1. Linkage disequilibrium in livestock populations

1.1 A brief history of QTL mapping

The vast majority of economically important traits in livestock and aquaculture production systems are quantitative, that is they show continuous distributions. In attempting to explain the genetic variation observed in such traits, two models have been proposed, the infinitesimal model and the finite loci model. The *infinitesimal model* assumes that traits are determined by an infinite number of unlinked and additive loci, each with an infinitesimally small effect (Fischer 1918). This model has been exceptionally valuable for animal breeding, and forms the basis for breeding value estimation theory (eg Henderson 1984).

However, the existence of a finite amount of genetically inherited material (the genome) and the revelation that there are perhaps a total of only around 20 000 genes or loci in the genome (Ewing and Green 2000), means that there is must be some *finite number of loci* underlying the variation in quantitative traits. In fact there is increasing evidence that the distribution of the effect of these loci on quantitative traits is such that there are a few genes with large effect, and a many of small effect (Shrimpton and Robertson 1998, Hayes and Goddard 2001). In Figure 1.1, the size of quantitative trait loci (QTL) reported in QTL mapping experiments in both pigs and dairy cattle is shown. These histograms are not the true distribution of QTL effects however, they are only able to observe effects above a certain size determined by the amount of environmental noise, and the effects are estimated with error. In Figure 1.1. B, the distribution of effects adjusted for both these factors is displayed. The distributions in Figure 1.1 B indicate there are many genes of small effect, and few of large effect. The search for these loci, particularly those of moderate to large effect, and the use of this information to increase the accuracy of selecting genetically superior animals, has been the motivation for intensive research efforts in the last two decades. Note that in this course *any* locus with an effect on the quantitative trait is a called a QTL, not just the loci of large effect.

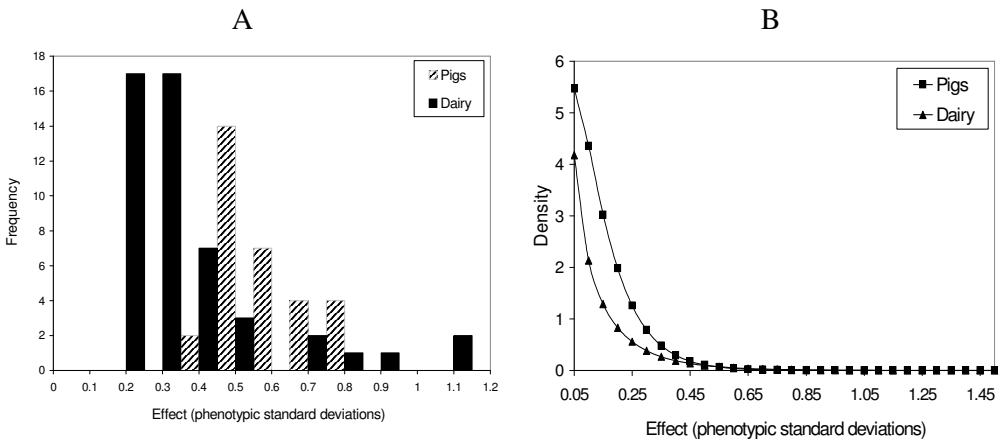


Figure 1.1 A. Distribution of additive (QTL) effects from pig experiments, scaled by the standard deviation of the relevant trait, and distribution of gene substitution (QTL) effects from dairy experiments scaled by the standard deviation of the relevant trait. B. Gamma Distribution of QTL effect from pig and dairy experiments, fitted with maximum likelihood.

Two approaches have been used to uncover QTL. The *candidate gene approach* assumes that a gene involved in the physiology of the trait could harbour a mutation causing variation in that trait. The gene, or parts of the gene, are sequenced in a number of different animals, and any variations in the DNA sequences, that are found, are tested for association with variation in the phenotypic trait. This approach has had some successes – for example a mutation was discovered in the oestrogen receptor locus (*ESR*) which results in increased litter size in pigs (Rothschild et al. 1991). For a review of mutations which have been discovered in candidate genes see Andersson and Georges (2004). There are two problems with the candidate gene approach, however. Firstly, there are usually a large number of candidate genes affecting a trait, so many genes must be sequenced in several animals and many association studies carried out in a large sample of animals (the likelihood that the mutation may occur in non-coding DNA further increases the amount of sequencing required and the cost). Secondly, the causative mutation may lie in a gene that would not have been regarded *a priori* as an obvious candidate for this particular trait.

An alternative is the QTL mapping approach, in which chromosome regions associated with variation in phenotypic traits are identified. QTL mapping assumes the actual genes which affect a quantitative trait are not known. Instead, this approach

uses neutral DNA markers and looks for associations between allele variation at the marker and variation in quantitative traits. A DNA marker is an identifiable physical location on a chromosome whose inheritance can be monitored. Markers can be expressed regions of DNA (genes) or more often some segment of DNA with no known coding function but whose pattern of inheritance can be determined (Hyperdictionary, 2003).

When DNA markers are available, they can be used to determine if variation at the molecular level (allelic variation at marker loci along the linkage map) is linked to variation in the quantitative trait. If this is the case, then the marker is linked to, or on the same chromosome as, a quantitative trait locus or QTL which has allelic variants causing variation in the quantitative trait.

Until recently, the number of DNA markers identified in livestock genome was comparatively limited, and the cost of genotyping the markers was high. This constrained experiments designed to detect QTL to using a linkage mapping approach. If a limited number of markers per chromosome are available, then the association between the markers and the QTL will persist only within families and only for a limited number of generations, due to recombination. For example in one sire, the *A* allele at a particular marker may be associated with the increasing allele of the QTL, while in another sire, the *a* allele at the same marker may be associated with the increasing allele at the QTL, due to historical recombination between the marker and the QTL in the ancestors of the two sires.

To illustrate the principle of QTL mapping exploiting linkage, consider an example where a particular sire has a large number of progeny. The parent and the progeny are genotyped for a particular marker. At this marker, the sire carries the marker alleles 172 and 184, Figure 1.2. The progeny can then be sorted into two groups, those that receive allele 172 and those that receive allele 184 from the parent. If there is a significant difference between the two groups of progeny, then this is evidence that there is a QTL linked to that marker.

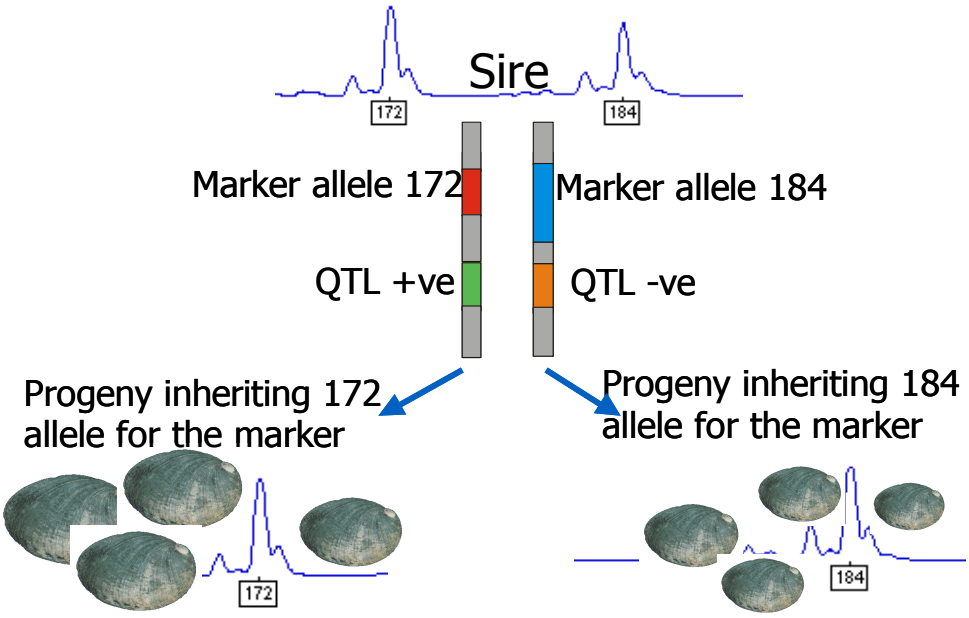


Figure 1.2. Principle of quantitative trait loci (QTL) detection, illustrated using an abalone example. A sire is heterozygous for a marker locus, and carries the alleles 172 and 184 at this locus. The sire has a large number of progeny. The progeny are separated into two groups, those that receive allele 172 and those that receive allele 184. The significant difference in the trait of average size between the two groups of progeny indicates a QTL linked to the marker. In this case, the QTL allele increasing size is linked to the 172 allele and the QTL allele decreasing size is linked to the 184 allele (Figure courtesy of Nick Robinson).

QTL mapping exploiting linkage has been performed in all nearly livestock species for a huge range of traits (for a review see Andersson and Georges 2004). The problem with mapping QTL exploiting linkage is that, unless a huge number of progeny per family or half sib family are used, the QTL are mapped to very large confidence intervals on the chromosome. To illustrate this, consider the formula that Darvasi and Soller (1997) gave for estimating the 95% CI for QTL location for simple QTL mapping designs under the assumption of a high density genetic map. The formula was $CI=3000/(kN\delta^2)$, where N is the number of individuals genotyped, δ allele substitution effect (the effect of getting an extra copy of the increasing QTL allele) in units of the residual standard deviation, k the number of informative parents per individual, which is equal 1 for half-sibs and backcross designs and 2 for F_2

progeny, and 3000 is about the size of the cattle genome in centi-Morgans. For example, given a QTL segregates on a particular chromosome within a half sib family of 1000 individuals, for a QTL with an allele substitution effect of 0.5 residual standard deviations the 95% CI would be 12 cM. Such large confidence intervals have two problems. Firstly if the aim of the QTL mapping experiment is to identify the mutation underlying the QTL effect, in a such a large interval there are a large number of genes to be investigated (80 on average with 20 000 genes and a genome of 3000cM). Secondly, use of the QTL in marker assisted selection is complicated by the fact that the linkage between the markers and QTL is not sufficiently close to ensure that marker-QTL allele relationships persist across the population, rather marker-QTL phase within each family must be established to implement marker assisted selection.

An alternative, if dense markers were available, would be to exploit linkage disequilibrium (LD) to map QTL. Performing experiments to map QTL in genome wide scans using LD has recently become possible due to the availability of 10s of thousands of single nucleotide polymorphism (SNP markers) in cattle, pigs, chickens and sheep in the near future (eg. (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/snp/Btau20040927/bovine-snp.txt). A SNP marker is a difference in nucleotide between animals (or an animals pair of chromosomes), at a defined position in the genome, eg.

Animal 1. ACTCGGGC

Animal 2. ACTTGGGC

Rapid developments in SNP genotyping technology now allow genotyping of a SNP marker in an individual for as little as 1c US.

1.2 Definitions and measures of linkage disequilibrium.

The classical definition of linkage disequilibrium (LD) refers to the non-random association of alleles between two loci. Consider two markers, A and B, that are on the same chromosome. A has alleles A1 and A2, and B has alleles B1 and B2. Four haplotypes of markers are possible A1_B1, A1_B2, A2_B1 and A2_B2. If the frequencies of alleles A1, A2, B1 and B2 in the population are all 0.5, then we would

expect the frequencies of each of the four haplotypes in the population to be 0.25. Any deviation of the haplotype frequencies from 0.25 is linkage disequilibrium (LD), ie the genes are not in random association. As an aside, this definition serves to illustrate that the distinction between linkage and linkage disequilibrium mapping is somewhat artificial – in fact linkage disequilibrium between a marker and a QTL is required if the QTL is to be detected in either sort of analysis. The difference is:

> *linkage analysis* only considers the linkage disequilibrium that exists within families, which can extend for 10s of cM, and is broken down by recombination after only a few generations.
>
> *linkage disequilibrium* mapping requires a marker to be in LD with a QTL across the entire population. To be a property of the whole population, the association must have persisted for a considerable number of generations, so the marker(s) and QTL must therefore be closely linked.

One measure of LD is *D*, calculated as (Hill 1981)

$$D = freq(A1\_B1)*freq(A2\_B2)-freq(A1\_B2)*freq(A2\_B1)$$

where freq (A1_B1) is the frequency of the A1_B1 haplotype in the population, and likewise for the other haplotypes. The *D* statistic is very dependent on the frequencies of the individual alleles, and so is not particularly useful for comparing the extent of LD among multiple pairs of loci (eg. at different points along the genome). Hill and Robertson (1968) proposed a statistic, r^2, which was less dependent on allele frequencies,

$$r^2 = \frac{D^2}{freq(A1) * freq(A2) * freq(B1) * freq(B2)}$$

Where freq(A1) is the frequency of the A1 allele in the population, and likewise for the other alleles in the population. Values of r^2 range from 0, for a pair of loci with no linkage disequilibrium between them, to 1 for a pair of loci in complete LD.

As an example, consider a situation where the allele frequencies are

freq(A1) = freq(A2) = freq (B1) = freq (B2) = 0.5

The haplotype frequencies are:

freq(A1_B1) = 0.1

freq(A1_B2) = 0.4

freq(A2_B1) = 0.4

freq(A2_B2) = 0.1

The $D = 0.1*0.1-0.4*0.4 = -0.15$

And $D^2 = 0.0225$.

The value of r^2 is then $0.0225/(0.5*0.5*0.5*0.5) = 0.36$. This is a moderate level of r^2.

Another commonly used pair-wise measure of LD is D' (Lewontin 1964). To calculate D', the value of D is standardized by the maximum value it can obtain:

$$D' = |D|/D_{max}$$

Where D_{max}= min[freq(A1)*freq(B2), -1*freq(A2)*freq(B1)] if D>0, else
= min[freq(A1)*freq(B1),--1*freq(A2)*freq*B2)] if D<0.

The statistic r^2 is preferred over D' as a measure of the extent of LD for two reasons. If we consider the r^2 between a marker and an (unobserved) QTL, *r^2 is the proportion of variation caused by the alleles at a QTL which is explained by the markers*. The decline in r^2 with distance actually indicates how many markers or phenotypes are required in initial genome scan exploiting LD are required to detect QTL. Specifically, sample size must be increased by a factor of $1/r^2$ to detect an ungenotyped QTL, compared with the sample size for testing the QTL itself (Pritchard and Przeworski 2001). D' on the other hand does a rather poor job of predicting required marker density for a genome scan exploiting LD, as we shall see in Section 2. The second reason for using r^2 rather than D' to measure the extent of LD is that D' tends to be inflated with small sample sizes or at low allele frequencies (McRae et al. 2002).

The above measures of LD are for bi-allelic markers. While they can be extended to multi-allelic markers such as microsatellites, Zhao et al. (2005) recommended the $\chi^{2'}$ measure of LD for multi-allelic markers, where

$$\chi^{2'} = \frac{1}{(l-1)} \sum_{i=1}^{k} \sum_{j=1}^{m} \frac{D_{ij}^2}{freq(A_i)\, freq(B_j)},$$

and $D_{ij} = freq(A_i \_ B_j) - freq(A_i)\, freq(B_j)$, *freq(Ai)* is the frequency of the i^{th} allele at marker A, *freq(Bj)* is the frequency of the j^{th} allele at marker B, and *l* is the minimum of the number of alleles at marker A and marker B. Note that for bi-allelic markers, $\chi^{2'} = r^2$.

Their investigations using simulation showed out of a number of multi-allelic pair-wise measures of LD $\chi^{2'}$ was the best predictor of useable marker-QTL LD (eg. the proportion of QTL variance explained by the marker).

While pair-wise measures of LD are important and widely used, are not particularly illuminating with respect to the causes of LD. For example, statistics such as r^2 consider only two loci at a time, whereas we may wish to calculate the extent of LD across a chromosome segment that contains multiple markers. An alternate multi-locus definition of LD is the **chromosome segment homozygosity** (**CSH**) (Hayes et al. 2003). Consider an ancestral animal many generations ago, with descendants in the current population. Each generation, the ancestor's chromosome is broken down, until only small regions of chromosome which trace back to the common ancestor remain. These chromosome regions are identical by descent (IBD). Figure 1.3 demonstrates this concept.

The CSH then is the probability that two chromosome segments of the same size and location drawn at random from the population are from a common ancestor (ie IBD), without intervening recombination. CSH is defined for a specific chromosome segment, up to the full length of the chromosome. The CSH cannot be directly observed from marker data but has to be inferred from marker haplotypes for segments of the chromosome. Consider a segment of chromosome with marker locus A at the left hand end of the segment and marker locus B at the other end of the

segment (as in the classical definition above). The alleles at A and B define a haplotype. Two such segments are chosen at random from the population. The probability that the two haplotypes are identical by state (IBS) is the haplotype homozygosity (HH). The two haplotypes can be IBS in two ways,

i. The two segments are descended from a common ancestor without intervening recombination, so are identical by descent (IBD), or

ii. the two haplotypes are identical by state but not IBD

The probability of i. is CSH. The probability of ii. is a function of the marker homozygosities, given the segment is not IBD. The probabilities of i. and ii. are added together to give the haplotype homozygosity (HH):

$$HH = CSH + \frac{(Hom_A - CSH)(Hom_B - CSH)}{1 - CSH}$$

Where Hom_A and Hom_B are the individual marker homozygosities of marker A and marker B. This equation can be solved for CSH when the haplotype homozygosities and individual marker homozygosities are observed from the data. For more than two markers, the predicted haplotype homozygosity can be calculated in an analogous but more complex manner.

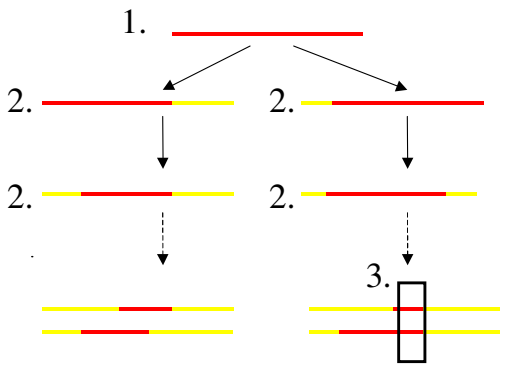


Figure 1.3 An ancestor many generations ago (1) leaves descendants (2). Each generation, the ancestors chromosome is broken down by recombination, until all that remains in the current generation are small conserved segments of the ancestor's chromosome (3). The chromosome segment homozygosity (CSH) is the probability that two chromosome segments of the same size and location drawn at random from the population are from a common ancestor.

Another justification for using multi-locus measures of LD is that they can be less variable than pair-wise measures. The variation in LD arises from two sampling processes (Weir and Hill 1980). The first sampling process reflects the sampling of gametes to form successive generations, and is dependent on finite population size. The second sampling process is the sampling of individuals to be genotyped from the population, and is dependent on the sample size, *n*. The first sampling process contributes to the high variability of LD measures. Marker pairs at different points in the genome, but a similar distance apart, can have very different r^2 values, particularly if the marker distance is small, Figure 1.4. This is because by chance there may have been an ancestral recombination between one pair of markers, but not the other.

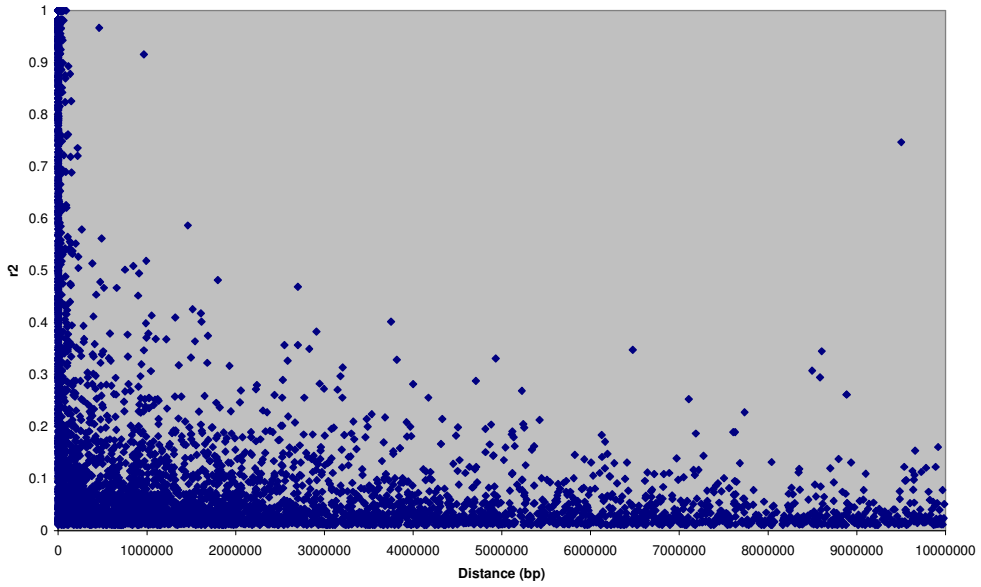


Figure 1.4. r^2 values against distance in bases between pairs of markers from 0 000 genome wide SNPs genotyped in a population of Holstein Friesian cattle. 1000000 bases is approximately 1cM.

Multi-locus measures of LD can have reduced variability because they accumulate information across multiple loci in an interval, thus averaging some of the effects of chance recombinations. Hayes et al. (2003) investigated the variability of r^2 and CSH using simulation. They simulated a chromosome segment of 10 cM containing 11 markers was simulated with a mutation-drift model, with a constant *N* of 1000. They

found CSH was less variable than r^2 provided at least four loci were included in the calculation of CSH, Figure 1.5.

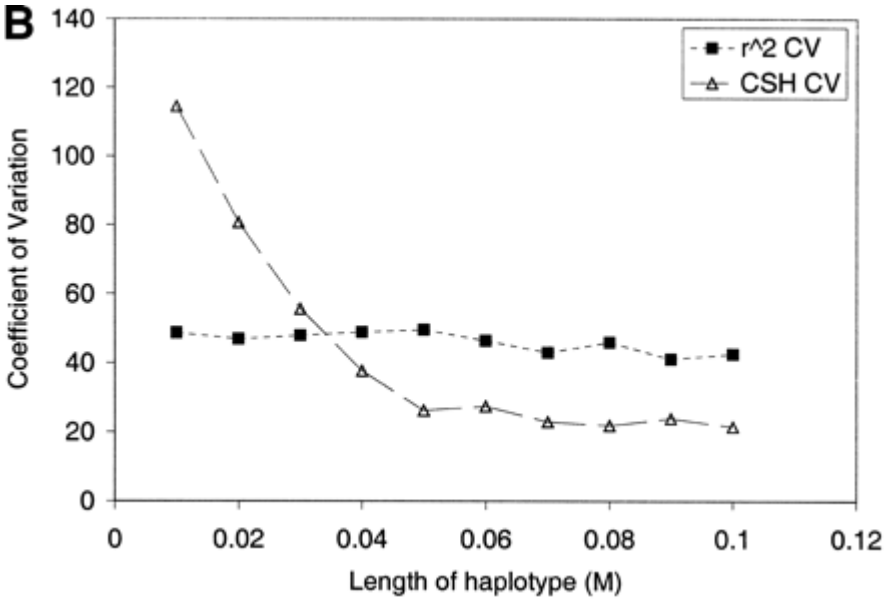


Figure 1.5 Coefficient of variation of r^2 and CSH in a simulated populations, over haplotype regions of the same length, across 200 replicates. There one marker per 0.01M (Hayes et al. 2003).

1.3 Causes of linkage disequilibrium in livestock populations

LD can arise due to migration, mutation, selection, small finite population size or other genetic events which the population experiences (eg. Lander and Schork 1994). LD can also be deliberately created in livestock populations; in an F2 QTL mapping experiment LD is created between marker and QTL alleles by crossing two inbred lines.

In livestock populations, finite population size is generally implicated as the key cause of LD. This is because

- effective population sizes for most livestock populations are relatively small, generating relatively large amounts of LD
- LD due to crossbreeding (migration) is large when crossing inbred lines but small when crossing breeds that do not differ as markedly in gene frequencies,

and it disappears after only a limited number of generations (eg. Goddard 1991)

- mutations are likely to have occurred many generations ago.
- while selection is probably a very important cause of LD, it's effect is likely to be localised around specific genes, and so has relatively little effect on the amount of LD 'averaged' over the genome. The use of LD measures to detect selected areas of the genome will be discussed briefly in section 1.8.

1.3.1 Predicting the extent of LD with finite population size

If we accept finite population size as the key driver of LD in livestock populations, it is possible to derive a simple expectation for the amount of LD for a given size of chromosome segment. This expectation is (Sved 1971)

$$E(r^2) = 1/(4Nc + 1)$$

where N is the finite population size, and c is the length of the chromosome segment in Morgans. The CSH has the same expectation (Hayes et al. 2003). This equation predicts rapid decline in LD as genetic distance increases, and this decrease will be larger with large effective population sizes, Figure 1.6.

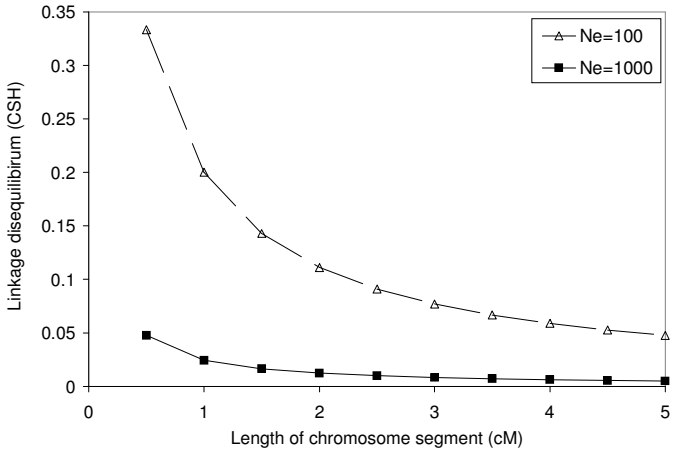
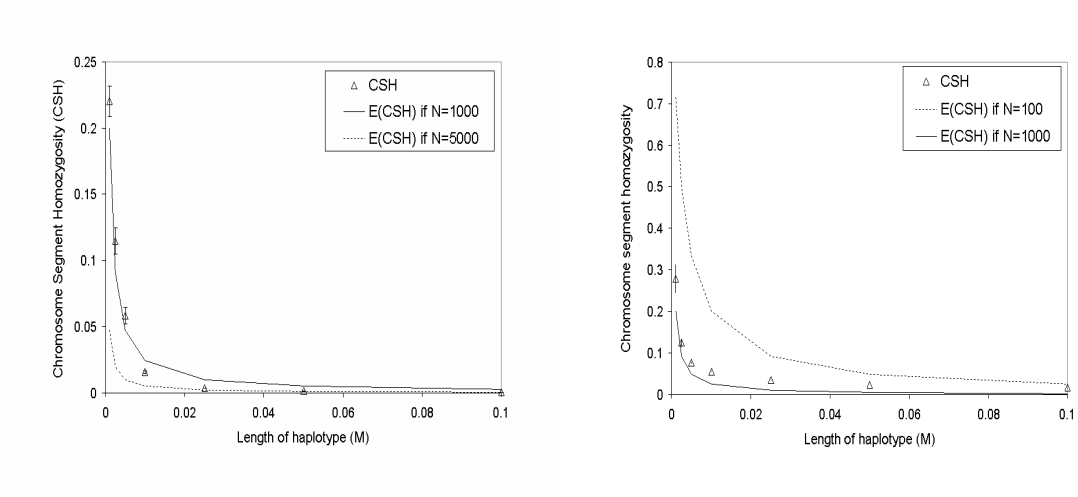


Figure 1.6. The extent of LD (as measured by chromosome segment homozygosity, CSH) for increasing chromosome segment length, for N_e=100 and N_e=1000. Note that r^2 has the same expectation as CSH.

