (1998). Figure 2.1 shows that predictions of power using our deterministic method (lines) match very well the empirical power (points) obtained via simulations. Power is shown as a function of c for 3 different allele frequencies (0.5, 0.3 and 0.1), denoted with circles (red), triangles (blue), and squares (green), respectively, averaging out D' and p. The non-centrality parameter for nested ANOVA (λ_N) can also be calculated following this method (all necessary elements are given in Appendix B and Table 2.3). In addition to the close match between deterministic and empirical power, two other features in Figure 2.1 are worth mentioning. First, power decayed more when p was dropped from 0.3 to 0.1, than when it was dropped from 0.5 to 0.3. Second, TDT_{Q5} was less powerful than TDT_R, whereas the contrary was true in Table 2.4. This can be explained by the fact that, in Figure 2.1, TDT_{Q5} was implemented as in Allison (1997), where only informative trios are used, and additive

2.3.3 Effect of sampling strategy on power

Different sampling strategies can result in the same number of phenotypes and/or genotypes being collected yet have radically different effects on power. For instance, one may sample *many and small* unrelated families, as when studying humans, or *few and large*, frequently related, families, as when studying domestic plants or animals. Which sampling strategy conveys more power to detect marker-QTL association with TDTs?

For a fixed number of progeny, there are fewer parents sampled with the latter strategy (*few and large* families) than with the former. The link between power and number of parents resides in the relationship between linkage disequilibrium in the parents ($D_{parents}$) and linkage disequilibrium in the progeny ($D_{progeny}$). In a large population, where drift can be assumed negligible, $D_{progeny} = D_{parents}(1-c)$, thus, if $D_{parents} = 0$ then there will be no association between loci. However, the variance of D is proportional to 1/2N (Weir 1996), therefore, even if D = 0 the actual value of D in any small sample is likely to be different from zero.

The effect of sampling strategy on power was studied via simulations. Assume a biallelic marker completely linked (c = 0), and in linkage equilibrium (D' = 0), with a biallelic QTL that explains 15% of the total phenotypic variance. Moreover, let us compare two very different sampling schemes: 1) each one of two unrelated males is mated to two unrelated females, and 75 progeny are born in each full-sib family (this mating system may be thought of as resembling a cattle breeding program), and 2) there are 150 nuclear families with 2 progeny each. In both cases 300 progeny were phenotyped and genotyped. In the former case only six parents were genotyped, whereas in the latter 300 parents were genotyped. Furthermore, assume that at least one parent in each family is heterozygous (i.e. all families are informative).

Table 2.6. Effect of sampling strategy on power of TDT

Strategy	One-way	TDT_R	TDT_{Q5}	TDT_G
I	49.1	64.5	42.9	42.3
II	6.2	5.1	5.6	5.5

Strategy I: Two unrelated males, each mated to 2 unrelated females, and 75 progeny per family
Strategy II: 150 nuclear families, and 2 progeny per family
Parameters: pM=pQ=0.5, c=0, D'=0 and a QTL effect explaining 15% of the phenotypic variance

Table 2.6 shows striking power differences in each test that can be explained by the sampling strategy. It is expected that association tests will not detect significant effects at a

marker locus if the population is in linkage equilibrium (i.e. D' = 0), even if this marker is completely linked (i.e. c = 0) to a gene that explains as much as 15% of the total phenotypic variance. However, there was a 10-fold increase in power when 6 parents where sampled (i.e. sampling 2 half-sib families twice), compared to the power when 300 parents were sampled (i.e. 150 full-sib families). The variance of linkage disequilibrium in the parental population, $D_{x\ parents}$, where x is the number of parents sampled, was ~50 times greater when x = 6 than when x = 300, e.g. $\sigma^2(D_{6parents}) \sim 1/6$ and $\sigma^2(D_{300parents}) \sim 1/300$, thus $\sigma^2(D_{6parents})/\sigma^2(D_{300parents}) \sim 50$. A large $\sigma^2(D_{6parents})$ meant that in any particular replicate values of $D_{6\ parents}$ very different from zero were likely, and hence, the sample analysed with TDT was no longer in linkage equilibrium.

The second striking feature in Table 2.6 is the different ranking of tests in terms of power. When only informative trios were analysed, TDT_R was the most powerful test, even ahead of one-way ANOVA. It is now clear that most of the advantage in power of one-way ANOVA with respect to TDT is that the latter cannot use families with two homozygous parents, and therefore analyse less data than one-way ANOVA after ascertainment has taken place. In Table 2.5, TDT_{Q5} was parameterised as in Allison (1997). However, such parameterisation is slightly less powerful than the one we proposed above and, hence, any power difference between TDT_{Q5} and TDT_G or TDT_R disappeared.

2.4 DISCUSSION

Complex traits, such as obesity and osteoporosis, are determined by multiple genetic factors (also known as quantitative trait loci or QTL), environmental factors, and potential interactions between both. These complex traits can be studied measuring quantitative intermediate risk factors such as blood pressure, cholesterol level, or bone mineral density. The greatest burden, both economically and in terms of human workload, for national health services is due to multifactorial disorders, such as infectious and parasitic diseases (23.4% of world-wide burden), neuropsychiatric disorders (11.5%) and cardiovascular diseases (10.3%), for which a polygenic component is usually hypothesized (The World Health Report 1999). On the other hand, monogenic diseases, although rare in humans, are comparatively less problematical to study from a genetic standpoint. For example, the most common monogenic disease among Caucasians is cystic fibrosis, affecting approximately 0.5-0.6 births every 1000, followed probably by phenylketonuria, which affects 0.2-0.5 births every 1000, all other monogenic diseases are even rarer (Underwood 1996).

Zhao (2000) comprehensively reviewed the family-based association methodology developed in the 1990s, providing more than 60 references of association tests for diseases caused by a single gene with clear pattern of Mendelian inheritance, and approximately six time less references of association tests for complex (i.e. quantitative) traits. It is rather paradoxical that most of the latest advances in statistical theory for gene mapping have focused on a relatively rare group of diseases in humans (e.g. Hastbäcka et al. 1992).

The transmission disequilibrium test (TDT) is a test for linkage and/or linkage disequilibrium that is being increasingly used to identify QTLs underlying complex diseases (Schaid 1998). TDT can be more powerful than other tests, e.g. affected-sib-pair linkage analysis, when markers are very close to responsible QTLs (Risch and Merikangas 1996). In addition, TDT is robust to spurious associations (Stephens et al. 1994) generated by common demographic events such as population stratification and/or admixture (e.g. Wilson and Goldstein 2000, Reich et al. 2001).

In this study, we have compared the power of three TDTs and two ANOVAs (analysis of variance) in detecting association between a marker and a QTL. We have assumed that both loci were biallelic, and shared the same allele frequencies. Moreover, we considered a continuously distributed trait genetically determined by a single additive QTL, without a polygenic component and no dominance. This simplistic scenario was chosen to facilitate the derivation of deterministic equations for predicting power. Nonetheless, we recognise that in order to get a more comprehensive picture of the properties of these TDTs, more realistic situations will have to be explored. For example, Page and Amos (1999) and Deng et al. (2001) concluded that polygenes have a negligible effect on power when using family trios. The latter authors concluded that, when using multiplex families, polygenes increase the power of TDT_G, and that the larger the family, the more the power of TDT_G increases compared to a case without polygenes. However, the change in power due to the presence of polygenic variance depends on the assumptions made regarding the size of the variance components, and their distribution within and between families. For example, assume a model where the phenotypic variance is the sum of QTL, polygenic, and residual variances, i.e. $\sigma_p^2 = \sigma_{QTL}^2 + \sigma_a^2 + \sigma_e^2$. Then, for the sake of simplifying mathematical modelling, for a fixed value of σ_{QTL}^2 one can fix either σ_p^2 or σ_e^2. Fixing σ_p^2, as in Deng et al. (2001), leads to the conclusion that polygenes increase power of TDT. If, for example, we sampled unrelated families with multiple progeny, then σ_a^2 would be equally distributed within and

between families, and σ_e^2 would reduce by σ_a^2 to keep σ_p^2 constant. Hence, the residual variance used in TDT analyses, i.e. the within family variance unexplained by the QTL, would be $\sigma_e^2 - \frac{\sigma_a^2}{2}$. If there are no polygenes acting on the trait then the power of TDT reduces because the residual variance used in TDT analyses will be σ_e^2 as opposed to $\sigma_e^2 - \frac{\sigma_a^2}{2}$. However, if σ_e^2, instead of σ_p^2, is fixed, the presence of polygenic variance will lead to a reduction of the power of TDT because the residual variance used in TDT analyses will be $\sigma_e^2 + \frac{\sigma_a^2}{2}$, as opposed to σ_e^2 when there is no polygenic variance.

Power was predicted via empirical simulations and deterministic equations, and both methods rendered very similar answers. The advantages of deterministic methods over stochastic ones are 1) ease of implementation, 2) instant predictions, and 3) direct appreciation of the relationship between population parameters and power. However, in complex scenarios where deriving deterministic methods becomes cumbersome, empirical simulations may be invaluable. The deterministic method proposed in this study consists in deriving non-centrality parameters (λ's) as functions of marker genotype contrasts specific to each test. These λ's can subsequently be used as input in computer routines to obtain power. A common feature across all λ's was the use of expected marker genotype means, conditional on family information, under the assumptions of random mating and no segregation distortion. These marker effects were functions of the standardised linkage disequilibrium (D'), the recombination rate (c), the allele frequencies (p_Q, p_M), and the additive gene effect (a). Allison (1997) derived a prediction of λ for TDT_{Q5}, simulation shows that this derivation can be considerable less accurate than that derived here. Rabinowitz (1997) derived λ for TDT_R although using parameters not included in his simulations, leading to some confusion in terms of interpretation and calculation of λ.

We have shown that our method is accurate and reliable, and that it contains a general part (Table 2.3) that can be used to calculate λ for other association tests. Moreover, our method can readily include dominant QTL effects, and a polygenic component. Extensions to cope with multiallelic markers are theoretically possible, however, future association studies in human populations are more likely to employ vast arrays (e.g. microarrays) of biallelic single nucleotide polymorphisms (SNPs) rather than multiallelic markers (Risch and Merikangas 1996, Weiss and Terwilliger 2000, Miller and Kwok 2001). Therefore, we think further

developments of this method ought to be directed, for instance, to cope with the problem of simultaneous testing of several loci (e.g. Goldgar and Easton 1997), and the study of haplotypes, rather than extending it to use multi-allelic loci.

The one-way ANOVA was usually the most powerful way of testing population association, followed by the TDTs and, finally, by the nested ANOVA. However, one-way ANOVA was also the only test having higher than expected levels of false positive results in the presence of spurious association (i.e. association without linkage). The TDTs were very similar in terms of power. However, the power of TDT_{Q5} was slightly improved by analysing all families, regardless of the number of heterozygous parents, without altering the type-I error rate in the presence of spurious association. In doing so, more data can be used to estimate the error mean squares, thus augmenting the residual degrees of freedom of the test. A further improvement in the power of TDT_{Q5} was still possible by dropping dominant effects off the model (e.g. when they seem negligible), and estimating only additive gene effects. In doing so, the numerator degrees of freedom of the test were reduced from 2 to 1. Although Xiong et al. (1998) showed that TDT_G is more powerful than TDT_{Q1} (Allison 1997), we considered that it would be fairer to compare TDT_G versus TDT_{Q5} because, as Allison pointed out, TDT_{Q5} is more powerful than TDT_{Q1}, and because, as we have shown, the power of TDT_{Q5} can be easily increased. In addition, Deng et al. (2001) noticed that analytical power results showed in Tables 1-3 in Xiong et al. (1998) were overestimates, although their deterministic formulae were correct.

Power was also affected by family structure. For a fixed number of phenotyped progeny, sampling multiplex families reduces the number of parents available for genotyping compared to sampling simplex families (i.e. trios). Imprecise estimates of parental linkage disequilibrium ($D_{parents}$) are expected when very few families are sampled because the variance of $D_{parents}$ is inversely proportional to twice the number of genotyped parents (viz. linkage disequilibrium, D, extends further chromosomal distances within families than across the entire population). This means that even when the population is in linkage equilibrium, single families may still show high (or low) $D_{parents}$ levels. Given a sufficiently large number of progeny $D_{progeny} = D_{parents}(1\text{-}c)$, and $D_{progeny}$ is a key parameters determining the power of TDT. Hence, even though power may increase, the mapping resolution is likely to decrease, i.e. a QTL may be equally associated with close and distant markers. Indeed, linkage analysis, a gene mapping method known to have low resolution (Boehnke 1994), is the limiting case where associations are investigated only within families. In our simulations, markers were found in association with a QTL when four large multiplex families were

sampled, despite having simulated a population in equilibrium. The same association was not detectable when 150 simplex families were sampled from the same population.

In summary, a new and accurate method has been developed for obtaining deterministic predictions of power of ANOVAs and TDTs for quantitative traits. We have shown that TDT_{Q5} is equivalent to a two-way ANOVA, where mating type and progeny genotype are the two factors in the model, and how a simple modification of TDT_{Q5} can increase power. Finally, we have shown that TDT_R may be the test of choice in certain circumstances, e.g. when the sample consists of multiplex families.

2.5 APPENDIX A: PROBABILITY OF QTL GENOTYPES OF A CHILD GIVEN MARKER GENOTYPES IN THE FAMILY TRIO

Table 2.A1. Probabilities of 4 parental haplotypes and expected frequency of QTL genotypes in progeny given Mm and Mm parents and MM, Mm or mm progeny.

Parents		Child→	MM			Mm			mm		
Mm	Mm	Prob	QQ	Qq	qq	QQ	Qq	qq	QQ	Qq	qq
QQ	QQ	$h_1^2h_2^2$	1	0	0	1	0	0	1	0	0
QQ	Qq	$h_1^2h_2h_4$	1-c	c	0	½	½	0	c	1-c	0
QQ	qQ	$h_1h_2^2h_3$	c	1-c	0	½	½	0	1-c	c	0
QQ	qq	$h_1h_2h_3h_4$	0	1	0	0	1	0	0	1	0
Qq	QQ	$h_1^2h_2h_4$	1-c	c	0	½	½	0	c	1-c	0
Qq	Qq	$h_1^2h_4^2$	$(1-c)^2$	$2c(1-c)$	c^2	$c(1-c)$	$c^2+(1-c)^2$	$c(1-c)$	c^2	$2c(1-c)$	$(1-c)^2$
Qq	qQ	$h_1h_2h_3h_4$	$c(1-c)$	$c^2+(1-c)^2$	$c(1-c)$	½ $[c^2+(1-c)^2]$	$2c(1-c)$	½ $[c^2+(1-c)^2]$	$c(1-c)$	$c^2+(1-c)^2$	$c(1-c)$
Qq	qq	$h_1h_3h_4^2$	0	1-c	c	0	½	½	0	c	1-c
qQ	QQ	$h_1h_2^2h_3$	c	1-c	0	½	½	0	1-c	c	0
qQ	Qq	$h_1h_2h_3h_4$	$c(1-c)$	$c^2+(1-c)^2$	$c(1-c)$	½ $[c^2+(1-c)^2]$	$2c(1-c)$	½ $[c^2+(1-c)^2]$	$c(1-c)$	$c^2+(1-c)^2$	$c(1-c)$
qQ	qQ	$h_2^2h_3^2$	c^2	$2c(1-c)$	$(1-c)^2$	$c(1-c)$	$c^2+(1-c)^2$	$c(1-c)$	$(1-c)^2$	$2c(1-c)$	c^2
qQ	qq	$h_2h_3^2h_4$	0	c	1-c	0	½	½	0	1-c	c
qq	QQ	$h_1h_2h_3h_4$	0	1	0	0	1	0	0	1	0
qq	Qq	$h_1h_3h_4^2$	0	1-c	c	0	½	½	0	c	1-c
qq	qQ	$h_2h_3^2h_4$	0	c	1-c	0	½	½	0	1-c	c
qq	qq	$h_3^2h_4^2$	0	0	1	0	0	1	0	0	1

Prob: Joint probability of 2 maternal and 2 paternal haplotypes under random mating
M & m: Marker alleles
Q & q: QTL alleles
c: Recombination rate
h_1, h_2, h_3, h_4: Frequencies of haplotypes QM, Qm, qM, and qm, respectively, where
$h_1=p_Qp_M+D$; $h_2=p_Qp_m-D$; $h_3=p_qp_M-D$; $h_4=p_qp_m+D$

Table 2.A2. Probabilities of 4 parental haplotypes and expected frequency of QTL genotypes in progeny given MM and Mm parents and MM or Mm progeny.

Parents		Child →	MM			Mm		
MM	Mm	Prob	QQ	Qq	qq	QQ	Qq	qq
QQ	QQ	$h_1^3 h_2$	1	0	0	1	0	0
QQ	Qq	$h_1^3 h_4$	1-c	c	0	c	1-c	0
QQ	qQ	$h_1^2 h_2 h_3$	c	1-c	0	1-c	c	0
QQ	qq	$h_1^2 h_3 h_4$	0	1	0	0	1	0
Qq	QQ	$h_1^2 h_2 h_3$	½	½	0	½	½	0
Qq	Qq	$h_1^2 h_3 h_4$	½ (1-c)	½	½ c	½ c	½	½ (1-c)
Qq	qQ	$h_1 h_2 h_3^2$	½ c	½	½ (1-c)	½ (1-c)	½	½ c
Qq	qq	$h_1 h_3^2 h_4$	0	½	½	0	½	½
qQ	QQ	$h_1^2 h_2 h_3$	½	½	0	½	½	0
qQ	Qq	$h_1^2 h_3 h_4$	½ (1-c)	½	½ c	½ c	½	½ (1-c)
qQ	qQ	$h_1 h_2 h_3^2$	½ c	½	½ (1-c)	½ (1-c)	½	½ c
qQ	qq	$h_1 h_3^2 h_4$	0	½	½	0	½	½
qq	QQ	$h_1 h_2 h_3^2$	0	1	0	0	1	0
qq	Qq	$h_1 h_3^2 h_4$	0	1-c	c	0	c	1-c
qq	qQ	$h_2 h_3^3$	0	c	1-c	0	1-c	c
qq	qq	$h_3^3 h_4$	0	0	1	0	0	1

Prob: Joint probability of 2 maternal and 2 paternal haplotypes under random mating
M & m: Marker alleles
Q & q: QTL alleles
c: Recombination rate
h_1, h_2, h_3, h_4: Frequencies of haplotypes QM, Qm, qM, and qm, respectively, where
$h_1=p_Q p_M+D$; $h_2=p_Q p_m-D$; $h_3=p_q p_M-D$; $h_4=p_q p_m+D$

Table 2.A3. Probabilities of 4 parental haplotypes and expected frequency of QTL genotypes in progeny given MM and MM, or mm and mm, or MM and mm parents and the marker genotype in their progeny.

Parents		Prob			Child		
		MM, MM	mm, mm	MM, mm	QQ	Qq	qq
QQ	QQ	h_1^4	h_2^4	$h_1^2 h_2^2$	1	0	0
QQ	Qq	$h_1^3 h_3$	$h_2^3 h_4$	$h_1^2 h_2 h_4$	½	½	0
QQ	qQ	$h_1^3 h_3$	$h_2^3 h_4$	$h_1^2 h_2 h_4$	½	½	0
QQ	qq	$h_1^2 h_3^2$	$h_2^2 h_4^2$	$h_1^2 h_4^2$	0	1	0
Qq	QQ	$h_1^3 h_3$	$h_2^3 h_4$	$h_1 h_2^2 h_3$	½	½	0
Qq	Qq	$h_1^2 h_3^2$	$h_2^2 h_4^2$	$h_1 h_2 h_3 h_4$	¼	½	¼
Qq	qQ	$h_1^2 h_3^2$	$h_2^2 h_4^2$	$h_1 h_2 h_3 h_4$	¼	½	¼
Qq	qq	$h_1 h_3^3$	$h_2 h_4^3$	$h_1 h_3 h_4^2$	0	½	½
QQ	QQ	$h_1^3 h_3$	$h_2^3 h_4$	$h_1 h_2^2 h_3$	½	½	0
QQ	Qq	$h_1^2 h_3^2$	$h_2^2 h_4^2$	$h_1 h_2 h_3 h_4$	¼	½	¼
QQ	qQ	$h_1^2 h_3^2$	$h_2^2 h_4^2$	$h_1 h_2 h_3 h_4$	¼	½	¼
QQ	qq	$h_1 h_3^3$	$h_2 h_4^3$	$h_1 h_3 h_4^2$	0	½	½
Qq	QQ	$h_1^2 h_3^2$	$h_2^2 h_4^2$	$h_2^2 h_3^2$	0	1	0
Qq	Qq	$h_1 h_3^3$	$h_2 h_4^3$	$h_2 h_3^2 h_4$	0	½	½
Qq	qQ	$h_1 h_3^3$	$h_2 h_4^3$	$h_2 h_3^2 h_4$	0	½	½
Qq	qq	h_3^4	h_4^4	$h_3^2 h_4^2$	0	0	1

Prob: Joint probability of 2 maternal and 2 paternal haplotypes under random mating
M & m: Marker alleles
Q & q: QTL alleles
c: Recombination rate
h_1, h_2, h_3, h_4: Frequencies of haplotypes QM, Qm, qM, and qm, respectively, where
$h_1=p_Q p_M+D$; $h_2=p_Q p_m-D$; $h_3=p_q p_M-D$; $h_4=p_q p_m+D$

2.6 APPENDIX B: NON-CENTRALITY PARAMETER FOR A TWO-WAY ANOVA

The non-centrality parameter (λ) of two-way can be expressed as (Searle 1971)

$$\lambda = \frac{(K'B)'\left[K'(X'X)^{-1}K\right]^{-1}(K'B)}{\sigma_e^2} \qquad [B1].$$

Let σ_e^2 be unity. Let B' be the vector $[\mu, f_1, f_2, f_3, g_1, g_2, g_3]$ of parameters in the model, where μ is the sample mean, f_i is the mean of the i^{th} family type, and g_j the mean of the j^{th}

marker genotype across all family types. Let K be a matrix of parameter contrasts reflecting the H_0 being tested, for example if H_0: $g_1 = g_2$ and $g_2 = g_3$, then

$$K' = \begin{bmatrix} 0 & 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}.$$

The matrix X'X is

$$\begin{bmatrix} n_{..} & n_{1.} & n_{2.} & n_{3.} & n_{.1} & n_{.2} & n_{.3} \\ n_{1.} & n_{1.} & 0 & 0 & n_{11} & n_{12} & n_{13} \\ n_{2.} & 0 & n_{2.} & 0 & n_{21} & n_{22} & n_{23} \\ n_{3.} & 0 & 0 & n_{3.} & n_{31} & n_{32} & n_{33} \\ n_{.1} & n_{11} & n_{21} & n_{31} & n_{.1} & 0 & 0 \\ n_{.2} & n_{12} & n_{22} & n_{32} & 0 & n_{.2} & 0 \\ n_{.3} & n_{13} & n_{23} & n_{33} & 0 & 0 & n_{.3} \end{bmatrix},$$

where n_{ij} is the number of records in the i^{th} family and j^{th} marker genotype class. X'X is a matrix of order 7 and rank 5, hence, there are 7 unknowns, and only 5 degrees of freedom (i.e. once 5 parameters are estimated, the remaining 2 become known). An appropriate generalisation of X'X is obtained deleting the first row and column, hence setting $\mu = 0$, and the last row and column, hence setting $g_3 = 0$ (Searle 1971, p264). Let G be the reduced X'X matrix. This G matrix can be partitioned as follows

$$G = \left[\begin{array}{c|c} G_{11} & G_{12} \\ \hline G_{21} & G_{22} \end{array}\right] = \left[\begin{array}{ccc|cc} n_{1.} & 0 & 0 & n_{11} & n_{12} \\ 0 & n_{2.} & 0 & n_{21} & n_{22} \\ 0 & 0 & n_{3.} & n_{31} & n_{32} \\ \hline n_{11} & n_{21} & n_{31} & n_{.1} & 0 \\ n_{12} & n_{22} & n_{32} & 0 & n_{.2} \end{array}\right].$$

Then, if $c = G^{-1}$, $K^{*\prime}$ is the matrix K' with the first and last columns deleted, and B^* is the vector B with the first and last elements deleted, then

$$\lambda = (K^{*\prime}B^*)'C_{22}^{-1}K^{*\prime}B^* \qquad [B2]$$

where $C_{22} = K^{*\prime}CK^* = \left(G_{22} - G_{21}G_{11}^{-1}G_{12}\right)^{-1}$, and $(K^{*\prime}B^*)' = [g_1 - g_2, g_2]$.