As the extent of LD that is observed depends both on recent and historical recombinations, not only the current effective population size, but also the past effective population size are important. Effective population size for livestock species may have been much larger in the past than they are today. For example in dairy

cattle the widespread use of artificial insemination and a few elite sires has greatly reduced effective population size in the recent past. In humans, the story is the opposite; improved agricultural productivity and industrialisation have led to dramatic increases in population size. How does changing population size affect the extent of LD? To investigate this, we simulated a population which either expanded or contracted after a 6000 generation period of stability. The LD, as measured by CSH, was measured for different lengths of chromosome segment, Figure 1.7. Results for r^2 would look very similar.



A B

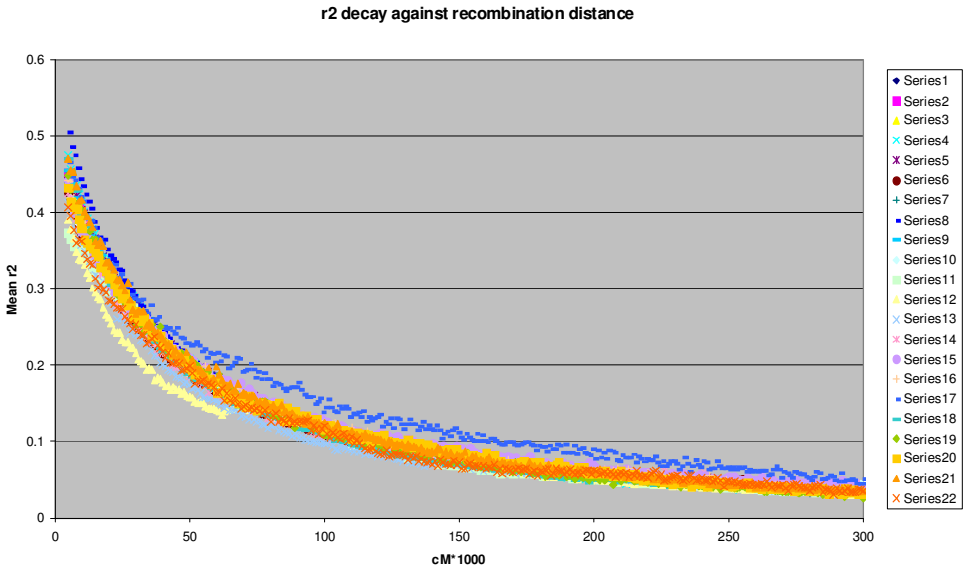
Figure 1.7. Chromosomal homozygosity for different lengths of chromosome (given the recombination rate) for populations: A. Linearly increasing population size, from N=1000 to N=5000 over 100 generations, following 6000 generations at N=1000. B. Linearly decreasing population size, from N=1000 to N=100 over 100 generations, following 6000 generations at N=1000.

The conclusion is that LD at short distances is a function of effective population size many generations ago, while LD at long distances reflects more recent population history. In fact, provided simplifying assumptions such as linear change in population size are made, it can be shown that the r^2 or CSH reflects the effective population size 1/(2c) generations ago, where c is the length of the chromosome segment in Morgans. So the expectation for r2 with changing effective population size can be written as $E(r^2) = 1/(4N_t c + 1)$ where $t = 1/2c$.

1.4 The extent of LD in livestock and human populations

If LD is a predominantly result of finite population size, then the extent of LD should be less in humans than in cattle, as in humans the effective population size is ~ 10000 (Kruglyak 1999) whereas in livestock where effective population sizes can be as low as 100 (Riquet et al. 1999). The picture is somewhat complicated by the fact that livestock populations have been very much larger, while the Caucasian effective population size has been very much smaller (following the out of Africa hypothesis). So what we could expect to see is that at long distances between markers, the r_2 values in livestock are much larger than in humans, while at short distances, the level of LD is more similar. This is in fact what is observed. Moderate LD (eg. $r^2 \geq 0.2$ in humans typically extends less than 5kb (~0.005cM), depending on the population studied (Dunning et al. 2000, Reich et al. 2001, Tenesa et al. 2007), Figure 1.8. In cattle moderate LD extends up to 100kb, Figure 1.8. However, very high levels of LD (eg. $r^2 \geq 0.8$ only extend very short distances in both humans and cattle.

It is interesting to compare the extent of LD in the different cattle populations. The Dutch and Australian Holstein populations had a very similar decline of LD, probably because these populations are highly related (eg. Zenger et al. 2007) and are similar in effective population size and history. The decline of LD in the Norwegian Reds was more rapid than in the Holstein populations. One explanation for this could be that the effective population size in Norwegian Red is higher than in Holstein, even though the global population is much smaller. Effective population size in Norwegian Reds is approximately 400 (Meuwissen et al. 2002), while for the global Holstein population effective population size is close to 150 (Zenger et al 2007), and a more limited extent of LD is expected with larger effective population size.



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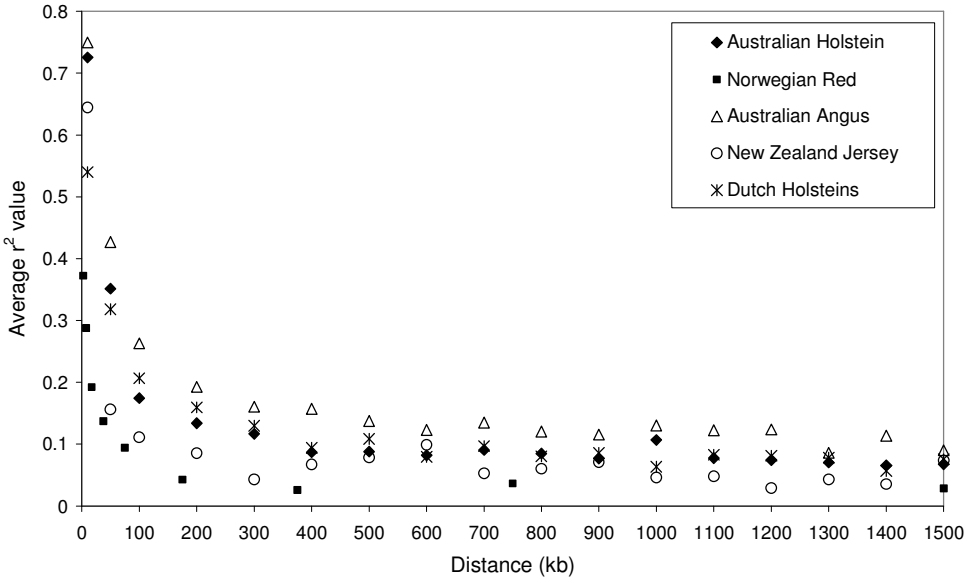


B

Figure 1.8. A. Average r^2 with distance in Caucasian humans (from Tenesa et al. 2007). 1cM is approximately 1000kb. B. Average r^2 value according to the distance between SNP markers in different cattle populations. Results are from 9918 SNPs distributed across the genome genotyped in 384 Holstein cattle or 384 Angus cattle, 403 SNPs genotyped in 783 Norwegian Red cattle, 3072 SNPs genotyped in 2430 Dutch Holstein cattle, or 351 SNPs genotyped in Jersey cattle. Norwegian red data kindly supplied by Prof. Sigbjorn Lien, Norwegian University of Life Sciences, New Zealand Jersey data kindly supplied by Dr. Richard Spelman, Livestock Improvement Co-operative.

Figure 1.8 implies that for the Holstein populations at least, there must be a marker approximately every 100kb (kilo bases) or less to achieve an average r^2 of 0.2. This level of LD between markers and QTL would allow a genome wide association study of reasonable size to detect QTL of moderate effect. As the bovine genome is approximately 3,000,000kb, this implies that in order of 30,000 evenly spaced markers are necessary in order that every QTL in the genome can be captured in a genome scan using LD to detect QTL. In Jerseys and Norwegian Reds, a larger number of markers would be required.

Du et al. (2007) assessed the extent of LD in pigs using 4500 SNP markers genotyped in six lines of commercial pigs. Only maternal haplotypes of the commercial pigs were used to evaluate r^2 between the SNPs, as the paternal haplotypes were over-represented in the population. The results from their study indicate there may be considerably more LD in pigs than in cattle. For SNPs separate by 1cM, the average value of r^2 was approximately of 0.2. LD of this magnitude only extends 100kb in cattle. In pigs at a 100kb the average r^2 was 0.371.

Heifetz et al. (2005) evaluated the extent of LD in a number of populations of breeding chickens. They used microsatellite markers and evaluated the extent of LD with the $\chi^{2'}$ statistic. In their populations, they found significant LD extended long distances. For example 57% of marker pairs separated by 5-10cM had an $\chi^{2'} \geq 0.2$ in one line of chickens and 28% in the other. Heifetz et al. (2005) pointed out that the lines they investigated had relatively small effective population sizes and were partly inbred, so the extent of LD in other chicken populations with larger effective population sizes may be substantially different.

McRae et al. (2002) evaluated the extent of LD in domestic sheep. They used the D' parameter rather than r^2, so comparison with results for other species given here is difficult. They found that high levels of LD extended for tens of centimorgans and declined with increasing marker distance. They also thoroughly investigated bias in D' under different conditions, and found that D' may be skewed when rare alleles are

present. They therefore recommended that the statistical significance of LD is used in conjunction with coefficients such as *D'* to determine the true extent of LD.

1.5 Extent of LD between populations and breeds.

Marker assisted selection exploiting LD relies on the phase of LD between markers and QTL being the same in the selection candidates as in the reference population where the QTL marker associations were detected. However as the reference population and the population in which MAS is applied become more and more diverged, for example different breeds, the phase is less and less likely to be conserved. The statistic r is a measure for LD between two markers in a population, but can also be used to measure the persistence of the LD phases between populations. While the r^2 statistic between two SNP markers at the same distance in different breeds or populations can be the same value even if the phases of the haplotypes are reversed, they will only have the same value and sign for the r statistic if the phase is the same in both breeds or populations. For marker pairs of a given distance, the correlation between r in two populations, corr(r1,r2), is equal to the correlation of the effects of the marker between both populations, for markers that have that same distance to a QTL (De Roos et al. 2007). If this correlation is 1, the marker effects are equal in both populations. If this correlation is zero, a marker in population 1 is useless in population 2. A high correlation between r values means that the marker effect persists across the populations. Calculating the correlation of r values across different breeds and populations as an indicator of how far the same marker phase is likely to persist between these breeds and populations (Goddard et al. 2006). This information can in turn be used to give an indication of marker density required to ensure marker-QTL phase persists across populations and or breeds, which would be necessary for the application LD-MAS or Genomic selection using the same marker set and SNP effects across the breeds or populations.

In Figure 1.9, the correlation of r values is given for a number of different cattle populations. The correlation of r values for Dutch Red-and-white bulls and Dutch Black-and-white bulls was 0.9 at 30kb. This indicates at this distance r^2 is high in both populations and the sign of r is the same in both populations, so the LD phase is the same in both populations. If one of these SNPs was actually an unknown mutation

affecting a quantitative trait, the other SNP could be used in MAS and the favourable SNP allele would be the same in both breeds. For Holstein and Angus breeds, the correlation of r is above 0.9 only at 10kb or less. For Australian Holsteins and Dutch Holsteins, the correlation of r values was above 0.9 up to 100kb, reflecting the fact that there are common bulls used in the two populations (eg. Zenger et al. 2007).

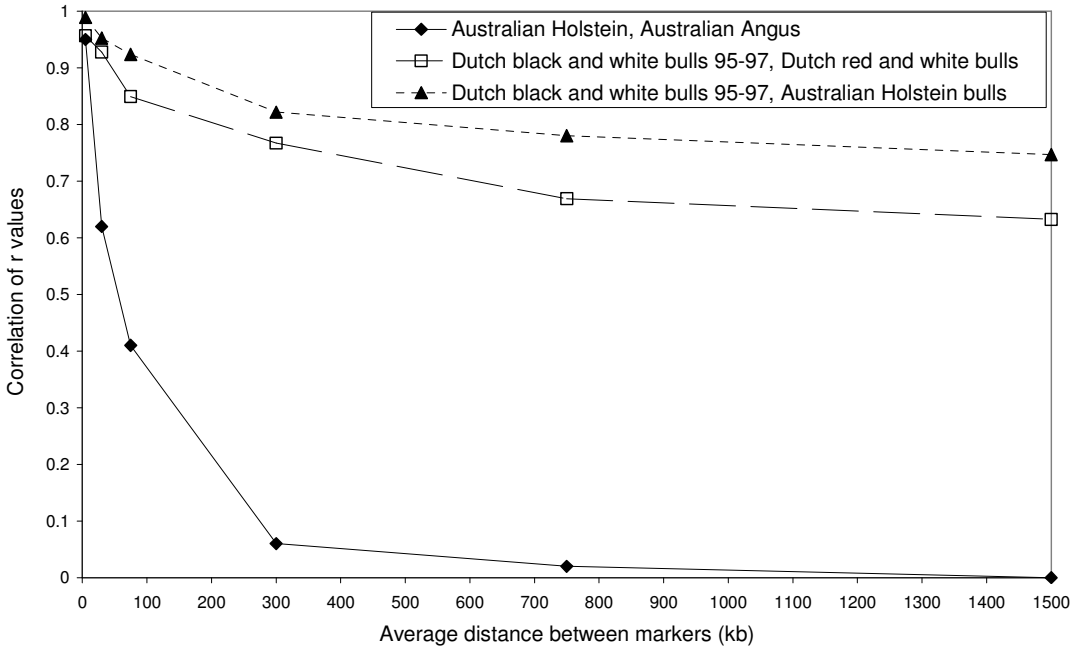


Figure 1.9. Correlation between r values for various cattle populations or sub-populations, as a function of marker distance (from De Roos et al. 2007).

1.6 Haplotype blocks and recombination hotspots

Recent studies of human populations using very high marker densities (eg. 7 million SNPs) suggest that there is an LD pattern of small segments of chromosome which have very high levels of LD between the markers defining the end of the segment, interspersed with boundaries where the markers across the boundary have very little LD. These chromosome segments have been termed haplotype blocks, and the boundaries are defined by recombination hot spots (for a review see Wall and Pritchard 2003). The requirement of recombination hot spots to define haplotype blocks was questioned by Phillips et al. (2003). They used evolutionary modelling of the data to demonstrate that recombination hot spots are not required to explain most of the observed blocks, providing that marker ascertainment and the observed marker

spacing are considered. In other words a proposed recombination hotspot could arise just due to an ancestral recombination. Whatever their origin, haplotype blocks have proved to be a useful concept in human genetics, as they allow tagging SNPs, that is a single SNP that identifies a haplotype block, to be identified, greatly reducing the total number of SNPs required for genome wide association studies.

In dairy cattle, Khatkar et al. (2007) investigated the number of SNPs that would be required to define haplotype blocks given the extent of LD. They concluded in the order of 250 000 SNPs would be required to elucidate haplotype block structures.

1.7 Optional topic 1. Brief note on haplotyping strategies

Calculations of LD parameters like r^2 and CSH assume that the genotypes of individuals can be phased into haplotypes (ie. which marker alleles belong on the paternally inherited chromosome and which marker alleles belong on the maternally inherited chromosome). If large half sib families are available, the sires haplotypes can fairly readily be reconstructed by determining which alleles are most often co-inherited from the sire. The haplotypes which the dam passed on the to the progeny can then be inferred by 'subtracting' the alleles transmitted from the sire from the progeny genotypes. Inferring haplotypes becomes more difficult in complex pedigrees, with missing marker information, or when there is very little pedigree information at all.

One method of inferring haplotypes in complex pedigrees is to run a *Markov Chain* on a set of *genetic descent graphs*. A genetic descent graph specifies the paths of gene flow (parents to offspring), but not the particular founder alleles travelling down the paths. See Sobel and Lange (1996) for more details on this procedure. This method is implemented in a freeware program called SimWalk (http://www.genetics.ucla.edu/software/simwalk_doc/).

In some cases, the individuals that are genotyped may be randomly sampled from the population, with no pedigree information available. Provided the markers which have been genotyped are closely spaced, it can be possible to estimate haplotypes based on linkage disequilibrium and allele frequency information alone. One such method was

proposed by Stephens et al. (2001). Suppose we have a sample of *n* diploid individuals from a population (these individuals are assumed to be unrelated). Let G = (G_1,….G_n) denote the (known) genotyped for the individuals, let H = (H_1,…,H_n) denote the (unknown) corresponding haplotype pairs, let F = (F_1,….,F_M) denote the set of unknown population haplotype frequencies, and let f = (f_1,….,f_M) denote the set of unknown sample haplotype frequencies (the M possible haplotypes are labelled 1,…,M). The haplotype reconstruction method of Stephens et al. (2001) regards the unknown haplotypes as unobserved random quantities and aims to evaluate their conditional distribution in light of the genotype data. To do this, they used MCMC, to obtain an approximate sample from the posterior distribution of H given G, eg. Pr(H|G). The steps in the algorithm are:

1. Start with an initial guess for H (the haplotype pairs of all individuals), H^0. This begins by listing all haplotypes that must be present unambiguously in the sample, that is those individuals who are homozygous at every locus or are heterozygous at only one locus. For the other individuals, who have ambiguous haplotypes, the haplotypes can be allocated at random from the genotypes.
2. Choose an individual, i, at random from all the ambiguous genotypes. Sample the haplotypes for this individual for the next iteration (H_i^{t+1}). These haplotypes are sampled from a distribution which assumes that the haplotypes in the haplotype pair H_i are likely to look either *exactly the same* or *similar to* a haplotype that has already been observed. This assumption is based on the existence of both LD and mutation – if the chromosome segment carrying the haplotypes is short enough, there will be considerable LD, greatly restricting the number of haplotypes. New haplotypes can be generated either by recombination or mutation at one of the markers. Formally, the distribution from which the new haplotypes are sampled is:

$$\pi(h \mid H) = \sum_{\alpha=1}^{M} \sum_{s=0}^{\infty} \frac{r\infty}{r} \left(\frac{\theta}{r+\theta} \right)^{s} \frac{r}{r+\theta} P_{\alpha h}^{s}$$

where r_α is the number of haplotypes of type α in the set H, r is the total number of haplotypes in H, θ is a scaled mutation rate (based on assumptions about population size, mutation rates at individual loci and length of the haplotype, relating to the expectation of LD described above), and P is mutation matrix (mapping the mutations onto markers in the haplotype). This corresponds to the next sampled haplotype, *h*,

being obtained by applying a random number of mutations, s, to a randomly chosen existing haplotype, α, where *s* is sampled from a geometric distribution.

The above algorithm is implemented in a program (again free) called PHASE. At least for short haplotypes (< 1cM) it appears to construct haplotypes very accurately. A nice feature of the algorithm is that an approximate probability of each haplotype for each animal being correct can be obtained from the posterior distribution. These probabilities could potentially be used in the QTL mapping procedure. The PHASE program is now widely used in human genetics, and is likely to be used to construct the bovine haplotype map as part of the bovine genome sequencing activity.

1.8 Optional topic 2: Identifying selected areas of the genome by linkage disequilibrium patterns.

While the average extent of linkage disequilibrium (LD) between closely spaced markers contains information about population history, including past population size, the extent of LD among markers within a given interval also reflects selection on genes within the interval. This is because selected alleles will increase the frequency in the population of a surrounding segment of chromosome as they are driven toward fixation, in selective sweeps (Maynard-Smith and Haigh 1967). However comparing the extent of LD between intervals is unlikely to be particularly informative with regard to selection history due to the extremely variable nature of LD (Hill 1980, Hill and Weir 1994). Another approach is to compare the LD surrounding the selected allele to the non-selected allele, as proposed by Voight et al. 2006. The LD surrounding the non-selected allele then acts as in internal control for the level of LD expected in the region. The measure that Voight et al. (2006) proposed for the detection of selection signatures was the standardized integrated extended haplotype homozygosity (iHS).

The next section describing how iHS is calculated is taken from Voight et al. (2006) "Our new test begins with the EHH (extended haplotype homozygosity) statistic proposed by Sabeti et al. (2002). The EHH measures the decay of identity, as a

function of distance, of haplotypes that carry a specified "core" allele at one end. For each allele, haplotype homozygosity starts at 1, and decays to 0 with increasing distance from the core site. When an allele rises rapidly in frequency due to strong selection, it tends to have high levels of haplotype homozygosity extending much further than expected under a neutral model. Hence, in plots of EHH versus distance, the area under the EHH curve will usually be much greater for a selected allele than for a neutral allele. In order to capture this effect, we compute the integral of the observed decay of EHH away from a specified core allele until EHH reaches 0.05. This *integrated* EHH (iHH) (summed over both directions away from the core SNP) will be denoted iHH_A or iHH_D, depending on whether it is computed with respect to the ancestral or derived core allele. Finally, we obtain our test statistic iHS using

$$\text{unstandardized } iHS = \ln\left(\frac{iHH_A}{iHH_D}\right). \qquad (1)$$

When the rate of EHH decay is similar on the ancestral and derived alleles, $iHH_A/iHH_D \approx 1$, and hence the unstandardized iHS is ≈ 0. Large negative values indicate unusually long haplotypes carrying the derived allele; large positive values indicate long haplotypes carrying the ancestral allele. Since in neutral models, low frequency alleles are generally younger and are associated with longer haplotypes than higher frequency alleles, we adjust the unstandardized iHS to obtain our final statistic which has mean 0 and variance 1 regardless of allele frequency at the core SNP:

$$iHS = \frac{\ln\left(\frac{iHH_A}{iHH_D}\right) - E_p\left[\ln\left(\frac{iHH_A}{iHH_D}\right)\right]}{\mathrm{SD}_p\left[\ln\left(\frac{iHH_A}{iHH_D}\right)\right]}. \qquad (2)$$

The expectation and standard deviation of $\ln(iHH_A/iHH_D)$ are estimated from the empirical distribution at SNPs whose derived allele frequency p matches the frequency at the core SNP. The iHS is constructed to have an approximately standard normal distribution and hence the sizes of iHS signals from different SNPs are directly comparable regardless of the allele frequencies at those SNPs. Since iHS is standardized using the genome-wide empirical distributions, it provides a measure of how *unusual* the haplotypes around a given SNP are, relative to the genome as a whole, and it does not provide a formal significance test".

An experiment was conducted to investigate selection signatures of bovine chromosome six in Norwegian Red dairy cattle. Four hundred and three SNPs were genotyped on BTA6 in 18 paternal half-sib families (18 sires and 716 sons). Using the pedigree information, the genotypes were resolved into paternal and maternal haplotypes. Both haplotypes of the sires and the maternal haplotypes of the progeny were retained for analysis. iHS scores were then calculated for the BTA6 SNPs. This required defining an ancestral allele at each SNP position. This was done by extracting the allele in the assembled bovine genome sequence based on a Hereford cow (Genbank accession number CM000182). The largest cluster of extreme iHS scores was in the interval 35-36.5Mb, Figure 1.10. This interval contains a QTL with a large effect on protein %, as reported in a number of QTL mapping and fine mapping experiments (eg. Olsen et al. 2005). Various mutations have been proposed as the mutation underlying the QTL effect, including a mutation in the ABCG2 gene (Cohen-Zindar et al. 2005).

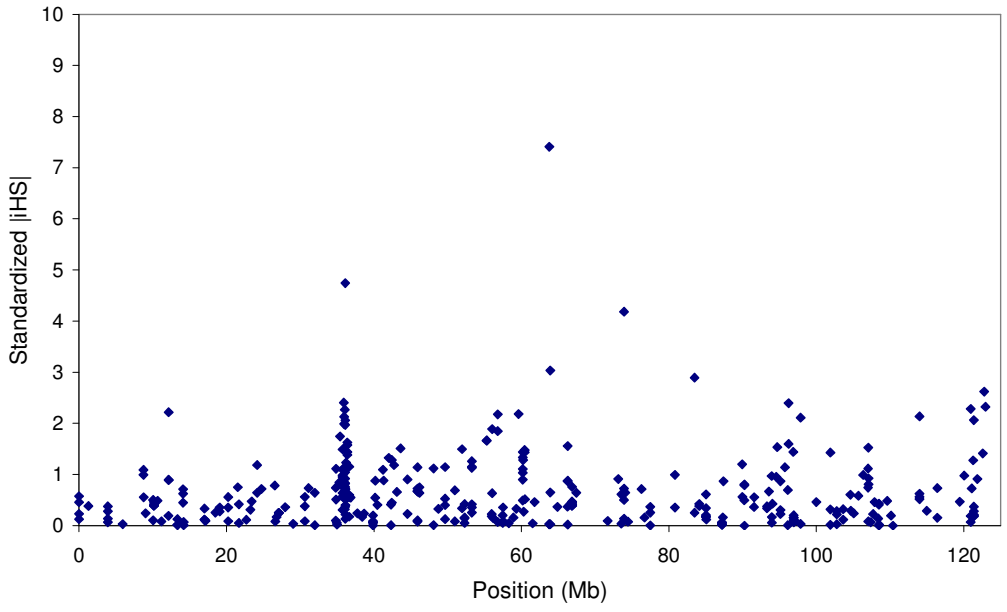


Figure 1.10. Value of |iHS| for individual SNPs across BTA6 in Norwegian Red cattle.

The results indicate that selection signatures can be detected in cattle populations at least with a medium density of SNPs. In highly selected livestock populations, detection of selection signatures may reveal QTL for selected traits.

2. Mapping QTL using Linkage Disequilibrium

2.1 Introduction

Linkage disequilibrium (LD) mapping of QTL exploits population level associations between markers and QTL. These associations arise because there are small segments of chromosome in the current population which are descended from the same common ancestor. These chromosome segments, which trace back to the same common ancestor without intervening recombination, will carry identical marker alleles or marker haplotypes, and if there is a QTL somewhere within the chromosome segment, they will also carry identical QTL alleles. There are a number of QTL mapping strategies which exploit LD, the simplest of these is the genome wide association test using single marker regression.

2.2 Genome wide association tests using single marker regression

In a random mating population with no population structure the association between a marker and a trait can be tested with single marker regression as

$$\mathbf{y} = \mathbf{1_n}\boldsymbol{\mu} + \mathbf{X}g + \mathbf{e}$$

Where **y** is a vector of phenotypes, $\mathbf{1_n}$ is a vector of 1s, **X** is a design matrix allocating records to the marker effect, g is the effect of the marker and **e** is a vector of random deviates $e_{ij} \sim N(0, \sigma_e^2)$, where σ_e^2 is the error variance. In this model the effect of the marker is treated as a fixed effect. Note that the g can actually be a vector of 2 times the number of marker alleles, if both additive and dominance effects are to be estimated. The underlying assumption here is that the marker will only affect the trait if it is in linkage disequilibrium with an unobserved QTL. This model ignores fixed effects other than the mean, however they can be easily included.

The null hypothesis is that the marker has no effect on the trait, while the alternative hypothesis is that the marker does affect the trait (because it is in LD with a QTL). The null hypothesis is rejected if $F > F_{\alpha,v1,v2}$, where F is the F statistic calculated

from the data for example by an analysis of variance (ANOVA), $F_{\alpha,v1,v2}$ is the value from an *F* distribution at α level of significance and *v1, v2* degrees of freedom.

Consider a small example of 10 animals genotyped for a single SNP. The phenotypic and genotypic data is:

Animal	Phenotpe	SNP allele 1	SNP allele 2
1	2.030502	1	1
2	3.542274	1	2
3	3.834241	1	2
4	4.871137	2	2
5	3.407128	1	2
6	2.335734	1	1
7	2.646192	1	1
8	3.762855	1	2
9	3.689349	1	2
10	3.685757	1	2

We need a design matrix X to allocate both the mean and SNP alleles to phenotypes. In this case we will use an X matrix with number of rows is equal to the number of records, and one column for the SNP effect. We will set the effect of the "1" allele to zero, so the SNP effect column in the **X** matrix is the number of copies of the "2" allele an animal carries (**X** matrix in bold):

Animal	$\mathbf{1_n}$	**X**, Number of "2" alleles
1	**1**	**0**
2	**1**	**1**
3	**1**	**1**
4	**1**	**2**
5	**1**	**1**
6	**1**	**0**
7	**1**	**0**
8	**1**	**1**
9	**1**	**1**
10	**1**	**1**

The mean and SNP effect can then be estimated as:

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'X} \\ \mathbf{X'1_n} & \mathbf{X'X} \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{X'y} \end{bmatrix}$$

Where **y** is the (number of animals x 1) vector of phenotypes.

In the above example the estimated of the mean and SNP effect are

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} 2.36 \\ 1.38 \end{bmatrix}$$

This is not far from the real value of these parameters. The data above was "simulated" with a mean of 2, a QTL effect of 1, an r^2 (a standard measure of LD) between the QTL and the SNP of 1, plus a normally distributed error term.

The F-value can be calculated as:

$$F = \frac{(n-1)\left(\hat{g}\,\mathbf{X}'\mathbf{y} - 1/n\mathbf{y}'\mathbf{y}\right)}{\mathbf{y}'\mathbf{y} - \hat{g}\,\mathbf{X}'\mathbf{y} - \hat{u}\,\mathbf{1_n}'\mathbf{y}}$$

Using the above values, the value of F is 4.56. This can be compared to the tabulated F-value at a 5% significance value and 1 and 9 (number of records -1) degrees of freedom is 5.12. So the SNP effect in this case is not significant (not surprisingly with only 10 records!).

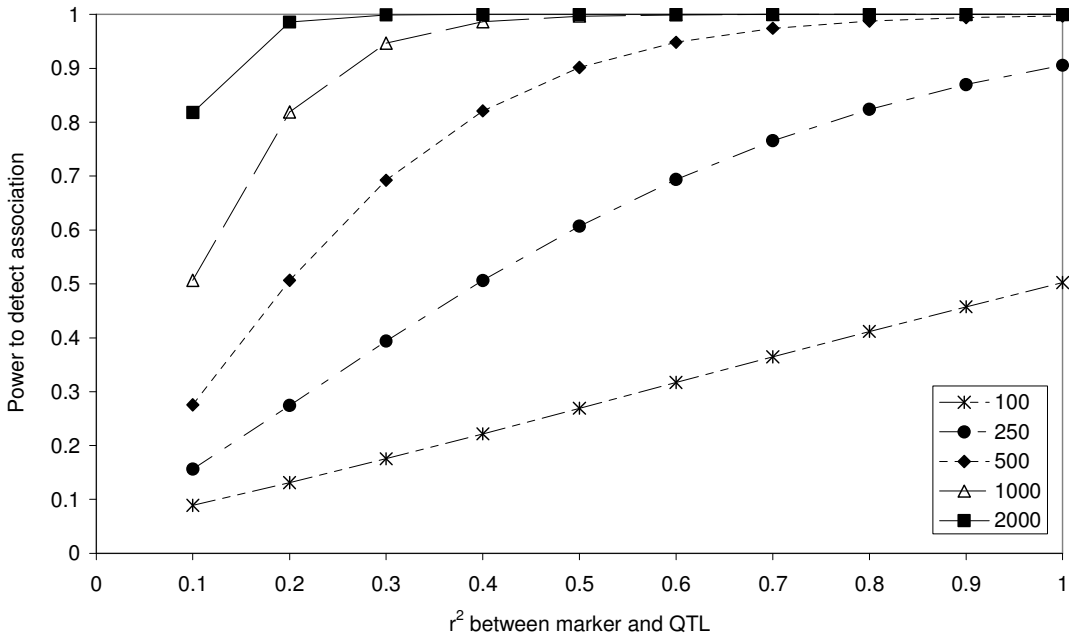
The power of the association test to detect a QTL by testing the marker effect depends on:

1. The r^2 between the marker and QTL. Specifically, sample size must be increased by a factor of $1/r^2$ to detect an ungenotyped QTL, compared with the sample size for testing the QTL itself (Pritchard and Przeworski 2001).
2. The proportion of total phenotypic variance explained by the QTL, termed h_Q^2.
3. The number of phenotypic records *n*
4. The allele frequency of the rare allele of the SNP or marker, *p*, which determines the minimum number of records used to estimate an allele effect. The power becomes particular sensitive to *p* when *p* is small (eg. <0.1).
5. The significance level α set by the experimenter.

The power is the probability that the experiment will correctly reject the null hypothesis when a QTL of a given size of effect really does exist in the population. Figure 2.1 illustrates the power of an association test to detect a QTL with different levels of r^2 between the QTL and the marker and with different numbers of phenotypic records (using the formula's of Luo 1998).

Using both this figure, and the extent of LD in our livestock species, we can make predictions of the number of markers required to detect QTL in a genome wide association study. For example, an r^2 of at least 0.2 is required to achieve power ≥ 0.8 to detect a QTL of $h_Q^2 = 0.05$ with 1000 phenotypic records. In dairy cattle, $r^2 \approx 0.2$ at 100kb. So assuming a genome length of 3000Mb in cattle, we would need at least 15 000 markers in such an experiment to ensure there is a marker 100kb from every QTL. However this assumes that the markers are evenly spaced, and all have a rare allele frequency above 0.2. In practise, the markers may not be evenly spaced and the rare allele frequency of a reasonable proportion of the markers will be below 0.2. Taking these two factors into account, at least 30 000 markers would be required.

To demonstrate the dependence of power on r^2 between a QTL and SNP in another way, consider the results of Macleod et al. (2007). They attempted to assess the power of whole genome association scans in outbred livestock with commercially available SNP panels. In their study, 365 cattle were genotyped using a 10,000 SNP panel while QTL, polygenic and environmental effects were simulated for each animal, with QTL simulated on genotyped SNPs chosen at random. The power to detect a QTL accounting for 5% of the phenotypic variance with 365 animals genotyped, was 37% ($p<0.001$). There was a strong correlation between the F-value of significant SNPs and their r^2 with the "QTL", Figure 2.2. The correlation of F-values with D' was almost zero.



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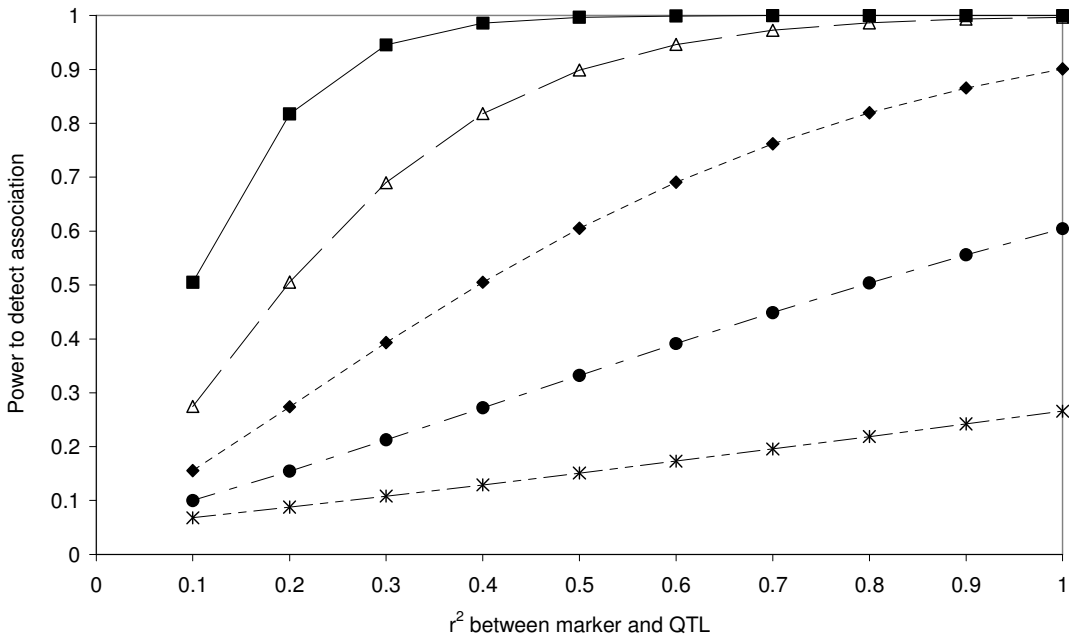


B

Figure 2.1 A. Power to detect a QTL explaining 5% of the phenotypic variance with a marker. B. Power to detect a QTL explaining 2.5% of the phenotypic variance with a marker, for different numbers of phenotypic records given in the legend and for different levels of r^2 between the marker and the QTL, with a P value of 0.05. Rare allele frequencies at the QTL and marker were both 0.2.

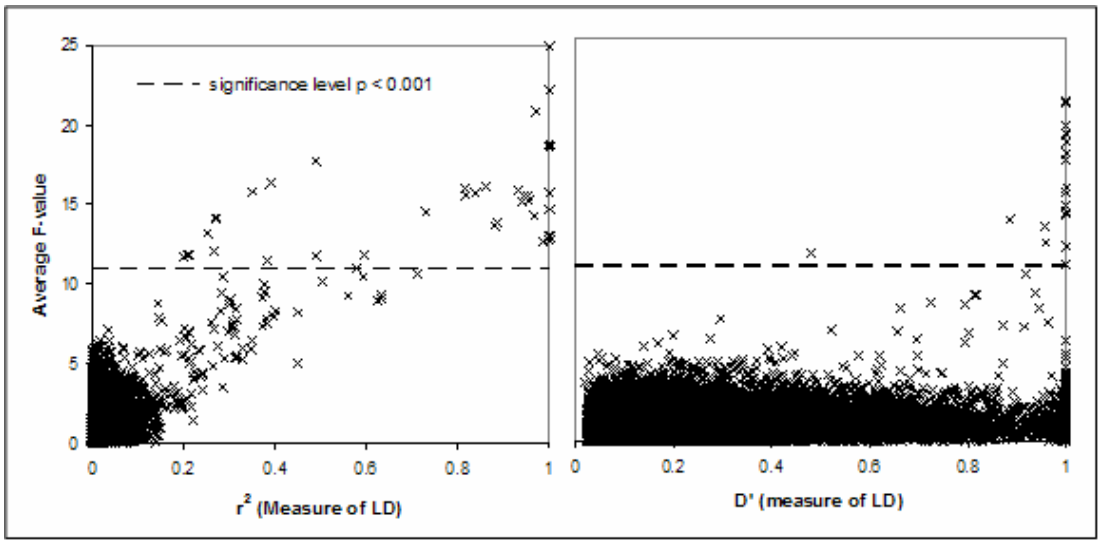


Figure 2.2 Plots of F-values of SNPs tested for association, against r^2 and D' of the tested SNP with the QTL. The QTL accounted for 5% of the phenotypic variance. From Macleod et al. 2007.

2.2.1 Choice of significance level

With such a large number of markers tested in genome wide association studies, an important question is what value of α to choose. In a genome wide association study, we will be testing 10s or possibly 100s of thousands of markers. A major issue in setting significance thresholds is the multiple testing problem. In most QTL mapping experiments, many positions along the genome or a chromosome are analysed for the presence of a QTL. As a result, when these multiple tests are performed the "nominal" significance levels of single test don't correspond to the actual significance levels in the whole experiment, eg. when considered across a chromosome or across the whole genome. For example, if we set a point-wise significance threshold of 5%, we expect 5% of results to be false positives. If we analyse 10 000 markers (assuming for the moment these points are independent), we would expect 10000*0.05 = 500 false positive results! Obviously more stringent thresholds need to be set. One option would be to adjust the significance level for the number of markers tested using a Bonferoni correction to obtain an experiment wise P-value of 0.05. However such a correction does not take account of the fact that 'tests' on the same chromosome may not be independent, as the markers can be in linkage disequilibrium with each other as well as the QTL. As a result, the Bonferoni

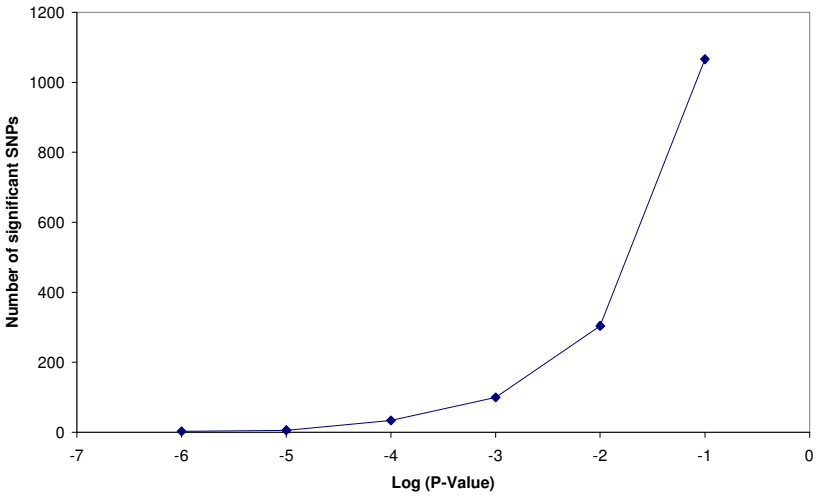
correction tends to be very conservative, and requires some decision to be made about how many independent regions of the genome were tested.

Churchill and Doerge (1994) proposed the technique of permutation testing to overcome the problem of multiple testing in QTL mapping experiments. Permutation testing is a method to set appropriate significance thresholds with multiple testing (eg testing many locations along the genome for the presence of the QTL). Permutation testing is performed by analysing a large number of simulated data sets that have been generated from the real one, by randomly shuffling the phenotypes across individuals in the mapping population. This removes any existing relationship between genotype and phenotype, and generates a series of data sets corresponding to the null hypothesis. Genome scans can then be performed on these simulated data-sets. For each simulated data the highest value for the test statistic is identified and stored. The values obtained over a large number of such simulated data sets are ranked yielding an empirical distribution of the test statistic under the null hypothesis of no QTL. The position of the test statistic obtained with the real data in this empirical distribution immediately measure the significance of the real dataset. . For example if we carry out 100 000 analyses of permuted data, the F value for the 5000^{th} highest value will represent the cut off point for the 5% level of significance. Significance thresholds can then be set corresponding to 5% false positives for the entire experiment, 5% false positives for a single chromosome, and so on. Permutation testing is an excellent method of setting significance thresholds in a random mating population. In populations with some pedigree structure however, randomly shuffling phenotypes across marker genotypes will not preserve any pedigree structure that exists in the data.

An alternative to attempting to avoid false positives is to monitor the number of false positives relative to the number of positive results (Fernando et al. 2004). The researcher can then set a significance level with an acceptable proportion of false positives. The false discovery rate (FDR) is the expected proportion of detected QTL that are in fact false positives (Benjamini and Hochberg 1995, Weller 1998). FDR can be calculated for a QTL mapping experiment as

$$mP_{max}/n,$$

where P_{max} is the largest P value of QTL which exceed the significance threshold, n is the number of QTL which exceed the significance threshold and m is the number of markers tested. Figure 2.3 shows an example of the false discovery rate in an experiment where 9918 SNPs were tested for the effect on feed conversion efficiency in 384 Angus cattle. As the significance threshold is relaxed, the number of significant SNPs increases. However, the FDR also increases.



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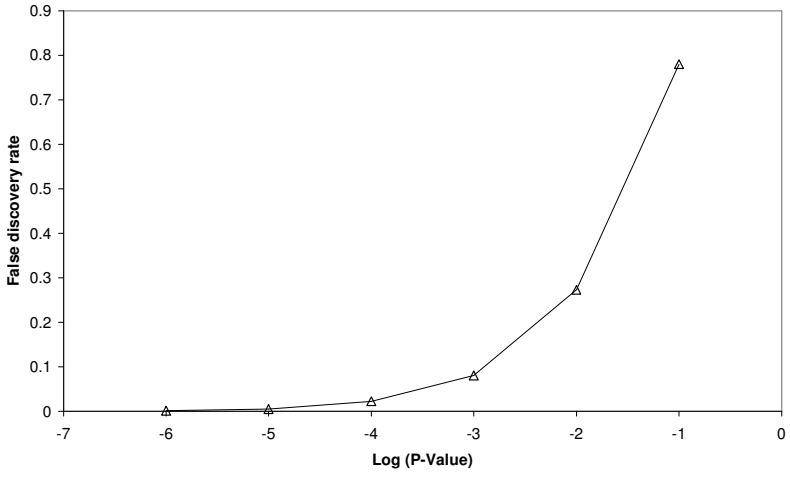

B

Figure 2.3 A. Number of significant markers at different P values in a genome wide association study with 9918 SNPs, using 384 Angus cattle with phenotypes for feed conversion efficiency. B. False discovery rate at the different P-values.

In this experiment, a P-value of 0.001 was chosen as a criteria to select SNPs for further investigation. At this P-value, there were 56 significant SNPs. So the false discovery rate was 9918*0.001/56 = 0.18. This level of false discovery was deemed acceptable by the researchers.

A number of other statistics have been proposed to control the proportion of false positives, including the proportion of false positives (PRP Fernando et al. 2004), and the positive false discovery rate (pFDR Storey 2002).

2.2.2 Confidence intervals.

There are few reports in the literature on methods to estimate confidence intervals in genome wide association studies. A method based on cross-validation is described here. To calculate approximate 95% confidence intervals for the location of QTL underlying the significant SNPs, a genome wide association study is first conducted as above. The data set is then split into two halves at random (eg. half the animals in the first data set, the other half in the second data set). The genome wide association study is then re-run for each half of the data. When each half of the data confirmed a significant SNP in the analysis of the full data (ie a significant SNP in almost the same location), the information is used in the following way. The position of the most significant SNP from each split data set was designated x_{1i} and x_{2i} respectively, for the i^{th} QTL position (taken as the most significant SNP in a region from the full data set). So for n pairs of such SNPs, the standard error of the underlying QTL is calculated as $se(\bar{x}) = \sqrt{\frac{1}{4n}\sum_{i=1}^{n} x_{1i} - x_{2i}}$. The 95% confidence interval is then the position of the most significant SNP from the full data analysis $\pm 1.96\, se(\bar{x})$.

Using this approach in a data set with 9918 SNPs genotyped on 384 Holstein-Friesian cattle, and for the trait protein kg, there were 24 significant SNP clusters (clusters of SNP putatively marking the same QTL, a cluster consists of 1 or more SNPs) in the full data, and the confidence interval for the QTL was calculated as 2Mb.

2.2.3 Avoiding spurious false positives due to population structure

The very simple model above for testing association of a marker to phenotype assumes there is no structure in the population, that is it assumes all animals are equally related. In livestock populations, or any population for that matter, this is unlikely to be the case. Multiple offspring per sire, selection for specific breeding goals and breeds or strains within the population all create population structure. Failure to account for population structure can cause spurious associations (false positives) in the genome wide association study (Pritchard 2000). A simple example is where the population includes a sire with a large number of progeny in the population. In this case the sire has a significantly higher estimated breeding value than other sires in the population. If a rare allele at a marker any where on the genome is homozygous in the sire, the sub-population made up of his progeny will have a higher frequency of the allele than the rest of the population. As the sires' estimated breeding value is high, his progeny will also have higher than average estimated breeding values. Then in the genome wide association study, if the number of progeny of the sire is not accounted for, the rare allele will appear to have a (perhaps significant) positive effect.

Spielman et al. (1993) proposed the transmission disequilibrium test (TDT) which requires that parents of individuals in the genome wide association study are genotyped to ensure the association between a marker allele and phenotype is linked to the disease locus, as well as in linkage disequilibrium across the population with it. In this way the TDT test avoids spurious associations due to population structure. However the TDT test has a cost in that genotypes of both parents must be collected, and this is often not possible in livestock populations.

An alternative is to remove the effect of population structure using a mixed model:

$$\mathbf{y} = \mathbf{1_n}'\mu + \mathbf{X}g + \mathbf{Zu} + \mathbf{e}$$

Where u is a vector of polygenic effect in the model with a covariance structure $u_i \sim N(0, \mathbf{A}\sigma_a^2)$, where **A** is the average relationship matrix built from the pedigree of the population, and σ_a^2 is the polygenic variance. **Z** is a design matrix allocating animals to records. In other words, the pedigree structure of the population is

accounted for in the model. Note that this is BLUP, with the marker effect and the mean as fixed effects and the polygenic effects as random effects.

In the study of Macleod (2007) described in section 2.2.1, they assessed the effect of including or omitting the pedigree on the number of QTL detected in the experiment, in a simulation where no QTL effects were simulated (so all QTL detected are false positives), Table 2.1. They found a significant increase in the number of false positives, when the polygenic effects were not fully accounted for.

Table 2.1 Detection of type I errors in data with no simulated QTL.

Analysis model	Significance level		
	p<0.005	p<0.001	p<0.0005
Expected type I errors	40	8	4
1. Full pedigree model	39 (SD=14)	9 (SD=5)	4 (SD=3)
2. Sire pedigree model	46* (SD=21)	11* (SD=7)	6* (SD=5.5)
3. No pedigree model	68** (SD=31)	18** (SD=11)	10** (SD=7)
4. Selected 27% - full pedigree	54** (SD=18)	12** (SD=6)	7** (SD=4)

The results indicate that the number of type 1 errors (significant SNPs detected when no QTL exist) is significantly higher when no pedigree is fitted, and even fitting sire does not remove all spurious associations due to population structure.

A problem arises if the pedigree of the population is not recorded, or is recorded with many errors. One solution in this case is to use the markers themselves to infer the average relationship matrix (Hayes et al. 2007) or population structure (eg. Pritchard 2000).

For a given marker single locus, a similarity index S_{xy} between two individuals x and y is calculated, where $S_{xy} = 1$ when genotype $x = ii$ (i.e. both alleles at loci l are identical) and genotype $y = ii$, or when $x = ij$ and $y = ij$. $S_{xy} = 0.5$ when $x = ii$ and $y = ij$, or vice versa, $S_{xy} = 0.25$ when $x = ij$ and $y = ik$, and $S_{xy} = 0$ when the two individuals have no alleles in common at the locus. The similarity as a result of chance alone was $s = \sum_{i=1}^{a} p_i^2$ where p_i is the frequency of allele i in the (random mating) population, and a is the number of alleles at the locus. Then the relationship between individuals x and y at locus l is calculated as $r_l = (S_{xy} - s)/(1 - s)$. The average relationship between the individuals is calculated as the r_l averaged over all loci.

With large numbers of markers, average relationship matrices derived from markers can be very accurate, and can even capture mendelian sampling effects (eg. Two full sibs may be more or less related than 0.5 because they have more or less paternal and maternal chromosome segments than expected by chance. This approach can also be used to correct for population structure across breeds or lines. In Figure 2.4, the average relationship matrix derived from markers is shown for a combined Angus Holstein Population.

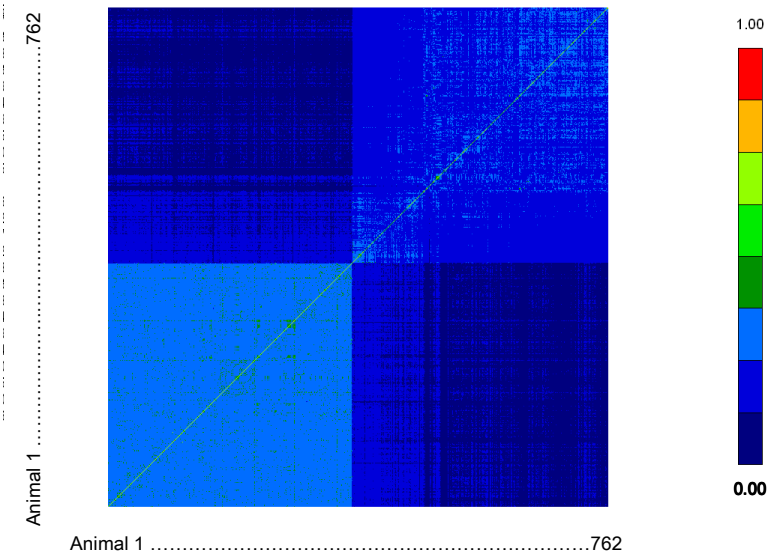

Figure 2.4. Average-relationship matrix derived from 9323 SNP loci where the population consists of two breeds. The diagonal element for the first Angus animal is in the bottom left hand corner and the element for the last Holstein animal in the top left hand corner.

There are a number of situations in which marker derived relationship matrices will be especially valuable. When there is limited or no pedigree recorded in a population, marker genotypes may be the only source of information available to build relationship matrices. For example, in livestock, there are many traits which can only be recorded in animals which are not candidates for selection, such as meat quality. If there is no recorded pedigree linking selection candidates and commercial animals on which the trait is recorded, marker derived relationship matrices could be used in estimation of QTL effects for marker assisted selection. Another example is populations where multiple sires are used in the same paddock of dams, such that recording pedigree is difficult. Finally, in multi-breed populations including crosses between breeds, the marker derived relationship matrix offers a way to account for the different breed composition of the animals.

2.3 Genome wide association experiments using haplotypes

Rather than using single markers, haplotypes of markers could be used in the genome wide association. The effect of haplotypes in windows across the genome would then be tested for their association with phenotype. The justification for using haplotypes is that marker haplotypes may be in greater linkage disequilibrium with the QTL alleles than single markers. If this is true, then the r^2 between the QTL and the haplotypes is increased, thereby increasing the power of the experiment.

To understand why marker haplotypes can have a higher r^2 with a QTL than an individual marker, consider two chromosome segments containing a QTL drawn at random from the population, which happen to carry identical marker haplotypes for the markers on the chromosome segment. There are two ways in which marker haplotypes can be identical, either they are derived from the same common ancestor so they are identical by descent (IBD), or the same marker haplotypes have been regenerated by chance recombination (identical by state IBS). If the "haplotype" consists only of a single SNP the chance of being identical by state is a function of the marker homozygosity. Now as more and more markers are added into the chromosome segment, the chance of regenerating identical marker haplotypes by chance recombination is reduced. So the probability that identical haplotypes carried

by different animals are IBD is increased. If the haplotypes are IBD, then the chromosome segments will also carry the same QTL alleles. As the probability of two identical haplotypes being IBD increases, the proportion of QTL variance explained by the haplotypes will increase, as marker haplotypes are more and more likely to be associated with unique QTL alleles.

Just as for single markers, the proportion of QTL variance explained by the markers can be calculated. Let q_1 be the frequency of the first QTL allele and q_2 be the frequency of the second QTL allele. The surrounding markers are classified into *n* haplotypes, with p_i the frequency of the i^{th} haplotype. The results can be classified into a contingency table:

	Haplotype			
	1	i	N	
QTL allele 1	$p_1q_1-D_1$	$p_iq_1-D_i$	$p_nq_1-D_n$	Q_1
QTL allele 2	$p_1q_2+D_1$	$p_1q_2+D_i$	p_nq_2+Dn	Q_2
	p_1	p_i	p_n	1

For a particular haplotype i represented in the data, we calculated the disequilibrium as $D_i = p_i(q_1)-p_iq_1$, where $p_i(q_1)$ is the proportion of haplotypes i in the data that carry QTL allele 1 (observed from the data), p_i is the proportion of haplotypes i, and q_1 is the frequency of QTL allele 1. The proportion of the QTL variance explained by the haplotypes, and corrected for sampling effects was then calculated as

$$r^2(h,q) = \frac{\sum_{i=1}^{n} \frac{D_i^2}{p_i}}{q_1 q_2}$$

For example, in a simulated population of Ne=100, and a chromosome segment of length 10cM, the proportion of the QTL variance accounted for by marker haplotypes when there were 11 markers in the haplotype was close to one, Figure 2.5. [Note that if the effective population size was larger, the proportion of genetic variance explained by a 10cM haplotype would be reduced (Goddard 1991).]