When testing the QTL, equation [B2] gives

$$\lambda = \sum_{j}^{3} n_{.j} g_j^2 - \sum_{i}^{3} \frac{\left(\sum_{j}^{3} n_{ij} g_j \right)^2}{n_{i.}} \qquad [B3]$$

where the first part of [B3] corresponds to the sum of squares due to genotype, and the second part of [B3] corresponds to the sum of squares due to mating type.

However, usually it is a linked marker, rather than the QTL, what is being tested. Thus, equation [B3] needs to accommodate this fact. Using Table 2.2 in the Materials and Methods section, the new λ can be written as

$$\lambda = \sum_{i=1}^{10} b_i^2 n_i I_i - \sum_{j=1}^{6} F_j^2 f_j I_j \qquad [B4]$$

where b_i is the expected (progeny) marker genotype effect in the i^{th} trio class, n_i the number of trios in class i, $I_{i(j)}$ an indicator variable equal to 1 if the trio is informative (i.e. having at least one heterozygous parent), and 0 otherwise. Table 2.B1 shows F_j, the mean value of the j^{th} family class, and f_j, the number of these families

Table 2.B. Family mean (F_i) and number (f_i)

i	F_i	f_i
1	b_1	n_1
2	$(b_2 + b_3) / 2$	$n_2 + n_3$
3	b_4	n_4
4	$b_6/2 + (b_5 + b_7) / 4$	$n_5 + n_6 + n_7$
5	$(b_8 + b_9) / 2$	$n_8 + n_9$
6	b_{10}	n_{10}

It is also possible to use all trios, thus setting $I_{i(j)} = 1$ for all i (j), without increasing the type-I error rate. By doing so, power increases slightly, through augmenting the residual degrees of freedom, and ascertainment of informative families becomes unnecessary.

This method of obtaining λ can be applied to derive the non-centrality parameter for nested ANOVA, however the algebra becomes more tedious. Although a simpler method for obtaining λ_o was given in the Materials and Methods section, the same equation [3] was obtained when applying the method described here.

2.7 APPENDIX C: TDT_{Q5} AS A TWO-WAY ANOVA

Let us consider two fixed effects, α and β, where α could represent the factor mating type (or family type), and β could represent the genotype of the progeny. Thus, the model can be written as $y_{ij} = \mu + \alpha_i + \beta_i + e_{ij}$, which corresponds to a two-way ANOVA model without interaction. We will now show that the original statistic $F_{2, N-5}$ for TDT_{Q5} (Allison 1997), is equivalent to the F-ratio for testing the effects of β after having corrected for the effects due to μ and α, using the previous model.

For a constant $k = \frac{2}{N-5}$, we can see that

$$kF_{2,n-5} = \frac{SS_F - SS_R}{1 - SS_F} = \frac{(SS_\mu + SS_\alpha + SS_\beta - SS_\mu - SS_\mu)/SS_T}{1 - (SS_\mu + SS_\alpha + SS_\beta)/SS_T} = \frac{SS_{\beta|\mu,\alpha}}{SS_e} \qquad [C1]$$

where $SS_{\mu\alpha}$ and $SS_{\mu\alpha\beta}$ are the sum of squares explained by a model that fits μ and α, and by a model that fits μ, α and β, respectively. The null hypothesis of interest is whether factor β explains a significant amount of phenotypic variance over and above the amount explained by μ and α jointly. The F-ratio that appropriately reflects this null hypothesis is given in equation [C1].

2.8 APPENDIX D: NON-CENTRALITY PARAMETER FOR TDT_R

Let assume T is a random variable following a t-distribution, and let σ_T be the standard deviation of T. A first order Taylor's approximation for λ is $\lambda = E\left(\frac{T}{\sigma_T}\right) \approx \frac{E[T]}{E[\sigma_T]}$ (Kendall and Stuart 1963, Lynch and Walsh 1998). In order to derive E[T] and $E[\sigma_T]$, we used the probabilities of the 10 different types of trios and the expected effects of marker genotypes in the progeny contained in Table 2.2. Hence, conditional on p_M, p_Q, c, and D', $E[T] = E\left[\sum_i^n (y_i - \bar{y}) w_i\right]$, and because all family trios are unrelated (i.e. independent) then $E[T] = NE[(y - \bar{y})w]$, where y, the phenotype, and w, a weighting factor (see Materials and Methods), are expectations for a single trio. Thus, the expected value of the numerator of TDT_R is approximately $E[T] = Np_M p_m \left[p_M^2 (b_2 - b_3) + p_M p_m (b_5 - b_7) + p_m^2 (b_8 - b_9)\right]$.

When analysing the QTL, and assuming no dominance, the previous equation simplifies to $E[T] = Np_Q p_q a$.

The expected variance of T, $E[\sigma_T^2]$, is the same regardless whether the locus being tested is the QTL or a marker. Equation A1.23a in Lynch and Walsh (1998) is $E[\sqrt{v}] \approx \sqrt{\mu_v} - \sigma_v^2 \frac{\mu_v^{-3/2}}{8}$, which reduces to $E[\sqrt{\sigma_T^2}] \approx \sqrt{E[\sigma_T^2]}$ if the second term is ignored. Hence, $E[\sigma_T^2] = E\left[\frac{1}{4}\sum_i^n (y_i - \bar{y})^2 H_i\right] = \frac{1}{4}E[(y-\bar{y})^2 H]$ and, as the expectation of a random variable X given another random variable Y is $E[X] = E[E[X \mid Y]]$ (Casella and Berger 1990), then $E[(y-\bar{y})^2 H] = \sum_{H=0}^{2} HP_H E(y-\bar{y})^2 = p_M p_m (\sigma_e^2 + \sigma_{QTL}^2)$. Finally, dividing E[T] by $\sqrt{E[\sigma_T^2]}$ we obtain $E[TDT_R] = \lambda_R \approx [p_M^2 (b_2 - b_3) + p_M p_m (b_5 - b_7) + p_m^2 (b_8 - b_9)] \sqrt{\frac{Np_M p_m}{\sigma_e^2 + \sigma_{QTL}^2}}$.

CHAPTER 3

Candidate gene analysis for quantitative traits using the transmission-disequilibrium test: the example of the Melanocortin 4-Receptor in pigs

3.1 INTRODUCTION

Population-wide associations between loci due to linkage disequilibrium can be used in high resolution mapping of quantitative trait loci (QTL). Spurious associations between markers and QTL can also arise as a consequence of population stratification, for example due to admixture of two different populations. Associations between a genotype and a trait have been frequently tested, after corrections, with simple one-way ANOVA models, e.g. testing mean genotype differences directly. However, these types of analyses are prone to false positive results due to confounding effects of population stratification/admixture (e.g. Deng et al. 2001). Spielman et al. (1993) developed an allele-trait association test called Transmission Disequilibrium Test (TDT) that is robust to these confounding effects. Different TDTs have since been developed for dichotomous traits (Schaid 1996, Horvath and Laird 1998, Martin et al. 2000, Lunetta et al. 2000, Zhao et al. 2000), and for quantitative traits (Allison 1997, Rabinowitz 1997, Szyda 1998). However, most of these TDTs have been formulated in a rather rigid form, hence reducing the scope for further statistical modelling.

This work describes the use of a TDT (Rabinowitz 1997) to obtain robust estimates of genetic effects within the statistically more flexible mixed linear model context. This approach allows maximum likelihood estimates of genetic effects to be obtained via REML. Additive allele substitution effects were estimated within (b_{TD} for Transmission Disequilibrium) and between families (b_{PD} for Population Disequilibrium) with two independent regression coefficients. Moreover, the rejection of the null hypothesis $b_{TD} = b_{PD}$ provides evidence for stratification/admixture and hence can be used to guard against false positive results.

The analysis of the MC4R locus on pig chromosome 1 constitutes a practical demonstration of this method. The interaction between melanocortins and their receptors (MC3R and

MC4R) at the hypothalamus is one of the main neuro-endocrinological pathways controlling energy balance (Wardlaw 2001). In humans, different allelic variants of both MC4R and MC3R have been associated with obesity (Vaisse et al. 1998, Yeo et al. 1998, Hinney et al. 1999, Li et al. 2000). In pigs, the seventh transmembrane region of the MC4R locus contains a mutation at codon 298 that causes a change of aspartic acid for asparagine, i.e. Asp298Asn (Kim et al. 1999). This region is highly conserved across all four types of melanocortin receptors in humans (Gantz et al. 1993), and it is also very conserved between pigs and humans (Kim et al. 2000). The Asp298Asn mutation has been associated with fatter and faster growing pigs, having significant effects on backfat, days to 110 kg, test daily gain and daily food intake in a study involving four different commercial pig lines from nucleus breeding farms (Kim et al. 1999, 2000). However, the original analyses were performed with methods that are potentially biased in the presence of population stratification. Here we analyse an augmented data set using the new methodology to confirm and extend the original findings.

3.2 MATERIAL AND METHODS

3.2.1 Data

Performance traits were recorded on four different commercial PIC pig lines in the same farm over a five year period (1993-1998). The traits were lifetime daily gain (LDG), test daily gain (TDG), daily food intake (DFI) and backfat depth (BF) at the 10^{th} rib. All pigs were performance tested for growth over a fixed period of 12 weeks, during which they were fed ad lib and weighed at the beginning (on-test) and at the end (off-test) of that period. TDG was calculated as off-test weight minus on-test weight divided by the number of days on test. LDG was calculated as off-test weight minus one (assumed the average birth weight) divided by the age of the pig (in days) at off-test. BF was measured ultrasonically in real time at off-test, and it was normalised with the natural log transformation. DFI was electronically recorded for some pigs over the testing period. The sample sizes by line and sex are given in Table 3.1. In this data set, there were 726 extra records of BF, 574 of TDG and 44 of DFI, with respect to the data set analysed by Kim et al. (2000). The Asp298Asn substitution mutation is located within a *Taq*I restriction enzyme recognition site (Kim et al. 1999), which was used to generate a codominant restriction fragment length polymorphism (RFLP) to distinguish all three genotypic classes (Kim et al. 2000).

Table 3.1. Number of pigs[a] (males/females/total) used in the analyses within each trait (BF, TDG, LDG or DFI), line (A, B, c or D) and MC4R genotype (11, 12 or 22).					
MC4R	LINE A	LINE B	LINE C	LINE D	All lines
Test Daily Gain (TDG)					
11	3/22/25	27/28/55	89/349/438	155/25/180	274/424/698
12	9/146/155	38/79/117	11/177/188	152/44/196	210/446/656
22	9/245/254	12/57/69	0/32/32	37/22/59	58/356/414
Total	21/413/434	77/164/241	100/558/658	344/91/435	542/1226/1768
Lifetime Daily Gain (LDG) and Backfat at 10^{th} rib (BF)					
11	3/37/40	27/52/79	89/504/593	155/25/180	274/618/892
12	9/266/275	38/145/183	11/250/261	152/44/196	210/705/915
22	9/392/401	12/117/129	0/50/50	37/22/59	58/581/639
Total	21/695/716	77/314/391	100/804/904	344/91/435	542/1904/2446
Daily Food Intake (DFI)					
11	3/0/3	27/0/27	87/0/87	21/0/21	138/0/138
12	9/0/9	38/0/38	10/0/10	44/0/44	101/0/101
22	9/0/9	12/0/12	0/0/0	15/0/15	36/0/36
Total	21/0/21	77/0/77	97/0/97	80/0/80	275/0/275
[a] Line A is a Landrace-based population. Line B is a Large White-based population. Line c is a synthetic population based on Duroc and Large White. Line D is a synthetic line based on several different populations including Landrace, Large White, Duroc, and Pietrain.					

3.2.2 Methods

The effects of the Asp298Asn mutation on pig production traits were estimated with the models used in Kim et al. (2000), which are not robust to population stratification/admixture, and with new robust models. The latter models were a combination of the former models and a TDT (Rabinowitz 1997).

ANOVA. The original models included sex, batch, line and genotype as fixed factors, and sire as random effect. Backfat (BF) records were analysed both in the original scale and in the log-transformed scale. The skewness and kurtosis of the untransformed distribution of BF were 0.88 (± 0.049) and 1.96 (± 0.099), respectively. After transformation the distribution of BF became more normal (skewness = -0.03 ± 0.049, kurtosis = 0.32 ± 0.099). All two-way interactions between fixed factors were also included in the analyses. Non-significant factors were dropped out from the models using a backwards elimination procedure. The coefficients for genotypes can be found in the column with the heading A in

Table 3.2. The analyses were performed with the REML procedure in GENSTAT (GENSTAT 4.2, 5th edition, 2000). This method of estimating allele effects is not robust to stratification/admixture (Hernández-Sánchez et al. 2002b). We will refer to this method as the ANOVA method.

Batches as random. There were 54 batch means to estimate when they were fitted as fixed effects in the models. In order to avoid this unnecessary loss of degrees of freedom, batches were fitted as a random term where direct comparisons were being made with the TDT, and their effect accounted for with cubic splines. This procedure was feasible because all trait means followed a yearly cycle when plotted against batches. The correction uses up only two degrees of freedom: one in fitting a linear regression across all batches, and a second in estimating the residual variance around the previous line. The software used to run models with batch as random splines was ASREML (Gilmour et al. 2001). This approach was also implemented in the TDT analyses (see below).

TDT. A robust analysis of the Asp298Asn mutation was performed with the same models but substituting genotype for two independent fixed covariates. One of these two covariates was based on a TDT (Rabinowitz 1997). Given a biallelic marker, each individual's genotype received a coefficient equal to $H_{♂}$ ($T_{♂}$-1/2) + $H_{♀}$ ($T_{♀}$-1/2); where $H_{♂}$ = 1 if the individual's sire was heterozygous, or 0 otherwise; $T_{♂}$ = 1 if the sire had transmitted allele 1 to the individual, or 0 otherwise; and likewise, $H_{♀}$ and $T_{♀}$ for the individual's dam. These coefficients can be found in the column TD in Table 3.2. The slope of this covariate, b_{TD} (TD for Transmission Disequilibrium), is a robust estimate of additive substitution effects of alleles at the locus. Allelic effects were also estimated via a second regression coefficient sensitive to the effects of population structure, b_{PD} (PD for Population Disequilibrium) (L. L. G. Janss personal communication). The appropriate coefficients to estimate b_{PD} were obtained subtracting the column A from the column TD in Table 3.2. We will refer to this method as the TDT method.

Table 3.2. Parameterisation of covariates A, TD, and PD given family genotypes.

G_f	G_m	G_o	A	TD	PD
11	11	11	1	0	1
11	12	11	1	½	½
		12	0	-½	½
11	22	22	0	0	0
12	12	11	1	1	0
		12	0	0	0
		22	-1	-1	0
12	22	12	0	½	-½
		22	-1	-½	-½
22	22	22	-1	0	1

G_f: Paternal genotype
G_m: Maternal genotype
G_o: Offspring genotype

One-way ANOVA

A = 1, 0, -1 if Go = 11, 12, 22, respectively

TDT

$TD = H_{♂}\,(T_{♂}-1/2) + H_{♀}\,(T_{♀}-1/2)$

where $H_{♂\,(♀)} = 1$ if $G_{f\,(m)} = 12$ and 0 otherwise

$T_{♂\,(♀)} = 1$ if offspring receives allele 1 from a 12 father (mother) and 0 otherwise

PD = A - TD

Generating parental genotype data. The TDT method requires parental genotype data, which in the MC4R data set were mostly missing. Nevertheless, missing parental genotypes could be generated using Gibbs sampling (e.g. Wang et al. 1994, Sorensen 1996). Gibbs sampling was equivalent to integrating over all genotype probabilities of parents with missing genotypes. Missing parental genotypes were sampled conditional on genotypes of progeny and other relatives. Convergence was reached after 10^3 realisations after a burn-in period of 100 realisations (see Appendix A). The autocorrelation in Gibbs sampling was minimised by sampling one realisation every 50 consecutive ones, hence a total of 50×10^4 realisations were generated. The actual integration over missing genotype probabilities was carried out by analysing the MC4R data set after each sampled realisation and averaging results (i.e. p-values) across all realisations.

Simulation. A simulation study was carried out to investigate the properties of b_{PD} and b_{TD}. Population stratification was generated sampling from two separated populations with different allele frequencies, and analysing the data jointly. Each population was characterised

by: 1) 15 unrelated full-sib families, and 4 offspring per family (60 progeny in total), 2) random mating, 3) a quantitative trait locus (QTL) and a linked neutral marker, both biallelic and with allele frequency fixed to 0.9 in one population, and frequencies, at both loci, of 0.9, 0.7, 0.5, 0.3 and 0.1 in the other population 4) the recombination rate between loci was c = 0 or ½, 5) the standardised linkage disequilibrium in parents (D' = D/D_{max}, Lewontin 1988) was either 0 or 1, 6) the residual variance was 1 and the polygenic variance 0, and 7) the QTL explained 5% (and in some cases 10%) of the total phenotypic variance. There were no inter-population matings and all analyses were performed at the marker locus.

3.3 RESULTS

3.3.1 Properties of b_{PD} and b_{TD} in analyses of simulated data

The power of estimating genetic effects through b_{PD} and b_{TD} in the simulated data is shown in Figure 3.1, where F-ratios are plotted against the level of stratification S (allele frequency difference between populations) across 4 different scenarios. Each dot in Figure 3.1 is the average of 100 replicates. The significance threshold is based on the tabulated nominal 5% threshold and is shown as a straight line. Figure 3.1a shows the results after analysing a marker totally unlinked (c = ½) and with no association in the population (D' = 0) to a QTL. In this situation, any significant effect is a type I error or due to bias. The F_{TD} (i.e. the F-ratio testing whether a significant amount of the total variation is explained by b_{TD}) is approximately 1 across all S values, which indicates that the marker did not have a significant effect on the trait. On the contrary, F_{PD} (i.e. the corresponding F-ratio test for b_{PD}) appeared positively correlated to the level of S. The effect was significant when $S \geq 0.6$. In this case, spurious disequilibrium increases as stratification increases, and b_{PD} cannot distinguish between this sort of disequilibrium and disequilibrium due to linkage.

Figure 3.1b shows the results after analysing a marker totally unlinked (c = 0.5) but in complete disequlibrium (D' = 1) in the parents. Here, F_{PD} is always greater than F_{TD}, and this difference increases with S. Moreover, the value of F_{PD} was greater than 1 (approximately 2) even without stratification (S = 0). This can be explained by considering that, on average, the level of disequilibrium among offspring was ½, because D' is expected to be halved every generation assuming no linkage and random mating. This feature suggests that b_{PD} could detect an effect given sufficient linkage disequilibrium between a marker and a QTL, even if these two loci are totally unlinked, whereas b_{TD} needs the joint occurrence of linkage and linkage disquilibrium in order to estimate an effect.

Figure 3.1c shows how F_{PD} rapidly increases for S > 0.4, whilst F_{TD} remains constant and equal to one regardless of S. The marker was totally linked to the QTL locus in this set of simulations (c = 0). Despite having simulated no linkage disequilibrium (D' = 0) within each population, mixing two populations with different allele frequencies will cause haplotype frequencies to depart from the expected equilibrium frequencies. In this simple scenario, the fact that F_{TD} = 1 across all values of S even when c = 0 demonstrates the robustness of the test based on b_{TD}.

Finally, Figure 3.1d shows the effect of effectively analysing the QTL itself (D' = 1 and c = 0). The power of estimating b_{PD} increases monotonically with S, a fact consistently observed in all previous graphs. However, the power of estimating b_{TD} reaches its maximum at intermediate levels of S. This is so because intermediate allele frequencies (e.g. at 0.5, represented by S = 0.9 (population 1) – 0.5 (population 2) = 0.4 in graph 1d) are associated with a higher proportion of heterozygous parents, providing better information to estimate b_{TD}.

Figure 3.1 F-ratios when testing b_{PD} (blue squares) or b_{TD} (dark triangles) given parental linkage disequilibrium (D'), recombination rate (c) and level of stratification (S). Threshold is shown in red.

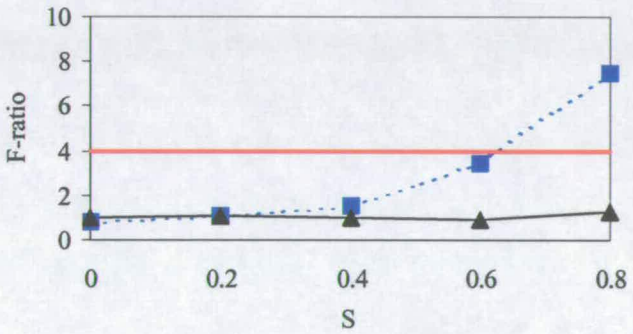


Figure 3.1a. D' = 0 and c = 0.5.

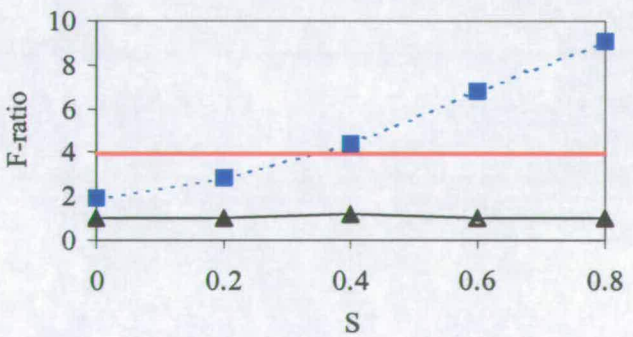


Figure 3.1b. D'=1 and c =0.5

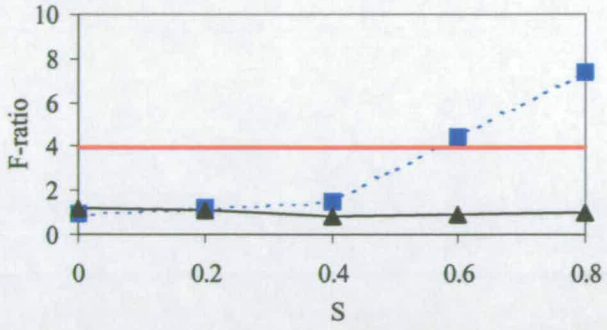


Figure 3.1c. D' = 0 and c = 0

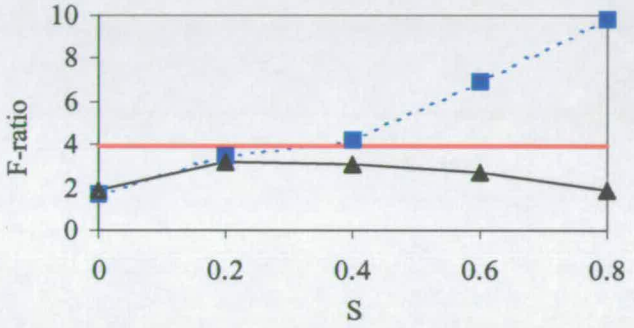


Figure 3.1d. D' = 1 and c = 0

3.3.2 Analyses of MC4R data

ANOVA method. Results were obtained from analyses of the data from the four lines separately and from an overall analysis of the combined data from all lines. Striking differences between genotypes were detected for BF ($p<0.001$), TDG ($p<0.001$) and LDG ($p<0.001$) in the overall (i.e. all lines together) analysis (Table 3.3). Batches were treated as fixed effects in these analyses in order to be able to compare results directly with the findings of Kim et al. (2000). Genotypic differences within all pig lines were confirmed for BF (ranging from $p<0.001$ in lines A and c to $p<0.02$ in line B), and also within some lines for TDG (ranging from $p<0.001$ in line c to $p>0.8$ in line B) and LDG (ranging from $p<0.01$ in line c to $p>0.5$ in line D). No significant effect of the Asp298Asn substitution mutation on DFI was found in this analysis, in spite of the significant difference of 0.17 kg between both homozygotes ($p<0.01$) reported by Kim et al. (2000). In this study, the estimated difference in DFI between the two homozygous genotypes, although not significant, was in the same direction as that previously reported at 0.1 kg (sed = 0.063) in the overall analysis.