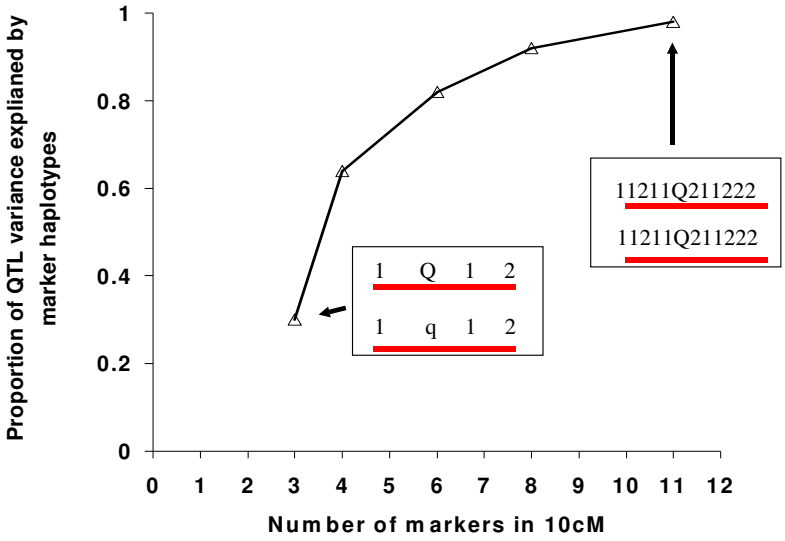

Figure 2.5. Proportion of QTL variance explained by marker haplotypes with an increasing number of markers in a 10 cM interval

A model for testing haplotypes in an association study could be similar to the model described above:

$$\mathbf{y} = \mathbf{1_n}'\mu + \mathbf{Xg} + \mathbf{Zu} + \mathbf{e}$$

However **g** is now a vector of haplotype effects rather than the effect of a single marker. The haplotypes could be treated as random, as there are likely to be many of them and some haplotypes will occur only a small number of times. The effect of treating the haplotypes as random is to "shrink" the estimates of the haplotypes with only a small number of observations. This is desirable because it reflects the uncertainty of predicting these effects. So $g_i \sim N(0, I\sigma_h^2)$ where *I* is an identity matrix and σ_h^2 the variance of the haplotype effects. The g can be estimated from the equations:

$$\begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{u}} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n}'\mathbf{1_n} & \mathbf{1_n}'\mathbf{Z} & \mathbf{1_n}'\mathbf{X} \\ \mathbf{Z}'\mathbf{1_n} & \mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z}'\mathbf{X} \\ \mathbf{X}'\mathbf{1_n} & \mathbf{X}'\mathbf{Z} & \mathbf{X}'\mathbf{X} + \mathbf{I}\lambda_2 \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \\ \mathbf{X}'\mathbf{y} \end{bmatrix}$$

Where $\lambda_1 = \frac{\sigma_e^2}{\sigma_a^2}$, and $\lambda_2 = \frac{\sigma_e^2}{\sigma_h^2}$. Note that this model assumes no-covariance between haplotype effects. In practise, the haplotype variance is unlikely to be known, so will need to be estimated . A REML program, such as ASREML (Gilmour et al 2002), can be used to do this.

2.4 IBD LD mapping

The IBD model is quite different from that used in single marker regression in that now the effect of a putative QTL itself is fitted, rather than the marker:

$$y_i = \mu + u_i + vp_i + vm_i + e_i$$

Where vp_i and vm_i are the effects of the QTL alleles carried on the ith animals paternal and maternal chromosome respectively. In this model, the assumption is that each animal carries two unique QTL alleles, and so there are two QTL effects fitted for each animal.

Then marker haplotype information is used to infer the probability that two individuals carry the same QTL allele at a putative QTL position. The existence of LD implies there are small segments of chromosome in the current population which are descended from the same common ancestor. These IBD chromosome segments will not only carry identical marker haplotypes; if there is a QTL somewhere within the chromosome segment, the IBD chromosome segments will also carry identical QTL alleles. Therefore if two animals carry chromosomes which are likely to be IBD at a point of the chromosome carrying a QTL, then their phenotypes will be correlated. We can calculate the probability the 2 chromosomes are IBD at a particular point based on the marker haplotypes and store these probabilities in an IBD matrix (**G**). Then the *v* are distributed $v \sim N(0, G\sigma_{QTL}^2)$, where σ_{QTL}^2 is the QTL variance. If the correlation between the animals is proportional to G there is evidence for a QTL at this position.

2.4.1 Building the IBD matrix from marker haplotypes

Consider a chromosome segment which carries 10 marker loci and a single central QTL locus. Three chromosome segments were selected from the population at random, and were genotyped at the marker loci to give the marker haplotypes

11212Q11211, 22212Q11111 and 11212Q11211, where Q designates the position of the QTL. The probability of being IBD at the QTL position is higher for the first and third chromosome segments than for the first and second or second and third chromosome segments, as the first and third chromosome segments have identical marker alleles for every marker locus.

This type of information can be used, together with information on recombination rate of the chromosome segment and effective population size, for calculating an IBD matrix, **G**, for a putative QTL position from a sample of marker haplotypes. Element G_{ij} of this matrix is the probability that haplotype *i* and haplotype *j* carry the same QTL allele. The dimensions of this matrix is (2 x the number of animals) x (2 x the number of animals), as each animal has two haplotypes.

Meuwissen and Goddard (2001) described a method to calculate the IBD matrix based on deterministic predictions which took into account the number of markers flanking the putative QTL position which are identical by state, the extent of LD in the population based on the expectation under finite population size, and the number of generations ago that the mutation occurred.

Now consider a population of effective population size 100, and a chromosome segment of 10cM with eight markers. Two animals are drawn from this population. Their marker haplotypes are 12222111, 11122111 for the first animal, and 12222111 and 11122211 for the second animal. The putative QTL position is between markers 4 and 5 (ie. in the middle of the haplotype). The **G** matrix could look something like:

			Animal 1		Animal 2	
			Hap 1	Hap 2	Hap 1	Hap 2
			12222111	11122111	12222111	11122211
Animal 1	Hap 1	12222111	1.00			
	Hap 2	11122111	0.30	1.00		
Animal 2	Hap 1	12222111	**0.90**	0.30	1.00	
	Hap 2	11122211	0.20	0.40	0.20	1.00

2.4.2 Estimation of the QTL variance and QTL mapping

To estimate the additive genetic variance, we could calculate the extent of the correlation between animals with high additive genetic relationships A_{ij}. In practise, we fit a linear model which includes additive genetic value (**u**) with $\mathbf{V(u)} = \mathbf{A}\sigma_a^2$, and then estimate σ_a^2. In a similar way, to estimate the QTL variance at a putative QTL position we fit the following linear model:

$$\mathbf{y} = \mathbf{1_n}\mu + \mathbf{Zu} + \mathbf{Wv} + \mathbf{e},$$

where ***W*** is a design matrix relating phenotypic records to QTL alleles, ***v*** is a vector of additive QTL effects, *e* the residual vector, where the random effects *v* are assumed to be distributed as $v \sim (0, \boldsymbol{G}\sigma_{QTL}^2)$. A REML program, such as ASREML (Gilmour et al. 2002), can be used to estimate the QTL variance and the likelihood of the data given the QTL and polygenic parameters.

QTL mapping then proceeds by proposing a putative QTL position at intervals along the chromosome. At each point, the QTL variance is estimated and the likelihood of the data given the QTL and polygenic parameters is calculated. The most likely position of the QTL is the position where this likelihood is a maximum.

The significance of the QTL at it's most likely position can then be tested using a likelihood ratio test by comparing the maximum likelihood of the model with the QTL fitted and without the QTL fitted:

$$LRT = -2(LogLikelihood_{no\_QTL\_fitted} - LogLikelihood_{QTL\_fitted})$$

This test statistic has a χ_1^2 distribution. The QTL is significant at the 5% level if LR > 3.84.

A method to assign confidence intervals to the QTL location is based on the change in LRT values across the chromosome (Lander and Bostein, 1989; Zeng, 1994; Zou, 2001). The confidence interval (CI) is calculated by moving sideward (left and right) of the most likely position to the locations corresponding to a decrease in the LRT score of one or two units. The total width corresponding to a one- or two-LRT drop-off is then considered as the 96.8 or 99.8%CI, respectively (Mangin *et al.,* 1994). In the Lander and Bostein method, estimates of QTL position and its effects are

approximately unbiased if there is only one QTL segregating on a chromosome (Zeng, 1994).

2.5 Comparisons with single markers

While the use of haplotypes seems initially attractive, there are a number of factors which potentially limit there value over single markers. These are:

- The requirement that the genotypes must be sorted into haplotypes. This may not be a trivial task, and is discussed briefly in section 1.7
- The number of effects which must be estimated increases. For a single marker there is one effect to estimate if an additive model is assumed, while for marker haplotypes there are potentially a large number of effects to estimate depending on the number of markers in the haplotype.
- Some simulation results which show benefits of marker haplotypes rely on increasing the density of markers in a given chromosome segment to achieve this. This may not be possible in practise.

Grapes et al. (2004), Grapes et al (2006) and Zhao et al (2007) compared single marker regression, regression on marker haplotypes and the IBD mapping approach for the power and precision of QTL mapping. Grapes et al (2004) and Grapes et al (2006) did this assuming a QTL had already been mapped to a chromosome region, Zhao et al (2007) did this in the context of a genome wide scan for QTL. All three papers compared the approaches using simulated populations. The conclusion from these papers was that single marker regression gives greater power and precision than regression on marker haplotypes, and was comparable to the IBD method. However these results contradict those of Hayes et al. (2007), who found that in real data (9323 SNPs genotyped in Angus cattle) using marker haplotypes would give greater accuracy of predicting QTL alleles than single markers. They also contradict the results of Calus et al (2007), who found that in genomic selection, use of the IBD approach gave greater accuracies of breeding values than using either single marker regression or regression on haplotypes, particularly at low marker densities (discussed further in section 4). The explanation for the contradictory results may be that Zhao et al (2007), Grapes et al. (2004) and Grapes et al. (2004) were simulating a situation where single markers had very high r^2 values with the QTL, in which case using

marker haplotypes would only add noise to the estimation of the QTL effect. Densities of markers required to give these high levels of r^2 may be obtainable in the near future in most livestock species, in which case the single marker approach becomes very attractive. Further work is needed in this area.

2.6 Combined LD-LA mapping

Authors investigating the extent of LD in both cattle and sheep were somewhat surprised/alarmed to find not only was LD highly variable across any particular chromosome, but there was even significant LD between markers which were not even on the same chromosome! (Farnir et al 2002, McRae et al. 2002). These authors (and others) have suggested that LD information be combined with linkage information to filter away any spurious LD likelihood peaks. This type of QTL mapping is referred to as LDLA, for linkage disequilibrium linkage analysis.

2.6.1 IBD matrix for LDLA mapping

The IBD matrix for LDLA mapping will have two parts, a sub-matrix describing IBD coefficients between the haplotypes of founder animals, and a second matrix describing the transmission of QTL alleles from the founders to later generations of genotyped animals.

So for example, if we have a half sib design, we will have two haplotypes per sire, a paternal haplotype for each progeny (the one he or she inherited from dad) and a maternal haplotype from each progeny (the marker alleles the progeny did not get from dad, so must have received from mum). The sire haplotypes and the maternal haplotypes of progeny provide the LD information, and the paternal haplotypes of progeny provide the linkage information. Table 2.2, from Meuwissen et al. (2002), describes the IBD matrix for LDLA for a half-sib design.

The calculation of blocks [a] is described in Meuwissen and Goddard (2001) (and above). The calculation of blocks [b] was described in Meuwissen *et al.* (2002), and are very similar to the standard linkage analysis calculations (eg. Fernando and Grossman 1989). Briefly, element of blocks [b] are $P_{IBD}(X(p);Y) = r \times P_{IBD}(S(p);Y) + (1-r) \times P_{IBD}(S(m);Y)$, where

$P_{IBD}(X(p);Y)$ is the IBD probability of the paternal QTL allele of progeny X, X(p), with any other QTL allele, Y.

S(p) and S(m) are the paternal and maternal alleles of sire S, respectively.
r or (1-r) is the probability that the progeny inherited the paternal or maternal QTL allele of the sire.

Table 2.2. The IBD matrix

	SH	MHP	PHP
SH	**[a]**	**[a]**	**[b]**
MHP	**[a]**	**[a]**	**[b]**
PHP	**[b]**	**[b]**	**[b]**

SH: sire haplotypes; MHP: maternal haplotypes of progeny; PHP: paternal haplotypes of progeny; [a] is calculated by the method of Meuwissen and Goddard (2001); [b] is calculated by the method of Meuwissen *et al.* (2002).

A variance component model similar to the one above can then be fitted.

2.6.2 Example of the twinning QTL

The power of combining LD and LA information to filter both spurious LD and spurious LA likelihood peaks was demonstrated in a study designed to map QTL for twinning rate in Norwegian dairy cattle (Meuwissen et al 2002). Figure 2.6A is the likelihood profile from linkage only, Figure 2.6B the likelihood profile from LD analysis only, and Figure 2.6C the likelihood profile from combined LDLA.

When LDLA is performed, both linkage and linkage disequilibrium information contribute to the likelihood profile. Any peaks due to LD or linkage alone are filtered from the profile. Using LDLA, Meuwissen et al. (2002) were able to map the QTL for twinning rate to a 1cM region.

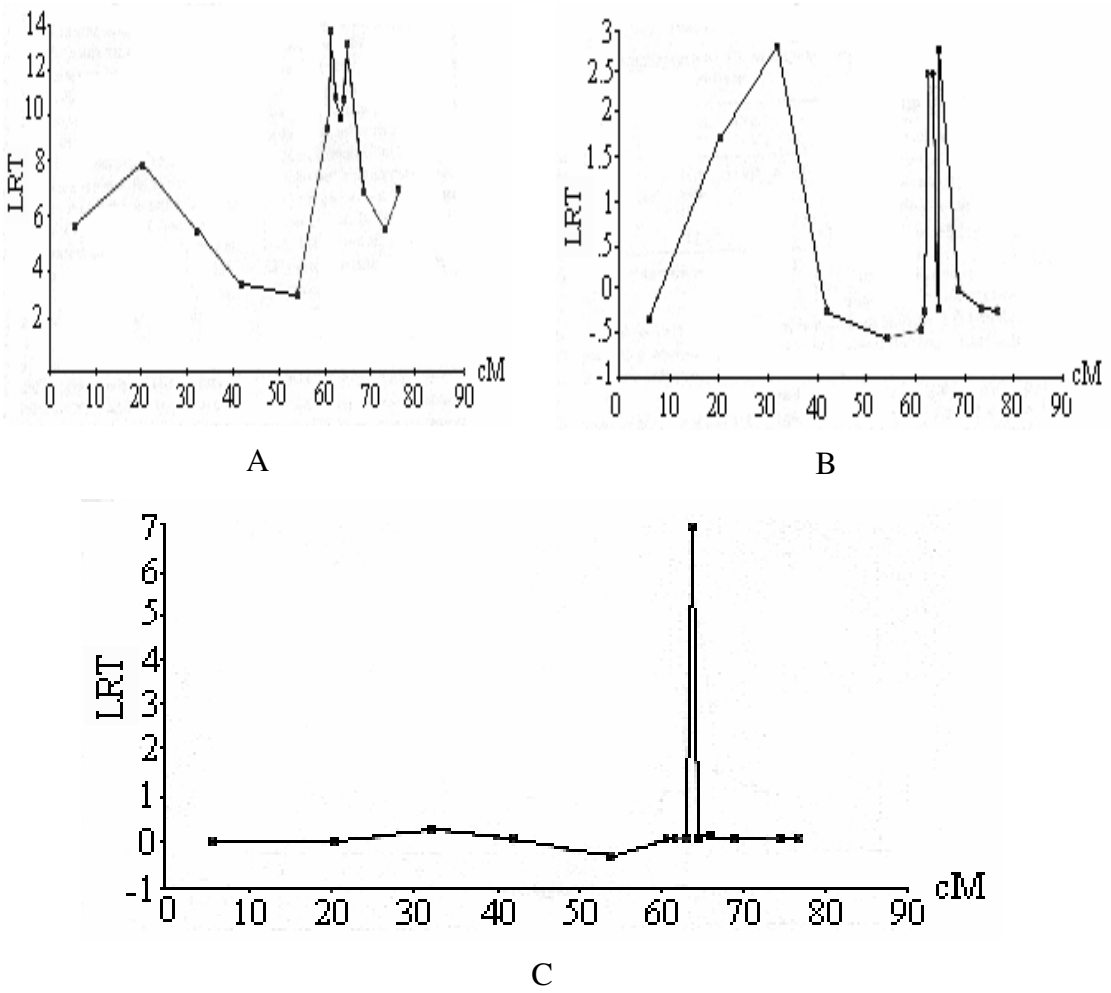

Figure 2.6. Likelihood profile from linkage analysis (A), Linkage disequilibrium analysis (B) and combined linkage disequilibrium linkage analysis (C) of marker data on chromosome 5 and twinning rate phenotypes in Norwegian dairy cattle. Meuwissen et al. (2002). Reproduced with permission from the authors.

2.6.3 Design of LD-LA experiments.

There are two design issues with LD-LA analysis. One is the density of markers required, which has already been discussed. The other is the population structure and size of experiment that is appropriate for LDLA.

An important question is 'are the large half-sib families that are common in linkage analysis also suitable for LDLA analysis'? Large half sib families are of course suitable for linkage studies. LD on the other hand is a population based method (eg. the association between QTL and marker haplotype must persist across the population to be detected). To maximise the LD information, a large number of different

haplotypes must be sampled, and there must be sufficient records per haplotype to estimate the effects of each haplotype accurately. In a half sib design, the total number of founder haplotypes sampled from the population will be the number of dams (the maternal haplotype for each progeny) plus twice the number of sires (two haplotypes per sire). The number of unique haplotypes in this sample will depend on the length of the chromosome segment and the number of markers. If the markers are all in a small interval (say a few cM) the number of unique haplotypes may be small (due to LD), and there will be a considerable number of records per unique haplotype. If on the other hand the markers are widely spaced and cover the whole chromosome, there will be almost as many unique haplotypes as haplotypes sampled. In this situation only the effect of haplotypes carried by the sires are estimated with any accuracy.

Results from a simulation with Ne=100, 1 marker per cM, and varying number of half sib families, show the accuracy of LDLA (in positioning the QTL) is increased slightly by increasing the number of half sib families, Figure 2.7, but not by a great deal (Lee and van der Werf 2004).

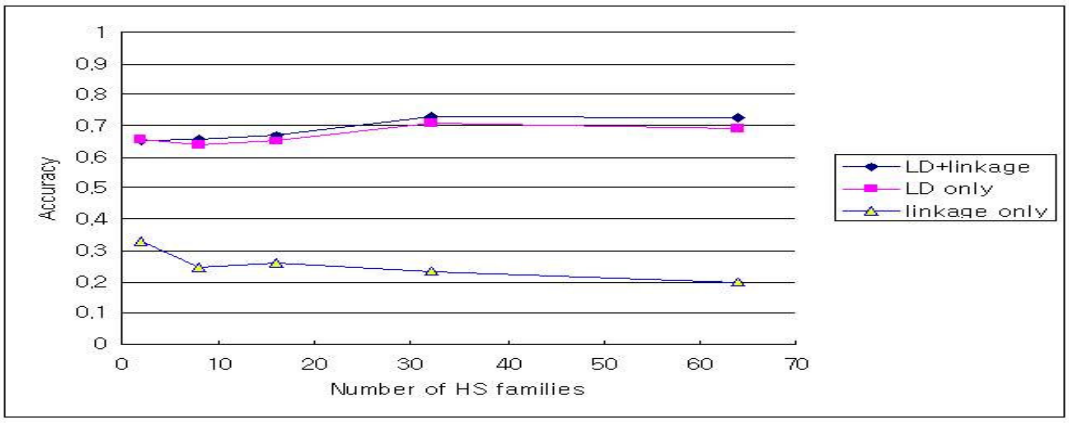

Figure 2.7 Accuracy of positioning a QTL (percentage of replicates positioning QTL in correct 1cM bracket) within a 10cM interval, with an increasing number of half sib families, 128 animals in each design. Linkage, linkage disequilibrium or combined linkage disequilibrium linkage analysis were used to position the QTL (Figure kindly provided by S. Lee).

An interpretation of this result is that the dam haplotypes are providing considerable LD information, in the designs with a small number of sires. The implications are that the designs we currently use for linkage studies should also be suitable for LDLA

studies. Of course, the marker density will have to be greatly increased for the LDLA studies.

This of requires more genotyping. Another good question is can we combine the advantages of LDLA analysis with selective genotyping, to come up with a relatively cheap but powerful experiment? A simulation study was conducted, with Ne=100, 10 markers in a 10cM interval containing a QTL, and either 15 sires mated to 200 dams, 30 sires mated to 100 dams or 60 sires mated to 50 dams each, and 10 progeny per dam (so the total number of progeny in each design was 3000). Selective genotyping was conducted such that 10% of the highest phenotype and 10% of the lowest phenotype progeny were genotyped in each family (600 progeny genotyped total). The results (Table 2.3) indicate some loss of power with selective genotyping, but still a relatively high probability of correctly positioning the QTL within a 3cM bracket.

Table 2.3 Precision of QTL position estimates from LDLA. For each strategy the first number is the proportion of the progeny genotyped (100 or 20, with the progeny with the highest 10% and lowest 10% of phenotypes genotyped within each family). The second number is the number of sires used to breed the resource population (15, 30 or 60). In each design there were 3000 progeny.

	Deviation (in 1cM bracket) of estimated from correct position				
	0	1	2	3	4
100%15	44	31	9	4	5
100%30	46	32	7	3	5
100%60	40	39	8	4	2
20%15	35	36	14	7	1
20%30	32	32	15	8	6
20%60	33	37	11	8	4

Without selective genotyping, there was not a great deal of difference in the accuracy of the three designs. When selectively genotyping was implemented, only 20% of the progeny population, the 15-sire design was most accurate in estimating the QTL position. The 30-sire and 60-sire designs may have lost some linkage information during selective genotyping, resulting in less precise estimation of the QTL position.

This experiment illustrated that accurate positioning of QTL is possible with relatively few genotypings (600 progeny) by combining selective genotyping and LDLA analysis.

3. Marker assisted selection with markers in linkage disequilibrium with QTL

3.1 Introduction

Traditionally selection of animals for breeding is based on two types of data – pedigrees and phenotypes. Best Linear Unbiased Prediction (BLUP) combines these to generate estimated breeding values (EBVs). A third type of data is based on DNA markers.

Marker assisted selection (MAS) can be based on DNA in linkage equilibrium with a quantitative trait locus (QTL) (LE-MAS), molecular markers in linkage disequilibrium with a QTL (LD-MAS), or based on selection of the actual mutation causing the QTL effect (Gene-MAS). All three types of MAS are currently being used in the livestock industries (Dekkers 2004). For example Plastow et al. (2003) report the use of LD-MAS and Gene-MAS for reproduction, feed intake, growth, body composition, meat quality in commercial lines of pigs and national genetic evaluation programs based on LE-MAS are available to dairy breeding organisations in both France (Boichard et al. 2002) and Germany (Bennewitz et al. 2003). LE-MAS is the most difficult to implement. With LE markers, the linkage between the markers and QTL is not sufficiently close to ensure that marker-QTL allele relationships persist across the population (as occurs with LD markers), rather marker-QTL phase within each family must be established before an increase in selection response can be realised.

In this section we will concentrate on LD markers for the following reasons; the optimum use of LE markers has been extensively discussed previously (eg Spelman et al. 1999), LE markers are not readily adopted by industry for the reasons given above, and because, with genome sequencing efforts in a number of species, very large numbers of single nucleotide polymorphism (SNP) markers suitable for LD mapping have recently become available.

In this section, we will describe the application of LD-MAS as a two step procedure. In Step 1, the effects of a marker or set of markers are estimated in a reference

population. In Step 2, the breeding values of a group of selection candidates are calculated using the marker information. In many cases, the selection candidates will have no phenotypic information of their own, eg young dairy bulls which are progeny test candidates. In some cases step 1 and step 2 may actually occur simultaneously, for example when LD-MAS is implemented using the IBD approach.

3.2 Applying LD-MAS with single markers

In section 2.2, a mixed model for estimation of marker effects was described. This results in the estimate of the effect of a marker $\hat{g}$, which is scalar if the marker is biallelelic and an additive model has been assumed, and a vector if otherwise. Then the marker breeding value can be predicted for a group of selection candidates with genotypes but no phenotypes as:

$$\mathbf{MEBV} = \mathbf{X}\hat{\mathbf{g}}$$

Where x is a design matrix allocating marker genotype to marker effects. For example, X_i could be 0 if the SNP genotype of animal i is 11 for SNP1, 1 if the SNP genotype of the animal is 1 2 and 2 if the genotype is 2 2.

In practise, a single marker is unlikely to account for a large proportion of the genotypic variance. This means that the accuracy of the MEBV, the correlation between the MEBV and the unobserved true breeding value, will be low.

There are two ways in which the proportion of the genetic variance captured in the MEBV can be increased. These are including a polygenic effect (the genetic effect not accounted for by the marker) and using multiple markers.

A polygenic effect can be included in the prediction of the **MEBV**:

$$\mathbf{MEBV} = \hat{\mathbf{u}} + \mathbf{X}\hat{\mathbf{g}}$$

Where $\hat{\mathbf{u}}$ is a vector of polygenic effects. These should be calculated simultaneously with the prediction of the marker effects, provided the selection candidates are progeny of animals in the reference population.

If the selection candidates do have their own phenotypes, the procedure to calculate MEBV would happen in single step using the mixed model equations to include all the information and weight this information appropriately.

Consider an example where we wish to calculate MEBV for a group of progeny which are the offspring of a group of phenotyped animals. The data was "simulated" with a mean of 2, a SNP effect of 1 for allele 2 and 0 for allele 1, true polygenic breeding value for animal 1 of 3 and animal 5 0f -3, and true polygenic breeding values for animals 2,3,4,6,7,8,9 and 10 of zero. Errors were randomly distributed with mean 0 and variance 1.

The genotype and phenotype data is:

Animal	Sire	Dam	Phenotpe	SNP allele 1	SNP allele 2
1	0	0	3.53	1	1
2	0	0	3.54	1	2
3	0	0	3.83	1	2
4	0	0	4.87	2	2
5	0	0	1.91	1	2
6	0	0	2.34	1	1
7	0	0	2.65	1	1
8	0	0	3.76	1	2
9	0	0	3.69	1	2
10	0	0	3.69	1	2
11	1	2	-	1	2
12	1	4	-	2	1
13	5	6	-	1	1
14	5	7	-	2	1
15	5	8	-	2	2

We wish to calculate the MEBV for animals 11 to 15. First we must calculate the SNP effect and the polygenic breeding values from the data using BLUP, eg. Fitting the model:

$$\mathbf{y} = \mathbf{1_n}'\mu + \mathbf{Xg} + \mathbf{Zu} + \mathbf{e}$$

To solve for the SNP effect and polygenic effects:

$$\begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n}'\mathbf{1_n} & \mathbf{1_n}'\mathbf{X} & \mathbf{1_n}'\mathbf{Z} \\ \mathbf{X}'\mathbf{1_n} & \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} \\ \mathbf{Z}'\mathbf{1_n} & \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1}\lambda \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n}'\mathbf{y} \\ \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \end{bmatrix}$$

Where $\mathbf{1_n}$ and $\mathbf{X}$ are both of dimensions (number of records x 1):

record	**1n**	**X**
1	1	0
2	1	1
3	1	1
4	1	2
5	1	1
6	1	0
7	1	0
8	1	1
9	1	1
10	1	1

The **Z** matrix allocates records to phenotypes:

		animal														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	1	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	2	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	3	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	4	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	5	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	6	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
record	7	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	8	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	9	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
	10	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**

And the **A** matrix is the matrix of average additive relationships:

		Animal														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	1	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0.5**	**0**	**0**	**0**
	2	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0**
	3	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	4	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0**
	5	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0.5**	**0.5**
	6	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**
animal	7	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0**
	8	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**
	9	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
	10	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
	11	**0.5**	**0.5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.25**	**0**	**0**	**0**
	12	**0.5**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0**	**0**	**0**	**0.25**	**1**	**0**	**0**	**0**
	13	**0**	**0**	**0**	**0**	**0.5**	**0.5**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.25**	**0.25**
	14	**0**	**0**	**0**	**0**	**0.5**	**0**	**0.5**	**0**	**0**	**0**	**0**	**0**	**0.25**	**1**	**0.25**
	15	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0**	**0.25**	**0.25**	**1**

And the $\lambda=1/2$ (error variance divided by the polygenic variance).

Solving the equation above gives:

$\hat{\mu}$		2.69
$\hat{g}$		0.87
$\hat{u}$	1	0.56
	2	-0.01
	3	0.19
	4	0.3
	5	-1.1
	6	-0.23
	7	-0.03
	8	0.14
	9	0.09
	10	0.09
	11	0.28
	12	0.43
	13	-0.67
	14	-0.56
	15	-0.48

Now to calculate MEBV for animals 11-15, we use the formula

$$\mathbf{MEBV} = \hat{\mathbf{u}} + \mathbf{X}\hat{\mathbf{g}}$$

Where X in this case is the matrix allocating SNP genotypes to the 5 animals:

Animal	**X**
11	1
12	1
13	0
14	1
15	2

The MEBV are

Animal	MEBV	TBV
11	1.14	1.75
12	0.99	1.75
13	-0.67	-0.75
14	-0.38	0.25
15	0.12	1.25

The true breeding values (TBV) from the simulation are also given (in this case just the number of 2 alleles at the SNP carried by the animal * the true SNP effect of 1, plus half the effect of the sire). The accuracy of the MEBV can be calculated as the correlation of the MEBV with the TBV. In this very simple example, the accuracy is very high, 0.93.

3.2 Applying MAS with multiple markers

When multiple markers from a genome wide association study used in the prediction of MEBV, we must account for the fact that some of the markers may be detecting the same QTL. If there are a number of markers in linkage disequilibrium with a large QTL, all these markers could have significant effects. There are a number of ways of accounting for this. The simplest is to use multiple regression, fitting all the markers simultaneously, eg fit the model:

$$\mathbf{y} = \mathbf{u} + \sum_{\mathbf{i}}^{\mathbf{p}} \mathbf{X}_{\mathbf{i}} g_{\mathbf{i}} + \mathbf{e}$$

Where *p* is the number of significant markers from the genome wide association study, **X** is a column of the design matrix relating to the i^{th} marker, and g_i is the effect of the i^{th} marker. Before the estimates of g from the multiple regression can be used in LD_MAS, there are two problems which must be overcome. The first is how many markers should be used, and the second is how to account for the over-estimation of the QTL effects?

3.2.1 How many QTL to use?

The advantage of MAS over non-MAS is approximately proportional to the percentage of the genetic variance accounted for by the marked QTL (Meuwissen and Goddard 1996, Spelman *et al*. 1999). The key questions then are how many QTL underlie the variation in quantitative traits, and how many of these QTL are necessary to explain the majority of the genetic variance for a typical quantitative trait. Results from powerful genome scans with thousands of SNP markers, exploiting linkage disequilibrium between the markers and the QTL, shed some light on these questions. In such a scan, as the significance threshold which QTL must exceed in the scan to be 'detected' is reduced, larger numbers of QTL are detected. However, an increasing proportion of these QTL will be false positive results. Additionally, some SNPs will be very close to each other on the genome and will therefore be detecting the same QTL. We can estimate the number of true QTL by correcting the number of significant SNPs for both these factors. A method of doing this is given below.

Let the number of QTL in one chunk of the genome, say 20 Mb long, be x, and assume x is Poisson distributed with mean μ. Then the expectation of x, E(x) is equal

to the variance of x is equal to μ (from the properties of a Poisson distribution). In the genome scan, each QTL is associated with m significant SNPs in one chunk. Let y = the number of SNPs truly associated with the QTL in the chunk, and z = the number of false positive SNPs per chunk. Then E(y)=mμ, Var(y)=m^2μ, E(z)=fmμ/(1-f), where f is the false discovery rate, and assuming z has the same type of distribution as y, Var(z)=m^2μf/(1-f). Then the number of significant SNPs observed per chunk is w =y + z, and E(w) = E(y)+E(z) = mμ/(1-f), and V(w) = V(y)+V(z)=m^2μ (1-f). Solving these equations, we can calculate the number of true QTL per genome chunk, say of 20Mb, as μ=[E(w)]^2(1-f)/V(w).

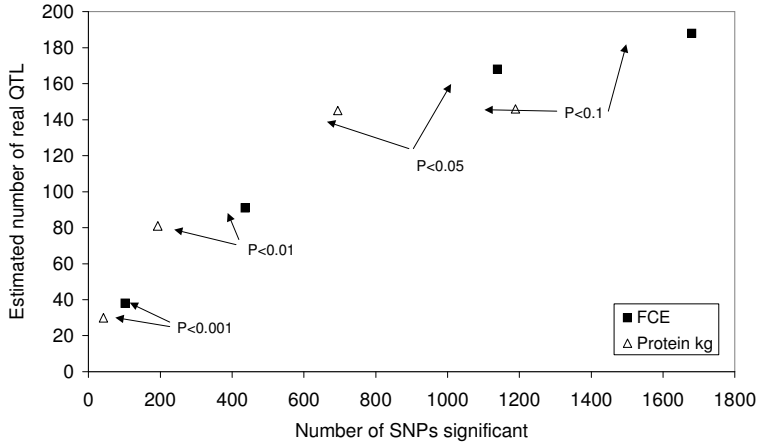

Figure 3.1. Estimated number of real QTL detected at decreasingly stringent significance thresholds in two genome scans based on dense SNP markers.

We have used this formula to calculate the estimated number of QTL from two genome scans (Figure 3.1). One experiment was conducted in beef cattle to detect QTL affecting net feed intake (NFI), and one experiment in dairy cattle to detect QTL affecting milk production traits. In each experiment 384 animals with extreme phenotypes were genotyped for 10 000 SNP markers, using the Parallele™ technology. Distances between SNPs were estimated by mapping the SNPs to the human genome (Goddard *et al*. 2006). The results indicate that as the stringency thresholds are relaxed, more QTL are detected, however the number of QTL appears to plateau at 145 and 188 for NFI and Protein kg respectively. So if we wanted to capture all the genetic variance with marked QTL, we would need markers surrounding between 145 and 188 QTL. However, given the distribution of QTL effects for a typical quantitative trait is likely to be such there are many QTL of small

effect, and few QTL of large effect (Hayes and Goddard 2001, Weller *et al*. 2005), it will only be necessary to consider a fraction of the QTL in MAS, as this fraction of the QTL will explain the majority of the genetic variance. For example, based on a meta-analysis of QTL detection experiments in dairy cattle and pigs, Hayes and Goddard (2001) estimated between 10 and 20% of the largest QTL would explain 50% of the genetic variance for a typical quantitative trait, Figure 3.2. The actual proportion of the genetic variation which should be captured by marked QTL can be determined by cost benefit analysis (Hayes and Goddard 2003). Profitability of exploiting each additional QTL (ordered by size) actually decreases, as the additional QTL explain successively less of the genetic variation but the markers bracketing them cost the same to genotype. With multiple trait breeding goals, more QTL will be needed to explain 50% of the genetic variance in the breeding goal, with the total number of QTL depending on extent of pleiotropy.

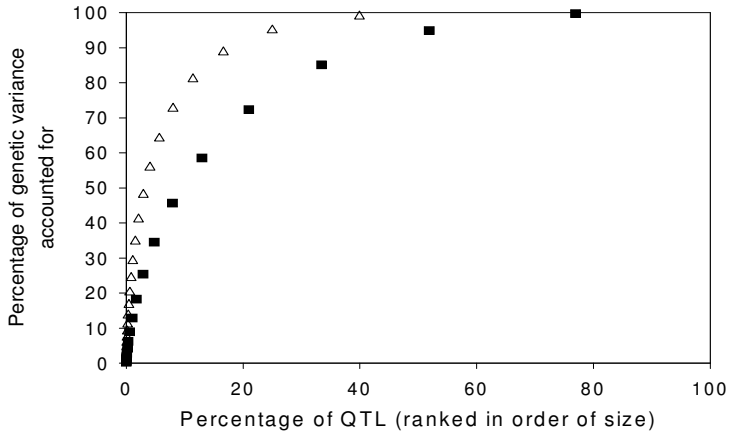

Figure 3.2. Proportion of genetic variance explained by QTL ranked in order of size of effect from a meta-analysis of QTL mapping experiments (■=pigs, and Δ=dairy cattle). Hayes and Goddard (2001).

So if we use 10-20 QTL per trait in our LD-MAS program, we will exploit a maximum of 50% of the genetic variance. This assumes however that we have perfect knowledge of the QTL alleles. This is only the case in Gene-MAS, while the proportion of genetic variance we can capture at each of the QTL in LD-MAS depends on the extent of linkage disequilibrium between the marker and the QTL.

3.2.2 Estimating the vector of marker effects

LD-MAS can be implemented by using a linear model that includes, as well as fixed effects and polygenic breeding values, the effect of each marker or marked-QTL (g). The total breeding value is calculated by adding the effect of marked-QTL and polygenic breeding value as above. If the marked-QTL effects are treated as fixed effects there is a strong tendency to overestimate them, as these effects will only exceed significance thresholds if the estimate is larger than the actual effect due to a large positive error term (Georges *et al*. 1995, Weller *et al*. 2005). This over-estimation is more pronounced in genome scans of low power, as in this case the positive error term must be large to overcome the significance threshold. If the QTL effect is over-estimated, the advantage of MAS can be eroded substantially (eg. Whittaker *et al*. 2000).

The best method to estimate avoid over-estimation of the QTL effects is to estimate their effects in a population which is completely independent of the sample used in the original genome scan where the QTL were first detected. This will also validate that the markers are not an artefact of the statistical model used in the genome scan or some unaccounted for population stratification.

However validation of markers in this way is expensive, as a new population must be genotyped. If it is not possible to estimate marker effects in a validation experiment, we must adjust the estimates of the marker effect from the original genome wide scan. Because such effects are overestimated in the original genome scan. strategies that shrink the estimate of **g** increase the accuracy of MAS. We can shrink the estimate of g according to the amount of data used to estimate g by treating g as a random effect. The less data there is to estimate g, the more the estimate will be shrunk towards the mean.

For example, $\hat{\mathbf{g}} = (\mathbf{X}'\mathbf{X} + \lambda\mathbf{I})^{-1}\mathbf{X}'\mathbf{y}$ will shrink the estimate of the g. In the absence of knowledge of λ (eg arbitrary choice of λ), this is ridge regression (Whittaker *et al*. 2000). Whittaker *et al*. (2000) found that response from MAS was improved by up to 7% when QTL effects were estimated by ridge regression. Alternatively if $\lambda = \sigma_e^2 / \sigma_{QTL}^2$, this is BLUP. To investigate the effect of shrinkage, lets re-visit the effect of the marker we calculated in section 2.2.

The prediction of the mean and effect of the marker is now from the BLUP mixed model equations.

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'X} \\ \mathbf{X'1_n} & \mathbf{X'X + I\lambda} \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{X'y} \end{bmatrix}$$

In the simulation, the QTL had a gene substitution effect of 1. The frequency of the 2 allele (p) in the data set was 0.4. Therefore the variance due to the QTL is $\sigma^2_{QTL} = 2p(1-p)a^2 = 2*0.4*0.6*1 = 0.48$. The environmental variance was 1. This gives us λ=2.08. Solving the equations gives $\hat{g} = 0.81$. This is closer to the true value of g (eg. 1) than the least squares estimate of 1.28 in section 2.2.

However when many, many markers are tested in the genome wide association study, even with BLUP the QTL with the largest variance will tend to have it's variance σ^2_{QTL} overestimated, and this will still decrease the accuracy of MAS.

Better estimates of breeding value can be obtained by methods that treat the QTL variance as sampled from a distribution. Weller *et al*. (2005) suggested a maximum likelihood (ML) method for estimating QTL effects, where least squares estimates were regressed according to a assumed known distribution of QTL effects. In simulations, their ML method estimated QTL effects that had a mean close to the mean simulated effect, while least squares estimates were more than twice the mean simulated effect. Both Meuwissen *et al*. (2001) and Gianola *et al*. (2003) suggested similar approaches, though in a Bayesian framework, where prior distribution of QTL effects was used. Meuwissen *et al*. (2001) demonstrated greatly improved accuracy of prediction of breeding values using haplotype variances estimated by their Bayesian approach compared to breeding values predicted with least squares estimates of QTL effects.

Figure 3.3 illustrates the shrinkage of QTL effects when the QTL effects are estimated using both the data and a prior distribution of QTL effects. The prior distributions in this case were gamma distributions Hayes and Goddard (2001).

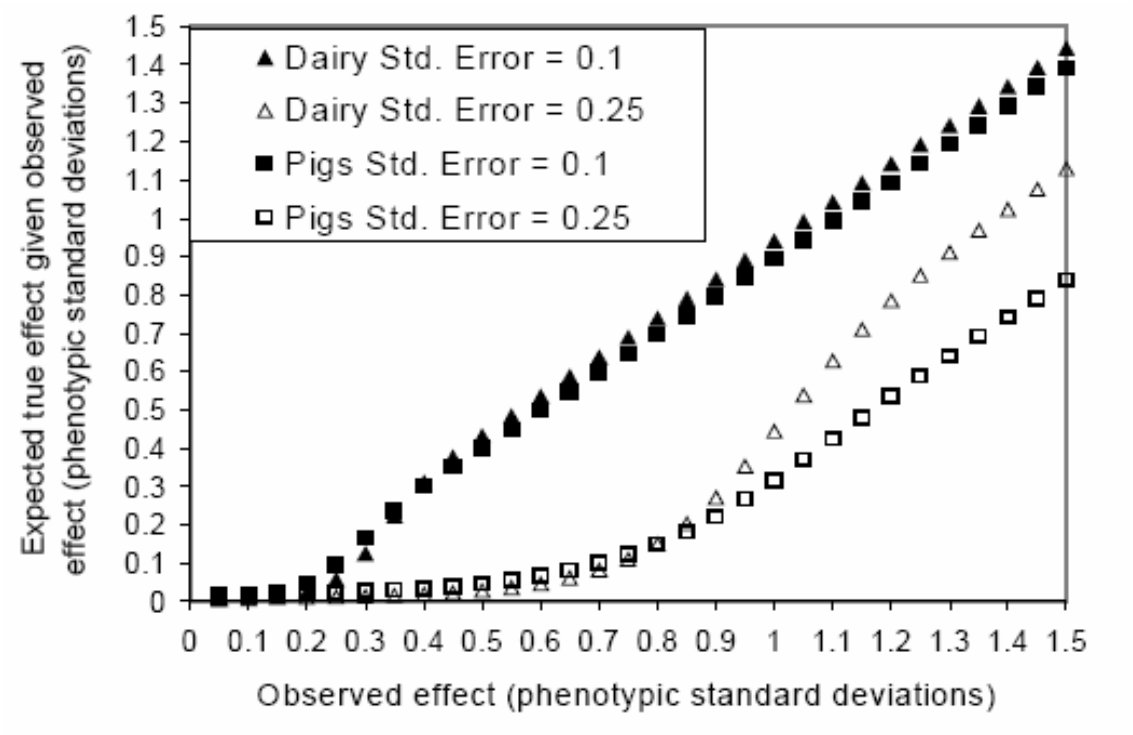

Figure 3.3 Expected true QTL effects given observed effect from a genome scan and the standard error of the QTL from a genome scan. The underlying distribution of QTL effects is assumed to be a gamma distribution, as described by Hayes and Goddard (2001).

When QTL effects are to be estimated from association studies with very large numbers of SNPs, another technique to correct for over-estimation of QTL effects is cross-validation. Suggested by Whittaker *et al* (1997) for calculating the covariance between true and estimated marker effects, cross validation involves splitting the data set in two and comparing estimates of QTL effects to determine the extent of shrinkage that is required to arrive at the "true" value.

If we let x1=the estimate from the first half sample, and x2=the estimate from the second half sample, and xt, the estimate from the full data set=the true value (u)+ error (e), then V(xt)=V(u)+V(e). As $b_{x1,x2}$ (the regression of solutions split 1 on split 2 = V(u)/V(xt), then, following some algebra, we can calculate $b_{u,xt}$, the regression of the true effects of the SNPs on the estimates from the full data set, as $2b_{x1x2}/(1+b_{x1x2})$.

We have attempted to determine the degree of over-estimation of QTL effects from the experiments described above (10,000 SNPs genotyped in 379 beef and 383 dairy

cattle). In each experiment, the data set was randomly split in two, and the effect of each SNP on NFI (beef experiment) and protein kg (dairy experiment) was estimated, correcting for fixed effects and average relationship. The results from both the dairy and beef experiments indicate the regression of solutions from one split of the data on another is about As $b_{x1,x2} = 0.3$. So $b_{u,xt}$ from both the dairy and the beef data will be 0.46, indicating the SNP solutions are over-estimated by a factor of two. Interestingly, this is the same answer to that obtained by Weller *et al* (2005) when using an ML approach to estimate QTL effects, assuming a known distribution of QTL effects.

3.3 Applying LD-MAS with marker haplotypes

An alternative to using single markers in LD-MAS is to use haplotypes of markers. The same models as described above can be used in the reference population to predict the haplotype effects, however the g (single marker effect) are replaced with **g**, a vector of haplotype effects. The dimensions of the **g** are the (number of unique haplotypes observed in the data x 1). The model described in section 2.3 can be used to estimate the haplotype effects. While the problems of over-estimation of haplotype effects when these effects are taken from a genome wide association study remains, treating the haplotypes as random effects would "shrink" the haplotype effect estimates.

The justification for using marker haplotypes in LD-MAS is that the haplotypes could be in greater linkage disequilibrium with the QTL (higher r^2), and therefore explain more of the QTL variance, as was discussed for QTL mapping. The benefit of the increase in proportion of QTL variance explained by marker haplotypes is countered by two "costs". The first is that the haplotypes need to be inferred from the genotype data, as discussed in section 1.8. The second is that when marker haplotypes are used there will generally be more than two haplotypes in the population, so a large number of effects need to be estimated than for single SNP markers.

As an example, Hayes et al. (2007) ccompared the accuracy of MAS using either single markers or marker haplotypes in an Angus cattle data set consisting of 9323 genome wide SNPs genotyped in 379 Angus cattle. The extent of LD in the data set

was such that the average marker-marker r^2 was 0.2 at 200kb. A marker was chosen at random from the 9323 to be a surrogate QTL. The single closest marker, and the 2, 4 and 6 marker haplotypes surrounding the QTL were evaluated for the proportion of QTL variance explained, the number of haplotype effects to be estimated, and the accuracy of predicting the QTL effect. The results are given in Table 3.1 and Figure 3.4.

Table 3.1. Proportion of QTL variance explained by marker haplotypes and observed number of unique haplotypes in the Angus data set .

	Proportion of QTL variance explained	Maximum number of haplotypes	Observed number of haplotypes
Nearest marker	0.10	2	2
Best marker	0.20	2	2
2 Marker haplotypes	0.15	4	3.4
4 Marker haplotypes	0.28	16	9.4
6 Marker haplotypes	0.55	64	20.8

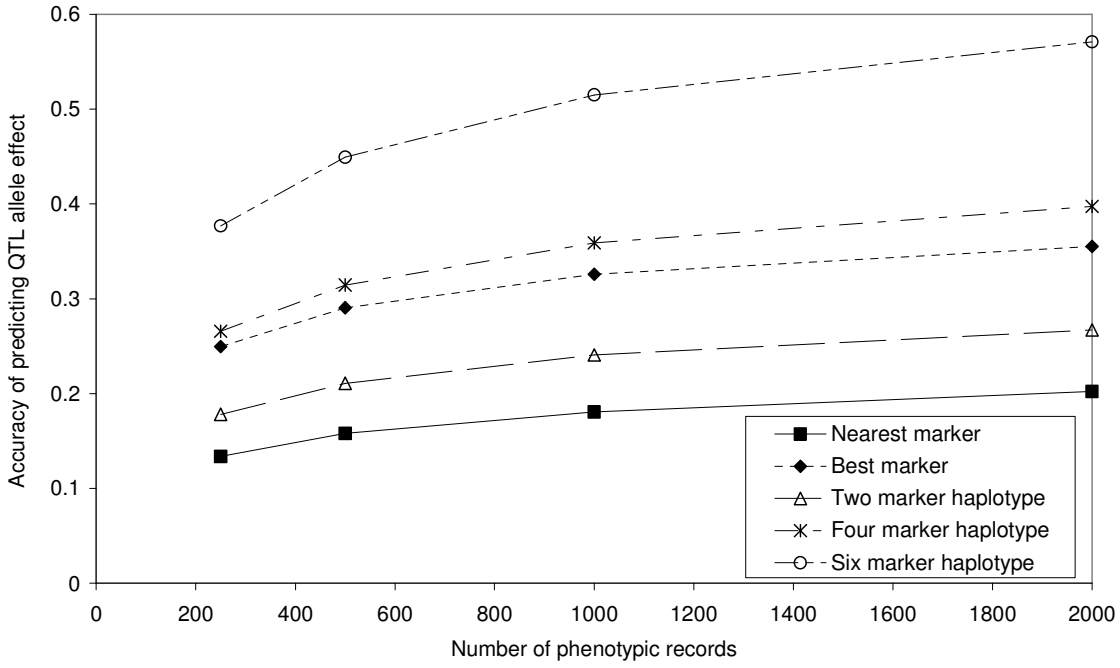

Figure 3.4. Accuracy of predicting QTL effects with an increasing number of markers in the haplotype and an increasing number of phenotypic records.

The accuracy of predicting the QTL effect increased as the number of markers in the haplotype surrounding the QTL increased, although only when the number of markers in the haplotype was 4 or greater did the accuracy exceed that achieved when the SNP in the highest LD with the QTL was used. A large number of phenotypic records (>1000) were required to accurately estimate the effects of the haplotypes.

3.4 Marker assisted selection with the IBD approach.

Another alternative to using single marker regression of fitting haplotype effects is to use the IBD approach discussed in section 2.4. In this approach the selection candidates would be included in the IBD matrix when it is built. If the QTL variance and additive genetic variance have been estimated previously, then **MEBV** can be predicted, including selection candidates with no phenotypic records as:

$$\mathbf{MEBV} = \hat{\mathbf{u}} + \hat{\mathbf{v}}$$

Where $\hat{\mathbf{v}}$ are the estimates of the QTL effects. The BLUP equations are

$$\begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{u}} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'Z} & \mathbf{1_n'W} \\ \mathbf{Z'1_n} & \mathbf{Z'Z} + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z'W} \\ \mathbf{W'1_n} & \mathbf{W'Z} & \mathbf{W'W} + \mathbf{G}^{-1} \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{Z'y} \\ \mathbf{W'y} \end{bmatrix}$$

Where **W** is a design matrix relating phenotypic records to QTL alleles,

The IBD approach for MAS is especially attractive if the IBD matrix is calculated using both linkage and linkage disequilibrium information. Particularly if there are large half sib families, and the density of markers is such that the average r2 between markers and QTL is < 0.2, the accuracy of such **MEBV**s may be substantially higher than using linkage disequilibrium alone.

3.5 Gene assisted selection

The greatest gains are achieved from use of marker information in breeding schemes if the causative mutation underlying the QTL effect is identified. In this case, the following procedure could be used:

1. Pre-correct the phenotypic record of each animal for the effect of the gene allele it carries.
2. Subtract the genetic variance associated with the gene from the additive genetic variance.
3. Solve the standard mixed model equations to predict EBVs.
4. Add to the EBV for each animal the estimated effect of the gene allele it carries.

Of course, the improvement in the rate of genetic gain of Gene-MAS compared with non-Gene-MAS will depend on how accurately the effect of the gene alleles are estimated – over-estimation of these effects will erode the advantage of Gene-MAS.

One factor that considerable complicate the application of Gene-MAS is that once the causative mutation has been discovered, it some cases it could become clear that the effect of the mutation is not strictly additive. For example, with mutations which only have an effect when they are inherited from the father, or mother. The mutation in the IGF2 gene which affects fatness operates in this way (Jeon et al. 1999). In this situation truncation selection (based on ranking the animals on their MEBVs and selecting the required proportion) may not be the optimum use of the QTL information. Special mating schemes to optimally exploit the QTL may be required.

3.6 Optimising the breeding scheme with marker information

The formula for the response to selection is not changed by the availability of molecular data ie , where G =genetic gain, i is the intensity of selection, r is the accuracy of selection, is the genetic standard deviation and L is the generation length. The potential to improve accuracy with molecular data has already been discussed.

The accuracy of traditional EBVs increase as an animal ages and it and its relatives acquire phenotypic data. However, animals can be typed for DNA markers at any age and so the gain in accuracy of EBV due to adding the marker data should be greatest at young ages. Consequently, if selection is optimised, marker data should lead to a decrease in generation length. This decrease might be limited by the minimum age of reproduction. However, if the reduction in generation length is biologically possible,

failure to implement it can reduce the gains from MAS. For instance, in dairy cattle selected for milk production, MAS leads to greater gains if selection of yearling bulls and cows is practiced than if a traditional progeny testing system is adhered to (Spelman et al., 1999). Technology that reduces the minimum age of reproduction increases the benefits from MAS leading to futuristic breeding schemes such as velogenetics (Georges and Massey, 1991) and whizzogenetics (Haley and Visscher, 1998).

3.6.1 Long term versus short term response from MAS

Simulations of MAS find that the extra selection response due to markers declines with multiple generations of selection because the variance at the marked QTL declines as the frequency of the favourable allele increases towards fixation (eg Meuwissen and Goddard 1996, Gibson 1994). Gibson (1994) found that use of markers even reduced long term response below that obtained by selection on phenotype, as a result of reduced selection intensity on the polygenic component of breeding value. However this has not been an inevitable finding in simulation studies (Dekkers 1999). Henshall and Goddard (1997) found that MAS retained an advantage over traditional BLUP selection even in the long term. They based selection on a full BLUP analysis of the phenotypic and marker data rather than selecting on phenotype within QTL genotype. This leads to more accurate estimation of the polygenic BV and thus may help to maintain the advantage of MAS over conventional selection.

Long term selection response may be maximised by giving less weight to the QTL than to the polygenic component of the EBV (Dekkers and van Arendonk, 1998) However an economic optimum also needs to consider short term gains and genotyping costs.