Table 3.3. Means by genotype class within and across lines (overall). The models were as in Kim et al. (2000), although containing only significant terms.

Trait	G	LINE A	LINE B	LINE c	LINE D	Overall
TDG (g/day)	11	960.8	852.1	942.3	895.0	910.5
	12	913.3	859.3	913.6	873.3	888.9
	22	898.5	851.6	889.3	877.8	878.6
	sed	13.88	14.6	14.14	14.61	6.41
	P	0.002	0.819	0.001	0.165	<0.001
LDG (g/day)	11	626.1	660	692.5	696.4	698.1
	12	613.4	656.9	681	677.6	685.4
	22	606.2	651.3	670	676.7	679.5
	sed	7.4	7.3	7.3	9.2	3.4
	P	0.043	0.522	0.005	0.022	<0.001
DFI (kg/day)	11	1.88	1.94	1.89	1.79 *	1.89
	12	1.81	1.84	1.83	1.8 *	1.82
	22	2	1.69	n.a.	1.74 *	1.79
	sed	0.298	0.108	0.126	0.105 *	0.063
	P	0.705	0.1	0.655	0.861 *	0.202
BF (log mm)	11	2.55	2.55	2.55	2.44	2.49
	12	2.46	2.50	2.48	2.36	2.42
	22	2.41	2.46	2.43	2.30	2.37
	sed	0.03	0.028	0.027	0.039	0.013
	P	<0.001	0.017	<0.001	0.002	<0.001
BF (mm)$^{+}$	11	12.8 (12.3-13.2)	12.8 (12.4-13.1)	12.8 (12.4-13.2)	11.5 (11.1-11.9)	12 (11.8-12.2)
	12	11.8 (11.3-12.2)	11.9 (11.6-12.2)	12.2 (11.8-12.5)	10.6 (10.2-11)	11.2 (11-11.4)
	22	11.2 (10.8-11.6)	11.3 (11.1-11.6)	11.7 (11.4-12.1)	9.9 (9.6-10.3)	10.7 (10.5-10.8)

G: Genotypes, 1 ~ Asp and 2 ~ Asn at codon 298 of the porcine MC4R
sed: Standard error of the difference between any pair of genotypic means
P: p-values
* Regarding DFI, only these analyses included sire as a random effect
$^{+}$ The confidence intervals around BF means on the back-transformed scale are asymmetric, thus they are shown within brackets
n.a.: data not available

TDT method. Only results from the overall analyses of the combined data set are shown. The estimation of b_{TD} and b_{PD} via REML was done on the basis of 10^3 replications, where

each replicate used a different population of parental genotypes generated with Gibbs sampling. The average p values across these analyses are shown in Table 3.4. The ASREML software does not perform hypothesis testing for fixed effects in the model although it provides t-values and residual degrees of freedom. The p-values associated with genotypic contrasts were obtained from the t-distribution. There were two independent null hypotheses of interest: H_0^1: $b_{PD} = 0$ and H_0^2: $b_{TD} = 0$, both were rejected for all traits except DFI (H_0^1: BF p<0.0001, TDG p<0.01, LDG p<0.01 and DFI p>0.5; H_0^2: BF p<0.0001, TDG p<0.001, LDG p<0.01 and DFI p>0.2). For the sake of comparison, Table 3.4 also incorporates results from the ANOVA method (reported as regression coefficients rather than genotype means as in Table 3.3). Both, the ANOVA and the TDT methods yielded similar results, although the former was a slightly more powerful analysis (the standard errors were generally smaller). Results in Tables 3 and 4 are of similar magnitude.

Table 3.4. Average effect of allele substitution for each trait using TDT (PD, TD) and ANOVA. Cycle was treated as random.

Trait	Estimation	b	s.e.(b)	T	DF	p-value
TDG	PD	16.1	5.84	2.76	2430	< 0.01
	TD	14.1	4.45	3.17		< 0.01
	ANOVA	14.8	3.66	4.05		< 0.0001
LDG	PD	9.1	3.31	2.75	2423	< 0.01
	TD	8.8	2.38	3.69		< 0.001
	ANOVA	8.9	2.01	4.42		< 0.0001
BF	PD	0.07	0.013	5.14	2423	< 0.0001
	TD	0.06	0.009	6.7		< 0.0001
	ANOVA	0.06	0.009	8.11		< 0.0001
DFI	PD	0.03	0.047	0.64	272	> 0.5
	TD	0.08	0.061	1.26		> 0.2
	ANOVA	0.05	0.037	1.28		> 0.2

PD: Population Disequilibrium (between families gene effect)
TD: Transmission-Disequilibrium (within families gene effect)
ANOVA: Genotype substituted covariates PD and TD
b: allele substitution effect
s.e. (b): standard error of b
T: t-statistic from testing b=0 vs. b≠0
DF: Nominal residual degrees of freedom
p-value: the N(0,1) was used as an approximation to the t-distribution for TDG, LDG and BF

3.4 DISCUSSION

The study demonstrated that the mean additive effect of allele substitution calculated with a one-way ANOVA model can be decomposed into the within and the between family effects. These two effects can be estimated via a flexible REML analysis (Patterson and Thompson 1971) as the regression coefficients b_{TD} and b_{PD}, respectively, using a mixed linear model that can also incorporate other fixed and random effects.

Parental genotypes are needed to estimate b_{TD} and b_{PD}. This information was not available for the MC4R data analysed, and was generated via Gibbs sampling (1000 realisations). The new methodology was tested via simulation and real data analysis of the effect of the MC4R gene on pig production traits.

The simulation results can be summarised in three main points. First, b_{TD} extracts information from the within and the b_{PD} from the between family genetic variances, σ^2_{WF} and σ^2_{BF} respectively. Second, b_{TD} is robust and b_{PD} is biased in the presence of population admixture/stratification. Third, there is generally more power to detect a significant $b_{PD} \neq 0$ than a significant $b_{TD} \neq 0$.

Population stratification/admixture increases σ^2_{BF} and not σ^2_{WF}. As a consequence, estimates of b_{PD} may be biased in the presence of stratification/admixture, whereas estimates of b_{TD} are not as the simulation results demonstrate. Tests such as the TDT are robust because they only exploit σ^2_{WF}. However, the one-way ANOVA model uses both σ^2_{WF} and σ^2_{BF}, from which a pooled estimate between b_{PD} and b_{TD} is obtained, and because of the latter component of variance, this pooled estimate of allelic effects may be biased if there is population admixture/stratification. In spite of this potential bias producing false positive results (e.g. Figures 1a and 1b), robust methods such as the TDT have seldom been used in animal breeding (some exceptions are Bink et al. 2000, and Hernández-Sánchez et al. 2002b).

The fact that b_{TD} and b_{PD} exploit different sources of information can be intuitively appreciated by inspecting the coefficients in Table 3.2. First, the coefficients required in the estimation of b_{TD} are weights given to all individuals with records (e.g. offspring) according to their genotypes and to the genotypes of their parents (i.e. family type). Hence, b_{TD} is the slope of the regression of phenotypes onto explanatory variables that combine both

offspring's genotypes and family type. Second, the coefficients required in the estimation of b_{PD} can alternatively be obtained as $\sum_{j=1}^{2} G_{ij}$, where G_{ij} = ½, 0 or –½ if the genotype of the j[th] parent in the i[th] family is 11, 12 or 22, respectively. Therefore b_{PD} is the slope of the regression of phenotypes onto family type.

More explicitly, let us model y_{ij}, the phenotype of the j[th] individual having the i[th] QTL genotype, as $y_{ij} = \mu + g_i + a_{ij} + e_{ij}$, where μ is the population mean, g_i the effect of the i[th] QTL genotype on the j[th] offspring, and a_{ij} and e_{ij} are the polygenic and residual random terms drawn from two independent normal distributions with zero means and variances σ_A^2 and σ_e^2, respectively. Let there be random mating, no population stratification, and only additive genetic effects at the QTL locus. Under these circumstances, the total additive genetic variance splits equally between and within families, therefore $E[\sigma_{BF}^2] = \sigma_G^2/2$, and $E[\sigma_{WF}^2] = \sigma_e^2 + \sigma_G^2/2$, where $\sigma_G^2 = \sigma_A^2 + \sigma_Q^2$, and σ_Q^2 is the variance due to the QTL. If a statistical model explains all σ_Q^2 then the additive genetic effects of the QTL will be fully accounted for. However, if the model estimates additive effects only through either b_{TD} or b_{PD} then σ_Q^2 will only be partially explained. For example, if b_{TD} is the only estimator of QTL effect then $E[\sigma_{WF}^2] = \sigma_e^2 + \sigma_A^2/2$ and $E[\sigma_{BF}^2] = \sigma_G^2/2$, as only within-family variation can be used to estimate b_{TD}. If, on the other hand, b_{PD} is the only estimator of QTL effect then $E[\sigma_{WF}^2] = \sigma_e^2 + \sigma_G^2/2$ and $E[\sigma_{BF}^2] = \sigma_A^2/2$, as only between-family variation can be used to estimate b_{PD}. These changes in the within and between family variances due to estimation of b_{TD} or b_{PD} were validated via computer simulations (data not shown).

The situation is more complex when there is stratification within a population. Appendix B shows how the proportion of the total phenotypic variation explained fitting a linear regression, differed when either b_{TD} or b_{PD} was estimated in the presence of population stratification.

Simulation results showed that testing the null hypothesis $b_{PD} = 0$ tends to produce higher F-ratios when it is not true than testing $b_{PT} = 0$ (Figure 3.1). Nevertheless, the analysis of real data showed the opposite effect (i.e. t-tests for b_{TD} were always higher than those for b_{PD} in Table 3.4). This result is possible when there is no admixture/stratification, because b_{TD} is expected to be equivalent to b_{PD}. Moreover, in simulations, the only between family component was ½ of the total QTL variance, whereas in reality other factors (e.g. litter and

sow effects) may also increase σ^2_{BF}. The analysis of real data showed that b_{TD} was very similar to b_{PD} across all traits, and furthermore, that both estimates were also similar to allelic effects obtained with ANOVA (Table 3.4). This suggests that there was no significant stratification/admixture in the population, and that there were equivalent amounts of genetic information between and within families.

Where estimates of b_{TD} and b_{PD} differ, a t-test could be used to test the null hypothesis $b_{TD} = b_{PD}$. If no evidence to reject the null hypothesis were found then a more powerful one-way ANOVA model could be safely implemented, i.e. there would be no evidence for admixture/stratification in the population. Otherwise, robust approaches such as the one proposed here, i.e. estimating b_{TD}, or other TDTs, should be considered as the only reliable methods of analysis.

A complication of this approach applied to many real data sets, including the MC4R data used as an example, will be generating missing parental genotypes. Nevertheless, a Gibbs sampling technique with 10^3 replications was simple to implement (and we know they were accurate enough because 10 times more replicates did not affect the outcome) in this simple scenario, i.e. a single biallelic marker, a maximum of three generations, few missing data. Other robust tests that do not require parental genotypes, e.g. sib-TDTs (e.g. Schaid and Rowland 1999, Spielman and Ewens 1998), were found to be less powerful than the method outlined here in additional analyses of simulated data, e.g. $F_{sib} = 5.5$ vs. $F_{TD\text{-}PD} = 7.7$ (based on 1000 replicates). Analysing more complex or unbalanced data sets will probably demand more realisations of the Gibbs sampler and it will present an additional problem: testing fixed effects in REML, because the asymptotic properties of t-test or Wald test could not be guaranteed (see Kenward and Roger 1997, Welham and Thompson 1997, Elston 1998).

This approach was also tested with real data. There is strong evidence that the substitution-mutation Asp298Asn in the MC4R gene affects production traits in the pig, e.g. backfat (BF), growth (TDG, LDG), and appetite (DFI) (Kim et al. 2000). These effects were re-estimated with extra records with respect to the original work. Significant effects were found for BF, TDG and LDG across all lines ($p < 0.001$), although not for all pig lines (ranging from $p < 0.001$ to $p > 0.8$). DFI was the only trait not significant in this study ($p > 0.2$), even after using the models described in Kim et al. (2000).

The lack of effect on DFI was unexpected given previously reported results ($p < 0.01$), and other reported associations of MC4R with appetite and feeding behaviour in macaques

(Koegler et al. 2001), rats (Todd et al. 1997), mice (Butler et al. 2001), and layer chicks (Tachibana et al. 2001). One possible explanation for not detecting an effect on DFI is the lack of power due to a small data set (n=275). Although there were less data (n=231) in the study of Kim et al. (2000) than in this study, the difference is small and additional statistical noise may have been introduced through factors such as data structure and/or chance. An independent study that used a larger data set (n=619), found a significant ($p<0.05$) additive gene effect of 0.075 kg/day on DFI (G. Plastow, personal communication). Further experiments are needed in order to ascertain whether the Asp298Asn substitution mutation at the MC4R locus is causative.

This study demonstrates that TDT can be implemented within the REML framework. As a guideline, one should test associations with ANOVA only after having checked that b_{TD} and b_{PD} are not significantly different from each other, i.e. making sure no false positive results are being caused by population stratification.

3.5 Appendix A: Gibbs sampling convergence when generating missing parental genotypes

Each missing parental genotype was stochastically generated with Gibbs sampling conditioning on genotypes of his/her relatives. A total of 50×10^4 consecutive realisations were obtained and only one every 50 was saved to reduce the autocorrelation of the chain. The traits backfat (BF), test daily gain (TDG), lifetime daily gain (LDG) were analysed with a TDT (Rabinowitz 1997) using the remaining 10^4 (independent) sets of parental genotypes. For each trait a distribution of log-transformed p-values was obtained (dist-0). Three different samples of size 10^3 were obtained from dist-0, they were: dist-1 containing the initial 10^3 log-p-values, dist-2 containing the last 10^3 log-p-values, and dist-3 containing 10^3 log-p-values evenly spaced in the chain (one every 10 consecutive ones). The distributions dist-1, 2, and 3 were compared against dist-0 with the non-parametric Kolmogorov-Smirnoff test (KOLMOG2 procedure in GENSTAT 4.1), which obtains the maximum absolute difference between two cumulative density functions. If the two distributions are the same, this difference follows a χ^2_2 distribution. There were no significant differences (Table 3.A) between Dist-1, 2 or 3 and Dist-0 in any trait at a significance level of 0.017 (Bonferroni correction for 3 comparisons within trait). These results ensure that 10^3 out of the 10^4 independent sets of parental genotypes provide an unbiased sample, and that if convergence was reached after 10^4 realisations, then it was also reached after 10^3 realisations.

Table 3.A. P-values of the Kolmogorov-Smirnoff test for differences between Dist-0 and Dist-1, 2, or 3			
	[a]BF	[b]TDG	[c]LDG
[d]Dist-1	0.02	0.13	0.66
[e]Dist-2	0.36	0.23	0.42
[f]Dist-3	0.05	0.51	0.20

[a] Backfat
[b] Test Daily Gain
[c] Lifetime Daily Gain
[d] The first 10^3 log-p-values from Dist-0
[e] The last 10^3 log-p-values from Dist-0
[f] 10^3 log-p-values from Dist-0, sampling 1 every 10 consecutive ones
Significant level = 0.05 / 3 = 0.017 (Bonferroni correction)

3.6 Appendix B: Impact of stratification on b_{TD} and b_{PD}

Population stratification affects both the estimation of the effect via b_{PD} and the power of that estimation (s.e. b_{PD}). We will assume the simplest scenario where a population is divided into two subpopulations of equal size, where mating is at random within each subpopulation, and there is no matings across subpopulations.

The expected mean square of a linear model that regresses Y onto a single explanatory variable X is $E[MSR] = \sigma_e^2 + B^2 \sum \left(X - \overline{X}\right)^2$, where B is the expected regression parameter, and σ_e^2 the residual variance (Sokal and Rohlf 1995). When n phenotypes (Y) are simulated with gene effect (B) and σ_e^2 equal to one, then $E[MSR] = 1 + E\left[\sum \left(X - \overline{X}\right)^2\right] = 1 + n\left\{E[X^2] - (E[X])^2\right\}$. Let us assume p_i is the frequency of allele i at the trait locus in subpopulation 1, and q_i the equivalent frequency in subpopulation 2. Furthermore, let us assume $p_{11} = (p_1)^2$ is the frequency of genotype 11 in subpopulation 1, and q_{11} the frequency of the equivalent genotype in subpopulation 2. Hence, it can be shown that $E[PD] = (p_1 - p_2 + q_1 - q_2) / 2$, and that $E[PD^2] = 0.5\left(p_{11}^2 + p_{22}^2 + q_{11}^2 + q_{22}^2\right) + 0.25\left(p_{11}(1 - p_{11}) + q_{11}(1 - q_{11})\right)$. The same process is followed to develop the expected mean squares when using TD, thus $E[TD^2] = \left(p_1^2 + q_1^2\right)/4$, and $E[TD] = 0$. These two predictions were very similar to simulation results (not shown). We can see the effect of stratification on $E[MSR]_{TD}$ and $E[MSR]_{PD}$ for n=100 in Figures 3.b1 and 3.b2. Figure 3.b1 takes a hill-type shape (seen from above) where the highest point is at the centre, and gradually decays in all directions away from the centre. Figure 3.b2 takes a valley-type shape (seen from above) where the lowest points are on the diagonal (in fact Figures 3.b1 and 3.b2 are identical on the diagonal) and

quickly rising away from the diagonal. If there is no stratification, e.g. on the diagonal passing through points $p_1 = q_1$, then $E[MSR]_{TD} = E[MSR]_{PD}$, thus both regression lines are identical. However, when there is stratification (e.g. $p_1 \neq q_1$), $E[MSR]_{PD}$ increases, and $E[MSR]_{TD}$ decreases. The effect on $E[MSR]_{TD}$ is not due to stratification itself but rather to a reduction of the information content due to a decrease in the frequency of heterozygous genotypes. Although, we have shown the effect of analysing the trait locus itself, this effect is transferred to a marker as a function of linkage disequilibrium between both loci.

Figure 3.b1 Expected mean squares (EMS) for b_{TD}

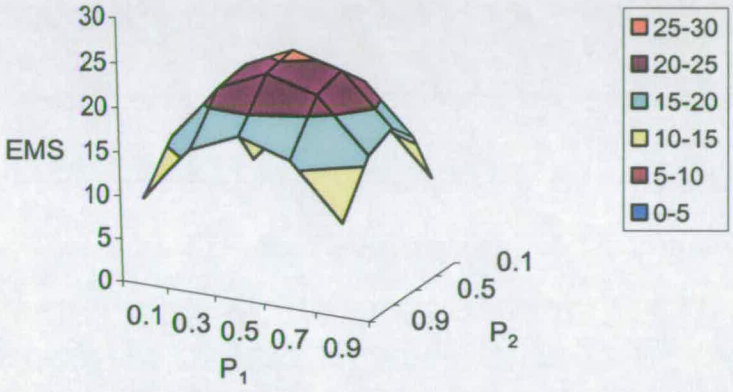
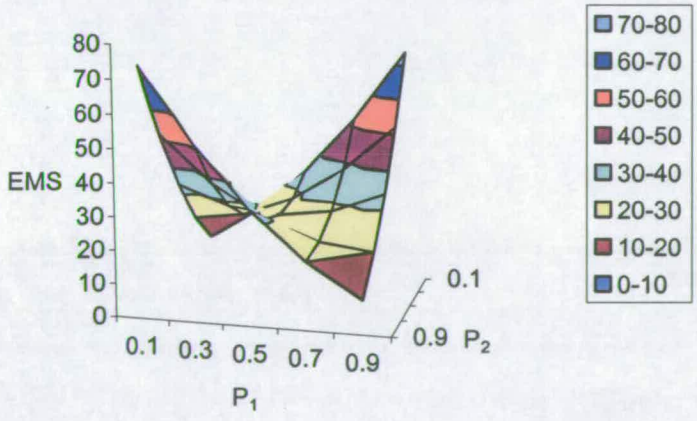


Figure 3.b2 Expected mean squares (EMS) for b_{PD}



CHAPTER 4

Genome-wide search for markers associated with Bovine Spongiform Encephalopathy

4.1 INTRODUCTION

Bovine Spongiform Encephalopathy (BSE) is a slowly progressing, inevitably fatal, neurodegenerative disorder characterised by deposition of an abnormal form of the host prion protein (PrP^{Sc}) in neurones, leading to a widespread sponge-like vacuolation of the brain (Hunter 1999, www.bseinquiry.gov.uk/index.htm). BSE belongs to a family of diseases known as Transmissible Spongiform Encephalopathies (TSEs) that include scrapie, in sheep, and Creutzfeldt-Jakob Disease (CJD), in humans. The prevailing hypothesis is that BSE is not a genetic disease, but that there could be a genetic component conferring resistance to the infectious agent (Ferguson et al. 1997, Donnelly et al. 1997). BSE can be experimentally transmitted across species via an isoform of the prion protein found in scrapie-affected sheep (PrP^{Sc}) (Bruce et al. 1994) and it appears that the bovine form of PrP^{Sc} is also responsible for a new variant of CJD (vCJD) (Bruce et al. 1997, Almond and Pattison 1997).

There is strong evidence that different polymorphisms in the PrP gene cause variable incubation periods (IP) and, possibly, degrees of resistance to scrapie in sheep (Goldmann et al. 1990, Goldmann et al. 1996) and to CJD in humans (Palmer et al. 1991). In particular codons 136, 154 and 171 in the PrP gene are strongly associated with incidence of scrapie (Goldmann et al. 1994). In humans, individuals homozygous at codon 129 of the PrP gene are over-represented in CJD cases, whereas heterozygous individuals seem to be more resistant to CJD (Palmer et al. 1991). All vCJD cases to date are homozygous for methionine at this codon (Bruce et al. 1997).

The PrP gene is less polymorphic in cattle than in sheep or humans. So far, only two polymorphisms, a variable number of an octapeptide repeat and an RFLP, have been found in the coding region of the bovine PrP gene (Goldmann et al. 1991b, Ryan and Womack 1993). Two case-control studies (Hunter et al. 1994b, Neibergs et al. 1994) found no association between the octapeptide-repeat and BSE, and only the latter study found a significant association between BSE and the RFLP.

Recently, there has been evidence of genes other than PrP affecting susceptibility to an experimental prion disease in mice. Stephenson et al. (2000) found Quantitative Trait Loci (QTL) partially accounting for differences of IP in F_2 mice, derived from a cross between two different parental strains with the same PrP gene, on Chr 9 and 11 using mouse-derived PrP^{Sc} inocula. In a similar experiment, Lloyd et al. (2001) found significant evidence for QTL influencing IP in mice on Chr 2, 11 and 12 plus suggestive evidence for QTL on Chr. 6 and 7. Manolakou et al. (2001) backcrossed F1 animals with their parental lines, identical only at the PrP locus, and used PrP^{Sc} inocula from cattle. They reported QTL influencing IP in mice on Chr 2, 4, 8 and 15, plus significant host environment factors (i.e. the age of the host's mother, the age of the host at infection, and an X-cytoplasm interaction in the host).

The purpose of the study reported here was to perform a genome wide scan for BSE susceptibility/resistance alleles in cattle through their associations with markers. The amount of association between two linked loci is a function of the underlying linkage disequilibrium (LD). In large and panmictic populations the expected range of LD for relatively old mutants is very short, and simulation studies have predicted that between 30,000 and 500,000 biallelic markers would be required in genome-wide QTL scans (Ott 2000, Kruglyak 1999). However, Riquet et al. (1999) predicted conserved chromosomal segments spanning 4.5-5 cM in one Holstein cattle population, and Farnir et al. (2000) found significant LD even between markers up to 50 cM apart. These results were probably due to a recent history of intensive artificial selection leading to high levels of inbreeding (Bradley and Cunningham 1999) and to a low effective population size (Roughsedge et al. 1999) leading to strong drift effects. Hence, relatively sparse marker maps may still be sufficient for genome-wide QTL scans in Holstein cattle.