4. Genomic selection

4.1 Introduction to genomic selection

One problem with LE-MAS, LD-MAS or Gene-MAS is that only a limited proportion of the total genetic variance is captured by the markers. An alternative to tracing a limited number of QTL with markers is to trace all the QTL. This can be done by dividing the entire genome up into chromosome segments, for example defined by adjacent markers, and then tracing all the chromosome segments. This method was termed genomic selection by Meuwissen et al. (2001). Genomic selection exploits linkage disequilibrium – the assumption is that the effects of the chromosome segments will be the same across the population because the markers are in LD with the QTL that they bracket. Hence the marker density must be sufficiently high to ensure that all QTL are in LD with a marker or haplotype of markers. Genomic selection has become possible very recently with the availability of 10s of thousands of markers and high throughput genotyping technology.

Implementation of Genomic selection conceptually proceeds in two steps, 1. Estimation of the effects of chromosome segments in a reference population and 2. Prediction of genomic EBVs (GEBVs) for animals not in the reference population, for example selection candidates. This second step is straightfoward: To predict GEBVs for animals with genotypes but no phenotypes. the effect of the chromosome segments they carry can be summed across the genome:

$$\mathbf{GEBV} = \sum_{\mathbf{i}}^{\mathbf{n}} \mathbf{X_i}\, \hat{\mathbf{g}}_\mathbf{i}$$

Where n is the number of chromosome segments across the genome, X_i is a design matrix allocating animals to the haplotype effects at segment i, and $\hat{\mathbf{g}}_\mathbf{i}$ is the vector of effects of the haplotypes within chromosome segment i.

The difficulty in step 1. is that a very large number of haplotype effects across the chromosome segments must be estimated (the $\hat{\mathbf{g}}_\mathbf{i}$), most likely from a data set where the number of phenotypic observations is less than the number of chromosome segment effects to be estimated.

It is important to note that genomic selection has the desirable property that because all chromosome segment effects are estimated simultaneously, the problem of over-estimation of QTL effects due to multiple testing described in section 3.2.2 does not occur.

Genomic selection can proceed using single markers, haplotypes of markers or using an IBD approach. The methodologies that will be described in section 4.2 can be applied to either single markers or haplotypes. The only difference will be in the number of effects to estimate per chromosome segment (ignoring the problems of inferring haplotypes). In the case of single markers, there will be one effect per segment (eg. $\hat{g}_i$ are scalars). In the case of marker haplotypes, there will be multiple effects per segment (eg. $\hat{\mathbf{g}}_\mathbf{i}$ are a vector). We will describe the IBD approach separately.

It is important to note that the following genomic selection procedures can be used to map QTL as well as predict GEBV. Procedures such as the LDLA approach as described yesterday assume one QTL per chromosome. Given the distribution of QTL effects, there are likely to be 100 or more QTL throughout the genome affecting a particular quantitative trait (eg. Hayes et al. 2006). Therefore most chromosomes will carry at least two QTL affecting the trait, though one of these may have a very small effect. Both estimates of effects and position of a QTL can be biased by other QTL on the same chromosome, especially if the QTL are closely linked. The worst case scenario is that two linked QTL cancel each others effects, so none of the QTL are detected. Alternatively, a 'ghost' QTL, with a very large confidence interval, can be positioned between two real QTL (Martinez and Curnow 1992). Because genomic selection approaches can fit all QTL simultaneously, they can remove the effect of the QTL in brackets adjacent to the true QTL position, giving tighter confidence intervals.

4.2 Methodologies for genomic selection

A number of approaches have been proposed for estimating the single marker or haplotype effects across chromosome segment effects for genomic selection. A key

difference between these approaches is the assumption they make about the variances of haplotype or single marker effects across chromosome segments.

The simpler assumption is that the variance of haplotype effects is equal across all chromosome segments. This is analogous to estimating breeding values where we assume that the breeding values are distributed $V(\mathbf{u}) \sim N(0, A\sigma_a^2)$. In the case of the chromosome segment effects, they would be distributed $V(\mathbf{g}) \sim N(0, I\sigma_g^2)$ where σ_g^2 is the variance of the effects across all segments.

However this assumption does not capture our "prior" knowledge that some chromosome segments will contain QTL with large effects, some chromosome segments will contain QTL with small effects, and some chromosome segments will contain no QTL. We can capture this prior knowledge by modelling the data at two levels. The first level is at the level of the data including the overall mean, the error variance and the chromosome segment effects. In this model, each chromosome segment has it's own variance of haplotype or marker effects $V(\mathbf{g}_i) \sim N(0, I\sigma_{gi}^2)$. The second model is at the level of the variance of chromosome segment effects, to allow these to be different for each approach.

We shall consider genomic selection approaches with the simpler assumption of equal variances of effects across chromosome segments first.

4.2.1.1 Least squares

The first approach actually makes no assumptions regarding the distribution of chromosome segment effects, because it treats these effects as fixed in a least squares approach. The approach is identical to that described for LD-MAS. As described by Meuwissen et al. (2001) least squares genomic selection proceeds in two steps.

1. Perform single segment regression analyses for every segment, *i*, using the model

$$\mathbf{y} = \mu\mathbf{1}_n + \mathbf{X}_i\mathbf{g}_i + \mathbf{e}$$

where $\boldsymbol{y}$ is the data vector; μ is the overall mean; $\mathbf{1}_n$ is a vector of n (n=number of records) ones; $\boldsymbol{g}_i$ represents the genetic effects of the haplotypes at the i^{th} 1-cM segment (the vector of values of $\hat{g}_{ij}$ for the different j but at the same i) ; $\boldsymbol{X}_i$ is the design matrix for the i^{th} segment; and $\boldsymbol{e}$ is the error deviation. The dimensions of $\mathbf{g_i}$ will be (number of haplotypes within chromosome segment i x 1), while the dimensions of $\mathbf{X_i}$ will be (number of records x number of haplotypes within chromosome segment i).

- *2*. Select the *m* most significant segments. Estimate the effects of the haplotypes at these positions simultaneously using multiple regression $\mathbf{y} = \mu\mathbf{1_n} + \sum_m \mathbf{X_i g_i} + \mathbf{e}$ where summation Σ_m is over all significant QTL positions. All other haplotype effects are assumed to be zero.

The least squares approach has two major problems. One is the choice of significance level (arguments such as FDR could be used). This can not be too lenient, or else the number of chromosome segment effects to estimate will be larger than the number of phenotypic records, in which case least squares cannot be used. The other is that in the least squares approach, there is a selection of which chromosome segment effects to include in the estimation of breeding values based on the effect of the chromosome segment estimated from single segment regression. As a result, the problem of over-estimation of segment effects due to multiple testing will be incurred.

4.2.2 Ridge regression and BLUP

To overcome the problem of over-estimation of segment effects in the context of marker assisted selection, Whittaker et al. (2000) applied ridge regression. In ridge regression, estimates of the $\mathbf{g_i}$ are shrunk towards the mean, in an attempt to avoid the over-estimation of these effects. This shrinkage can also allow all effects to be estimated simultaneously. In ridge regression, all $\mathbf{g_i}$ have a common variance. Ridge regression can be applied to genomic selection:

$$\hat{\mathbf{g}} = (\mathbf{X}'\mathbf{X} + \lambda\mathbf{I})^{-1}\mathbf{X}'\mathbf{y}$$

where $\mathbf{X}$ is a matrix allocating all marker genotypes or haplotypes to phenotypes, and y is a vector of phenotypes. The difficulty with ridge regression is that the choice of λ is arbitrary. Further, if a very small value λ is chosen, there may not be a unique

solution for the model with the large number of $\mathbf{g_i}$ fitted. Methods for selecting values of λ are given in Xu (2003) and Whittaker et al. (2000). Xu (2003) concluded that ridge regression was not a viable choice for QTL mapping if the model includes markers across the entire genome. There reason was that ridge regression treats all effects equally across all loci, whereas in fact many markers have negligible effects. However ridge regression may still perform reasonably well in the context of estimating genomic breeding values, as the effects are accumulated across many segments.

If $\lambda=\sigma_e^2 / \sigma_g^2$ in the equation for ridge regression, this is in fact BLUP as used by Meuwissen et al. (2001). The BLUP assumes the variance of haplotype effects at each chromosome segment is the same.

An important question is what value of σ^2_g should be used in the BLUP (eg. the variance of haplotype effects at a chromosome segment). Meuwissen et al. (2001) dealt with this problem by calculating the genetic variance expected from a genetic drift-mutation model, and assuming the distribution of QTL effects was as given by Hayes and Goddard (2001). See their paper in the appendix for details.

Another way of estimating σ^2_g would be to first estimate the total additive genetic variance (using REML for example) then divide by the number of chromosome segments.

An example of genomic selection using BLUP follows. Consider the following data set for animals with a single chromosome, with 4 markers defining three chromosome segments. The markers are SNPs, so there are 4 possible haplotypes per segment. Phenotypes were "simulated" with an overall mean of 2, an effect of haplotype 1 in the first segment of 1, an effect of haplotype 1 in the second segment of -0.5, and a normally distributed error term with mean 0 and variance 1. The data is as follows:

	Haplotype segment 1		Haplotype segment 2		Haplotype segment 3		Phenotype
Animal	Paternal	Maternal	Paternal	Maternal	Paternal	Maternal	
1	1	1	2	2	1	1	3.41
2	1	2	1	2	1	1	2.47
3	2	2	1	1	1	2	2.32
4	1	3	2	3	2	1	2.32
5	1	4	1	3	2	1	1.75

Note that there are 9 haplotypes observed in total (4 for the first segment, 3 for the second segment, and 2 for the third segment), while there are only 5 phenotypic records.

The design matrix (**X**) for this data set is (in bold):

	Segment 1 haplotypes				*Segment 2 haplotypes*			*Segment 3 haplotypes*	
Animal	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*1*	*2*
1	**2**	**0**	**0**	**0**	**0**	**2**	**0**	**2**	**0**
2	**1**	**1**	**0**	**0**	**2**	**0**	**0**	**2**	**0**
3	**0**	**2**	**0**	**0**	**0**	**2**	**0**	**1**	**1**
4	**1**	**0**	**1**	**0**	**0**	**1**	**1**	**1**	**1**
5	**1**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**1**

The vector $\mathbf{1_n}$' is [1 1 1 1 1]'

The mixed model equations are

$$\begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'X} \\ \mathbf{X'1_n} & \mathbf{X'X + I\lambda} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\mu}} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{X'y} \end{bmatrix}$$

Where $\lambda = \frac{\sigma_e^2}{\sigma_g^2}$ and ***I*** is an Identity matrix (total number of haplotypes x total number of haplotypes).

Assuming a value of 1 for λ, the mixed model equations with our data are:

$$\begin{bmatrix} 5 & 5 & 3 & 1 & 1 & 3 & 5 & 2 & 7 & 3 \\ 5 & 8 & 1 & 1 & 1 & 3 & 5 & 2 & 8 & 2 \\ 3 & 1 & 6 & 0 & 0 & 2 & 4 & 0 & 4 & 2 \\ 1 & 1 & 0 & 2 & 0 & 0 & 1 & 1 & 1 & 1 \\ 1 & 1 & 0 & 0 & 2 & 1 & 0 & 1 & 1 & 1 \\ 3 & 3 & 2 & 0 & 1 & 6 & 0 & 1 & 5 & 1 \\ 5 & 5 & 4 & 1 & 0 & 0 & 10 & 1 & 7 & 3 \\ 2 & 2 & 0 & 1 & 1 & 1 & 1 & 3 & 2 & 2 \\ 7 & 8 & 4 & 1 & 1 & 5 & 7 & 2 & 12 & 3 \\ 3 & 2 & 2 & 1 & 1 & 1 & 3 & 2 & 3 & 4 \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} 12.28 \\ 13.37 \\ 7.12 \\ 2.32 \\ 1.75 \\ 6.7 \\ 13.79 \\ 4.07 \\ 18.17 \\ 6.39 \end{bmatrix}$$

Giving the following estimates of the mean and Haplotype effects:

Effect		Estimate
Mean		2.05
Segment 1	Haplotype 1	0.2
	Haplotype 2	-0.06
	Haplotype 3	0
	Haplotype 4	-0.14
Segment 2	Haplotype 1	-0.07
	Haplotype 2	0.21
	Haplotype 3	-0.14
Segment 3	Haplotype 1	0.19
	Haplotype 2	-0.19

With so few records, the accuracy of estimating the haplotype effects is low.

Now if we genotype a group of young animals we can estimate their GEBV from the haplotypes they carry:

$$\mathbf{GEBV} = \mathbf{X}\hat{\mathbf{g}}$$

Consider the following animals:

	Haplotype segment 1		Haplotype segment 2		Haplotype segment 3	
Animal	Paternal	Maternal	Paternal	Maternal	Paternal	Maternal
6	1	2	1	2	1	1
7	1	1	2	2	1	2
8	2	3	2	2	1	2
9	1	4	3	1	1	2
10	2	4	2	2	1	2

The **X** matrix for the new animals is:

	Segment 1 haplotypes				*Segment 2 haplotypes*			*Segment 3 haplotypes*	
Animal	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*1*	*2*
6	1	1	0	0	1	1	0	2	0
7	2	0	0	0	0	2	0	1	1
8	0	1	1	0	0	2	0	1	1
9	1	0	0	1	1	0	1	1	1
10	0	1	0	1	0	2	0	1	1

Using the values of $\hat{\mu}$ and $\hat{\mathbf{g}}$ from above gives the following vector of GEBV

Animal	**GEBV**	**TBV**
6	2.72	0.5
7	2.88	2
8	2.42	0
9	1.9	0.5
10	2.28	0

As the data was simulated, we also have a true breeding value (TBV) for these animals (the sum of the true haplotype effects described above). We can correlate the GEBV and TBV to get the accuracy of genomic selection in this case, which is 0.58 in this case.

With BLUP the chromosome segment (or QTL) with the largest variance will tend to have it's variance over-estimated, and this will still decrease the accuracy of genomic selection somewhat although much less than when the **g** are treated as a fixed effect. Better estimates of breeding value can be obtained by methods that allow the variance of the chromosome segment effects to vary between chromosome segments.

4.2.4 Bayesian methods

If we adopt a Bayesian approach, we can capture our prior knowledge that there are some chromosome segments containing QTL of large effects, some segments with moderate to small effects, and some segments with no QTL at all when we estimate the effects of haplotypes (or single markers) within the chromosome segments.

4.2.4.1 Optional topic: Bayesian statistics refresher

Bayes theorem uses a simple rule about conditional probabilities

$$P(x \mid y) = P(xandy) / P(y) = P(y \mid x)P(x) / P(y)$$

This can be understood with an example. Suppose I have a jar of coins in which 99% are fair coins and 1% are double headed coins. I take a coin at random and toss it three times and observe three heads. What is the probability the coin is a double headed coin? Let y = the data, eg. 3 heads from 3 tosses, x is this is a double headed coin, x' this is a fair coin. Then P(x)=0.01,P(x')=0.99, P(y|x)=1.0 and P(y|x') =0.125 (eg. 0.5^3). Then the outcomes of the experiment can be represented in a table:

	P(x or x')	P(y|x or x')	P(y|x)*P(x)
Fair coin	0.99	0.125	0.124
Double headed coin	0.01	1.0	0.01
P(y)			0.134

Therefore the probability that this is a double headed coin given I observed three heads from three tosses is $P(x \mid y) = P(y \mid x)P(x) / P(y)$ =1.0*0.01/0.134 = 0.075. That is despite the outcome of three heads there is only a small probability of the coin being double headed because doubled headed coins are so rare.

Bayes theorem is useful because often it is easy to calculate P(y|x), while it is more difficult to calculate P(x|y), as in the above example.

After the experiment has been done, the P(y) will be a constant in all calculations we do. So we can also write Bayes theorem as

$$P(x \mid y) \propto P(y \mid x)P(x)$$

Where the symbol $\propto$ indicates is proportional to. This is useful because the calculation of P(y) may be difficult.

The probability P(x|y) is called the posterior probability because it is the probability after the experiment has been done. It is calculated from two terms. P(y|x) is the likelihood used by frequentists. P(x) is called the prior probability because it is the probability of x before the experiment was conducted. This allows us to incorporate prior knowledge into the estimate of x.

In practise, calculating the posterior distribution (and integrating out nuisance parameters) may be difficult to do. Often it is impossible to find a formula that gives the solution. Bayesians have developed a number of approaches to overcome this problem.

- Choose priors that make the algebra easy. So called conjugate prior distributions have the property that, when combined with a particular distribution for the data, they yield a recognised distribution for the posterior. For instance if the data are normally distributed, and a normal prior is used for a parameter affecting the data, then the posterior distribution of that parameter will be normally distributed.
- Numerical integration. If you can calculate the height of the posterior distribution at every point, you can integrate it over nascence parameters using numerical integration such as Simpsons rule.
- Simulation. If you can draw samples from the posterior distribution, you can use the samples to approximate the distribution. For example the mean of many samples is a good approximation to the mean of the distribution. This is what Markov Chain Monte Carlo (MCMC) methods such as Gibbs sampling do.

4.2.4.2 Bayesian method with a prior that assumes many QTL have a small effect and few have a large effect

If we allow the variance of the effects across chromosome segments to vary, then the variances $V(\mathbf{g_i}) = \sigma^2_{gi}$ must be estimated. Meuwissen et al. (2001) described a

Bayesian method they termed Bayes Method A to estimate chromosome segments effects and their variances simultaneously.

The method modelled the data at two levels. The first is at the level of the data as above:

$$\mathbf{y} = \mu\mathbf{1}_n + \mathbf{X}_i\mathbf{g}_i + \mathbf{e}$$

The prior distribution of the error variance σ^2_e was $\chi^{-2}(-2, 0)$, which yields an uninformative prior (eg the prior receives little or no weight in the calculation). The prior distribution of the mean μ was uniform and uninformative, while the prior distribution of haplotype effects within chromosome segment *i* was $\mathbf{g}_i \sim N(0, \sigma^2_{gi})$. Note that this is equal to BLUP estimation of the chromosome segment effects with different variances for each segment.

The second level of model is at the variances of chromosome segment effects. In Meuwissen et al (2001), the prior distribution of the variances of effects across chromosome segments was consistent with many QTL of small effect and few of large effect. The prior distribution was the scaled inverted chi-square distribution, $\Pr ior(\sigma^2_{gi}) \sim \chi^{-2}(v, S)$, where *S* is a scale parameter and v is the number of degrees of freedom. The values of *v* and *S* were chosen as v=4.012 and S =0.002. These values were chosen to give a distribution similar to what would be expected from the distribution of QTL effects derived by Hayes and Goddard (2001) and the expected heterozygosity of QTL under the neutral model (see Appendix for details).

The posterior distribution of σ^2_{gi} combines information from the prior and the data. Information from the data is included by conditioning on the chromosome segment effects, eg. $P(\sigma^2_{gi} \mid \mathbf{g}_i)$. An advantage of using an inverted chi-square distribution as a prior for the variances is that with normally distributed data, the posterior is also inverted chi-squared. In fact if the prior for our chromosome segment variances has the scale parameter *S*, and degrees of freedom *v*, then the posterior for σ^2_{gi} given the chromosome segment effects, $P(\sigma^2_{gi} \mid \mathbf{g}_i)$ is an inverted chi-squared scaled by $S+\mathbf{g}_i'\mathbf{g}_i$ and $v+n_i$ degrees of freedom:

$$P(\sigma^2_{gi} \mid \mathbf{g_i}) = \chi^{-2}(v + n_i, S + \mathbf{g_i}'\mathbf{g_i})$$

where n_i is the number of haplotype effects at segment *i*.

We cannot use this posterior distribution directly for estimating the σ^2_{gi} because it is conditional on the unknown $\boldsymbol{g_i}$ effects. Meuwissen et al. (2001) therefore used Gibbs sampling to estimate effects and variances.

The Gibbs chain could proceed as follows:

Step 1. Initialise the vectors of haplotype effects for each vector of chromosome segment effects $\mathbf{g_i}$ for j=1,n_i where n_i is the number of haplotypes at the chromosome segment, with a small positive number. The overall mean μ must also be initialised.

Step 2. Update the σ^2_{gi} for the i^{th} chromosome segment by sampling it from the fully conditional distribution $\chi^{-2}(v + n_i, S + \mathbf{g_i}'\mathbf{g_i})$, where v is 4.012 and S is 0.002, and n_i is the number of haplotype effects at the i^{th} chromosome segment.

Step 3. Given the $\mathbf{g_i}$ and μ calculate the values for $\boldsymbol{e}$ as $\mathbf{e} = \mathbf{y} - \mathbf{Xg} - 1_n'\mu$, where $\mathbf{X} = [\mathbf{X_1}\ \mathbf{X_2}\ \mathbf{X_3}\ ...]$ is the design matrix of all haplotype effects; and $\boldsymbol{g}$ is a vector of all haplotype effects across chromosome segments. Then update the error variance, σ^2_e by drawing a single sample from $\chi^{-2}(n-2, \mathbf{e_i}'\mathbf{e_i})$

Step 4. Sample the overall mean μ given the updated error variance from a normal distribution with mean $\frac{1}{n}\left(\mathbf{1_n}'\mathbf{y} - \mathbf{1_n}'\mathbf{Xg}\right)$ and variance σ^2_e / n, where $\mathbf{X} = [\mathbf{X_1}\ \mathbf{X_2}\ \mathbf{X_3}\ ...]$ is the design matrix of all haplotype effects; and $\boldsymbol{g}$ is a vector of all haplotype effects.

Step 5. Sample all the haplotype effects g_{ij} given the newly sampled μ, σ^2_e and σ^2_{gi} from a normal distribution with mean $\frac{\mathbf{X_{ij}}'\mathbf{y} - \mathbf{X_{ij}}'\mathbf{Xg_{(ij=0)}} - \mathbf{X_{ij}}'\mathbf{1_n}\mu}{\mathbf{X_{ij}}'\mathbf{X_{ij}} + \sigma^2_e / \sigma^2_i}$, where $\boldsymbol{X_{ij}}$ is column of $\boldsymbol{X}$ of effect g_{ij}; $\mathbf{g_{(ij=0)}}$ equals $\boldsymbol{g}$ except that the effect of g_{ij} is set to zero, and variance $\sigma^2_e / (\mathbf{X_{ij}}'\mathbf{X_{ij}} + \sigma^2_e / \sigma^2_{gi})$.

Step 6. Repeat Step 2 (using the updated $\mathbf{g_i}$) to Step 5 for a large number of cycles.

Other authors have published similar methods but with different priors used for the variance of chromosome segment effects**.** In Xu (2003) this was $1/\chi_1^2$ (eg. an inverted chi-square distribution with 1 degree of freedom). Xu (2003) also described their method for single SNP markers, rather than marker haplotypes. Therefore the matricies $\mathbf{X_i}$ are the design matricies for the effect of a single marker, so X_{ij} =1 if the i^{th} SNP genotype for individual *j* is a_1a_1, X_{ij}=0 if the i^{th} SNP genotype for individual *j* is a_1a_2, and X_{ij}=-1 if the i^{th} SNP genotype for individual *j* is a_2a_2. The implicit assumption in Xu (2003) is that the partial regression coefficient, g_i, (the effect of marker i on the trait), will absorb partly the effects of all QTL located between markers i-1 and i+1. The validity of this assumption will depend on the LD between the markers and the QTL.

Ter Braak et al. (2005) argued that prior used by Xu (2003) would result in an improper posterior distribution, in particular a posterior of g_i with infinite mass near zero. To ensure a valid posterior, they altered the prior distribution of variance of chromosome segment effects to be $1/\chi^2_{0.998}$.

Xu (2003) actually proposed their method for QTL mapping rather than genomic selection, claiming that the method gave more precise estimates of QTL location than single QTL models. This was because the effect of a QTL was removed in adjacent marker brackets so the QTL were mapped to a smaller interval. The approach also gave more accurate estimates of QTL effect, as the problem of over-estimating the QTL effect due to multiple testing were avoided. Xu (2003) describe applications for plant populations for QTL mapping such as backcross, double haploid, or F2.

Meuwissen et al. (2001) pointed out that in reality, the distribution of genetic variances across chromosome segments is that there are many chromosome segments which contain no QTL, and relatively few chromosome segments which do contain QTL. However, the prior density of method BayesA does not actually reflect this, the prior does not have a density peak at $\sigma^2_{gi} = 0$; in fact its probability of $\sigma^2_{gi} = 0$ is infinitesimal. Meuwissen et al. (2001) addressed this in their Method BayesB.

Method BayesB used a prior that has a high density, π, at $\sigma^2_{gi} = 0$ and has an inverted chi-square distribution for $\sigma^2_{gi} > 0$; . The prior distribution was

$$\sigma^2_{gi} = 0 \text{ with probability } \pi,$$
$$\sigma^2_{gi} \sim \chi^{-2}(\nu, S) \text{ with probability } (1-\pi),$$

where $\nu = 4.234$ and S = 0.0429 yield the mean and variance of σ^2_{gi} given that $\sigma^2_{gi} > 0$ (see Appendix for derivation of *v* and *S* values).

Figure 4.1 Illustrates the difference between the prior distribution of variances of chromosome segment effects used in method Bayes B and that used in method BayesA.

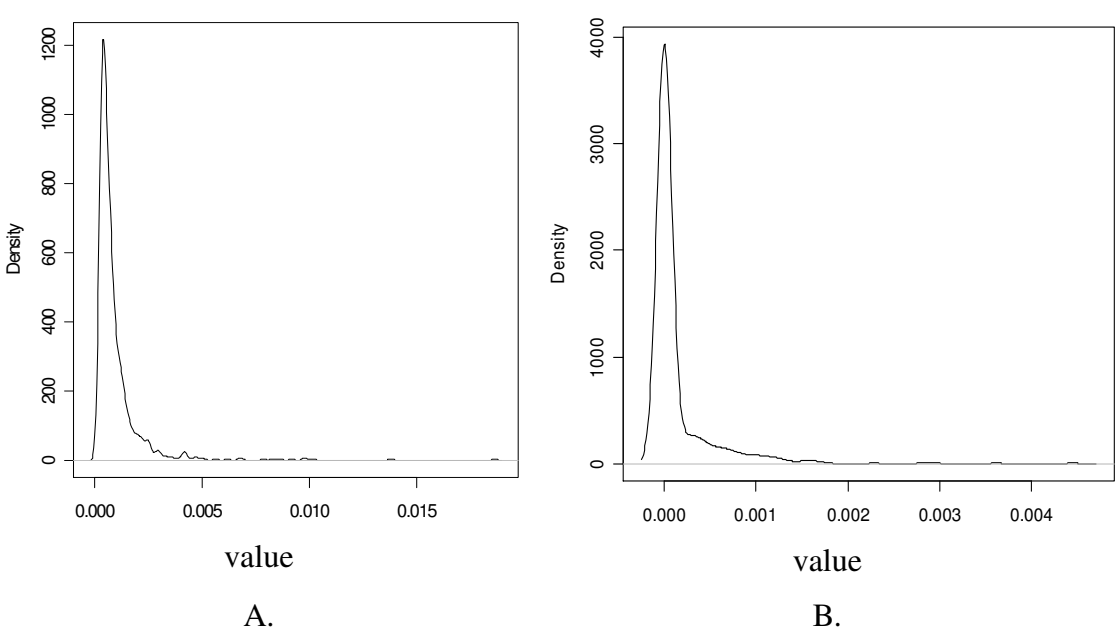

Figure 4.1 A. Prior distribution of variances of chromosome segment effects used in method BayesA, and B. Prior distribution of variances of chromosome segment effects used in method BayesB in Meuwissen et al. (2001), for 20% of chromosome segments containing QTL.

The figure illustrates the infinitesimal density of the prior used in BayesA at 0, and the much higher mass near (and actually at) zero for the prior used in BayesB. The Gibbs sampler described in Method BayesA cannot be used in method BayesB, as it will not move through entire sampling space. This is because the sampling of $\sigma^2_{gi} = 0$

from the posterior distribution of Var(of σ^2_{gi}) is not possible if $\boldsymbol{g}'_i\boldsymbol{g}_i > 0$, which it will never be as $\mathbf{g_i} = 0$ has an infinitesimal probability if $\sigma^2_{gi} > 0$. This problem was resolved by sampling σ^2_{gi} and $\boldsymbol{g_i}$ simultaneously using a Metropolis-Hastings algorithm (see Appendix for details).