Transmission-Disequilibrium Tests (TDTs) are tests for linkage in the presence of LD between a marker and a trait locus that are robust to spurious associations (i.e. association without linkage) due to population stratification. TDTs identify preferential transmission of particular alleles from heterozygous parents to affected offspring across a sample of families. Such a pattern of transmission is also known as segregation distortion, because alleles do not segregate at random. Although the original TDT (Spielman et al. 1993, Terwilliger and Ott 1992) and its extensions for multiple alleles (Bickeböller and Clerget-Darpoux 1995) are conceptually simple and easy to implement, more versatile TDTs have been developed based on logistic regressions (Sham and Curtis 1995, Waldman et al. 1999). In this paper, we use TDTs to assess the association between BSE and markers spanning the Holstein genome in half-sib families.

4.2 MATERIAL AND METHODS

4.2.1 Samples and genotyping

Blood samples were obtained from a total of 358 BSE-affected and 172 BSE-unaffected half-sib offspring from 4 Holstein sires throughout the UK (Table 4.1). Animals in both disease categories were sampled at the same time from the same farms and were age and sex-matched. None of the controls were recorded in the BSE case database of DEFRA at a later date. Micro-satellite markers were selected from the published bovine linkage maps (http://spinal.tag.csiro.au/ and http://www.marc.usda.gov) to give uniform coverage of all bovine chromosomes at approximately 20 centimorgan (cM) intervals. The markers were tested across a limited number of samples to estimate heterozygosity in the sires (DNA was not available from all sires). Markers with low heterozygosity were rejected and replaced by adjacent markers when available. A final panel of 166 markers was chosen for the study. These markers were genotyped on all samples using an ABI373 DNA sequencer and genotypes of the sires were inferred from the daughters' genotypes (genotyping was conducted by GeneSeek Inc., Nebraska, USA). Sires used in this study were found not to be heterozygous for 20 of the markers, therefore the total number of markers analysed was 146. The genotypes of the dams were unknown. Sex-linked loci could not be analysed with TDT in this study because the probability of allele transmission from sires to daughters assuming no association with BSE (i.e. under H_0) was a function of the recombination rate between Chr X and Chr Y at every specific locus.

Table 4.1. Total number of BSE-affected offspring and controls per sire

Sire	1	2	3	4	Total
BSE-Affected	124	88	93	53	358
Control	44	44	56	28	172

Only data that conformed to specific criteria could be analysed with TDT. First, only families with heterozygous sires for a marker are informative for that marker. Second, progeny with the same genotype as the sire were excluded from the analysis because it was ambiguous which allele had been transmitted (Sham et al. 2000). And third, estimated segregation ratios are biased when alleles within a locus have different frequencies, one parental genotype is missing and only offspring sharing the known parental genotype are

excluded from the analysis (Curtis and Sham 1995). In these circumstances, homozygous offspring were excluded from the analysis.

4.2.2 Statistical tests

The statistical tests used in this study derived from the McNemar-type TDT proposed by Spielman et al. (1993) adapted for multi-allelic loci (Bickeböller and Clerget-Darpoux 1995) and re-parameterisations of those TDTs within a logistic regression framework (Sham and Curtis 1995, McCullagh and Nelder 1983). The latter procedure was further extended for testing the interaction between transmission rate and disease status. Allelic transmission probabilities can be studied within and across genotypes. The genotypic-TDT statistic, *g-TDT*, assesses departures from random segregation of alleles within genotypes. Data from sires with identical genotypes is pooled together. The *g-TDT* is asymptotically distributed as a χ^2_{df} distribution with degrees of freedom (df) equal to the number of different heterozygous sires. The allelic-TDT statistic, *a-TDT*, is used to test departures from random segregation of alleles across genotypes. Asymptotically, *a-TDT* follows a χ^2_{df} distribution with df = a - 1, where a is the number of different alleles across all sires in the sample. Both *a-TDT* and *g-TDT* were also implemented using logistic regressions for parameter estimation and log-likelihood ratios for hypothesis testing assuming data to be binomially distributed. These tests were named *log-LR*$_a$ and *log-LR*$_g$, respectively. All likelihood ratios under H_0 are distributed as χ^2_{df} with df equal to the difference between the number of parameters estimated under H_1 (i.e. number of transmission probabilities estimated from data) and H_0 (i.e. no parameters estimated because all transmission probabilities are assumed to be ½). The use of theoretical reference distributions (i.e. χ^2) for obtaining p-values was empirically validated through simulations for all markers and two TDTs, *g-TDT* and *log-LRa*. For each marker and each sire family, a particular allele was sampled from a binomial B (n, p), where n was the total number of BSE-affected offspring within a sire family and p = ½, then both TDTs analysed the sample and the process was repeated 1,000 times.

Logistic regressions could not be solved when a particular allele was not transmitted to any offspring. Paradoxically, such observations are the most conclusive signals supporting a BSE-marker association. In order to analyse that valuable information, an *ad hoc* solution was to use 0.5 rather than 0 for an allele never transmitted to offspring, and N-0.5 rather than N for the allele always transmitted to offspring (N is the number of BSE-affected offspring in the analysis) (Cox 1970).

The basic tests described above only looked at transmission of sire alleles to affected progeny. Therefore, an interaction test was constructed to compare allele transmission rates to BSE affected and control offspring within the framework of logistic regression. If a marker allele was truly associated with a disease susceptibility allele then it should be over-transmitted to affected individuals and under-transmitted to control individuals. A log-likelihood ratio test for an interaction can be written as

$$\log\text{-}LR_{INT} = 2\sum_{ijk} N_{ijk} \ln\left(P_{ijk}/P_{ij}\right) \qquad [1]$$

where a sire with genotype ij ($i \neq j$) transmits allele i N_{ij1} times to BSE-affected offspring and N_{ij2} times to BSE-unaffected offspring, the probabilities of allele transmission within each disease category are P_{ijk} (obtained after solving logistic regressions) and the overall transmission probability across both disease categories is $P_{ij} = \left(N_{ij1} + N_{ij2}\right)/\left(N_{ij1} + N_{ji1} + N_{ij2} + N_{ji2}\right)$. The interaction test assumes that the transmission rate for a given marker is the same for both affected and control animals and, hence, a significant interaction indicates that the transmission rate differs between the two classes as might occur if a resistance/susceptibility locus in linked, and in LD, to the marker in question.

This would prevent false positive results from arising if there when there is a BSE-unrelated population-wide segregation distortion if both disease categories are equally distorted, as it could be the case testing only BSE affected individuals.

All statistical thresholds were adjusted using the Bonferroni's correction for multiple tests. However, this correction was rather conservative as it assumed all tests were independent, i.e. unlinked marker loci.

4.3 RESULTS

In selecting the appropriate data for the TDT some data were lost for all loci. This data loss caused a reduction of statistical power for detecting marker-BSE associations and was severe for 9 markers, which were removed from the analysis. The criterion for excluding a marker from the analysis was a non-significant *g-TDT* statistic when testing the most extreme allele distribution compatible with the data. Such an extreme distribution was obtained within each marker simulating a data set of the same size where only one allele was transmitted from sires. The list of markers used in this study is available in Table 4.A (Appendix).

Three marker loci (BM315, INRA107 and INRA36 on Chr 5, 10 and 20, respectively) showed a significant segregation distortion in BSE-affected individuals across all tests (Table 4.2). The *log-LR$_a$* and *log-LR$_g$* tests gave equivalent results and are shown as a single column in Table 4.2. The strongest evidence for segregation distortion across all tests was for marker INRA107. This marker was also the only one showing a significant departure from random segregation of alleles when analysing control individuals on their own with TDT.

Table 4.2. P-values of significant marker loci using a Bonferroni's threshold for 137 tests

Marker	*g-TDT*	*a-TDT*	*Log-LR$_a$*	*Log-LR$_{int}$*	Chromosome
BM315	$1.1x10^{-4}$	$1.6x10^{-5}$	$4x10^{-5}$	0.99	5
INRA107	$1.4x10^{-10}$	$1.2x10^{-9}$	$1.3x10^{-11}$	0.39	10
INRA36	$3.4x10^{-4}$	$5x10^{-4}$	$1.6x10^{-4}$	0.39	20

g-TDT : TDT based on the genotype model
a-TDT : TDT based on the allele model
log-LR$_a$: log-likelihood ratio based on the allelic model (similar results obtained with *log-LR$_g$*)
log-LR$_{int}$: log-likelihood ratio for interaction

In order to validate these results, two markers flanking each of the markers in Table 4.2 were genotyped. Marker BMS1658, at an estimated 2 cM from BM315 on Chr 5, was significant at a 0.01 level (Bonferroni's threshold set for 5 independent tests). The other flanking marker at this locus (BM8230) was uninformative because none of the sires was heterozygous. The results for the flanking markers are shown in Table 4.3.

Table 4.3. P-values of new markers closely linked to markers in Table 4.2

Old Markers (cM)	New Markers	*g-TDT*	*a-TDT*	*Log-LR$_a$*	*Log-LR$_{int}$*	cM
BM315 (100.1) Chr 5	BMS1658	0.0033	0.0019	0.0013	0.91	103.5
INRA107 (47) Chr 10	BM875	0.61	0.32	0.31	0.96	46.5
	BM888	0.83	0.52	0.52	0.99	50.4
INRA36 (59*) Chr 20	BMS2361	0.87	0.7	0.7	0.35	46
	AGLA29	0.57	0.49	0.35	0.72	51

g-TDT : TDT based on the genotype model
a-TDT : TDT based on the allele model
log-LR$_a$: log-likelihood ratio based on the allelic model (similar results obtained with *log-LR$_g$*)
log-LR$_{int}$: log-likelihood ratio for interaction
All distances have been obtained from the USDA98 map (http://www.marc.usda.gov) except (*) that was obtained from the Barendse97 map (http://locus.jouy.inra.fr)

Sample sizes and allele distribution among BSE-affected and control offspring for the 3 selected markers and their flanking markers are shown in Table 4.4.

Table 4.4. Counts of the number of times a particular allele (A or B) is transmitted from heterozygous sires to BSE-affected and control daughters for markers in Table 4.3

	BM875					INRA107					BM888				
	Genotype	BSE		Control		Genotype	BSE		Control		Genotype	BSE		Control	
Sire	A/B	A	B	A	B	A/B	A	B	A	B	A/B	A	B	A	B
1	127/127	?	?	?	?	180/179	39	3	13	0	192/190	20	18	4	6
2	127/127	?	?	?	?	180/179	29	8	9	1	192/190	12	13	10	6
3	127/127	?	?	?	?	?	?	?	?	?	196/192	13	8	2	1
4	127/125	3	6	1	3	?	?	?	?	?	192/190	12	10	7	4
	BMS2361					INRA36					AGLA29				
	Genotype	BSE		Control		Genotype	BSE		Control		Genotype	BSE		Control	
Sire	A/B	A	B	A	B	A/B	A	B	A	B	A/B	A	B	A	B
1	146/146	?	?	?	?	210/188	8	11	1	3	178/170	31	19	7	11
2	150/142	26	30	13	6	210/188	9	6	4	5	178/166	20	22	10	6
3	144/140	23	24	5	9	190/188	27	5	10	1	174/170	27	23	5	4
4	148/140	13	19	9	4	210/188	7	1	0	4	174/166	13	15	8	4
	BM8230					BM315					BMS1658				
	Genotype	BSE		Control		Genotype	BSE		Control		Genotype	BSE		Control	
Sire	A/B	A	B	A	B	A/B	A	B	A	B	A/B	A	B	A	B
1	?	?	?	?	?	142/124	25	30	10	9	102/94	0	19	0	8
2	?	?	?	?	?	?	?	?	?	?	106/102	28	23	7	7
3	?	?	?	?	?	138/126	30	5	7	1	102/94	9	12	1	1
4	?	?	?	?	?	?	?	?	?	?	92/92	?	?	?	?

A/B genotype of sire
A number of transmissions of allele A
B number of transmissions of allele B
? data not available because sire was either homozygote or had not been genotyped

Similar results were obtained using either theoretical χ^2 or empirical distributions as reference distributions (results not shown). None of the 137 markers showed any significant interaction between transmission and disease status.

4.4 DISCUSSION

The objective of this study was to identify markers associated with BSE by screening the entire bovine genome in a population of UK Holstein cows. TDTs were used because they are robust to spurious associations due to population stratification or admixture (Warren and Spielman 1995, Schork et al. 2001). A marker is associated with BSE when alleles at that marker did not segregate at random in a sample of affected individuals. Segregation distortion was tested within and across genotypes with *g-TDT* and *a-TDT*, respectively. These two tests gave similar results, and were equivalent when none of the sires had alleles in common. Both tests were also implemented within a logistic regression framework because this facilitated statistical modelling. For instance, logistic regressions were extended to analyse interactions between allele transmission rates and disease status. Moreover, additional variables such as age and farm could be more easily incorporated in logistic regressions than in *g-TDT* or *a-TDT*. In principle, logistic regressions would also allow testing loci on Chr Y, however the estimation of allelic transmission probabilities under H_0 would have been inaccurate because genetic distances were not estimated in this study and vary from population to population (Leach 1996).

Asymptotically, all these TDTs follow χ^2 distributions. However, there was a reduction in sample size because, in this study, TDTs could only be used to analyse heterozygous offspring from sires with a different heterozygous genotype. Therefore, the use of χ^2 as reference distribution was verified through simulations for *g-TDT* and *log-LRa*. The p-values obtained from empirical distributions in both TDTs were similar to p-values obtained from χ^2 distributions (data not shown). It was not necessary to check *a-TDT* and *log-LRg* because they are alternative parameterisations of *log-LRa* and *g-TDT*, respectively.

Three marker loci showed significant departures from random allelic segregation in BSE-affected individuals and were selected for further study. The strongest evidence (lowest p-value) came from marker INRA107 on Chr 10. Two sires with the same genotype were informative at this locus and the same allele was over-represented in their affected offspring. Furthermore, two candidate genes linked to INRA107 have homologues located on a region of mouse Chr 9 that showed suggestive evidence of QTL affecting IP following an experimental scrapie challenge (Stephenson et al. 2000). The homology data for this paper were obtained from the Mouse Genome Database (MGD). One of these genes encodes for the enzyme hexosaminidase A (HEXA), which is related to a progressive and lethal neurodegenerative human disorder called Tay Sachs (Bach et al. 2001). Whether variation in

the HEXA gene is partly responsible for controlling IP of a PrP^{Sc}-induced disorder in mice has yet to be tested, however the phenotypic effects on target tissues for TSEs makes it a candidate for further study.

INRA107 was also the only marker for which a significant segregation distortion was found in the control group, with the same allele being over-represented in both disease groups. Thus, there may be a common underlying cause of segregation distortion affecting both BSE-affected and control individuals, which may arise from sampling bias unrelated to BSE. Indeed, the second linked gene from the mouse study is the cytochrome P450 family XIX (CYP19). This gene is implicated in several human disorders such as reduced fertility and sexual organ development (Genissel and Carreau 2001, Carreau 2001), neonatal hypothyroidism (Ando et al. 2001), slow bone maturation and shorter adult height (Wickman et al. 2001), and breast cancer (Kuerer et al. 2001). It is possible that alleles at the CYP19 locus have a detrimental effect on fitness, leading to differential survival and, hence, give a spurious association of INRA107 with BSE.

Two flanking markers were genotyped, at an estimated 1.5 and 3.4 cM from INRA107, but neither was significantly associated with disease status (Table 4.3). These latter results were unexpected, considering that the probability of a recombination event between INRA107 and either of the two flanking markers in one generation is small. However, this apparent inconsistency can be explained by the fact that different families, different individuals within families and, hence, different sample sizes within a family, were used in the analysis of each of the markers. It should also be noted that the flanking markers were selected from the published genetic maps, and in some cases the position of markers was estimated between different maps. Thus, it is possible that the flanking markers were in fact further from the original marker than the estimated distance.

A similar inconsistency was observed between the marker INRA36 on Chr 20 and its two flanking markers. Despite a significant segregation distortion at the INRA36 locus, alleles at both flanking markers segregated at random. Again, the fact that different individuals were informative for the three loci may explain this discrepancy. Several QTL influencing milk traits have already been mapped on Chr 20 (Arranz et al. 1998) and low productivity is an important reason for culling, only second to reproduction problems (Young et al. 1983). Therefore the observed distortion at INRA36 could be through culling and selecting within herds favouring higher yielding individuals, rather than by association with BSE.

Two closely linked marker loci flanking the third significant locus, BM315 on Chr 5, were also genotyped on the samples. One of the markers was totally uninformative, but the other, at an estimated 2 cM from BM315, showed a significant effect at a 0.01 level. The number of homologies between the bovine Chr 5 and the murine Chr 6 (reported by Lloyd et al. 2001) and Chr 15 (reported by Manolakou et al. 2001) are 5 and 3, respectively (MGD). However, none of these genes seem to be functionally associated to BSE. Moreover, these genes are outside the 95% confidence interval for the location of QTLs in the mouse studies, and their distances from BM315 and BMS1658 cannot be precisely estimated as they only appear in cytogenetic cattle maps. However the confirmation of an association with two linked markers makes this chromosomal region worthy of further study.

As the PrP locus is involved in IP and development of TSEs in other species it is surprising that none of the markers genotyped on Chr 13, where the PrP gene is located, were associated with incidence of BSE in this analysis. To date there is little evidence that PrP polymorphisms are involved in BSE susceptibility, and the lack of significant results on Chr 13 in this study also suggests that the PrP alleles present in cattle do not add variation to BSE susceptibility.

It is necessary to interpret results obtained with TDT with care. TDTs cannot distinguish between a marker associated with a disease and a marker where alleles segregate non-randomly for other reasons. Indeed, if a marker is associated with a disease locus then its alleles will show a segregation distortion within a sample of affected individuals. Such distortion is entirely due to sample bias (testing only affected individuals) and needs not exist at population level. However, if distortion exists at population level then we recommend testing for an interaction because, unlike only testing affected individuals, it would be an unbiased test if the same degree of disease-independent segregation distortion was present in both affected and control individuals. It should be noted that although affected animals had evidently been exposed to PrP^{Sc} in sufficient quantities to induce BSE, controls may either be genetically resistant to BSE or may have not received sufficient challenge to become infected despite being age, sex and cohort matched sibs from the same farms as affected samples. The controls may also include animals with longer IP for the disease. We checked the BSE case database at a later date and did not identify any of the controls being reported as BSE cases. However, animals may have been culled for management reasons prior to the onset of BSE. This uncertainty may have reduced the power for detecting interaction effects in this study. Additionally, the total sample size of controls was smaller

than that of BSE-affected individuals, and neither more BSE-affected or more control individuals in these families could be sampled due to the retrospective nature of this study.

We have demonstrated that TDTs can be used in QTL genome scans with low-density marker maps. However, the success of the search depends, among other things, on having a minimum marker coverage, e.g. at least one marker per region of relatively constant LD (Goldstein 2001), and on the experimental design. Firstly, the minimum number of markers needed in genome-wide QTL analyses is still unclear (e.g. Lander and Schork 1994, Risch and Merikangas 1996, Chakravarti 1998, Terwilliger and Weiss 1998, Johnson et al. 2001) but it certainly depends on the LD pattern in the population. Secondly, this study sheds light on how the experimental design may impact on statistical power of TDTs, for example, not knowing maternal genotypes restricted the amount of information that could be used. We are also currently developing new statistical tests that use more of the information than TDTs, and studying the level of LD in this cattle population.

In conclusion, 3 marker loci (Chr 5, 10 and 20) were associated with BSE in the TDT analysis of a low-density genome scan. When these loci were tested further using flanking markers one locus closely linked to BM315 on Chr 5 also showed significant association with BSE. Neither of the loci on Chr 10 or 20 were confirmed by analysing flanking markers. However these three loci should be studied in more detail by increasing the density of markers in the regions of interest, and by examining transmission distortion in haplotypes.

4.5 APPENDIX A: ADDITIONAL DATA

Table 4.A1. List of all tested markers

Locus	[a] Cr	[b] cM	Locus	[a] Cr	[b] cM	Locus	[a] Cr	[b] cM
TGLA49	1	1.9	DIK106*	8	47	BM1233	17	98.6
TGLA57	1	46.2	TGLA13	8	51.4	INRA25	17	110
INRA128*	1	82	HEL9	8	76.7	IDVGA31	18	0
CSSM32	1	88.2	DIK74	8	77	INRA121	18	31.8
BM864	1	88.2	CSSM47	8	110.5	ABS13*	18	38
TGLA130	1	98.2	HUJ174*	8	121	HAUT14	18	44.8
CSSM19	1	108.3	BM757	9	0.6	ILSTS2	18	55.9
BM1824	1	108.6	ETH225	9	8.1	DIK67	18	70
BM3205	1	113.8	BM2504	9	25.2	AFL361	19	7
MAF46	1	118.1	UWCA9	9	44.9	HEL10	19	15.9
TGLA431	2	9.1	INRA84	9	84.3	CSSM65	19	65.7
CSSM42	2	34.4	MM12E6**	9	87	ETH3	19	81.5
BM4440	2	55	TGLA131	10	19.3	IDVGA44	19	90
TGLA226	2	80	BM875	10	46.5	BM3517	20	0
BM2113	2	106.2	INRA107	10	47	BMS2361	20	45.5
IDVGA2	2	118	BM888	10	50.4	AGLA29	20	50.6
ILSTS96	3	29.7	TGLA327	11	36.8	DIK15**	20	59
DIK69	3	33.7	ILSTS100	11	55.9	INRA36**	20	59
INRA123	3	66.2	TGLA272	11	86.8	BM5004	20	64.3
IDVGA35	3	102.9	CSSM46	11	92.9	HEL5	21	13
IOBT250*	3	104	CSRM60*	11	116	TGLA337	21	56.3
IDVGA27	3	123	TGLA36	12	6.8	IDVGA39	21	75.1
RM188	4	24.7	BM6108	12	15.8	CSSM26	22	0
MAF50	4	47.4	RM162	12	46	INRA26	22	2.9
INRA37	4	69.9	BM6404	12	56	INRA130**	22	23
DIK26*	4	87	IDVGA3	12	76.9	BM3628	22	44.5
RM88	4	94.8	HUJV174	12	85.4	UWCA49*	22	93
MGTBG4B*	4	128	L6003	13	20	IOBT528	23	0
BM6026	5	6.7	RM178	13	23.5	CSSM5	23	7.2
RM103	5	28.6	HUJ616	13	43.8	UWCA1	23	22.1
BR2936	5	64.3	INRA5	13	83.1	DRB3	23	43
ETH10	5	70	INRA209*	13	125	BM1905	23	64.3

BM1819	5	77.6	DIK93*	13	144	TGLA351	24	8.6
BM8230	5	88.4	CSSM66*	14	17	CSSM23	24	18.4
BM315	5	100.1	RM11	14	27.7	INRA90	24	53.2
BMS1658	5	102	BM4630	14	30.1	BM4005	25	12.3
ETH2	5	108.5	PZ271**	14	59	TGLA40*	25	25
BM2830	5	113.5	DIK54**	14	81	INRA222*	25	56
ILSTS93	6	0	ABS10*	14	114	ABS12*	26	0
BM1329	6	35.5	BR3510	15	1	HEL11	26	20.7
DIK82*	6	67	JAB1	15	20.8	RM26	26	37.3
RM28	6	74.3	IDVGA10**	15	52	BM4505	26	39.7
AFR227	6	90.4	FSHB	15	59.9	RM209	27	15
BM2320	6	120.7	BM4513	15	62.5	BM203	27	64.1
BM7160	7	0	TGLA53	16	40.6	BP23	28	4.7
BP41	7	13.9	ETH11	16	56.5	IDVGA29	28	8.7
BM1853	7	85	BM719	16	78	JAB5*	29	0
ILSTS6	7	116	HUJ625	16	89.8	RM44	29	23.3
INRA53	7	123.5	PZ510	17	16	TGLA86*	29	25
IDVGA11	8	8.8	TGLA231*	17	50			
BM4006	8	41.7	IDVGA40	17	67			

[a] Chromosome
[b] CentiMorgans
Primary sources of information were http://spinal.tag.csiro.au/ and http://www.marc.usda.gov, and secondary sources of information were http://www.thearkdb.org (*) and http://locus.jouy.inra.fr (**)

Table 4.A2. Marker losses due to TDT data restrictions

Locus	**[a] Cr**	**[b] cM**
TGLA49	1	1.9
BM3205	1	113.8
BP41	7	13.9
TGLA13	8	51.4
BM2504	9	52.2
L6003*	13	20
ETH11	16	56.5
UWCA1	23	22.1
INRA30**	X	183

See Table 4.A for a definition of: a, b, *, **

Table 4.A3. Marker losses due to lack of informativeness		
Locus	[a] **Cr**	[b] **cM**
HUJ117	3	87
AGLA293	5	32
BP7	6	91.2
CSSM29**	7	86
INRAMTT180	8	67
BM716	11	9.5
INRA177	11	26
TGLA6**	13	12
ETH7	13	54.4
URB48	17	0
CSSM33	17	75
BM1225	20	8
TGLA126	20	31.2
ETH131	21	32
ILSTS101	24	34
BM226	24	75
BM3507	27	0
CSSM43	27	34
HAU37**	X	116
See Table 4.A for a definition of: a, b, *, **		

CHAPTER 5

Prediction of Identity By Descent based on Marker Information and Linked Gene Flow Theory: Potential Applications for Fine Mapping Quantitative Trait Loci

5.1 INTRODUCTION

The linkage disequilibrium (LD) framework has become the new paradigm for fine quantitative trait loci (QTL) mapping (Terwilliger and Weiss 1998, Cardon and Bell 2001, Ardlie et al. 2002). Within this framework, families that are unrelated by a common pedigree are assumed to share common ancestors in the past. Hence, in LD mapping, historical recombination events are tracked down in the form of population-wide associations. In contrast, in QTL mapping via linkage analysis, only those recombination events recorded within known pedigrees are used (Hoeschele et al. 1997). As a consequence, linkage signals can spread over long chromosomal distances, whereas association signals are expected only over much shorter distances.