4.2.5 Evaluation of accuracy of genomic selection methods

To evaluate their methods (least squares, BLUP, Bayes A and Bayes B), a genome of 1000 cM was simulated with a marker spacing of 1 cM. The markers surrounding every 1-cM region were combined into marker haplotypes. Due to finite population size ($N_e = 100$), the marker haplotypes were in linkage disequilibrium with the QTL located between the markers. The effects of the chromosome segments were predicted in one generation of 2000 animals, and the breeding values for the progeny of these animals were predicted based only on the markers which they carried, Table 4.1.

Table 4.1. Comparing estimated *vs.* true breeding values in progeny with no phenotypic records (from Meuwissen et al. (2001). Chromosome segments were estimated in a population of 2000 animals.

	$r_{TBV;EBV}$ + SE	$b_{TBV.EBV}$ + SE
LS	0.318 ± 0.018	0.285 ± 0.024
BLUP	0.732 ± 0.030	0.896 ± 0.045
BayesA	0.798	0.827
BayesB	0.848 + 0.012	0.946 + 0.018

Mean of five replicated simulations LS, least squares; BLUP, best linear unbiased prediction; Bayes, Bayesian method with inverse chi-square prior distribution and where the prior density of having zero QTL effects was increased; $r_{TBV;EBV}$, correlation between estimated and true breeding values (equals accuracy of selection); $b_{TBV;EBV}$, regression of true on estimated breeding value.

The least squares method does very poorly, primarily because the haplotype effects are over-estimated. The increased accuracy of the Bayesian approach occurs because this method sets many of the effects of the chromosome segments to close to zero in BayesA or zero in BayesB, and "shrinks" the estimates of effects of other chromosome segments based on a prior distribution of QTL effects.

4.2.6 An IBD approach to genomic selection

In the above models, the covariance between haplotype effects for haplotypes within a chromosome segment are assumed to be zero, eg. $\mathbf{g_i} \sim N(0, \mathbf{I}\sigma^2_{gi})$. The assumption is that each haplotype carries a unique QTL allele. In reality, it is possible that different haplotypes may carry the same QTL allele. We can model this by setting the covariance between two haplotypes to the probability that at a putative QTL position, the two haplotypes are identical by descent, and therefore carry the same QTL allele. This approach can be used in Genomic selection if we consider many putative QTL positions across the genome simultaneously (DeRoos et al. 2007). In fact Meuwissen and Goddard (2004) described this approach considering one chromosome at a time, but their method could include all chromosomes at once. De Roos et al. (2007) used this approach to predict GEBV for fat % of progeny tested bulls, and reported an accuracy of GEBV of 0.75.

4.2.7 Optional topic 1: Genomic selection with LDLA

Meuwissen and Goddard (2004) describe an approach to mapping multiple QTL with multi-trait data, incorporating the IBD matrix for LDLA. They make a key simplification which make their method tractable. The first is that correlations among QTL effects at a single gene are either +1 or –1, ie the QTL either increases both traits, or it increases one trait and decreases the other. This assumption is strictly valid only if there are two alleles segregating at the QTL. They also assumed there was one QTL per marker bracket, and considered only one putative QTL per bracket.

The multi-trait multi-QTL model, for the vector of *m* phenotypic records of animal *i* is:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b} + \mathbf{u}_i + \sum_j \left(q_{ij1} + q_{ij2}\right)\mathbf{v}_j + \mathbf{e}_i$$

where y_i is a *m*x*1* vector of phenotypic records for animal *i*; $X_i b$ is a *m*x*1* vector of non genetic fixed effects corrections for the traits of animal *i*; u_i=(*m*x*1*) vector of the effects of background genes (polygenic effect); e_i is a *m*x*1* vector of environmental effects on each of the traits, Σj denotes summation over all putative QTL positions on the chromosome (or genome for genomic selection); v_j is a *m*x*1* vector of the direction of the effects of the QTL alleles on different traits at position *j*; and q_{ij1} (q_{ij2}) is the size of the QTL effect for the paternal (maternal) allele of animal i at position j along the direction v_j. This v_j is the same for all animals at QTL position *j*. For example, if $(q_{ij1} + q_{ij2}) = 2$ and v_j =[1 2]' this gives a genotypic effect of 2 and 4 for traits 1 and 2 respectively.

At each marker bracket, there was an indicator variable I_j, which was one if there was a QTL in the bracket and zero if there was no QTL in the bracket.

What is needed is the posterior probability density of the parameters **b, u,v,q, I** and **R** (the covariance between the errors of the traits) . Using Bayes theorem:

$$p(\mathbf{b,u,v,q...,I,R} \mid \mathbf{y,A,H}) \propto \prod_i \left[p(y_i \mid \mathbf{b}, u_i, \mathbf{v}, q_i ...., \mathbf{I,R})\right] p(\mathbf{b,v,u,q...,v,R})$$

The **H** here is the IBD matrix described for LDLA in section 2.6.1 and **A** is the average relationship matrix. $\prod_i \left[p(y_i \mid \mathbf{b}, u_i, \mathbf{v}, q_i ...., \mathbf{I,R})\right]$ is the likelihood of the data and $p(\mathbf{b,v,u,q...,v,R})$ are the prior distributions of these parameters.

Meuwissen and Goddard (2004) describe a Gibbs sampling scheme for sampling from the joint posterior distribution $\prod_i \left[p(y_i \mid \mathbf{b}, u_i, \mathbf{v}, q_i ...., \mathbf{I,R})\right] p(\mathbf{b,v,u,q...,v,R})$.

De Roos et al. (2007) in real data and Calus et al. (2007) in simulated data demonstrated that using the method of Meuwissen and Goddard (2004) can lead to high accuracies of GEBV.

4.3 Factors affecting the accuracy of genomic selection

While the simulations demonstrate genomic selection has huge potential to increase rates of genetic gain, several key questions remain regarding it's implementation. These are

1) How many markers are required, determined by the extent of LD.
2) How many phenotypic records are required in the initial experiment estimating the effect of chromosome segments
3) How do non-additive effects affect the accuracy of genomic selection.

4.3.1 Extent of linkage disequilibrium and number of markers required

The arguments here are similar to those given in chapter 3 for the number of markers required for LD-MAS. For genomic selection to work, the haplotypes or single markers must be in sufficient LD with the QTL such that the haplotype or single markers will predict the effects of the QTL across the population. For genomic selection to be as successful as in the simulations of Meuwissen et al. (2001), the level of LD between adjacent markers should be r^2>=0.2, as this was the level of LD there simulations generated. Solberg et al. (2006) used simulation of a population with N_e 100 to assess the effect of marker spacing on the accuracy of genomic selection (with BayesMethodB). They found a drop in accuracy of 20% as marker spacing was increased from one marker every 0.5cM to one marker every 4cM. Calus et al. (2007) used simulation to assess the effect of the average r^2 between adjacent marker pairs on the accuracy of genomic selection (where the accuracy was the correlation of true breeding values and GEBV for a group of un-phenotyped animals). They found that accuracy increased dramatically as the average r^2 between adjacent markers increased, from 0.68 when the average r^2 between adjacent markers was 0.1, to 0.82 when the average r^2 between adjacent markers was 0.2, Figure 4.2.

In dairy cattle populations, an average r^2 of 0.2 between adjacent markers is only achieved when markers are spaced every 100kb. As the bovine genome is approximately 3 000 000kb, this implies that in order of 30 000 markers are required for genomic selection to be successful!

4.3.2 Haplotypes or single markers

Closely related to the effect of the extent of linkage disequilibrium on the accuracy of genomic selection is the effect of using single markers rather than haplotypes. The advantage of haplotypes over single markers in genomic selection is dependent on how accurately the haplotypes identify identical by descent chromosome segments compared to the accuracy with which single markers identify identical by descent chromosome segments. This can be quantified as the proportion of QTL variance which is explained by the haplotype effects compared to the proportion of QTL variance which is explained by single marker effects, as discussed in section 2.3. Calus et al. (2007) compared the accuracy of GEBV for progeny without phenotypic records from genomic selection using single markers or marker haplotypes, in simulated data.

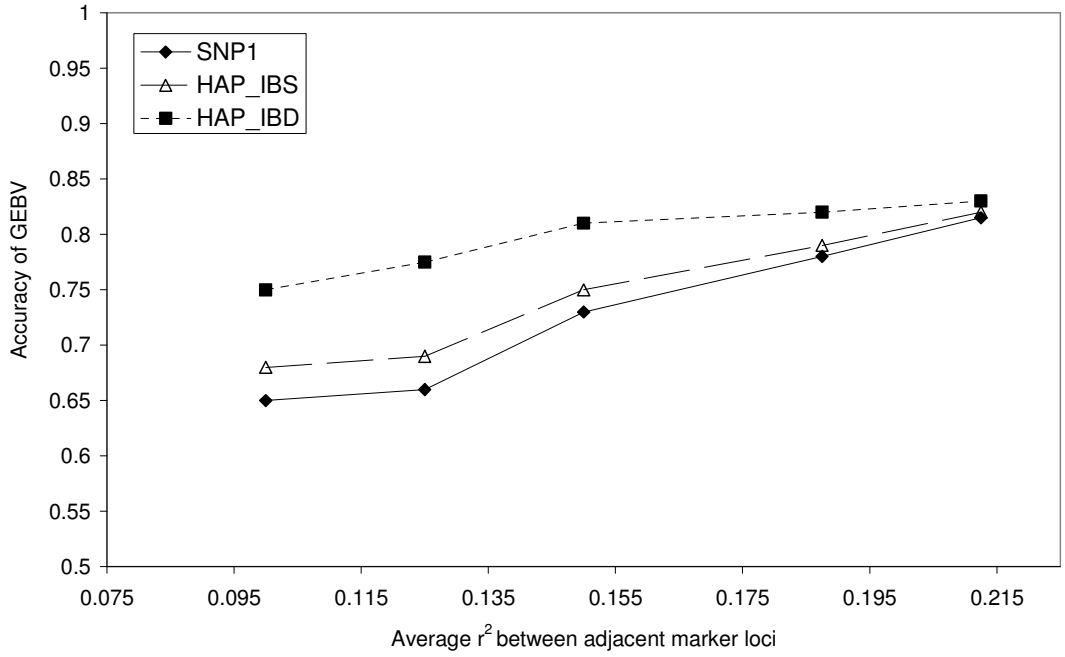

Figure 4.2 Accuracies of genomic breeding values estimated for animals with no phenotypic information with three different models of genomic selection: SNP1, using the single marker approach of Xu (2003), with the addition of a polygenic effect in the model; HAP_IBS using haplotypes of adjacent markers and method BayesB of Meuwissen et al (2001) to estimate haplotype effects, with the addition of a polygenic effect; HAP_IBD using windows of haplotypes of 10 markers in the approach of Meuwissen and Goddard (2004). With permission from the authors., Calus et al. (2007).

They constructed haplotypes from the two adjacent markers defining each chromosome segment. They found that the advantage of using haplotypes increased at lower marker densities (or lower r^2 values between adjacent makers). When the r^2 between adjacent markers was 0.2 or greater, there was little advantage in using marker haplotypes, Figure 4.2 Presenting the accuracy as a function of the average r^2 between adjacent markers, as Calus et al. (2007) do, is appealing as the results can be used to infer the number of markers required to achieve a desired accuracy of genomic selection given the extent of LD observed in the livestock species in question. However, in all cases the accuracy achieved with the IBD approach was higher than regression on single markers or markers haplotypes. This was particularly true at low densities of markers, probably due to the contribution from linkage.

4.3.3 Number of phenotypic records in the reference population

The accuracy of genomic selection will depend on the number of haplotype effects at the chromosome segments, and the number of phenotypic records per unique haplotype, or per marker allele if single markers are used. The more phenotypic records available, the more observations there will be per haplotype and the higher the accuracy of genomic selection. There are also large differences between statistical methodologies in the accuracy achieved with a low number of records. Meuwissen *et al*. (2001) compared the accuracy of least squares, BLUP and BayesB with different numbers of phenotypic records, Table 4.2. Their results also suggest that in the order of 2000 phenotypic records are required to accurately estimate the haplotype effects. In their simulation, a heritability of 0.3 was used. If the heritability were higher, so that phenotype was a more accurate predictor of genotype, fewer records may be required. For example, in dairy cattle, daughter yield deviations (DYDs) are often used as the phenotype. DYDs can have an accuracy of 0.99.

Table 4.2: Correlations between true and estimated breeding values when the number of phenotypic records is varied (from Meuwissen et al. 2001, with permission from the authors)

	No. of phenotypic records		
	500	1000	2200
Least squares	0.124	0.204	0.318
Best linear unbiased prediction (BLUP)	0.579	0.659	0.732
BayesB	0.708	0.787	0.848

4.4 Non additive effects in genomic selection

While breeding values by definition should include only additive effects (genetic merit which is passed from one generation to the next), in some cases it may be desirable to predict genetic merit which better predict an animals actual phenotype, for example through the inclusion of dominance and epistatic effects. If phenotypes are used in the estimation of chromosome segment effects (rather than DYDs for example), inclusion of epistatic and dominance effects in the model could improve the accuracy of estimating the additive effect of the chromosome segment effects. Further, dominance and epistatic effects can be exploited to produce sets of progeny with maximum genetic merit, through mate selection for example (Kinghorn 1998).

Estimates of dominance effects with single markers is straight forward, requiring extension of the genetic model to estimate two effects per SNP, rather than one:

$$y_j = \mu + \sum_i^p x_{ij} g_i + \sum_i^p w_{ij} d_i + e_i$$

Where x_{ij} and w_{ij} are defined as $x_{ij} = \sqrt{2}$ and $w_{ij} = -1$ for genotype A_1A_1, $x_{ij} = 0$ and $w_{ij} = 1$ for A_1A_2 and , $x_{ij} = -\sqrt{2}$ and $w_{ij} = -1$ for A_2A_2. If G_{11}, G_{12} and G_{22} are the genotypic coefficients for the three genotypes, then $g_i = G_{11} - G_{22}$ for the additive effect and $d_i = 2G_{12} - G_{22} - G_{11}$. The x and w coded in this way are independent and

each has a zero expectation and unity variance. The Bayesian estimation method of Xu (2003) can then be extended to estimate d_i as well as g_i.

Estimation of epistatic effects is more difficult, due to extremely large number of marker by marker interactions in the single marker approach, or haplotype by haplotype interactions in the haplotype approach. Xu (2007) extended the single marker Bayesian approach in Xu (2003) to account for epistatic effects.
A model including epistatic effects can be written as:

$$y = \sum_{l=1}^{k} g_l \alpha_l + \sum_{l'>l}^{k} (g_l \times g_{l'}) \alpha_{ll'} + \varepsilon$$

Where $g_l \times g_{l'}$ is the element wise multiplication of vectors g_l and $g_{l'}$, α_l is the main effect of locus l, and $\alpha_{ll'}$ is the epistatic effect between locus l and l'. The model can be simplified to fit into the methodology of Xu (2003) by using j to index the j^{th} genetic effect for $j=1,q$, where $q=k(k+1)/2$. The model can then be re-written

$$\mathbf{y} = \sum_{\mathbf{j=1}}^{\mathbf{q}} \mathbf{Z_j} \gamma_\mathbf{j} + \boldsymbol{\varepsilon}$$

For example, $Z_j=g_l$ and $\gamma_j=\alpha_l$ if the j^{th} effect is a main effect, and $Z_j= g_l \times g_{l'}$ and $\gamma_j=\alpha_{ll'}$ if the j^{th} effect is an epistatic effect.

Xu (2007) used a similar approach to that in Xu (2003) to estimate the γ_j. A normal prior was assigned to the γ_j, where $\gamma_j \sim N(0, \sigma_j^2)$. The prior assigned to the σ_j^2 was $\sigma_j^2 \sim 1/\chi^2_{(\tau,\omega)}$. For details on this prior distribution see Xu (2007).

Xu (2007) showed that epistatic effects could be estimated both in simulated data with this approach using 600 records in a back-cross design. They also applied the method to real data from a barley backcross experiment.

Gianola et al. (2006) presented semi-parametric procedures for genomic selection which allowed them to estimate interactions between potentially hundreds of thousands of markers. Their methods included kernel regression, which regress marker effects according to a smoothing parameter h, embedded into the standard mixed model equations. Their model treated the variance of effects across

chromosome segments as equal. In a small example, they achieved accuracies of up to 0.85 for predicted genotypic values in selection candidates with no phenotypes, when both dominance and epistasis were simulated. For more details see their paper.

4.5 Genomic selection with low marker density

The IBD methodology for genomic selection is particularly suited to cases where marker density is low, as in this case there will be some advantage in including the linkage information in the estimation of chromosome segment effects carried by each animal. Calus et al. (2007) demonstrated that use of the IBD approach can achieve high accuracies of genomic selection even with levels of r^2 between adjacent markers as low as 0.1, Figure 4.2. This result is however dependent on population structure. For example large sire half sib groups in the population will allow accurate estimation of sire haplotypes, such that linkage information contributes considerably to the accuracy of genomic selection.

In LD-MAS, a polygenic breeding value is included in the GEBV to pick up genetic variance not captured by the markers. In genomic selection as specified by Meuwissen et al. (2001), a polygenic component is not included in the prediction of GEBVs. However if the available marker density is not sufficient to ensure all QTL are in high LD with a marker of haplotype, inclusion of a polygenic component in the GEBV from genomic selection would recapture some of the effects of the QTL which are not in sufficient LD with markers.

Even with a sparse marker map, genomic selection can also be used to increase the efficiency of development of composite lines (Piyasatian et al. 2006). Crosses between breeds will exhibit much greater levels of LD than within breed populations. Piyasatian et al. (2006) demonstrated that the genetic merit of composite lines can be improved by using genomic selection to capture chromosome segments with the largest effects from the contributing breeds, even with a sparse marker map.

4.6 Genomic selection across populations and breeds

In practise Genomic selection is always applied in a population that is different to the reference population where the marker effects are estimated. It might be that the

selection candidates are from the same breed, but are younger than the reference population, or they could be from a different selection line or breed. Genomic selection relies on the phase of LD between markers and QTL being the same in the selection candidates as in the reference population. However as the two populations diverge, this is less and less likely to be the case, especially if the distance between markers and QTL is relatively large. In section 1.5 we used the correlation between r in two populations, corr(r_1,r_2), to assess the persistence of LD across populations. No if the chromosome segment effects are estimated in population 1, and GEBVs in that population can be predicted with an accuracy x_1, then the GEBVs of animals population 2 may be predicted from the chromosome segment effects of population 1 with an accuracy $x_2 = x_1$*corr(r_1,r_2). For each set of populations, one can work out the marker density that is required to obtain a corr(r_1,r_2) = 0.9 (De Roos et al. 2007).

In the above, we have assumed that effect of QTL alleles are similar in different breeds and populations. For some QTL which have been traced to known mutations, the alleles do act reasonably similarly in different breeds and populations. For example, the A allele of the DGAT1 gene results in increased fat yield and reduced protein yield and milk volume in New Zealand Holstein-Friesians, Jersey's and Ayshires (Spelman et al. 2002). However while the size of the effects are consistent for protein and milk volume in the Holstein-Friesian and Jersey breeds, the size of the fat response in Holstein-Friesians is nearly double that for Jerseys (Spelman et al. 2002). Another problem is that we have assumed that the same mutations affecting production traits are polymorphic in different breeds. This is true for some well characterised mutations such as the K232A mutation in DGAT1, which is polymorphic in Holsteins, Jerseys, Aryshires and some *Bos indicus* breeds (Spelman et al. 2002, Kaupe et al. 2004). Other mutations, such as some of the functional mutations in the myostatin gene, appear to breed specific (Dunner et al. 2003). One solution would be to use a multi-breed reference population, so that all the genetic variants are captured. Finally, genotype by environment interaction may also reduce the accuracy of predicted GEBV when the chromosome segment effects are estimated from animals in another population.

4.7 How often to re-estimate the chromosome segment effects?

If the markers used in genomic selection were actually the underlying mutations causing the QTL effects, the estimation of chromosome segment effects could be performed once in the reference population. GEBVs for all subsequent generations could be predicted using these effects. A more likely situation in practise is that there will be markers with low to moderate levels of r2 with the underlying mutations causing the QTL effect. Over time, recombination between the markers and QTL will reduce the accuracy of the GEBV using chromosome segment effects predicted from the original reference population. Meuwissen et al. (2001) used simulations to investigate the change in accuracy of GEBV with an increasing number of generations between the reference population and the population for which GEBV were estimated, Table 4.3.

Table 4.3. The correlation between estimated and true breeding values in generations 1003–1008, where the estimated breeding values are obtained from the BayesB marker estimates in generations 1001 and 1002. From Meuwissen et al. (2001).

Generation	$r_{TBV;EBV}$
1003	0.848
1004	0.804
1005	0.768
1006	0.758
1007	0.734
1008	0.718

The generations 1004–1008 are obtained in the same way as 1003 from their parental generations.

After five generations, the decline in accuracy of GEBV was large. This suggests that with the levels of LD simulated in Meuwissen et al. (2001), re-estimation of the chromosome effects should take place every 3 generations.

De Roos et al (2007) investigated the same issue using real SNP data from both Dutch and Australian Holstein Bulls. They calculated the correlation of r values at different marker distances for sub-divisions of the same population across time, as an indicator of persistency of marker-QTL phase across generations. They found correlation of r values between Dutch Holstein bulls before 1995 and Dutch Holstein calves born in 2006 is 0.9 at 135kb. They concluded from this data that with 20,000 markers, the predictions of chromosome segment effects should be usable for two generations, as accuracy will be reduced only slightly (by a factor 0.9) by breakdown of LD phase over this time.

4.8 Cost effective genomic selection

Depending on the genotyping technology used, the cost of genotyping animals for ~ 30 000 SNPs may be $500, while the cost of genotyping animals for ~ 50 SNPs may be as low as $20. If the number of markers required to apply genomic selection can be reduced, this could represent a large saving to the breeding program (and may make the difference between applying or not applying genomic selection).

There are two possibilities to reduce the number of markers in genomic selection. When the method BayesB of Meuwissen et al. (2001) is applied to estimate chromosome segment effects in the reference population, many of the chromosome segment effects will be set to close to zero. So genotyping the markers in these segments in animals where GEBVs are to be predicted using generations has no value. In other words only the subset of markers in chromosome segments with a non-zero effect need be genotyped. One problem with this approach occurs when genomic selection is extended to multiple traits. If the selection criteria includes say 30 traits, and there are 50 markers per trait with non-zero effects, then the total number of markers to be genotyped may be ~ 1500. For most genotyping platforms, the cost of genotyping 1500 markers is close to the cost of genotyping 30000 markers!

4.9 Optimal breeding program design with genomic selection

Genomic selection allows prediction of very accurate EBVs for young animals. The effect of such information on the optimal breeding program design for the different livestock industries could be profound.

In dairy cattle breeding, progeny testing is currently used to identify bulls of high genetic merit. A good description of the progeny test scheme was given by Schaeffer et al. (2006) "In the progeny test scheme, a number of elite cows are identified each year as the dams of young bulls, and these cows are mated to specific sires". At one year of age, the young bulls are test mated to a large number of cows in the population, in order that they will have about 100 daughters with their first EBVs for production and other traits. Approximately 43 months later the daughters from these matings complete their first lactations and the young bull EBVs for production are produced with an accuracy of approximately 75%. At this point the young bull is proven or returned to service." As suggested by Schaeffer et al. (2006), genomic selection allows GEBVs with an accuracy of 0.75 or greater to be calculated for bull calves. Bull calves can therefore be selected at this stage, rather than following progeny testing. This reduces the generation interval by at least half. Further genetic gains can be made by genotyping the elite bull dams and selecting a smaller number for mating to specific sires. Schaffer (2006) suggested the effect of genomic selection may be to shift the structure of the dairy cattle breeding industry to a model similar to that used by the poultry and swine industries, where companies maintain a nucleus of elite animals "within house". Another effect of genomic selection may be more appropriate balance in the direction of genetic gain. Currently in the dairy industry, large gains are made for production traits, while the gains in fertility are relatively smaller, in part due to the lower accuracy of fertility EBVs (and also because production and fertility are unfavourable correlated). Genomic selection could increase the accuracy of fertility EBVs, if sufficient records were taken in the initial experiment to estimate chromosome segment effects, allowing greater contribution of this trait to the total breeding objective.

In the pig. sheep and poultry industries, a major impact of genomic selection is likely to be increased genetic gain for hard to select for traits. This would include traits like disease resistance in poultry and meat quality in pigs.

5. Practical Exercises

5.1 Haplotyping with the PHASE program

The above exercise assumes that the genotypes of each animal have already been sorted into haplotypes. In a real data set, this will not be the case. If the population has large half sib family structure, resolving the genotypes is relatively straightforward. In some situations pedigree information may no be available, or you may deliberately choose to randomly sample animals from the population for LD mapping. With this type of data it is possible to use the PHASE program (Stephens et al 2001). The program is available for free download at http://www.stat.washington.edu/stephens/software.html. Note that the program is only designed to resolve short range haplotypes, eg many markers in a single cM.

Exercise 3.2.1. Haplotyping with the PHASE program.
The casein genes in goats are good candidates for harbouring a mutation affecting milk production, as casein constitutes around 80% of caprine milk protein. You have found 10 SNPs in the goat casein genes, and want to sort the genotypes into haplotypes for LD analysis. 205 goats have been genotyped for the 10 SNPs.

The PHASE input file (goat_geno.txt) has the following format:

```
205
10
P       264     866     888     1105    1169    1379    1470    6075    6091    9889
SSSSSSSSSS
38362
        A       C       G       G       G       C       G       T       C       C
        A       C       G       G       G       C       G       T       C       C
38393
        A       C       G       G       G       T       G       ?       ?       T
        A       C       A       A       A       C       A       ?       ?       C
38421
        A       C       G       ?       G       T       G       ?       G       T
        A       C       A       ?       A       C       A       ?       C       C
38452
        ?       C       G       G       G       T       G       T       ?       C
        ?       C       A       A       A       C       A       C       ?       C
```

Where the first line is the number of animals in the analysis, the second line is the number of SNPs, the third line is the position of the SNPs (begin this line with P) in bases, the next line is the type of marker for each marker (S=SNP,M=microsatellite). Missing alleles are coded as ?.

Then for each animal, there is an ID, followed by the genotypes at each SNP
Marker1 allele1, marker2 allele 1 …..
Marker1 allele2, marker2 allele 1……

To run the phase program, you will need to type the following:

PHASE <filename.inp> <filename.out> <number of iterations> <thinning interval> <burn in>.

For the data set goat_geno.txt, 100 iterations with thinning interval 2 and burn in 10 iterations should be sufficient.

Run the PHASE program. Go to output file. How many unique haplotypes are there? Do they have the same frequency in the population?.

The PHASE program usually predicts a number of haplotypes with very low frequency. What we want to now if the probability that these haplotypes really exist. So, take one of the rare haplotypes from the *.out file. Then, in the *.pairs file, you can see the probability for each animal of carrying a certain haplotype configuration. Are you satisfied that your rare haplotype really exists?

5.2 Estimating the extent of linkage disequilibrium

The GOLD program (for Graphical Overview of Linkage Disequilibrium) calculates linkage disequilibrium statistics from haplotype data ((Abecasis and Cookson, 2000). The statistics calculated are D, r^2 (which the authors call delta) and D'. The program also gives a nice graphical picture of the extent of linkage disequilibrium. The program is freely available from http://www.sph.umich.edu/csg/abecasis/GOLD/.

We will use the **haploxt** program from GOLD to calculate the extent of linkage disequilibrium between pairs of SNPs in practical 5.1. The inputs to the program are marker haplotypes (eg output from the phase program) and a map of the markers.

The file map.gm should look like (the header must be included):

```
MARKERID    NAME       LOCATION
       1    SNP1   266
       2    SNP2   864
       3    SNP3   888
……….
```

You can create this file from the positions line in the file goat_geno.txt

Next you will need to create a file with a list of haplotypes, called HAPLO.LST.

The format of this file is:

```
  HAPLO_1    1 1 1 2 1 2 1
  HAPLO_2    1 2 2 1 2 1 2
  HAPLO_3    2 1 1 2 1 0 0
```

Eg. one line for each haplotype. You can create this file from the list of best pairs from PHASE, using excel for example. Note that in PHASE, we have used A,C,T and G to code the SNP alleles. These must be replaced with 1,2,3 and 4 in the *.lst file. Save your file to a location on your c: drive. If you used excel to create the file, save it as a text file, and then remove the .txt extension.

Open a DOS window, and go to the directory containing the *.lst file. Run the program **haploxt** in this directory by typing **haploxt**.

The program will produce a file called LD.XT. This a table of LD values for each marker pair. Open this file. Plot the values D' and r^2 against each other in excel. Is the value of D' usually larger or smaller than r^2?

Now open the gold program (click on the gold icon). Load the disequilibrium data (the file you have just produced, LD.XT). The load the map file (map.gm). View the LD across the segment with the delta squared statistic. Are there any regions of very high LD? Why do you think this is? In general, what is the relationship between distance between the SNPs and LD? Are there any exceptions to this across the chromosome segment?

Another useful program in the GOLD package is **ldmax**. This program estimates r^2 values from genotypes. So there is no need to haplotype the data first. The "cost" of using this program could be less accurate estimates of r^2 values. To investigate the effect of using genotype data to estimate r^2, we will use the genotype data from practical 5.1 The data format for ldmax is

```
<famid> <pid> <fatid> <motid> <sex> <genotype_1> ... <genotype_n>
```

<famid> is a unique identifier for each family, and within each family **<pid>** is a unique identifier for an individual. **<fatid>** and **<motid>** identify the individuals father and mother (if this line refers to a founder, these should be set to zero). **<sex>** denotes the individuals sex, using the convention 1=male, 2=female. Each **<genotype>** is encoded as two integer allele numbers. The pedigree columns should be separated by spaces.

An example pedigree file fragment, describing a single nuclear family genotyped at 3 markers would be:

```
100 1 0 0 1  1 2  1 2  1 2
100 2 0 0 2  1 2  1 2  1 2
100 3 1 2 1  1 1  2 2  1 1
```

This describes a family (labelled 100), contains two founders (1 and 2), and their single offspring (3). The founders are heterozygous at all marker loci, while the offspring is homozygous at all loci.

In the case of the goat genotype data, we will assume all animals are founders. The input file for **ldmax**, *qtdt.ped* has already been made for you. First rename your LD.XT file so you don't lose it. Then run the **ldmax** program, which also produces the file LD.XT. Now plot the delta^2 values from **haploxt** and **ldmax** (using excel for example). How similar are they? Do you think ldmax is giving reliable estimates of r^2 in this case?

5.3 Power of association studies

As we discussed in section 2, the power of association studies depends on the r^2 between the QTL and the marker we are trying to detect the QTL with, the frequency of the rare allele of the marker and the QTL, the number of phenotypic records, and the significance level we are testing the association at.

There is a program which calculates the power of an association study given all these parameters called ldDesign. The package is written in the R language.
By way of background, R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. We will use R in a windows environment. R provides a wide variety of statistical (linear and nonlinear modelling, classical statistical tests, time-series analysis, classification, clustering, ...) and graphical techniques. There are a very large number of "packages" available for R, and one of these is the ldDesign pack.

Before we use this design pack, lets take a moment to get acquainted with R. We will use a simple example of multiplication of two matricies to obtain another matrix.

Open the R graphical user interface by clicking on it. You should see the command prompt.

Lets multiply two matricies a and b to get a third matrix c.

The matrix a is a two by two matrix with elements:
1 1
2 2
The matrix b is a two by three matrix with elements:
1 2 2
2 3 4

We can input these matricies into the computer memory as:
```
>  a   <- matrix(c(1,1,2,2),ncol=2,byrow=TRUE)
>  b   <- matrix(c(1,2,2,2,3,4),ncol=3,byrow=TRUE)
```

To check the dimensions of a and be are correct type:
```
> dim(a)
> dim(b)
```

You can print a matrix at any time, eg
```
> print(a)
```

Now lets multiple matricies a and b to get a new matrix c:
```
> c <- a%*%b   (%*% is the symbol for matrix multiplication)
```

Check the dimensions of c are correct,
```
> dim(c)
```
And that the c matrix has the correct elements:
```
> print(c)   (you can compare this to the result in excel for example)
```

A matrix can be transposed using t(a), eg
> d <-t(a)

Now we will return to the ldDesign package. Hit the "packages" button on the top of the screen. Then click on ldDesign. If the package does not appear, you can install it by typing

```
> install.packages("ldDesign")
```

The documentation for the ldDesign package can be found here: (http://bg9.imslab.co.jp/Rhelp/R-2.4.0/src/library/ldDesign.html)

We will use the **luo.ld.power** function in the ldDesign package. This function performs a classical deterministic power calculation for power to detect linkage disequilibrium between a bi-allelic QTL and a bi-allelic marker, at a given significance level in a population level association study. This is based on the 'fixed model' power calculation from Luo (1998, Heredity 80, 198–208), with corrections described in Ball (2003).

To run the function:
> luo.ld.power(n, p, q, D, h2, phi, Vp = 100, alpha)

Where:

- *n* The sample size, i.e. number of individuals genotyped and tested for the trait of interest
- *p* Bi-allelic marker allele frequency
- *q* Bi-allelic QTL allele frequency
- *D* Linkage disequilibrium coefficient
- *h2* QTL `heritability', i.e. proportion of total or phenotypic variance explained by the QTL
- *phi* Dominance ratio: phi = 0 denotes purely additive, phi = 1 denotes purely dominant allele effects
- *Vp* Total or phenotypic variance: and arbitrary value may be used
- *alpha* Significance level for hypothesis tests

The function returns the power, or probability of detecting an effect, with the given parameters, at the given significance level.

One problem we will have is that the program takes as an input D instead of r^2, which is more useful to us. We can run the program at a desired level of r^2 between the marker and QTL by inputting for the value of $D = \sqrt{p(1-p)(q(1-q)r^2}$ where p and q are defined above.

For example, if we want to evaluate power at a level of r^2 of 0.2, with p=q=0.2, we would use a value of $\sqrt{0.2*(1-0.2)*0.2*(1-0.2)*0.2} = 0.072$. Now say we have n= 500 phenotypic records, the QTL explains 2.5% of the phenotypic variance, the QTL is purely additive (phi=0), and alpha is 0.05. Assume of a value of Vp of 100, though the value assumed will not affect the calculations. Then the power of the experiment is:

> luo.ld.power(500, 0.2, 0.2, 0.072, 0.025, 0, 100, 0.05)
Which should return a value of 0.277.

Now run the program with 1000 phenotypic records, p=q=0.2,h2=0.025,phi=0,Vp=100 an alpha =0.05 for r^2=0.1,0.2,0.3-1.0.

You can either do this by calculating the value of D at each level of r2 and rerunning the program, or you can write a small "script" which loops through the values of r2.

You can write such a script in notepad. The script might look like:

Script to calculate power at different levels of r2.

```
# Script to calculate power at different levels of r2.
n <- 1000
p_val <- 0.2
q_val <- 0.2
h2 <- 0.025
phi <- 0
Vp <- 100
alpha <- 0.05
for (i in 1:10) {
 r2 <- i/10
 D <- sqrt(p_val*(1-p_val)*q_val*(1-q_val)*r2)
 luo.ld.power(n, p_val, q_val, D, h2, phi, Vp, alpha)
}
```

Save your script with a *.R extension, eg power.R. To open the script, click the file tab and select "open script". You can run the script by clicking the edit tab and selection "Run all".

At what level of r^2 does the power reach 0.9 with these parameters? To determine this, you can plot the power against the level of r^2 in excel for example.

Now plot the power with 500 and 2000 records as well. What does the level of r2 need to be to get a power of 0.9 if 500 records are used. If 2000 records are used?

The next exercise is to determine the number of phenotypic records necessary to detect a QTL with power 0.9 with different levels of r^2. You can do this by looping through different numbers of phenotypic records (increments of 100 for example) in your script and keeping the r^2 constant. Plot the minimum number of records required to reach a power of 0.9 with r^2=0.1,0.2,0.3,0.4….1.0. (eg r^2 on the x axis, and number of phenotypic records required to reach a power of 0.9 with this level of r^2 on the y axis).

Do the results agree with the statement that the number of records must be increased by a factor of $1/r^2$ in order to achieve the same power as observing the QTL itself?

5.4 Building the IBD matrix from linkage disequilibrium Information

Background
In this practical, we will extract the LD information contained in marker haplotypes to build IBD matricies between animals sampled from a population. We will use the algorithm of Meuwissen and Goddard (2001). This algorithm calculates the IBD matrix based on deterministic predictions which take into account the number of markers flanking the putative QTL position which are identical by state, the extent of LD in the population based on the expectation under finite population size, and the number of generations ago that the mutation occurred.

The data files for the LD program are h5.dat, h5.ibs, and h5.dis

"h5.dat"
For two haplotypes, the first line of this file tells the program how many markers are identical by state. There is also one column for the putative QTL position. So if we were comparing two haplotypes,
112112
222222, with the putative QTL position on the middle of the haplotype, the first line of the h5.dat file would be:
0010001, with the circle indicating the position of the QTL. The second line of the file is identical, except with a one in the QTL position.

"h5.ibs"
The second file tells the program the probability that each marker is identical by state, which is very similar to the marker homozygosity (eg. 1-heterozygosity) for each marker, with a zero in the QTL position.

"h5.dis"
The third file tells the program the distance between the markers. The first number is the distance between markers one and two, etc. Usually the QTL is positioned in the middle of a marker bracket. So for example if we had four evenly spaced markers in 10cM, a putative QTL in the middle of the haplotype, the h5.dis file would be 0.033, 0.0167, 0.0167, 0.03.

To run the program, click on h5.exe. A small window should come up, asking for the number of loci, which will be the number of markers + one for the QTL. You will then be asked for the position of the QTL. The next prompt is the effective population size, followed by the number of generations ago that the QTL mutation occurred (we will assume 100 for all our examples).

Exercises
Exercise 3.1.1 Effect of segment length and effective population size on IBD coefficients.
Consider a chromosome segment 10cM long, containing 4 markers. Two animals are drawn from at random from the population and genotyped for the four markers. Two of the four haplotypes carry identical marker alleles (1222). There is a putative QTL in the middle of this haplotype.

What is the probability that the two identical haplotypes carry the same QTL allele (eg, are IBD at the QTL position)?

If the four markers were in a 1cM segment, what is the IBD probability? Why has it changed?

If the effective population size was 1000, what would the IBD probability (4 markers in 1cM). How does this result relate to the result of the first question and the predictive equation LD=1/(4Nc+1).

Now for a 10cM haplotype and Ne=100, increase the number of markers (6,8,11). In each case, work out the IBD coefficient for two identical haplotypes. Plot the IBD coefficient against the number of markers in the 10cM interval (use excel). What happens to the IBD coefficients as the number of markers increases?

Exercise 3.1.2. Building the IBD matrix from LD information.
A further 3 animals are genotyped for the four markers (effective pop size 100, chromosome segment length 10cM). The marker haplotypes are:
Animal 1. 1122, 2122
Animal 2. 1122, 1221
Animal 3. 1222, 1222

For a putative QTL in the middle of the marker haplotype, build the lower diagonal of the IBD matrix (dimensions 6 x 6). Graph the IBD coefficients against the length of the haplotype that is identical by state surrounding the QTL.

This IBD matrix could be inverted, then used in a variance component analysis in ASREML. Of course, for a large number of haplotypes, you would implement the algorithm of Meuwissen and Goddard (2001) in some sort of code, so you did not have to type in the haplotypes, etc, by hand.

5.5 Marker assisted selection with linkage disequilibrium

In this practical, we will investigate the accuracy of LD-MAS with single markers. We will predict MEBV for animals without phenotypes, as

$$\mathbf{MEBV} = \hat{\mathbf{u}} + \mathbf{X}\hat{g}$$

Where $\hat{\mathbf{u}}$ is a vector of polygenic effects, X is a design matrix, and g is the estimate of the effect of the marker.

You can use either excel or R for this practical, whichever you are more comfortable with.

A genome wide association study has identified a marker with a significant effect. We wish to calculate MEBV for a group of progeny which are the offspring of a group of phenotyped animals. The progeny are genotyped, but not phenotyped. The data was "simulated" with a mean of 1, a SNP effect of 1.5 for allele 2 and 0 for allele 1, true polygenic breeding value for animal 1 of 1 and animal 5 0f -1, and true polygenic breeding values for animals 2,3,4,6,7,8,9 and 10 of zero. Errors were randomly distributed with mean 0 and variance 1.

The genotype and phenotype data is:

Animal	Sire	Dam	Phenotpe	SNP allele 1	SNP allele 2
1	0	0	1.27	1	1
2	0	0	2.52	1	2
3	0	0	1.67	1	2
4	0	0	5	2	2
5	0	0	1.5	1	2
6	0	0	2.02	2	1
7	0	0	0.68	1	1
8	0	0	4.09	2	2
9	0	0	3.33	1	2
10	0	0	2.43	1	2
11	1	2	-	1	2
12	1	4	-	2	1
13	5	6	-	1	1
14	5	7	-	2	1
15	5	8	-	2	2

We will fit the model to the data:

$$\mathbf{y} = \mathbf{1_n}'\mu + \mathbf{Xg} + \mathbf{Zu} + \mathbf{e}$$

To solve for the SNP effect and polygenic effects:

$$\begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'X} & \mathbf{1_n'Z} \\ \mathbf{X'1_n} & \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{Z'1_n} & \mathbf{Z'X} & \mathbf{Z'Z} + \mathbf{A}^{-1}\lambda \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix}$$

Where $\mathbf{1_n}$ and $\mathbf{X}$ are both of dimensions (number of records x 1).

The steps are

1. Build the 1n and X (you can follow the example in section 3.2).
2. Build the Z matrix, which allocates records to phenotypes and has the dimensions (number of records 10 x number of animals 15).
3. Build the **A** matrix, the matrix of average additive relationships (number of animals x number of animals):
4. Build the coefficient matrix a block at a time (eg. 1n'1n first). To do this you can use the transpose(matrix1) and mmult(matrix1,matrix2) functions in excel. Assume λ=1.6.
5. Solve the equation to get estimates of the mean, marker effect and polygenic effects.
6. Calculate MEBV for animals 11-15, we using the formula

   $\mathbf{MEBV} = \hat{\mathbf{u}} + \mathbf{X}\hat{\mathbf{g}}$, where the X matrix in this case only refers to animals 11-15.
7. Calculate the accuracy of the MEBV, the correlation between true breeding values and estimated breeding values, where the TBV for animals 11-15 are 2, 2, -0.5, 1 and 2.5 respectively.

What is the accuracy of the MEBV?

Now we will treat the marker as a random effect rather than a fixed effect.
The equations to predict the marker effect and polygenic effect are now:

$$\begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'X} & \mathbf{1_n'Z} \\ \mathbf{X'1_n} & \mathbf{X'X} + \mathbf{I}\lambda_{QTL} & \mathbf{X'Z} \\ \mathbf{Z'1_n} & \mathbf{Z'X} & \mathbf{Z'Z} + \mathbf{A}^{-1}\lambda \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix}$$

Where $\lambda_{QTL} = \sigma_e^2 / \sigma_{QTL}^2 = 0.3$.

Solve these new equations. Is the estimate of the marker effect increased or reduced in absolute value when it is treated as a random effect? is it closer to it's true value (1.5). Is the accuracy of MEBV improved?

5.6 Genomic selection using BLUP

In this practical you will perform genomic selection in a small data set using BLUP. The data set consists of a reference population of 325 bulls with daughter yield deviations (DYDs) for protein %. This phenotype is an accurate predictor of genotype, eg the heritability is close to one. The bulls have been genotyped for 10 SNPs.

Then there are a set of 31 calves who are selection candidates for this years progeny test team. They are genotyped for the same 10 markers. Your task is to predict GEBV for these 31 selection candidates. To do this we will need to predict the effects of the 10 SNPs in the reference population, using the equations:

$$\begin{bmatrix} \mathbf{1_n'1_n} & \mathbf{1_n'X} \\ \mathbf{X'1_n} & \mathbf{X'X + I\lambda} \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1_n'y} \\ \mathbf{X'y} \end{bmatrix}$$

Where g are the SNP effects, 1n is a vector of ones (325 x 1, **X** is a design matrix allocating SNP genotype to records, μ is the overall mean. We will use R to solve these equations. The **X** matrix has already been built for you, and is contained in the file xvec_day4.inp. The y matrix is contained in the file yvec_day4.inp.

What you need to do is write a small R script to solve the equations. This can be done by starting the script in notepad, then opening it in the R console.

The first lines should declare the parameters number of markers and number of records. A this point we will also specify the value of lamda as 10.

```
nmarkers <- 10      #number of markers
nrecords <- 325     #number of records
lamda    <- 10     #value for lamda
```

Next we will read in the files. Change the path to the location where you have stored the files. Note that these statements should all be on one line. Have a look at these files before opening them.

```
x <-
matrix(scan("d:/iowacourse/practicals/day4/realDataExample/xvec_day4.
inp"),ncol=nmarkers,byrow=TRUE)
y <-
matrix(scan("d:/iowacourse/practicals/day4/realDataExample/yvec_day4.
inp"),byrow=TRUE)
```

So now we have the matrix x, the vector y. We still need a vector of ones and a identity matrix dimension number of markers x number of markers…..

```
ones <- array(1,c(nrecords))
ident_mat <-diag(nmarkers)
```

The next step is to build the coefficient matrix. This can be done in blocks, eg….

```
coeff <- array(0,c(nmarkers+1,nmarkers+1))
coeff[1:1, 1:1] <- t(ones)%*%ones
coeff[1:1,2:(nmarkers+1)] <- t(ones)%*%x
```

You will need to build the other blocks. You will also need to build the right hand side of the equation.

The solutions can be obtained easily by using the inbuilt function solve,

```
solution_vec <- solve(coeff,rhs)
```

Print out this vector of solutions (eg print(solution_vec)). What is the solution for the mean? Which SNP has the largest effect?

Next we want to print GEBV for the selection candidates. This is done with the equation:

$$\mathbf{GEBV} = \mathbf{X}\hat{\mathbf{g}}$$

The g_hat are the solutions for the SNP effects you have just solved. The xvector for the selection candidates is in the file xvec_prog.inp. Can you write a small R script to calculate the GEBV?

Fours years later, all the selection candidates receive a phenotypic record from a progeny test. The results are in the file yvec_prog.inp. What is the correlation between your GEBV and the TBV? (Don't expect this to be to high with only 10 SNPs).

5.7 Genomic selection using a Bayesian approach

For the first exercise, we will analyse a small data set using the method BayesA of Meuwissen et al. (2003). We will analyse the data with a script written in the R language, meuwissenBayesA.R. The script considers single markers rather than marker haplotypes, but would be easy to extend to haplotypes. The script estimates single marker effects (**g**), a variance for each of these effects (**gvar**), and overall mean **mu** and the error variance (**vare**). A description of the program is given here (descriptions in bold).

R coding of genomic selection from Meuwissen et al. (2001)

Set the number of markers, the number of markers and the number of iterations #

```
nmarkers <- 3      #number of markers
nrecords <- 25     #number of records
numit    <- 1000   #number of iterations
```

The next section reads in the data from two files. The first is the x vector, with -1 for the 1 1 SNP genotype, 0 for 1 2 and 1 for 2 2. The second file is a vector of phenotypic records. Set the path to the location of your files.

```
x <-
matrix(scan("d:/iowacourse/practicals/day5/smallExample/xvec.inp"),nc
ol=nmarkers,byrow=TRUE)
y <-
matrix(scan("d:/iowacourse/practicals/day5/smallExample/yvec.inp"),by
row=TRUE)
```

Set up some storage vectors and matricies to store parameter values across iterations

```
gStore <- array(0,c(numit,nmarkers))
gvarStore <- array(0,c(numit,nmarkers))
vareStore <- array(0,c(numit))
muStore <- array(0,c(numit))
ittstore <- array(0,c(numit))
```

The Gibbs cycles begin.

Step 1. Initialization of g and mu, declaration of other arrays.

```
g <- array(0.01,c(nmarkers))
mu <- 0.1
gvar <- array(0.1,c(nmarkers))
ones <- array(1,c(nrecords))
e <- array(0,c(nrecords))
```

Begin the iterations

```
for (l in 1:numit) {
```

Step 2. Sample the gvar from the inverse chi square posterior

```
      for (j in 1:nmarkers) {

#       gvar[j] <- (0.002+g[j]*g[j])/rchisq(1,4.012+1)  # Meuwissen
#et al. (2001) prior
#       gvar[j] <- (0.002+g[j]*g[j])/rchisq(1,1)        # Xu (2003)
#prior
        gvar[j] <- (0.002+g[j]*g[j])/rchisq(1,0.998)    # Te Braak et
# al. (2006) prior
      }
```

Step 3. Sample vare from an inverse chi-square posterior

```
      e <- y - x%*%g - mu   # First calculate the vector of residuals
      vare <- (t(e)%*%e)/rchisq(1,nrecords-2)
```

Step 4 Sample the mean from a normal posterior

```
      mu <- rnorm(1,(t(ones)%*%y -
t(ones)%*%x%*%g)/nrecords,sqrt(vare/nrecords))
```

Step 5 Sample the g from a normal distribution

```
      z <- array(0,c(nrecords))
      gold <- g
      for (j in 1:nmarkers) {
       gtemp <- gold
       gtemp[j] <- 0
       for (i in 1:nrecords) {
        z[i] <- x[i,j]
       }
       mean <- ( t(z)%*%y-t(z)%*%x%*%gtemp-t(z)%*%ones*mu ) /
(t(z)%*%z+vare/gvar[j])   # Calculating the mean of the distribution
       g[j] <- rnorm(1,mean,sqrt(vare/(t(z)%*%z+vare/gvar[j])))
      }
```

The final step in each iteration is to store the parameter values

```
      for (j in 1:nmarkers) {
        gStore[l,j] <- g[j]
        gvarStore[l,j] <- gvar[j]
      }
      vareStore[l] <- vare
      muStore[l] <- mu
      ittstore[l] <- l
}
```

This is the end of the program.

Consider a data set with three markers. The data set was simulated as: the effect of a 2 allele at the first marker is 2, the effect of a 2 allele at the second marker is 0, and the effect of a 2 allele at the third marker was -0.5. The mu was 3 and the vare was 1. The data set is:

Animal	Phenotype	Marker1 allele 1	Marker1 allele 2	Marker2 allele 1	Marker 2 allele 2	Marker3 allele 1	Marker 3 allele 2
1	7.34	2	2	2	1	1	1
2	6.02	2	2	2	2	2	2
3	4.92	1	2	2	2	2	2
4	2.89	1	1	2	1	1	1
5	5.62	2	1	1	1	1	1
6	4.85	2	1	2	1	2	2
7	7.01	2	2	2	1	2	2
8	7.61	2	2	2	2	1	1
9	2.14	1	1	2	2	1	2
10	8.68	2	2	2	2	1	1
11	4.22	1	2	1	2	2	1
12	7.15	2	2	1	1	1	2
13	6.57	2	2	1	2	1	1
14	2.47	1	1	2	2	2	2
15	4.51	2	1	1	1	1	1
16	5.98	1	2	2	1	1	1
17	5.37	2	1	2	1	1	1
18	4.51	1	2	2	1	1	2
19	7.53	2	2	2	2	2	2
20	3.33	1	1	2	1	1	2
21	7.32	2	2	1	2	1	1
22	5.87	2	2	2	1	1	2
23	3.19	1	1	2	2	2	1
24	3.87	1	1	2	1	2	1
25	2.72	1	1	2	2	2	2

The first step is to make the files yvec.inp and xvec.inp. In the case of yvec.inp, this is simply the list of phenotypes (no headers or identifiers). For xvec.inp, the number of 2 alleles at each marker for each animal, as a 25 x 3 matrix. The first line of this file would be (for animal 1) "2 1 0".

Save these files in a convenient location. Next open the R graphical interface, and open the script "meuwissenBayesA.R". Check the number of markers is set to 3, and the number of records 25. You will have to change the path of the files as well.

Choose a number of iterations, say 1000.

Run the script using the run all command. As the script runs, it stores values for g, gvar, mu and vare for each iteration. After the script has run, you can use the plotting facilities in R to investigate changes in the parameters over iterations.

For example, to look at the effect of the third marker across iterations, you would enter the command

```
> plot(ittstore[1:1000],gStore[1:1000,1])
```

Use this command to investigate each of the parameters in turn, and determine if they appear to be fluctuating about the correct values.

We can also plot the posterior distribution, for example for the effect of the third marker. We would discard the first 100 iterations of the program as "burn in":

```
> plot(density(gStore[100:1000,1]))
```

Does the distribution appear to be normal? What about the distributions of the other parameters?

To get the mean of the distribution, you would type:
mean(gStore[100:1000,1])
Do the means of the parameters agree with the true value of these parameters?

Now a new set of animals (selection candidates without phenotypes) are genotyped for the three markers. Their genotypes are:

Animal	Marker1 allele 1	Marker1 allele 2	Marker2 allele 1	Marker2 allele 2	Marker3 allele 1	Marker3 allele 2	TBV
26	2	2	2	1	2	1	3.5
27	2	1	1	2	2	1	1.5
28	1	1	1	2	2	2	-1
29	1	2	2	2	2	1	1.5
30	1	1	2	2	1	2	-0.5
31	2	1	1	2	2	1	1.5
32	2	2	2	2	2	2	3
33	2	2	2	2	1	2	3.5
34	2	2	2	1	1	2	3.5
35	1	1	1	2	2	2	-1

Calculate the GEBV for these animals as:

$$\mathbf{GEBV} = \mathbf{X}\hat{\mathbf{g}}$$

What is the correlation with the True breeding values ? (given in the table above, TBV).

Next we will use the script to estimate SNP effects in the reference population in practical 5.6. So you will need to read in the x matrix in xvec_day4.inp, the y vector in yvec_day4.inp. The number of markers in the program will need to be changed to 10 and the number of records to 325.

Run the script.

The next thing you want to do is extract SNP solutions. After the script has run, you can do this by typing:
> mean(gStore[100:1000,1]
This will give you the mean value of the SNP effect for SNP 1 from iterations 100 to 1000 (eg, excluding burn in). So for SNP 6 you would type
>mean(gStore[100:1000,6].

Compare your SNP solutions from the Bayes program to those from BLUP (practical 5.6). One of the reasons for using the Bayesian approach is to allow different variances of SNP effect across chromosome segments. In particular, the Bayes approach should set some variances (and so SNP effects) to very close to zero. Does this seem to have happened? How many QTL would you say are on the chromosome segment?

Can you predict GEBV for the selection candidates in practical 5.6 using the SNP solutions from the Bayesian approach? Are they more highly correlated with the TBV than the GEBV from the BLUP approach?

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