Meuwissen and Goddard (2000a) developed an original methodology for fine mapping QTL by modelling the history of haplotypes, assuming a genetically homogeneous population evolving by drift. They estimated the variance of QTL (σ^2_{QTL}) using mixed linear models (e.g. Grignola et al. 1996, George et al. 2000). The novelty of their approach was to model the covariance due to the QTL as $\sigma^2_{QTL}H_p$, where H_p is a matrix of IBD probabilities between all pairs of haplotypes at point p, estimated using identity-by-state (IBS) information from surrounding markers. Although H_p was initially obtained stochastically, Meuwissen and Goddard (2001) have also derived H_p deterministically based on Sved (1971). Meuwissen and Goddard (2000b) put this method in practice and were able to map a QTL for twinning rate in Norwegian cattle within a 1.3 cM region on chromosome 5, which is considerably more precise than would have been expected with a linkage analysis (Boehnke 1994).

The strengths of the method of Meuwissen and Goddard can be used together with a novel and more flexible way of estimating IBD probabilities based on linked gene flow (LGF) theory. This theory is an extension of the long-term genetic contributions theory used for predicting genetic gain when practising artificial selection whilst restricting rates of inbreeding (Bijma 2000).

In this work, we tested new developments in LGF theory with simulations, proved them worth pursuing, and applied them to calculate inbreeding at a particular genomic location i for a particular individual j (F_{ij}). A multiple regression equation allowed us to use neighbouring marker information when estimating F_{ij}. We also demonstrated that the utilisation of linked markers for predicting F_{ij} increases the accuracy of prediction.

5.2 MATERIALS AND METHODS

5.2.1 Joint Inbreeding (F_J)

Approach I. Under the assumption of a constant effective population size (N_e) over generations, the rate of accumulation of inbreeding per generation is $\Delta F = \frac{1}{2N_e}$ (equation [4.1] in Falconer and Mackay 1996). The average level of inbreeding in the population at generation t can be calculated as (equation [3.12] in Falconer and Mackay 1996)

$$F(t) = 1 - (1 - \Delta F)^t \qquad [1]$$

Alternatively, Woolliams and Bijma (2000) expressed $F(t)$ in terms of genetic contributions (r_i) and Mendelian sampling terms (a_i) of ancestors, as follows:

$$F(t) = \sum_{alleles} \sum_{i} r_{i,0}(m,t-1) r_{i,0}(f,t-1) A_{i,0}^2 + \sum_{alleles} \sum_{u=1}^{t-1} \sum_{i} r_{i,u}(m,t-1) r_{i,u}(f,t-1) a_{i,u}^2 \qquad [2]$$

where the first sum is over all alleles present at generation 0 ($2N$ assuming unrelated founders, where N is the number of founders), $A_{i,0}$ is the frequency of each allele present at generation 0 within the ith founder (e.g. for allele 1, $A_{i,0}$ = ½ for one founder and zero for all other individuals), $r_{i,0}(s,t-1)$ is the genetic contribution of founder i to parents of sex s at generation t-1 ($s = m, f$ denoting mother and father, respectively), and $a_{i,u}$ is the Mendelian sampling term of ancestor i at generation $u \geq 1$, i.e. $a_{i,u} = A_{i,u} - \frac{1}{2}(A_m + A_f)$. Nevertheless,

the choice of the founder generation is arbitrary as long as it is a distant one, and therefore, without loss of generality, we can let the population at generation 1 be the founders, and hence equation [2] simplifies to

$$F(t-1)=\sum_{alleles}\sum_{u=0}^{t-2}\sum_{i} r_{i,u}(m,t-2)r_{i,u}(f,t-2)a_{i,u}^2$$

and taking expectations

$$E[F(t-1)]=\sum_{i} E\left[\sum_{alleles}\sum_{u=0}^{t-2} r_i^2\right]\sum_{u=0}^{t-2} E\left[\sum_{alleles}\sum_{i} a_{i,u}^2\right]=\sum_{i}\hat{r}_i^2\sum_{u=0}^{t-2}\frac{1}{4}(1-\Delta F)^u \qquad [3]$$

Note that: 1) under random mating and no selection, an ancestor is expected to contribute the same proportion of genes to both parents, i.e. $E[r_{i,u}(m,t-1)]=E[r_{i,u}(f,t-1)]$, 2) at any given generation u, genetic contributions $r_{i,u}$ are independent from Mendelian sampling terms $a_{i,u}$, 3) the long-term genetic contributions of any ancestor are constant, i.e. $\lim_{t\to\infty} r_{i,t}=\hat{r}_i$, and $\Delta F=\frac{1}{4}\sum_{i}\hat{r}_i^2$, and 4) see Appendix A for a demonstration of $E\left[\sum_{alleles}\sum_{i} a_i^2\right]=\frac{1}{4}(1-\Delta F)$. Finally, equation [3] simplifies to

$$F(t)=\Delta F\sum_{u=0}^{t-1}(1-\Delta F)^u \qquad [4]$$

which is identical to equation [1] (using $\sum_{i=0}^{k} x^i=\frac{1-x^{k+1}}{1-x}$). The term $(1-\Delta F)^u$ represents the proportion of Mendelian sampling variance remaining at generation u.

The previous derivations apply to a single locus system. The study of a 2-loci system requires modelling the co-segregation of alleles on haplotypes due to linkage. The term $(1-2c)^t$ models the proportion of Mendelian sampling variance of haplotypes explained by linkage (c is the recombination rate between the loci). Equation [4] can be extended as follows

$$F_J(t)=\frac{1}{4}\sum_{i} r_i^2\sum_{u=0}^{t-1}(1-\Delta F)^{u-1}(1-2c)^{u-1}=\Delta F\left[\frac{1-(1-\Delta F)^t(1-2c)^t}{1-(1-\Delta F)(1-2c)}\right] \qquad [5]$$

where $F_J(t)$ is the joint inbreeding at generation t. Formally, $F_J(t)$ is the probability of sampling two haplotypes (i.e. gametes) in generation t containing alleles that were originally together on the same founder haplotype. This probability includes only founder haplotypes, i.e. those that have not recombined since the founding of the population or have recombined with other IBD haplotypes. Founder-like haplotypes may have also appeared at any generation u, where $0 < u < t$, due to recombination, although these are not included in the probability calculations. The relative importance of the latter type of haplotypes will be discussed in the light of simulation results.

Of particular interest is the steady-state equilibrium, i.e. where $F(t) = F(t-1)$ for any sufficiently large t. Letting t go to infinity we obtain (using $\sum_{i=0}^{\infty} x^i = \frac{1}{1-x}$ when $-1 < x < 1$)

$$F_J(t \to \infty) = \frac{1}{1+4N_e c - 2c} \approx \frac{1}{1+4N_e c} \qquad [6]$$

where the approximation holds when c is small and N_e large. Equation [6] has already been derived previously (e.g. Sved 1971). The parameter space of $F_J(t \to \infty)$ is bounded between ΔF and 1, for $c = ½$ and $c = 0$, respectively.

Approach II. Alternatively, Sved (1971) defined Q as the conditional probability of joint IBD at two loci, given that one of them is IBD, moreover $Q = E[r^2]$, where r^2 is the square correlation between allele frequencies at two loci (Hill 1975). Sved clearly stated that he considers only founder haplotypes, those without historical recombinations. This model is an adaptation of a recurrent formula for predicting inbreeding at a locus under the assumption of an infinite allele mutational model, i.e. neutral alleles are generated at a constant rate μ each generation, and each allele is different from all previous alleles (Hartl and Clark 1997 p175, Falconer and Mackay 1996 p79). The model is

$$Q(t) = (1-\Delta F)(1-c)^2 Q(t-1) + \Delta F(1-c)^2 \qquad [7]$$

where ΔF is equivalent to the probability of sampling twice the same haplotype, $(1 - \Delta F)Q(t-1)$ is the probability of sampling two different haplotypes that were identical in the previous generation, and $(1-c)^2$ is the probability of zero or an even number of recombination events

in both haplotypes. Equation [6] can also be written as

$$Q(t) = \Delta F(1-c)^2 \sum_{u=0}^{t-1} \left[(1-\Delta F)(1-c)^2\right]^u = \Delta F(1-c)^2 \frac{1-\left[(1-\Delta F)(1-c)^2\right]^t}{1-(1-\Delta F)(1-c)^2} \quad [8]$$

At the steady-state equilibrium, equation [7] is

$$Q(t \to \infty) = \frac{(1-c)^2}{2N_e c(2-c)+(1-c)^2} \approx \frac{1}{1+4N_e c} \quad [9]$$

Hence, both approaches predict the same equilibrium point when c is small and N_e large (equations [6] and [8]). However, the parameter space of Q is bounded between $\frac{1}{6N_e+1}$ and 1, for c = ½ and c = 0, respectively, hence slightly differing from F_J.

5.2.2 Simulations

We checked the validity of equation [5] via computer simulations. We followed the flow of haplotypes through generations in an ideal population, and counted how many times two haplotypes identical to a haplotype at generation t = 0 were inherited by an individual at generation t, considering independently both ancestral (without historical recombinations) and ancestral-like haplotypes (with historical recombinations).

Let us consider two neutral loci and unrelated founders, so that the first founder had haplotypes {1,1} and {2,2}, the second founder haplotypes {3,3} and {4,4}, et cetera. At each generation, haplotypes were allowed to recombine before sampling among them at random to create the parents for the next generation. Selfing was also permitted. The population size (N_e) remained constant over time, and the generations were discrete. We explored different combinations of parameters c and N_e, each one being replicated 1000 times.

5.2.3 Inbreeding per individual and locus

Two alleles that look alike but descend from different founders are said to be Identical-by-state (IBS). The joint probability of two loci containing IBS alleles is (denoted by the area shared between the circles in Figure 5.1)

$$P_{IBS} = F_J + (F_1 - F_J)\sum_j P_{2j}^2 + (F_2 - F_J)\sum_j P_{1j}^2 + (1 - F_1 - F_2 + F_J)\sum_j P_{1j}^2 \sum_j P_{2j}^2 \qquad [10]$$

where F_J is the probability of joint inbreeding, F_i is the inbreeding coefficient at locus i calculated with equation [1], p_{ij} is the frequency of allele j at locus i, and $\sum_j p_{ij}^2$ is the observed homozygosity at locus i. Equation [3] can be simplified as, on average, $F_1 = F_2 = F$, and using $\Pi_i = \sum_j p_{ij}^2$

$$P_{IBS} = F_J + (F - F_J)(\Pi_1 + \Pi_2) + (1 - 2F + F_J)\Pi_1\Pi_2 \qquad [11]$$

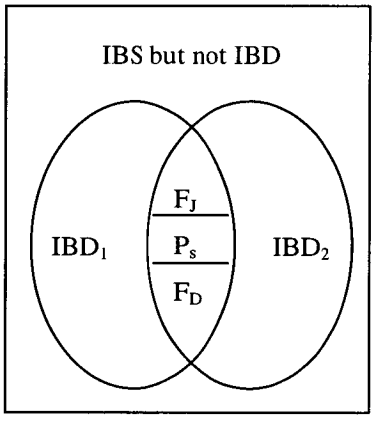


Figure 5.1. Different classes within homozygous genotypes at two loci. The area within the square excluding both circles denotes the 'IBS but not IBD' class. The area within the left (right) circle only, denotes IBD at locus 1 (locus 2) only. The area common to both circles denotes IBD at both loci. Within this area there are three subclasses: 1) Joint Inbreeding (F_J), or the probability of sampling twice the same ancestral (non-recombinant) haplotype, 2) Pseudo Joint Inbreeding (Ps), or the probability of sampling twice the same recombinant haplotype that is IBS with an ancestral haplotype, and 3) Disjoint Inbreeding (F_D), or the probability of sampling twice the same recombinant haplotype that is not IBS with an ancestral haplotype.

The covariance matrix between two loci in terms of IBD and IBS is given in Table 5.1 (see derivation in Appendix B). The inbreeding at locus i (i.e. the probability of IBD alleles at locus i) in the j^{th} individual is F_{ij}, and can be predicted using IBS information from neighbouring marker loci via a multiple regression technique (e.g. Draper and Smith 1966). The linear model is

$$F_{ij} = F + \sum_{k=1}^{n_k} b_{ik}\left(IBS_{kj} - \overline{IBS}_k\right) + e_j \qquad [12]$$

where F is the average expected inbreeding in the population (equation [1]), n_k is the number of segregating marker loci for which IBS (i.e. homozygosity) is known, IBS_{kj} is 1 if

individual j is homozygous at locus k, or 0 otherwise, $\overline{IBS}_k$ is the observed mean IBS at locus k in the population, or alternatively, its expectation: $E[IBS_k] = F + (1-F)\Pi_k$), and b_{ik} the regression coefficient relating IBS at locus k with IBD at locus i. If IBS information comes from a single marker m the regression coefficient would be

$$b_{im} = \frac{\sigma_{IBD(i),IBS(m)}}{\sigma^2_{IBS(m)}} = \frac{(F_J - F^2)}{(1-F)(F+(1-F)\Pi_m)} \qquad [13]$$

For k markers, the vector β with regression coefficients b_{ik} ($k = 1 \ldots n_k$) can be calculated as $\beta = V^{-1}G$, where V^{-1} is the inverse of the variance-covariance matrix of IBS among markers, i.e. bottom right quadrant in Table 5.1, and G is a vector containing the covariances ($F_J - F^2$)($1-\Pi_j$), for $j = 1 \ldots k$, linking IBS at locus j with IBD at locus i.

Table 5.1. Co-variance matrix of IBD and IBS between and within two loci

		IBD		IBS	
	Locus	1	2	1	2
IBD	1	F(1–F)	F_J–F^2	F(1–F)(1-Π_1)	(F_J–F^2)(1-Π_2)
	2	F_J–F^2	F(1–F)	(F_J–F^2)(1-Π_1)	F(1–F)(1-Π_2)
IBS	1	F(1–F)(1-Π_1)	(F_J–F^2)(1-Π_2)	(1–F)(1-Π_1)(F+(1–F)Π_1)	(F_J–F^2)(1-Π_1-Π_2+$\Pi_1\Pi_2$)
	2	(F_J–F^2)(1-Π_1)	F(1–F)(1-Π_2)	(F_J–F^2)(1-Π_1-Π_2+$\Pi_1\Pi_2$)	(1–F)(1-Π_2)(F+(1–F)Π_2)

IBD: Identity By Descent
IBS: Identity By State
F: Inbreeding coefficient within a locus, equation [1]
F_J: Joint inbreeding at 2 loci, equation [2]
Π_j: Homozygosity at locus j

5.2.4 Prediction error variance of F_{ij}

The prediction error variance of F_{ij}, $PEV(F_{ij})$, was obtained via simulations. IBS was simulated labelling alleles 0 or 1 at random in the founder population. Regarding IBS, the founder population was in linkage equilibrium, i.e. D = 0. The frequency of allele 1 at $u = 0$ was ½, and the changes in IBS and IBD throughout generations were recorded. IBD was predicted at locus i in each generation using equation [11]. The PEV was calculated as

$$PEV(F_{ij}) = \frac{\sum_{k=1}^{n_r}\sum_{j=1}^{N_e}\left(F_{ij}^k - \hat{F}_{ij}^k\right)^2}{n_r \cdot N_e} \qquad [14]$$

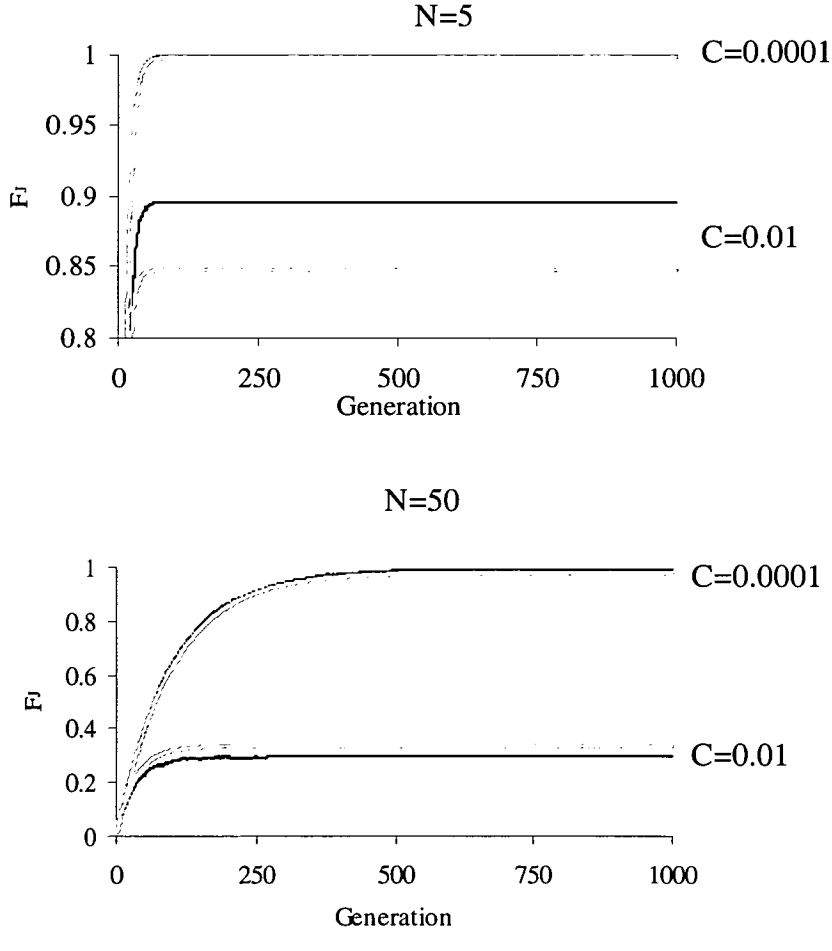
where n_r = 1000 were the number of replicates, F_{ij}^k was the observed and $\hat{F}_{ij}^k$ the predicted value of F_{ij} in the k[th] replicate, respectively.

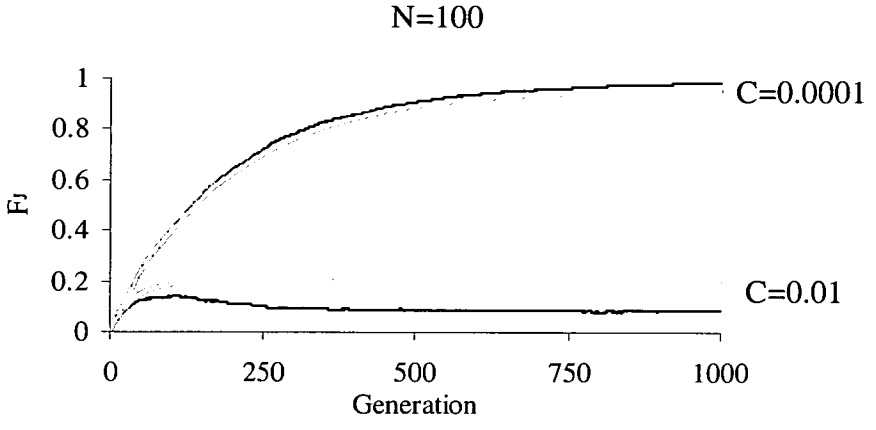
5.3 RESULTS

5.3.1 Validating F_J

F_J has been defined as the probability of sampling two identical founder haplotypes at any given generation. At equilibrium, F_J can be also interpreted as the probability that a population is fixed for any founder haplotype. Changes of F_J over generations were monitored for different combinations of parameters, N_e = [5, 50, 100] and c = [0.01, 0.0001]. There were 1000 replicates per parameter combination (Figure 5.2). Deterministic predictions of F_J using formula [5] were closer to empirical results during early generations with low c. The greatest difference between deterministic and empirical F_J occurs when equilibrium is reached.

Figure 5.2. Changes of F_J over generations, results with equation [5] shown in grey, simulation results shown in black.





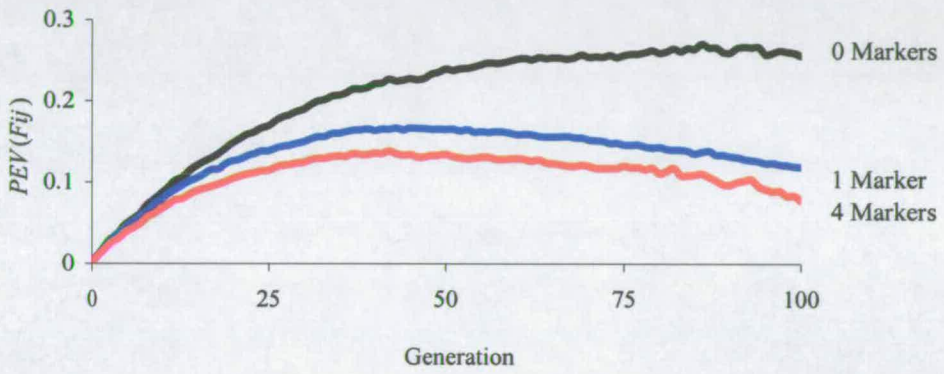
5.3.2 Impact of marker information on the $PEV(F_{ij})$

The amount of information to predict $F_{ij}(t)$ at locus i within individual j at generation t, abbreviated as F_{ij}, increases with the number of genotyped markers, providing that they segregate in the population, and with the degree of linkage between markers and locus i. The prediction error variance of F_{ij}, $PEV(F_{ij})$ (Equation [14]), is a measure of how much information is available for predicting F_{ij}, e.g. large $PEV(F_{ij})$ values correspond to little information and vice-versa. In the absence of markers F_{ij} is predicted by equation [1], and with marker information F_{ij} is predicted by equation [5]. Note that F_{ij} may be outside the range [0,1] when using marker information, however, rejecting those replicates in which $F_{ij} > 1$ did not have a serious impact on $PEV(F_{ij})$. Figure 5.3 shows the changes in $PEV(F_{ij})$ across 100 generations when using 0, 1 or 4 markers. In these simulations, the locus i was surrounded by four markers, two on each side, with c values between each marker and locus i equal to 0.2, 0.01, 0.001, and 0.1, from left to right, respectively. There were 1000 replicates per generation.

We always observed the same general pattern of changes in $PEV(F_{ij})$ over generations, regardless of the number of markers used. At generation $u = 0$ $PEV(F_{ij}) = 0$ because none of the individuals in the population were inbred. For $u > 0$, $PEV(F_{ij})$ increases until reaching a peak, and steadily decreasing thereafter (Figure 5.4). The height of the peak depends on the number of markers. In the long run, e.g. $u \rightarrow \infty$, $PEV(F_{ij}) = 0$, as all individuals become

eventually inbred. It is important to notice that, except at the extremes ($u = 0$ or $u \rightarrow \infty$), $PEV(F_{ij})$ showed always the lowest curve when using 4 marker loci, and the highest when using no markers. The relative drop in $PEV(F_{ij})$ was larger when a single tightly linked marker ($c = 0.001$) was used compared to a situation without marker information, than when three markers, loosely linked to locus i ($c = 0.01, 0.1, 0.2$), were added to a system that already contained a marker tightly linked ($c = 0.001$) to locus i. This result is highlighting the fact that not only marker density is important for predicting F_{ij}, but also tight linkage.

Figure 5.3. $PEV(Fij)$ over generations, using none, one ($c = 0.001$) or four markers ($c = 0.001, 0.01, 0.1, 0.2$)



5.4 Discussion

The linked gene flow (LGF) theory provides information on identity-by-descent (IBD) in a simple deterministic fashion. In this study we extend the existing theory based on Woolliams et al. (1999), to encompass a finite number of linked loci. This methodology is the only methodology that has predicted gene flows accurately over multiple generations in selected populations. Bijma et al. (1999) demonstrated its accuracy and Bijma and Woolliams (2000) demonstrated the weakness of a previous gene flow model (Hill 1974) to cope with gene flow with selection. The gene flow model (Woolliams and Bijma 2000) has an added benefit in that it naturally decomposes the population into genetic contributions and Mendelian sampling terms. The former are sufficient statistics for IBD arising through pedigree development, and are independent from the Mendelian sampling terms. Therefore it is a natural choice as a basic model since it has the ability to grow with the development of the applications.

This study demonstrates the accuracy of LGF theory in predicting IBD at a locus using neighbouring marker information, for a typical range of parameters (e.g. $t < 100$), under

random mating conditions. We have, nevertheless, observed that when t → ∞ certain parameter combinations, e.g. $N_e = 100$ and $c = 0.005$, empirical and deterministic predictions of F_J are much more similar than for other parameter combinations, e.g. $N_e = 5$ and $c = 0.01$. Although the theory predicts F_J better when c is small, the nature of this apparent discrepancy between theory and simulations in certain situations requires further study. F_J is explaining the proportion of Mendelian sampling variance due to allelic co-segregation via linkage. However, genetic drift can generate allelic co-segregation independently from linkage, by means of reducing the Mendelian sampling variance. We think that the inability of the current theory in predicting F_J when c is large is solvable by developing another term to take into account co-segregation due to drift. Meanwhile, F_J predicted with formula [5] coincides with Q predicted with formula [8] when c is small, and both are very close to simulation results. Yet, neither approach is satisfactory when $c > 0.1$.

An advantage of LGF theory over the theory used by Meuwissen and Goddard is that it provides the framework from which more complete models can be developed, e.g. including selection and/or mutation. For example, Wall et al. (2002) used LGF theory to estimate a multivariate Mendelian sampling term to predict IBD in the course of gene introgression on both QTL carrier and non-carrier chromosomes. Although introgression considers only relatively short pedigrees it is nevertheless a process of selection based upon markers. We wish to develop a linkage disequilibrium mapping method that utilizes the strengths of Meuwissen and Goddard (2000) by complementing it with the strengths of LDF theory. Hence, the basic mixed model methodology of George et al. (2000) will be extended to use a haplotype model with an IBD covariance matrix at its heart, derived from LGF theory. Additionally, we will extend the methodology to rapid calculation of IBD matrices in complex pedigrees using the methodology of Pong-Wong et al. (2001).

We have shown how the prediction error variance (PEV) of estimates of point IBD within an individual decreases as more marker information is used. Most of the information for predicting F_{ij} comes from tightly linked markers, although elucidating the optimum number and distances to locus i needs further work. Given optimum marker coverage, accurate multipoint IBD predictions along a chromosome (or genome) can be obtained. This information could be used, for instance, to investigate the impact of selection in determining patterns of localised inbreeding, and to increase the accuracy of estimation of average relationship matrices.

The power of this novel methodology will be compared against the power of existing QTL mapping methods, especially that proposed by Meuwissen and Goddard (2000), when 1) QTL genotypes are known, and 2), QTL genotypes are unknown but the current haplotypes surrounding the QTL are known. The robustness of the methodology will be assessed when assumptions are not fulfilled, and new theoretical enhancements will allow modelling more realistic situations, e.g. including mutation, migration and/or selection. Finally, we envisage LGF theory could aid the estimation of genetic relationships between individuals in natural populations where pedigrees are not recorded.

5.5 APPENDIX A: CO-VARIANCE OF IBD-IBS

The co-variance matrix of IBD-IBS, between and within loci, will be derived next. Let x and y be two variables representing IBS and IBD, respectively. The following procedure applies to both x and y, so let us consider x alone. Let x be 1 if two alleles chosen at random from a locus within an individual are IBS, and 0 otherwise. The variance of x within any locus is $\sigma^2(x)$, and the covariance of IBS between loci i and j will be denoted as $\sigma(x_i,x_j)$. The variance of x can be obtained from $\sigma^2(x)=E[x^2]-(E[x])^2$, where $E[x]=E[x^2]=P(x=0)\cdot 0+P(x=1)\cdot 1=P(x=1)$, which is the probability of IBS within a locus, hence

$$\sigma^2(x)=P(x=1)\cdot(1-P(x=1)) \qquad [A1]$$

The covariance of IBS between loci i and j is $\sigma(x_i,x_j)=E[x_ix_j]-E[x_i]E[x_j]$, where

$$E[x_ix_j]=\sum_{m=0}^{1}\sum_{n=0}^{1}P(x_i=m\cap x_j=n)\cdot m\cdot n=P(x_i=1\cap x_j=1)$$

and hence,

$$\sigma(x_i,x_j)=P(x_i=1\cap x_j=1)-P(x_i=1)\cdot P(x_j=1) \qquad [A2]$$

Likewise, the variance of IBD within a locus is

$$\sigma^2(y)=P(y=1)\cdot(1-P(y=1)) \qquad [A3]$$

and the covariance of IBD between loci is

$$\sigma(y_i,y_j)=P(y_i=1\cap y_j=1)-P(y_i=1)\cdot P(y_j=1) \qquad [A4]$$

The covariance between IBD and IBS is

$$\sigma(x_i y_j) = P(x_i = 1 \cap y_j = 1) - P(x_i = 1) \cdot P(y_j = 1) \qquad [A5]$$

where $i, j = 1, 2$.

In what follows, we will drop '$= 1$' in equations [A1] to [A5] for clarity, as we are interested in either IBS or IBD events, and not in the events non-IBS or non-IBD. Let us calculate the following probabilities $P(y)$, $P(x)$, $P(x_i \cap x_j)$, $P(y_i \cap y_j)$ and $P(x_i \cap y_j)$, where $i \neq j$. Figure 5.1 may help appreciating the following derivations.

The probability that alleles at a locus are IBD is precisely the inbreeding coefficent (F), hence $P(y) = F$. At a locus, IBD alleles are also IBS alleles, although the opposite is not necessarily true. Hence, the probability of simultaneous IBD and IBS at a locus is $P(x_i \cap y_i) = F$. The probability of IBS alleles at a locus is the probability of that locus being homozygous. Two mutually exclusive events can generate homozygosity at a locus: either alleles are IBD, so they are automatically IBS, or alleles are not IBD although they are IBS. Hence $P(x) = F + (1 - F)\Pi$, where $\Pi = \sum p_i^2$, and p_i is the frequency of allele i. The probability of joint IBD at two loci is $P(y_i, y_j) = F_J$, for $i \neq j$, where F_J is given in equation [5].

We have now all the necessary elements for calculating the co-variances between and within loci for IBD and IBS, and they have been summarised in Table 5.A.

Table 5.A. IBD and IBS variances and covariances between and within loci.
$\sigma^2(x) = (1-F)\cdot(1-\Pi)\cdot(F+(1-F)\Pi)$
$\sigma^2(y) = F\cdot(1-F)$
$\sigma(y_1,y_2) = F_J - F^2$
$\sigma(x_1,x_2) = (F_J - F^2)\cdot(1-\Pi_1-\Pi_2+\Pi_1\cdot\Pi_2)$
$\sigma(x_1,y_2) = (F_J - F^2)\cdot(1-\Pi_2)$
x: Variable for IBS y: Variable for IBD F: Population inbreeding, equation [1] F_J: Joint inbreeding, equation [5] Π_i: Homozygosity at locus i

5.6 APPENDIX B: VARIANCE OF MENDELIAN TERMS

Assume the frequency of allele b in the i[th] individual is the breeding value of that individual (B_i). Let B_i take the value 1, ½ or 0 if the genotype of individual i is bb, $b\cdot$ or $\cdot\cdot$, respectively ($\cdot$ represents any allele other than b). The Mendelian sampling term of individual i can be calculated as $M_i = B_i - (B_f + B_m)/2$, where f and m stand for father and mother of i, respectively.

Table 5.B. Mendelian sampling terms							
Parents		Progeny					
Genotypes	[a] P	bb	[b] P	$b\cdot$	[b] P	$\cdot\cdot$	[b] P
bb x bb	p^4	0	1				
bb x $b\cdot$	$2p^3q$	¼	½	-¼	½		
bb x $\cdot\cdot$	p^2q^2			0	1		
$b\cdot$ x $b\cdot$	$4p^2q^2$	½	¼	0	½	-½	¼
$\cdot\cdot$ x $b\cdot$	$2pq^3$			¼	½	-¼	½
$\cdot\cdot$ x $\cdot\cdot$	q^4					0	1
[a] Probability of joint parental genotypes [b] Probability of progeny genotypes p Frequency of allele b, and q = 1 – p							

The variance of Mi is $V(M_i) = E[M_i^2] - E[M_i]^2 = E[M_i^2]$, as the expectation of the individual Mendelian sampling term is zero. Hence, $E[M_i^2] = \sum_j f_j E[M_{ij}^2 \mid j]$, where f_j is the probability of the j[th] set of parents and $E[M_{ij}^2 \mid j]$ is the expected square Mendelian sampling term of individual i conditional on the j[th] set of parents (Table 5.B). Finally, $V[M_i] = \frac{pq}{4}$, if there is no inbreeding, otherwise $V[M_i] = \frac{pq}{4}(1 - \Delta F)$, where (1 - ΔF) reflects the decay of Mendelian sampling variance in one generation. When considering all alleles together $V[M_i] = \frac{1}{4}\sum_j \left(p_j \sum_{k \neq j} p_k \right)(1 - \Delta F) \approx \frac{(1 - \Delta F)}{4}$, as the number of alleles is large, i.e. j is large, $\sum_j \left(p_j \sum_{k \neq j} p_k \right) \approx 1$.

CHAPTER 6

6.1 Discussion

Genetics is revolutionising the breadth and depth of agriculture, basic biology, biomedicine and biotechnology. The accelerated rate of gene discovery is allowing geneticists to unravel the genetic architecture of traits. This goal usually involves four steps: 1) localisation of chromosomal regions likely to contain the gene of interest, 2) positional cloning of this gene, 3) the study of functional polymorphisms within the gene, and 4) the study of gene products. Steps 1 and 2 comprise the area of gene mapping; steps 3 and 4 are the realm of genomics, proteomics and physiology.

The benefits of one decade of gene mapping can already be seen, for example, in health care, where mapping the cystic fibrosis gene (Kerem et al. 1989) has led to the development of gene therapy treatments (e.g. Blair et al. 1998), and where drug design is now taking into account both human evolutionary adaptations (Hofbauer and Huppertz 2002) and genetic differences between individuals (Ensom et al. 2001). In agriculture, food production has increased, diversified and become more efficient because of artificial selection based mainly on individual performance (e.g. Bichard 2002, Khush 1999, Conway and Toenniessen 1999). However, performance records can be difficult, time consuming and costly to collect, and the accuracy of selection is reduced when environmental and genetic effects are confounded. Hence, faster genetic gains are expected by direct selection of favourable combinations of genes.

The scope of this thesis falls into step number one mentioned above, i.e. the localisation of chromosomal regions likely to contain genes of interest. Specifically, we were interested in the study and development of statistical techniques for refining the location of genes within these chromosomal regions.

Genome-wide linkage analyses have rendered evidence for chromosomal regions harbouring genes of interest, albeit with broad confidence intervals (CI) for their location estimates. Fine mapping is concerned with reducing these CI in order to facilitate positional cloning of genes. One of the strategies in fine gene mapping is searching for population-wide associations between markers and traits. This approach relies on the existence of sufficiently strong LD between neutral and causal polymorphisms. However, errors in association studies

have led in many cases to lack of reproducible results. The commonest errors are: small sample size, subgroup analysis, multiple testing, poorly matched control group, failure to attempt study replication, failure to detect LD with adjacent loci, overinterpreting results, positive publication bias, and unwarranted 'candidate gene' declaration after identifying association in an arbitrary region (Cardon and Bell 2001).

In this thesis, we have focused our attention on a group of related statistics known as transmission-disequilibrium tests (TDTs), for which errors due to poorly matched control groups do not occur. TDTs are tests for linkage in the presence of association. If the sample consists of family trios, or sib-pairs, TDTs are also tests for linkage disequilibrium (LD), i.e. joint linkage and association. LD is the non-random association of alleles at different loci on the same haplotype. The original TDT (Terwilliger and Ott 1992, Spielman et al. 1993) was designed to detect segregation distortion in alleles transmitted from heterozygous parents to affected progeny. Later, new TDTs were developed to analyse quantitative traits, sibships, and extended pedigrees. The most attractive feature of TDTs is their robustness in the presence of spurious disequilibrium, i.e. disequilibrium without linkage, which can produce a high rate of false positive results in other tests, e.g. case-control studies. This robustness derives from the fact that TDTs contrast different transmitted alleles within families, i.e. family-based controls, rather than using external controls.

The four main areas of research in this thesis were the following:

1. A comparative study of the power between different association tests both empirically and deterministically. Given the myriad of association tests currently available in the literature, studying their statistical properties, mainly power and robustness, is essential in order to make the best choice. We compared five different tests: a) a pure association test based on one-way classification analysis of variance (one-way ANOVA), where progeny genotype was the only classification factor, and b) four different TDTs, three of which were obtained from the literature (Rabinowitz 1997, Allison 1997, Szyda et al. 1998, Xiong et al. 1998), whilst the fourth was based on a nested design analysis of variance (nested ANOVA), where progeny genotypes were nested within mating types. The one-way and the nested ANOVAs were the most and the least powerful tests across all scenarios, respectively. The other TDTs shared a similar power and ranked between the two ANOVAs. The one-way ANOVA was the only non-robust test in the presence of population stratification, i.e. the rate of false positives was greater than the nominal 5% under

the null hypothesis of no linkage in the presence of association. All deterministic predictions of power were validated empirically.

2. The estimation, using TDT (Rabinowitz 1997), of the effect of a point mutation within the melanocortin 4-receptor on production traits in pigs. Data had to be pre-corrected for environmental influences such as sex and month of birth prior to use of the TDT. However, it is more efficient to estimate all parameters simultaneously. Hence, a set of dummy variables was extracted from the TDT to model the covariance between the mutation and each trait. In order to model this covariance, parental genotypes were required, but as most of them were missing, a Gibbs sampling technique was used to recover them. This covariance was estimated within a sire mixed linear model that also included other fixed and random factors, and, under an additive genetic model, it provided a direct estimate of allelic effects. This estimator uses only within-family genetic variation. A complementary estimator of allelic effects that uses between-family genetic variation was also developed and included in the model. Under the null hypothesis of neither linkage nor association, both estimators should be the same. Population stratification, a factor that generates association without linkage, increases the between-family genetic variation, and therefore has a proportional effect on the estimator that uses it. The within-family variation is unaffected by the presence of population stratification, and therefore the estimator that uses it remains robust to spurious association. Finally, we suggested relying on the robust estimator only, if spurious association was detected, otherwise, both estimators should be combined together in an overall and more powerful estimator (e.g. derived from a one-way ANOVA model). There was no evidence for admixture/stratification in this population, however this possibility should always be checked to avoid spurious association results.

3. A genome-wide search of markers associated with bovine spongiform encephalopathy (BSE) using TDTs. Humans that had been infected with the agent that induces BSE, the presumptive prion protein, developed a lethal, early onset variant of the Creutzfeldt-Jakob disease. Although it has been suggested that association tests should be used mainly for testing candidate genes (as in the case of MC4R), or for fine mapping within a chromosomal region for which there is prior evidence of linkage, because of the large number of markers that would be required otherwise, we have demonstrated here that a successful genome-wide scan with a sparse marker map is also possible. The data were analysed with several different

TDTs (Sham and Curtis 1995, Bickeböller and Clerget-Darpoux 1995, Waldman et al. 1999), and the same profile of p-values was obtained when using either reference distributions (i.e. appealing to the asymptotic properties of these TDTs) or permutation testing. We found a significantly higher risk of BSE associated with three markers (on chromosomes 5, 10 and 20). In a subsequent and independent study, a new marker adjacent to the one on chromosome 5 was also found significantly associated with incidence of BSE. These promising results are currently being used at the Roslin Institute and other research groups to pinpoint the genes associated with BSE susceptibility.

4. A multiple regression technique was developed to predict identity-by-descent (IBD) at specific chromosomal locations, within each individual, using marker information. The regression coefficients were obtained using the theory of long-term genetic contributions (Bijma 2000) and its extensions as linked gene flow (LGF) theory, which takes into account the history of a population. It is reassuring that, in the simplest case of a population evolving solely due to drift, LGF theory coincides with classical quantitative theory (e.g. Sved 1971). Nevertheless, the benefit of using LGF theory is that it provides a solid framework in which to explore the effects of mutation and selection in estimating IBD. This work sprang from two key papers (Meuwissen and Goddard 2000, 2001) in which a novel method for fine mapping quantitative trait loci (QTL) utilising haplotype information was proposed. At the core of this methodology there is the estimation of IBD by means of modelling the history of a population under simplifying conditions. This section of the thesis could be viewed as a successful preliminary study that encourages us to pursue this venture even further.

Overall, this thesis covers the contemporary area of research encompassing gene mapping via studying population-wide association, and explores in detail interesting statistical issues ranging from power and robustness to evolutionary modelling, all within the context of fine gene mapping. It is likely that the success of TDT as a LD-based method for gene mapping has been due to its reputed robustness in the presence of population stratification/admixture, and to its simplicity, which has facilitated understanding and prompted further developments, e.g. including quantitative traits and any family structure. Despite the relative success of LD methods in dealing with simple traits, complex multi-factorial traits have been much more challenging. Therefore, as Terwilliger and Weiss (1998) put it, it may be possible that "too many people are concentrating on simple mathematically tractable models that

assume the only difference between simple disease and complex disease is related to effect size of a single allele per locus, whereas there is a looming danger that there is also a substantial increase in complexity in both allelic and non-allelic heterogeneity, gene by environment interactions, epistasis, pleiotropy, and variable expressivity of different alleles in the same gene".

Despite this pessimistic view, agricultural populations may still be more amenable to LD mapping than human populations for several reasons. Firstly, most of the current breeds/strains with economic interest have been recently created after hybridising local ones (e.g. Jones 1998), which is an ideal population history for LD mapping (e.g. Stephens et al. 1994). Secondly, the large population sizes and controlled mating designs in agriculture provide greater power to detect meaningful associations than nuclear families in human studies.

Notwithstanding the advantages of TDT, one of its problems continues to be the single-marker analysis approach, which requires corrections, sometimes too conservative, to account for multiple testing. In theory, TDT can be extended to analyse haplotype transmission rates, e.g. Zhao et al. (2000) developed a TDT for multiple markers completely linked. However, in the absence of complete linkage, the number of haplotype classes increases geometrically, up to a maximum equal to the product of the number of alleles across all loci. In order to keep the problem tractable, one could consider two markers at a time, and look for preferential transmission of a joint pair of alleles over other pairs. In principle, this approach should provide a better estimate of QTL location compared to a single marker analysis, because the QTL could be located within two neighbouring markers. The downside of testing haplotypes, as opposed to single markers, is the potential reduction in power due to the increase in number of possible contrasts for a fixed sample size. One option could be detecting significant markers using a single-marker TDT, followed by a haplotype TDT analysis to discern which side the QTL is more likely to be.

Another problem is that TDT restricts its use of available information, e.g. discarding families without heterozygous parents, in order to exploit only within-family variation, the keypoint of the robustness of TDT. I have suggested in Chapter 3 a more powerful analysis, instead of TDT, unless significant spurious disequilibrium is detected.

The theoretical limit of resolution of TDT, and of any other statistical method that relies on population-wide LD, is a region with conserved maximum LD that includes the causative mutation. For this reason, although still able to render higher mapping resolution than

linkage tests, LD-based tests may have difficulties differeciating between a causative polymorphism and a marker in complete LD with it. Moreover, without information about genetic diversity across populations and levels of usable LD across genomes, testing population-wide associations may not be robust, and results can be hard to interpret.

The patterns of LD, e.g. measured as the chromosomal region in which LD between a locus and other loci dacays to half the maximum value, vary within chromosomal regions across populations, and across chromosomal regions within populations. Expected levels of LD have been calculated assuming a uniform recombination rate across the genome, over generations. However, phenomena such as non-uniform recombination rates, drift, ethnic diversity, population admixture, and mating structures cause LD patterns to diverge from their expectation. Therefore, it is preferable to assess LD empirically in each chromosomal region within each population before carrying out a disease-mapping study, rather than extrapolating from other chromosomal regions and/or experiments.

New molecular approaches (e.g. comparing the phenotypes between inbred mice lines genetically different only at the QTL region, or the molecular activity between cell strains with different candidate genes in the QTL region) and biotechlological advances (e.g. low density microsatellite maps, medium and high density SNP maps, radiation hybrid maps, expression sequence tags (EST) and cDNA transcript maps, yeast and bacterial artificial chromosomes (YAC and BAC) libraries, microarrays, and fluorescent in sity hybridisation (FISH)) are currently available for dissecting QTL into individual genetic components.

Even without a full dissection of a QTL into individual loci components, information on QTL position and effect can be used for practical purposes. For example, in artificial selection, QTL information has been incorporated in selection indeces as genetic scores that assess the genetic value of prospective parents. This is particularly useful when phenotypic data are difficult to collect, e.g. disease resistance, or the phenotype is not expressed in the candidate, e.g. milk yield in bulls. Some areas in which partial QTL information has been utilised are marker assisted selection (MAS), marker assisted introgression (MAI), conservation programs, product identification, and crossbred performance prediction. Some successful examples genes being used in animal breeding programs are the KIT gene (white coat colour in pigs), the RYR1 or halothane gene (high lean tissue growth and maligna hypertermia), the RN- gene (high lean tissue growth and poor quality of processed meat), the IGF2 gene (an imprented gene responsible for high lean tissue growth), the callipyge gene (double muscling in sheep), the miostatin gene (double muscling in cattle), the Inverdale

gene (increased ovulation rate in sheep), and the Booroola gene (increased ovulation rate and litter size in sheep) (Anderson 2001).

However, at this early stage of application of molecular information into animal and plant breeding programs, Dekkers and Hospital (2002) recommend 'cautious optimism', because phenotypic information will still be the most important factor in selection decisions, and because imprecise estimates of QTL locations and effects can diminish the response to selection. Undoubtedly, a better knowledge of the genetic basis of traits will lead to a more efficient design of breeding programs.

Finally, the future of statistical gene mapping may not lie within the mathematical domain of TDT, but rather in more sophisticated statistical approaches for analysing complex traits using genomic information, although QTL results should be confirmed using several independent methods and experiments, and rely less on single statistical analyses (Makcay 2000). There are several pitfalls in QTL mapping (e.g. Flint and Mott 2001, Doerge 2002) but genomic information is increasing both quantitatively and qualitatively as individual genotyping becomes less costly thanks to high throughput DNA sequencing technology. This has led to the accumulation of hundreds of thousands of polymorphisms publicly available to be used in searching for associations and causal mutations (Ellsworth et al. 1997, Collins et al. 1998).

Chapter 5 of this thesis explores the basis of a novel and promising venture, where a multi-marker approach will be integrated together with evolutionary models to maximise the use of all available information. However, this methodology is yet to be thoroughly assessed, especially with regard to robustness to spurious association and power of QTL detection.

BIBLIOGRAPHY

Abecasis GR, Cardon LR, Cookson WO (2000) A general test of association for quantitative traits in nuclear families. American Journal of Human Genetics 66:279-292

Allison DB (1997) Transmission-disequilibrium tests for quantitative traits. American Journal of Human Genetics 60:676-690

Allison DB, Heo M, Kaplan N, Martin ER (1999) Sibling-based tests of linkage and association for quantitative traits. American Journal of Human Genetics 64:1754-1764

Almond G, Pattison J (1997) Human BSE. Nature 389:437-438

Anderson L (2001) Genetic dissection of phenotypic diversity in farm animals. Nature Reviews Genetics 2:130-138

Ando S, Sirianni R, Forastieri P, Casaburi I, Lanzino M, Rago V, Giordano F, Giordano C, Caprino A, Pezzi V (2001) Aromatase expression in prepuberal Sertoli cells: effect of thyroid hormone. Molecular and Cell Endocrinology 178:11-21

Ardlie KG, Liu-Cordero SN, Eberle MA, Daly M, Barrett J, Winchester E, Lander ES, Kruglyak L (2001) Lower-than-expected linkage disequilibrium between tightly linked markers in humans suggests a role for gene conversion. American Journal of Human Genetics 69:582-589

Ardlie KG, Kruglyak L, Seielstad M (2002) Patterns of linkage disequilibrium in the human genome. Nature Reviews 3:299-309

Arranz JJ, Coppieters W, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Mezer C, Riquet J, Simon P, Vanmanshoven P, Wagenaar D, Georges M (1998) A QTL affecting milk yield and composition maps to bovine Chromosome 20: a confirmation. Animal Genetics 29:107-115

Avery OT, MacLeod CM, McCarty M (1944) Studies on the chemical nature of the substance inducing transformation of *pneumococcal* types. Journal of Experimental Medicine 98:451-460

Avery PJ, Hill WG (1979) Distribution of linkage disequilibrium with selection and finite population size. Genetical Research 33:29-48

Bach G, Tomczak J, Risch N, Ekstein J (2001) Tay-Sachs screening in the Jewish Ashkenazi population: DNA testing is preferred procedure. American Journal of Medical Genetics 99:70-75

Balter M (2001) In search of the first Europeans. Science 291:1722-1725

Baltimore D (2001) Our genome unveiled. Nature News and Views 209:814-816

Baret PV, Hill WG (1997) Gametic disequilibrium mapping: potential applications in livestock. Animal Breeding Abstracts 65:309-318

Barley J, Blackwood A, Miller M, Markandu ND, Carter ND, Jeffer S, Cappuccio FP, MacGregor GA, Sagnella GA (1996) Angiotensin converting enzyme gene I/D polymorphism blood pressure and the rennin-angiotensin system in Caucasian and Afro-Caribbean peoples. Journal of Human Hypertension 10:31-35

Begun DJ, Aquadro CF (1992) Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. Nature 356:519-520

Betensky RA, Rabinowitz D (2000) Simple approximations for the maximal transmission/disequilibrium test with a multi-allelic marker. Annals of Human Genetics 64:567-574

Bickeböller H, Clerget-Darpoux F (1995) Statistical properties of the allelic and genotypic transmission/disequilibrium test for multiallelic markers. Genetic Epidemiology 12:865-870

Bijma P (2000) Long-term genetic contributions: prediction of rates of inbreeding and genetic gain in selected populations. Doctoral Thesis, Department of Animal Sciences, Wageningen University, The Netherlands

Bijma P, Woolliams JA (1999) Prediction of genetic contributions and generation intervals in populations with overlapping generations under selection. Genetics 151:1197-1210

Bijma P, Woolliams JA (2000) A note on the relationship between gene flow and genetic gain. Genetics, Selection and Evolution 32:99-104

Bink MCAM, Pas MFWT, Harders FL, Janss LLG (2000) A transmission/disequilibrium test approach to screen for quantitative trait loci in two selected lines of Large White pigs. Genetical Research 75:115-121

Boehnke M (1994) Limits of resolution of genetic linkage studies: implications for the positional cloning of human disease genes. American Journal of Human Genetics 55:379-390

Boehnke M (2000) A look at linkage disequilibrium. Nature Genetics 25:246-247

Boehnke M, Langefeld CD (1998) Genetic association mapping based on discordant sib pairs: the discordant-alleles test. American Journal of Human Genetics 62:950-961

Bovenhuis H, van Arendonk JAM, Davis G, Elsen JM, Haley CS, Hill WG, Bartet PV, Hetzel DJS, Nicholas FW (1997) Detection and mapping of quantitative trait loci in farm animals. Livestock Production Science 52:135-144

Bradley DG, Cunningham EP (1999) *Genetic aspects of domestication* in *The genetics of cattle* Eds. Fries R and Ruvinsky A, CAB International 15-31

Brown AHD (1975) Sample sizes required to detect linkage disequilibrium between two or three loci. Theoretical Population Biology 8:184-201

Bruce ME, Chree A, McConnell I, Foster J, Pearson G, Fraser H (1994) Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. Philosophical Transactions of the Royal Society of London Series B- Biological Sciences 343: 405-411

Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389:488-501

(The) BSE enquiry: www.bseinquiry.gov.uk/index.htm

Bulmer MG (1971) The effect of selection on genetic variability. American Naturalists 105:201-211

Bulmer MG (1976) The effect of selection on genetic variability: a simulation study. Genetical Research 28:101-117

Butler AA, Kesterson RA, Khong K, Cullen MJ, M. A. Pelleymounter MA et al. (2000) A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141:3518-3521

Cardon LR, Bell JI (2001) Association study designs for complex diseases. Nature Reviews: Genetics 2:91-99

Carreau S (2001) Germ cells: a new source of estrogens in the male gonad. Molecular and Cell Endocrinology 178:65-72

Casella G, Berger RL (1990) Multiple random variables. In: Barndorff-Nielsen OE, Bickel PJ, Cleveland WS, Dudley RM (eds) Statistical inference. Wadsworth & Brooks/Cole Advanced Books & Software, CA, pp 128-200

Cavalli-Sforza LL, Menozzi P, Piazza A (1993) Demic expansions and human evolution. Science 259:639-646

Chakravarti A (1998) It's raining SNPs, hallelujah? Nature Genetics 19:216-217

Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH (1984) Nonuniform recombination within the human β-globin gene cluster. American Journal of Human Genetics 36:1239-1258

Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH (1986) Nonuniform recombination within the human β-globin gene cluster: a reply to B.S. Weir and W.G. Hill. (Letter to the Editor) American Journal of Human Genetics 38:779-781

Chapman NH, Wijsman EM (1998) Genome scans using linkage disequilibrium tests: optimal marker characteristics and feasibility. American Journal of Human Genetics 63:1872-1885

Charlesworth B, Morgan MT, Charlesworth D (1993) The effect of deleterious mutations on neutral molecular variation. Genetics 134:1289-1303

Clayton D (2000) Linkage disequilibrium mapping of disease susceptibility genes in human populations. International Statistical Review 68:23-43

Clayton D (2001) Population association. In Balding DJ, Bishop M, Cannings C (Eds) Handbook of statistical genetics. John Wiley & Sons ltd., pp 519-540

Clayton D, Jones H (1999) Transmission/disequilibrium tests for extended marker haplotypes. American Journal of Human Genetics 65:1161-1169

Collins A, Morton NE (1998) Mapping a disease locus by allelic association. Proceedings of the National Academy of Sciences USA 95:1741-1745

Copenhaver GP, Browne WE, Preuss D (1998) Assaying genome-wide recombination and cenromere functions with *Arabidopsis* tetrads. Proceedings of the National Academy of Sciences USA 95:247-252

Coperman JB, Cucca F, Hearne CM, Cornall RJ, Reed PW, Rønningen KS, Undlien DE et al. (1995) Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (IDDM7) to chromosome 2q31-q33. Nature Genetics 9:80-85

Cox DR (1970) *Analysis of binary data*. Methuen & Co Ltd.

Crow JF (1990) Mapping functions. Genetics 125:669-671

Curtis D (1997) Use of siblings as controls in case-control association studies. Annals of Human Genetics 61:319-333

Curtis D, Miller MB, Sham PC (1999) Combining the sibling disequilibrium test and transmission/disequilibrium test for multiallelic markers. American Journal of Human Genetics 64:1785-1786

Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES (2001) High-resolution haplotype structure in the human genome. Nature Genetics 29:229-232

Dean M, Stephens JC, Winkler C, Lomb DA, Ramsburgh M, Boaze R, Stewart C et al. (1994) Polymorphic admixture typing in human ethnic populations. American Journal of Human Genetics 55:788-808

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nature Reviews Genetics 3:22-32

Deng HW, Li J, Recker RR (2001) Effect of polygenes on Xiong's transmission disequilibrium test of a QTL in nuclear families with multiple children. Genetic Epidemiology 21:243-265

Deng HW, Chen WM and Recker RR (2001) Population admixture: detection by Hardy-Weinberg test and its quantitative effects on linkage-disequilibrium methods for localising genes underlying complex traits. Genetics 157:885-897

D'Errico A, Taioli E, Chen X, Vineis P (1996) Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. Biomarkers 1:149-173

Devlin B, Risch N (1995) A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 29:311-322

Devlin B, Risch N, Roeder K (1996) Disequilibrium mapping: composite likelihood for pairwise disequilibrium. Genomics 36:1-16

Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nature Reviews Genetics 3:43-51

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294

Donnelly CA, Ferguson NM, Ghani AC, Wilesmith JW, Anderson RM (1997) Analysis of dam-calf pairs of BSE cases: confirmation of a maternal risk enhancement. Proceedings of the Royal Society of London, Series B, 264:1647-1656

Draper NR, Smith H (1966) *Applied regression analysis.* John Wiley & Sons Inc., NY, USA

Du FX, Sorensen P, Thaller G, Hoeschele I (2002) Joint linkage disequilibrium and linkage mapping of quantitative trait loci. 7th World Congress of Genetics Applied to Livestock, Montpellier, France

Eaves IA, Merriman TR, Barber RA, Nutland S, Tuomilehto-Wolf E, Tuomilehto J, Cucca F, Todd JA (2000) The genetically isolated populations of Finland and Sardinia may not be a panacea for linkage disequilibrium mapping of common disease genes. Nature Genetics 25:320-323

Elston DA (1998) Estimation of denominator degrees of freedom of F-distributions for assessing Wald statistics for fixed-effect factors in unbalanced mixed models. Biometrics 54:1085-1096

Elston RC (1990) Models for discrimination between alternative modes of inheritance. In D. Gianola and K. Hammond (Eds.), *Advances in statistical methods for genetic improvement in livestock*, pp. 41-55. Springer-Verlag, Berlin

Ewens WJ, Spielman RS (1995) The transmission-disequilibrium test: history, subdivision and admixture. American Journal of Human Genetics 57:455-464

Ewens WJ, Spielman RS (2001) The transmission-disequilibrium test. In Balding DJ, Bishop M, Cannings C (Eds.) *Handbook of statistical genetics.* John Wiley & Sons ltd., pp 507-518

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. 4th Edition, Longman Group Ltd, England

Falk CT, Rubinstein P (1987) Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. Annals of Human Genetics 51:227-233

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 (2000) Extensive genome-wide linkage disequilibrium in cattle. Genome Research 10:220-227

Farrall M, Keavney B, McKenzie C, Delepine M, Matsuda F, Lathrop GM (1999) Fine mapping of an ancestral recombination breakpoint in DCP1. Nature Genetics 23:270-271

Ferguson NM, Donnelly CA, Woolhouse MEJ, Anderson RM (1997) A genetic interpretation of heightened risk of BSE in offspring of affected dams. Proceedings of the Royal Society of London Series B, 24:1445-1455

Flint J, Mott R (2001) Finding the molecular basis of quantitative traits: successes and pitfalls. Nature Reviews Genetics 2:437-445

Fulker DW, Cherny SS, Sham PC, Hewitt JK (1999) Combined linkage and association sib-pair analysis for quantitative traits. American Journal of Human Genetics 64:259-267

Genissel C, Carreau S (2001) Regulation of the aromatase gene expression in mature Leydig cells. Molecular and Cell Endocrinology 178:141-146

GENSTAT 5 Release 4.2, 5th Edition (2001) Lawes Agricultural Trust, England

George AW, Visscher PM, Haley CS (2000) Mapping quantitative trait loci in complex pedigrees: a two-step variance component approach. Genetics 156:2081-2092

George V, Tiwari HD, Zhu X, Elston RC (1999) A test of transmission/disequilibrium for quantitative traits in pedigree data, by multiple regression. American Journal of Human Genetics 65:236-245

Georges M (2001) Recent progress in livestock genomics and potential impact on breeding programs. Theriogenology 55:15-21

Gerhard DS, Kidd KK, Kidd JR, Egeland JA, Housman DE (1984) Identification of a recent recombination event within the human β-globin gene cluster. Proceedings of the National Academy of Sciences USA 81:7875-7879

Gibbons A (2001) The riddle of coexistence. Science 291:1725-1729

Gilmour AR, Cullis BR, Welham SJ and Thompson R (2001) *ASREML reference manual.* ftp.res.bbsrc.ac.uk in pub/aar

Goldgar DE, Easton DF (1997) Optimal strategies for mapping complex diseases in the presence of multiple loci. American Journal of Human Genetics 69:1222-1232

Goldmann W, Hunter N, Foster JD, Salbaum JM, Beyreuther K, Hope J (1990) Two alleles of a neural protein gene linked to scrapie in sheep. Proceedings of the National Academy of Sciences USA 87:2476-2480

Goldmann W, Hunter N, Martin T, Dawson M, Hope J (1991) Different forms of the bovine PrP gene have five or six copies of a short, G-C-rich element within the protein coding exon. Journal of General Virology 72:201-204

Goldmann W, Hunter N, Smith G, Foster J, Hope J (1994) PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. Journal of General Virology 75:989-995

Goldmann W, Martin T, Foster J, Hughes S, Smith G, Hughes K, Dawson M, Hunter N (1996) Novel polymorphisms in the caprine PrP gene: a codon 142 mutation associated with scrapie incubation period. Journal of General Virology 77:2885-2891

Goldstein DB (2001) Islands of linkage disequilibrium. Nature Genetics 29:109-111

Guo SW (1997) Linkage disequilibrium measures for fine-scale mapping: a comparison. Human Heredity 47:301-314

Guo SW (1997) Estimating the age of mutant disease alleles based on linkage disequilibrium. Human Heredity 47:35-337

Graham J, Thompson E (1998) Disequilibrium likelihoods for fine-scale mapping of a rare allele. American Journal of Human Genetics 63:1517-1530

Grapes L, Fernando RL, Rothschild MF (2002) Analysis of methods for fine mapping quantitative trait loci using linkage disequilibrium. 7th World Congress of Genetics Applied to Livestock, Montpellier, France

Grignola FE, Hoeschele I, Tier B (1996) Mapping quantitative trait loci in outcross populations via residual maximum likelihood: I. Methodology. Genetics Selection Evolution 28:479-490

Haley CS (1995) Livestock QTLs – bringing home the bacon? Trends In Genetics 11:488-492

Hanson L, Elston RC, Petitt DJ, Bennett PH, Knowler WC (1995) Segregation analysis of non-insulin-dependent diabetes mellitus in Pima indians: evidence for a major-gene effect. American Journal of Human Genetics 57:160-170

Harley JB, Moser KL, Neas BR (1995) Logistic transmission modelling of simulated data. Genetic Epidemiology 12:607-612

Heshey AD, Chase M (1952) Independent functions of viral protein and nucleic acid in growth of bacteriophage. Journal of Genetic Physiology 36:39-56

Harding RM, Sajantila A (1998) Human genome diversity – a Project? Nature Genetics 18:307-308

Hartl DL, Clark AG (1997) *Principles of population genetics*. Sinauer Associates, Inc., Sunderland, Massachusetts

Haseman JK, Elston RC (1972) The investigation of linkage between a quantitative trait and a marker locus. Behavioral Genetics 2:3-19

Hästbacka J, de la Chapelle A, Kaitila I, Sistonen P, Weaver A, Lander E (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. Nature Genetics 2:204-211

Hästbacka J, de la Chapelle A, Mahanti MM, Clines G, Reeve-Daly MP, Daly M, Kaitila I, Lander E (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage. Cell 78:1073-1087

Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A, Coloma A, Lovett M, Buckler A, Kaitila I, Lander E (1994) The Diastrophic Dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78:1073-1087

Hernández-Sánchez J, Visscher PM, Plastow G, Haley CS (2002) Candidate gene analysis for quantitative traits using the transmission disequilibrium test: the example of the Melanocortin 4-Receptor in pigs. Genetics, in press

Hernández-Sánchez J, Waddington D, Wiener P, Haley CS, Williams JL (2002) Genome-wide search for markers associated with Bovine Spongiform Encephalopathy. Mammalian Genome 13:164-168

Hernández-Sánchez J, Haley CS, Visscher PM (2002) Power of association and transmission disequilibrium tests. 7th World Congress of Genetics Applied to Livestock Production, Montpellier, France

Hill AP (1975) Quantitative linkage: a statistical procedure for its detection and estimation. Annals of Human Genetics 38:439-449

Hill WG (1974) Prediction and evaluation of response to selection with overlapping generations. Animal Production 18:117-139

Hill WG (1975) Linkage disequilibrium among multiple neutral alleles produced by mutation in finite populations. Theoretical Population Biology 8:117-126

Hill WG (1977) Correlation of gene frequencies between neutral linked genes in finite populations. Theoretical Population Biology 11:239-248

Hill WG, Robertson A (1968) Linkage disequilibrium in finite populations. Theoretical and Applied Genetics 38:226-231

Hill WG, Weir BS (1988) Variances and covariances of squared linkage disequilibria in finite populations. Theoretical Population Biology 33:54-78

Hill WG, Weir BS (1994) Maximum-likelihood estimation of gene location by linkage disequilibrium. American Journal of Human Genetics 54:705-714

Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I et al. (1999) Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. Journal of Clinical Endocrinology and Metabolism 84:1483-1486

Hoeschele I, Uimari P, Grignola FE, Zhang Q, Cage KM (1997) Advances in statistical methods to map quantitative trait loci in outbred populations. Genetics 147:1445-1457

Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. Genetics 132:1199-1210

Horvath S, Laird NM (1998) A discordant-sibship test for disequilibrium and linkage: no need for parental data. American Journal of Human Genetics 63:1886-1897

Hovatta I, Terwilliger JD, Lichtermann D, Mäkikyrö T, Suvisaari J, Peltonen L, Lönnqvist J (1997) Schizophrenia in the genetic isolate of Finland. American Journal of Medical Genetics 74:353-360

Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajävi R, Juovinen H, Kokko Sahin ML, Väisänen L et al. (1998) A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation suggesting multiple susceptibility loci. American Journal of Human Genetics 81:453-454

Hudson RR (1985) The sampling distribution of linkge disequilibrium under an infinite allele model without selection. Genetics 109:611-631

Hunter N, Goldmann W, Smith G, Hope J (1994) Frequencies of PrP gene variants in healthy cattle and cattle with BSE in Scotland. Veterinary Record 135:400-403

Hunter N (1999) Molecular Biology and Genetics of Bovine Spongiform Encephalopathy in *The Genetics of Cattle*. Eds. Fries R and Ruvinsky A, CAB International 229-246

(The) Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971-983

(The) International SNP Map Working Group (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409:928-933

Jayakar SD (1970) On the detection and estimation of linkage between a locus influencing a quantitative character and a marker locus. Biometrics 26:451-464

Jeffreys AJ, Kauppi L, Neumann R (2001) Intensely punctuated meiotic recombination in the class II region of the major histocompatibility complex. Nature Genetics 29:217-222

Jin K, Speed TP, Klitz W, Thomson G (1994) Testing for segregation distortion in the HLA complex. Biometrics 50:1189-1198

Jin L, Underhill PA, Doctor V, Davis RW, Shen P, Cavalli-Sforza LL, Oefner PJ (1999) Distribution of haplotypes from a chromosome 21 region distinguishes multiple prehistoric human migrations. Proceedings of the National Academy of Sciences USA 96:3796-3800

Johnson GCL, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H et al. (2001) Haplotype tagging for the identification of common disease genes. Nature Genetics 29:233-237

Jones GF (1998) Genetics aspects of domestication, common breeds and their origin. In Rothschild and Ruvinsky, (Eds.) p 17-50. *The genetics of the pig*. CABI, UK

Jorde LB (1995) Linkage disequilibrium as a gene-mapping tool. American Journal of Human Genetics 56:11-14

Kaplan NL, Weir BS (1992) Expected behaviour of conditional linkage disequilibrium. American Journal of Human Genetics 51:333-343

Kaplan NL, Hill WG, Weir BS (1995) Likelihood methods for locating disease genes in non-equilibrium populations. American Journal of Human Genetics 56:18-32

Kaplan NL, Weir BS (1995) Are moment bounds on the recombination fraction between a marker and a disease locus too good to be true? Allelic association mapping revisited for simple genetic diseases in the Finnish population. American Journal of Human Genetics 57:1486-1498

Kaplan NL, Martin ER, Weir BS (1999) Power studies for the transmission/disequilibrium tests with multiple alleles. American Journal of Human Genetics 60:691-702

Karlin S, McGregor JL (1968) Rates and probabilities of fixation for two locus random mating finite populations without selection. Genetics 58:141-159

Kendall MG, Stuart A (1963) *The advanced theory of statistics. Volume 1: distribution theory.* Charles Griffin & Co ltd. London

Kenward MG, Roger JH (1997) Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983-997

Kerem BS, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC (1989) Identification of the Cystic Fibrosis gene: genetic analysis. Science 245:1073-1080

Kim JJ, Farnir F, Coppieters W, Johnson D, Georges M (2002) Evaluation of a new QTL fine-mapping method exploiting linkage disequilibrium on BTA14 and BTA20 in a dairy cattle. 7th World Congress of Genetics Applied to Livestock, Montpellier, France

Kim KS, Larsen HJ, Rothschild MF (1999) Rapid communication: linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene. Journal of Animal Science 78:791

Kim KS, Larsen N, Short T, Plastow G, Rothschild MF (2000) A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth and feed intake traits. Mammalian Genome 11:131-135

Kingman J (1982) The coalescent. Stochastic Procedures and Applications 13:235-248

Knapp M (1999) A note on power approximations for the transmission/disequilibrium test. American Journal of Human Genetics 64:1177-1185

Knapp M (1999) The transmission/disequilibrium test and parental-genotype reconstruction: the reconstruction-combined transmission/disequilibrium test. American Journal of Human Genetics 64:861-870

Knott SA, Haley CS, Thompson R (1991a) Methods of segregation analysis for animal breeding data: parameter estimates. Heredity 68:313-320

Knott SA, Haley CS, Thompson R (1991b) Methods of segregation analysis for animal breeding data: a comparison of power. Heredity 68:299-311

Knowler WC, Williams RC, Pettitt DJ, Steinberg AG (1988) Gm3-5,13,14 and type-2 diabetes mellitus: an association in American-Indians with genetic admixture. American Journal of Human Genetics 43:520-526

Koegler FH, Grove KL, Shiffmacher A, Smith MS, Cameron JL (2001) Central melanocortin receptors mediate changes in food intake in the rhesus macaque. Endocrinology 142:2586-2592

König IR, Schäfer H, Müller HH, Ziegler A (2001) Optimised group sequential study designs for tests of genetic linkage and association in complex diseases. American Journal of Human Genetics 69:590-600

Kruglyak L (1997) What is significant in whole-genome linkage disequilibrium studies? American Journal of Human Genetics 61:810-812

Kruglyak L (1999) Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nature Genetics 22:139-144

Laan M, Pääbo S (1997) Demographic history and linkage disequilibrium in human populations. Nature Genetics 17:435-438

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037-2048

Leach DRF (1996) *Genetic recombination.* Blackwell Science Ltd

Lehesjoki AE, Koskiniemi M, Norio R, Tirrito S, Sistonen P, Lander E, de la Chapelle A (1993) Localization of the EPM1 gene for progressive myoclonus epilepsy on chromosome 21: linkage disequilibrium allows high resolution mapping. Human Molecular Genetics 8:1229-1234

Lewontin RC (1988) On measures of gametic disequilibrium. Genetics 120:849-852

Lewontin RC, Kojima KI (1960) The evolutionary dynamics of complex polymorphisms. Evolution 14:458-472

Li WD, Joo EJ, Furlong EB, Galvin M, Abel K et al. (2000) Melanocortin 3 receptor (MC3R) gene variants in extremely obese women. International Journal of Obesity and Related Metabolic Disorders 24:206-210

Lloyd SE, Onwuazor ON, Beck JA, Mallinson G, Farrall M, Targonski P, Collinge J, Fishcer EMC (2001) Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. Proceedings of the National Academy of Sciences USA 98:6279-6283

Long AD, Langley CH (1999) The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. Genome Research 9:720-731

Lonjou c, Collins A, Ajioka RS, Jorde LB, Kushner JP, Morton NE (1998) Allelic association under map error and recombinational heterogenity: a tale of two sites Proceedings of the Natioanal Academy of Sciences USA 95:11366-11370

Lonjou C, Collins A, Morton NE (1999) Allelic association between marker loci. Proceedings of the National Academy of Sciences USA 96:1621-1626

Lunetta KL, Faraone SV, Biederman J, Laird NM (2000) Family-based tests of association and linkage that use unaffected sibs, covariates, and interactions. American Journal of Human Genetics 66:605-614

Luria SE, Delbruck M (1943) Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28:491-511

Lynch M, Deng HW (1994) Genetic slippage in response to sex. The American Naturalist 144:242-261

Lynch M, Walsh B (1998) *Genetics and analysis of quantitative traits.* Sinauer Associates, Inc. Sunderland, Massachusetts, USA

MacDonald ME, Lin C, Srinidhi L, Bates G, Altherr M, Whaley WL, Lehrach H, Wasmuth J, Gusella JF (1991) Complex patterns of linkage disequilibrium in the Huntington disease region. American Journal of Human Genetics 49:723-734

Mackay TFC (2001) Quantitative trait loci in *Drosophila.* Nature Reviews Genetics 2:11-20

Manolakou K, Beaton J, McConnell I, Farquar C, Manson J, Hastie N, Bruce M, Jackson I (2001) Genetic and environmental factors modify bovine spongiform encephalopathy incubation period in mice. Proceedings of the National Academy of Sciences USA 98:7402-7407

Martin ER, Kaplan NL, Weir BS (1997) Tests for linkage and association in nuclear families. American Journal of Human Genetics 61:439-448

Martin ER, Monks SA, Warren LL, Kaplan NL (2000) A test for linkage and association in general pedigrees: the pedigree disequilibrium test. American Journal of Human Genetics 67:146-154

McCullagh P, Nelder JA (1983) *Generalised Linear Models: monographs on statistics and applied probability.* 2nd edition. Chapman and Hall

Mendel G (1866) Versuche über Pflanzenhybriden. In *Experiments in plant hybridisation*, Editor J. H. Bennett, English version commented by R. A. Fisher, Oliver & Boyd, Edinburgh and London, 1965

Merriman T, Twells R, Merriman M, Eaves I, Cox R, Cucca F, McKinney P et al. (1997) Evidence by allelic association-dependent methods for a type 1 diabetes polygene (IDDM6) on chomosome 18q21. Human Molecular Genetics 6:1003-1010

Meuwissen THE, Goddard ME (2000) Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155:421-430

Meuwissen THE, Goddard ME (2000) Combined linkage and linkage disequilibrium for fine scale mapping of QTL. 51th Annual Meeting of the European Association for Animal Production, The Hague, Netherlands

Meuwissen THE, Goddard ME (2001) Prediction of identity by descent probabilities from marker-haplotypes. Genetics, Selection and Evolution 33:605-634

Meuwissen THE, Goddard ME (2002) Mapping multiple QTL by combined linkage disequilibrium / linkage analysis in outbred populations. 7th World Congress of Genetics Applied to Livestock, Montpellier, France

Miller MB (1997) Genomic scanning and the transmission/disequilibrium test: analysis of error rates. Genetic Epidemiology 14:851-856

Miller RD, Kwok PY (2001) The birth and death of human single-nucleotide polymorphisms: new experimental evidence and implications for human history and medicine. Human Molecular Genetics 10:2195-2198

Monks SA, Kaplan NL, Weir BS (1998) A comparative study of sibship tests of linkage and/or association. American Journal of Human Genetics 63:1507-1516

Monks SA, Kaplan NL (2000) Removing the sampling restrictions from family-based tests of association for a quantitative-trait locus. American Journal of Human Genetics 66:576-592

Morris AP, Curnow RN, Whittaker JC (1997a) Randomization tests of disease-marker associations. Annals of Human Genetics 61:49-60

Morris AP, Whittaker JC, Curnow RN (1997b) A likelihood ratio for detecting patterns of disease-marker association. Annals of Human Genetics 61:335-350

Morton NE (1982) Estimation of demographic parameters from isolation by distance. Human Heredity 32:37-41

Morton NE, Zhang W, Taillon-Miller P, Ennis S, Kwok PY, Collins A (2001) The optimal measure of allelic association. Proceedings of the National Academy of Sciences USA 98:5217-5221

(The) Mouse Genome Database (MGD), Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine, http://www.informatics.jax.org.

Nachman MW, Churchill GA (1996) Heterogeneity in rates of recombination across the mouse genome. Genetics 142:537-548

Neale MC, Cherny SS, Sham PC, Whitfield JB, Heath AC, Birley AJ, Martin NG (1999) Distinguishing population stratification from genuine allelic effects with MX: association of ADH2 with alcohol consumption. Behavioral Genetics 29:233-243

Neibergs HL, Ryan AM, Womack JE, Spooner RL, Williams JL (1994) Polymorphism analysis of the prion gene in BSE-affected and unaffected cattle. Animal Genetics 25:313-317

Nordborg M et al. (2002) The extent of linkage disequilibrium in *Arabidopsis thaliana*. Nature Genetics 30:190-193

Noor MAF, Cunningham AL, Larkin JC (2001) Consequences of recombination rate variation on quantitative trait locus mapping studies: simulations based on the *Drosophila melanogaster* genome. Genetics 159:581-588

Nsengimana J, Baret PV (2002) Linkage disequilibrium in inbred populations: a geostatistical approach. 7th World Congress of Genetics Applied to Livestock, Montpellier, France

Ohashi J, Tokunaga K (2001) The power of genome-wide association studies of complex disease genes: statistical limitations of indirect approaches using SNP markers. Journal of Human Genetics 46:478-482

Ohta T, Kimura M (1969) Linkage disequilibrium due to random genetic drift. Genetical Research 13:47-55

Olson JM, Wijsman EM (1994) Design and sample-size considerations in the detection of linkage disequilibrium with a disease locus. American Journal of Human Genetics 55:574-580

Ott J (1996) Complex traits on the map. Nature 379:772-773

Ott J (2000) Predicting the range of linkage disequilibrium. Proceedings of the National Academy of Sciences 97:2-3

Ott J, Rabinowitz D (1997) The effect of marker heterozygosity on the power to detect linkage disequilibrium. Genetics 147:927-930

Page GP, Amos CI (1999) Comparison of linkage-disequilibrium methods for localisation of genes influencing traits in humans. American Journal of Human Genetics 64:1194-1205

Palmer MS, Dryden AJ, Hughes JT, Collinge J (1991) Homozygous prion protein genotype predisposes to sporadic Creutzfelt-Jakob disease. Nature 352:340-342

Parsch J, Meiklejohn CD, Hartl DL (2001) Patterns of DNA sequence variation suggest the recent action of positive selection in the janus-ocnus region of *Drosophila simulans*. Genetics 159:647-657

Patil N, Berno AJ, Hinds DA, Barrett WA, Doshi JM, Hacker CR, Kautzer CR et al. Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. Science 294:1719-1722

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545-554

Payne F (1918) The effect of artificial selection on bristle number in *Drosophila ampelophila* and its interpretation. Proceedings of the National Academy of Sciences USA 4:55-58

Pong-Wong R, Woolliams JA (1998) Response to mass selection when an identified major gene is segregating. Genetic Selection Evolution 30:313-337

Pong-Wong R, George AW, Woolliams JA, Haley CS (2001) A simple and rapid method for calculating identity-by-descent matrices using multiple markers. Genetic Selection Evolution 33:1-19

Przeworski M, Wall JD (2001) Why is there so little intragenic linkage disequilibrium in humans? Genetical Research 77:143-151

Rabinowitz D. (1997) A transmission disequilibrium test for quantitative trait loci. Human Heredity 47:342-350

Rannala B, Slatkin M (1999) Likelihood analysis of disequilibrium mapping, and related problems. American Journal of Human Genetics 62:459-473

Reich DE, Cargili M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T et al. (2001) Linkage disequilibrium in the human genome. Nature 411:199-204

Reich DE, Schaffner SF, Daly MJ, McVean G, Mullikin JC, Higgins JM, Richter DJ, Lander ES, Altshuler D (2002) Human genome sequence variation and the influence of gene history, mutation and recombination. Nature Genetics 32:135-142

Rice JP, Neuman RJ, Hoshaw SL, Daw EW, Gu C (1995) TDT with covariates and genomic screens with mod scores: their behavior on simulated data. Genetic Epidemiology 12:659-664

Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T et al. (2001) Nature Genetics 29:223-228

Riquet J, Coppieters W, Cambisano N, Arranz JJ, Berzi P, Davis SK, Grisart B, Farnir F, Karim L, Mni M, Simon P, Taylor JF, Vanmanshoven P, Wagenaar D, Womack JE, Georges M (1999) Fine-mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. Proceedings of the National Academy of Sciences USA 96:9252-9257

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516-1517

Roughsedge T, Brotherstone S, Visscher PM (1999) Quantifying genetic contributions to a dairy cattle population using pedigree analysis. Livestock Production Science 60:359-369

Rubinstein P, Walker M, Carpenter C et al. (1981) Genetics of HLA disease associations: the use of the haplotype relative risk (HRR) and the 'haplo-delta' (Dh) estimates in juvenile diabetes from three racial groups. Human Immunology 3:384

Ryan AM, Womack JE (1993) Somatic cell mapping of the bovine prion protein gene and restriction fragment length polymorphism studies in cattle and sheep. Animal Genetics 24:23-26

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552-560

Schaid DJ (1996) General score tests for associations of genetic markers with disease using cases and their parents. Genetic Epidemiology 13:423-449

Schaid DJ (1998) Transmission disequilibrium, family controls, and great expectations. American Journal of Human Genetics 63:935-941

Schaid DJ, Rowland CM (1999) Quantitative trait transmission disequilibrium test: allowance for missing parents. Genetic Epidemiology 17:307-312

Schaid DJ, Rowland CM (2000) Robust transmission regression models for linkage and association. Genetic Epidemiology 19:78-84

Schaid DJ, Sommer SS (1994) Comparison of statistics for candidate-gene association studies using cases and parents. 55:402-409

Schmidt S, Van Hooft IM, Grobbee DE, Ganten D, Ritz E (1993) Polymorphism of the angiotensin I converting enzyme gene is apparently not related to high blood pressure: Dutch hypertension and Offspring Study. Journal of Hypertension 11:345-348

Schork NJ, Cardon LR, Xu X (1998) The future of genetic epidemiology. Trends In Genetics 14:266-272

Schork NJ, Fallin D, Thiel B, Xu X, Broeckel U, Jacob HJ, Cohen D (2001) The future of genetic case-control studies. In *Advances in Genetics: Genetic dissection of complex traits*, Vol. 42 Edited by Rao DC and Province MA, Ch.14: 191-212 Academic Press

Searle SR (1971) Linear models. John Wiley & Sons, NY

Semino O, Passarino G, Brega A, Fellous M, Santachiara-Benerecetti AS (1996) A view of the Neolithic demic diffusion in Europe trhough two Y chromosome-specific markers. American Journal of Human Genetics 59:964-968

Sethuraman B (1997) Topics in statistical genetics. University of California, Berkley, USA

Sham PC, Curtis D (1995) An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. Annals of Human Genetics 59:323-336

Sham PC, Cherny SS, Purcell S, Hewitt JK (2000) Power of linkage versus association analysis of quantitative traits, by use of variance-component models, for sibship data. American Journal of Human Genetics 66:1616-1630

Sham PC, Zhao JH, Waldman I, Curtis D (2000) Should ambiguous trios for the TDT be discarded? Annals of Human Genetics 64:575-576

Slatkin M (1994) Linkage disequilibrium in growing and stable populations. Genetics 137:331-336

Slatkin M (1999) Disequilibrium mapping of a quantitative-trait locus in an expanding population. American Journal of Human Genetics 64:1765-1773

Snarey A, Thomas S, Schneider MC, Pound SE, Barton N, Wright AF, Somlo S, Germino GG, Harris PC, Reeders ST, Frischauf AM (1994) Linkage disequilibrium in the region of the autosomal dominant polycystic kidney disease gene (PKDI). American Journal of Human Genetics 55:365-371

Sokal RR, Rohlf FJ (1995) Biometry. 2nd Edition. WH Freeman and Co., NY, USA

Sorensen D (1996) *Gibbs sampling in quantitative genetics.* Intern Rapport of the Danish Institute of Animal Science n. 82

Spielman RS, McGinnis RE, Warren JE (1993) Transmission test for linkage disequilibrium: the Insulin gene region and Insulin-dependent Diabetes Mellitus (IDDM). American Journal of Human Genetics 52:506-516

Spielman RS, Ewens WJ (1996) The TDT and other family-based tests for linkage disequilibrium and association. American Journal of Human Genetics 59:983-989

Spielman RS, Ewens WJ (1998) A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. American Journal of Human Genetics 62:450-458

Stengard JH, Weiss KM, Sing CF (1998) An ecological study of association between coronary heart disease mortality rates in men and the relative frequencies of common allelic variations in the gene coding for apolipoprotein E. Human Genetics 103:234-241

Stephens JC, Briscoe D, O'Brien SJ (1994) Mapping by admixture linkage disequilibrium in human populations: limits and guidelines. American Journal of Human Genetics 55:809-824

Stephenson DA, Chiotti K, Ebeling C, Groth D, DeArmond SJ, Prusiner SB, Carlson GA (2000) Quantitative trait loci affecting prion incubation time in mice. Genomics 69:47-53

Stuart A (1955) A test of homogeneity of the marginal distribution in a two-way classification. Biometrika 42:412-416

Sturtevant AH (1913) The linear arrangement of six sex-linked factors in drosophila, as shown by their mode of association. Journal of Experimental Zoology 14:43-59

Sun FZ, Flanders WD, Yang QH, Khoury MJ (1998) A new method for estimating the risk ratio in studies using case-parental control design. American Journal of Epidemiology 148:902-909

Sun FZ, Flanders WD, Yang QH, Khoury MJ (1999) Transmission disequilibrium test (TDT) when only one parent is available: the 1-TDT. American Journal of Epidemiology 150:97-104

Sun FZ, Flanders WD, Yang QH, Zhao HY (2000) Transmission/disequilibrium tests for quantitative traits. Annals of Human Genetics 64:555-565

Sved JA (1971) Linage disequilibrium and homozygosity of chromosome segments in finite populations. Theoretical Population Biology 2:125-141

Sved JA, Feldman MW (1973) Correlation and probability methods for one and two loci. Theoretical Population Biology 4:129-132

Svejgaard A, Ryder LP (1994) HLA and disease associations: detecting the strongest association. Tissue Antigens 43:18-27

Szabo CI, King MC (1997) Population genetics of BRCA1 and BRCA2. American Journal of Human Genetics 60:1013-1020

Szyda J, Liu Z, Wild V (1998) Application of the transmission-disequilibrium test to detection of major genes. European Association of Animal Production, Warsaw, Poland

Tachibana T, Sugahara K, Ohgushi A, Ando R, Kawakami S et al. (2001) Intracerebroventricular injection of agouti-related protein attenuates the anorexigenic effect of alpha-melanocyte stimulating hormone in neonatal chicks. Neuroscience Letters 305:131-134

Tanksley SD, Ganal MW, Prince JP, deVicente MC, Bonierbale MW et al. (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160

Templeton AR (1995) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. V. Analysis of case/control sampling designs. Alzheimer's disease and the apoprotein E locus. Genetics 140:403-409

Teng J, Risch N (1999) The relative power of family-based and case-contro designs for linkage disequilibrium studies of complex human diseases. II. Individual Genotyping. Genome Research 9:234-241

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. American Journal of Human Genetics 56:777-787

Terwilliger JD, Ott J (1992) A haplotype-based 'haplotype relative risk' approach to detecting allelic associations. Human Heredity 42:337-346

Terwilliger JD, Zollner S, Laan M, Paabo S (1998) Mapping genes through the use of linkage disequilibrium generated by genetic drift: 'Drift Mapping' in small populations with no demographic expansion. Human Heredity 48:138-154

Terwilliger JD, Weiss KM (1998) Linkage disequilibrium mapping of complex disease: fantasy or reality? Current Opinion in Biotechnology 9:578-594

Thomson G (1995) Mapping disease genes: family based association studies. American Journal of Human Genetics 57:487-498

Thompson E, Neel J (1997) Allelic disequilibrium and allele frequency distribution as a function of social and demographic history. American Journal of Human Genetics 60:197-204

Underhill PA, Jin L, Zemans R, Oefner PJ, Cavalli-Sforza LL (1996) A pre-Columbian Y chromosome-specific transition and its implications for human evolutionary history. Proceedings of the National Academy of Sciences USA 93:196-200

Underwood JCE (1996) Genetic and environmental causes of disease. In Underwood JCE (Ed.) *General and systematic pathology.* Churchill Livingstone, London, pp 31-60

Vaisse C, Clement K, Guy-Grand B, Froguel P (1998) A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nature Genetics 20:113-114

Valdes AM, McWeeney S, Thomson G (1997) HLA Class II DR-DQ amino acids and Insulin-Dependent Diabetes Mellitus: application of the haplotype method. American Journal of Human Genetics 60:717-728

van Heelsum AM, Visscher PM, Haley CS (1997a) Marker assisted introgression using non-unique marker alleles I: selection on presence of linked marker alleles. Animal Genetics 28:181-187

van Heelsum AM, Visscher PM, Haley CS (1997b) Marker assisted introgression using non-unique marker alleles I: selection on probability of presence of the introgressed allele. Animal Genetics 28:188-194

Waldman ID, Robinson BF, Rowe DC (1999) A logistic regression based extension of the TDT for continuous and categorical traits. Annals of Human Genetics 63:329-340

Wall EE, Visscher PM, Woolliams JA (2002) Homozygosity due to identity-by-descent around the target locus in gene introgression programmes. 7th World Congress of Genetics Applied to Livestock Production, Montpellier, France

Wang CS, Rutledge JJ, Gianola D (1994) Bayesian analysis of mixed linear models via Gibbs sampling with an application to litter size in Iberian pigs. Genetic Selection Evolution 26:91-115

Wang D, Sun F (2000) Sample sizes for the transmission disequilibrium tests: TDT, S-TDT and 1-TDT. Communications in Statistics- Theory and Methods 29:1129-1142

Watkins WS, Zenger R, O'Brien E, Nyman D, Eriksson AW, Renlund M, Jorde LB (1994) Linkage disequilibrium patterns vary with chromosomal location: a case study from the von Willebrand factor region. American Journal of Human Genetics 55:348-355

Watson JD, Crick FHC (1953) Molecular structure of nucleic acids. Nature 171:737-738

Wardlaw SL (2001) Obesity as a neuroendocrine disease: lessons to be learned from proopiomelanocortin and melanocortin receptor mutations in mice and men. Journal of Clinical Endocrinology and Metabolism 86:1442-1446

Warren JE, Spielman RS (1995) The Transmission/Disequilibrium test: history, subdivision and admixture. American Journal of Human Genetics 57:455-464

Weir BS, Cockerham c (1978) Testing hypotheses about linkage disequilibrium with multiple alleles. Genetics 88:633-642

Weir BS, Cockerham C (1989) Complete characterization of disequilibrium at two loci. P. 86-110 in M. E. Feldman (Editor) *Mathematical Evolutionary Theory*, Princeton University Press, Princeton

Weir BS, Hill WG (1986) Nonuniform recombination within the human β-globin gene cluster (Letter to the Editor). American Journal of Human Genetics 38:776-778

Weir BS (1996) *Genetic data analysis II*. Sinauer Associates, Inc. Sunderland, Massachusetts, USA

Weiss KM, Terwilliger JD (2000) How many diseases does it take to map a gene with SNPs? Nature Genetics 26:151-157

Welham SJ, Thompson R (1997) Likelihood ratio tests for fixed model terms using residual maximum likelihood. Journal of the Royal Statistical Society, Series B, 59:701-714

Whittaker JC, Curnow RN, Haley CS, Thompson R (1995) Using marker-maps in marker-assisted selection. Genetical Research 66:255-265

Wilson JF, Goldstein B (2000) Consistent long-range disequilibrium generated by admixture in a Bantu-Semitic hybrid population. American Journal of Human Genetics 67:926-935

Woolliams JA, Bijma P, Villanueva B (1999) Expected genetic contributions and their impact on gene flow and genetic gain. Genetics 153:1009-1020

Woolliams JA, Bijma P (2000) Predicting rates of inbreeding in populations undergoing selection. Genetics 154:1851-1864

(The) World Health Report (1999) Part Three: Statistical Annex. World Health Organisation. www.who.int/whr/1999/en/report.htm

Wright AF, Carothers AD, Pirastu M (1999) Population choice in mapping genes for complex diseases. Nature Genetics 23:397-404

Xiong M, Guo SW (1998) Fine-scale mapping based on linkage disequilibrium: theory and applications. American Journal of Human Genetics 60:1513-1531

Xiong M, Krushkal J, Boerwinkle E (1998) TDT statistics for mapping quantitative trait loci. Annals of Human Genetics 62:431-452

Young GB, Lee GJ, Waddington D, Sales DI, Bradley JS, Spooner RL (1983) Culling and wastage in dairy cows in East Anglia. The Veterinary Record 113:107-111

Yang Q, Rabinowitz D, Isasi C, Shea S (2000) Adjusting for confounding due to population admixture when estimating the effect of candidate genes on quantitative traits. Human Heredity 50:227-233

Yeo GS, Farooqui IS, Aminian S, Halsall DJ, Stanhope A et al. (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nature Genetics 20:111-112

Yu CE, Oshima J, Goddard KAB, Miki T, Nakura J, Ogihara T, Poot M et al. (1994) Linkage disequilibrium and haplotype studies of chromosome 8p 11.1-12.1 markers and Werner Syndrome. American Journal of Human Genetics 55:356-364

Yu A, Zhao c, Fan Y, Jang W, Mungall AJ, Deloukas P, Olsen A, Doggett NA, Ghebranious N, Broman KW, Weber JL (2001) Comparison of human genetic and sequence-based physical maps. Nature 409:951-953

Zhang SL, Zhao HY (2001) Quantitative similarity-based association tests using population samples. American Journal of Human Genetics 69:601-614

Zhao H (1999) The interpretation of the parameters in the transmission/disequilibrium test. American Journal of Human Genetics 64:326-328

Zhao H (2000) Family based association studies. Statistical Methods in Medical Research 9:563-587

Zhao H, Zhang S, Merikangas KR, Trixler M, Wildenauer DB et al. (2000) Transmission/disequilibrium tests using multiple tightly linked markers. American Journal of Human Genetics 67:936-946

Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, McKenzie CA, Luke A, Chen G, Elston RC, Ward R (2001) Linkage and association analysis of Angiotensin I-Converting Enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. American Journal of Human Genetics 68:1139-1148

Zhu X, Elston RC (2000) Power comparisons of regression methods to test quantitative traits for association and linkage. Genetic Epidemiology 18:322-330

Zhu X, Elston RC (2001) Transmission/disequilibrium test for quantitative traits. Genetic Epidemiology 20:57-74