

**Mapping genes for birth weight in a
wild population of red deer (*Cervus elaphus*)**

Jon Slate

**A thesis submitted to The University of Edinburgh
in application for the degree of Doctor of Philosophy**

December 1999



Preface

This thesis has been composed by me, is the result of my own research and contains no work done in collaboration except where stated otherwise. The text does not exceed 100,000 words. No part of this thesis has been submitted to any other university in application for a higher degree.

Abstract

Evolutionary genetics theory predicts that additive genetic variation for fitness should be depleted by natural selection. Consequently it is expected that genes of large effect on fitness related traits should be rapidly selected to fixation. With the recent discovery of highly polymorphic molecular markers it has become possible to detect quantitative trait loci (QTL). This thesis describes the first attempt to detect QTL in an unmanipulated wild population.

The red deer (*Cervus elaphus*) on the Isle of Rum, Inner Hebrides, Scotland have been the focus of an intensive study for thirty years and constitute a suitable population for a QTL search. I performed a power analysis in which it was determined that QTL of large effect might be detectable in a complex pedigree descending from a recently introduced stag, 'MAXI'. Primers for 354 bovine microsatellite markers were obtained and tested for polymorphism in red deer. Informative markers were placed on the deer genetic map, of which 84 were screened in 364 deer descended from MAXI. I performed a genome wide search for QTL for birth weight, using a recently developed variance components approach. Birth weight is under directional selection and known to influence overall fitness in the study population, yet does retain some additive genetic variance ($h^2 \sim 0.18$). Three regions explained significant evidence for birth weight QTL if treated as single statistical tests, but none were significant at an experiment-wide threshold of $p < 0.05$. One of these regions has previously been reported to be associated with birth weight in a farmed pedigree derived from hybridised red deer and Père David's deer (*Elaphurus davidianus*). All three regions explained around 25% of the variation in birth weight, suggesting that genes of large effect on a fitness related trait can segregate in the wild. The large panel of markers screened also enabled me to derive a likelihood method to test and confirm the accuracy of the parentage inference program CERVUS.

A further theoretical prediction is that selection will erode additive genetic variation for fitness traits faster than for morphometric traits. Thus a greater proportion of genetic variation in fitness traits should be attributable to dominance variance. Testing this hypothesis is difficult, as measuring dominance variance directly is not trivial. One solution is to attempt to detect inbreeding depression or heterosis, as a trait can only experience these phenomena if its genetic architecture includes a component of dominance variance. Previous analyses in the study population have detected inbreeding depression or heterosis using the related genetic variables multilocus heterozygosity (MLH) and mean d^2 . I used generalised linear modelling techniques to determine whether these genetic variables explained significant variation in life history and morphometric traits. In accordance with predictions, dominance variance appeared to be more important in fitness-related traits than morphometric traits, especially in males.

Acknowledgements

The work presented in this thesis would not have been possible without the help of a great number of friends and colleagues. I have been fortunate enough to work with two excellent field workers on Rum – initially Ailsa Curnow, and then Sean Morris. Their dedication, expertise and willingness to work outdoors in horrendous weather is inspirational. This also seems a good place to acknowledge the role of Tim Clutton-Brock, Steve Albon and Fiona Guinness in setting-up and maintaining the long-term Rum study. Thank you to SNH for allowing the work on Rum, and BBSRC and The James Rennie Bequest for financial support.

A number of scientists contributed microsatellite primers, but special mention must go to John Williams, who provided the panel of primers that allowed the project to gather some momentum. Steven Kappes provided me with unpublished data describing an attempt to map cattle microsatellites in sheep. I have enjoyed a fruitful collaboration with Mike Tate during the course of this thesis. Mike provided DNA from the deer mapping herd and added my microsatellites to the map with the data I sent him. He was also an excellent host on a memorable earlier visit to Dunedin.

ICAPB has provided an exciting environment in which to study evolutionary genetics, and I'm grateful to everybody who helps make it this way. Past and present members of the group have provided great company and stimulating discussion. Tristan Marshall deserves a big thank you for his earlier work developing CERVUS. Loeske Kruuk and Dave Coltman have offered sensible and clear statistical advice without being all that nerdy. Antonis Rokas, Graeme Swanson, Jo Girvan, Judith Smith, Kate Oddie, Martin Dallimer, Nick Colegrave, Rachel Atkinson, Rebecca Wilson, Simon Goodman and Steve Paterson have all been good value in the lab or office. An extra special mention goes to Ashleigh Griffin and her 'naughty pixie' antics that made lab work fun when it should have been a necessary chore. Ashleigh not only tolerated my Hank Williams tapes but seemed to enjoy them. A number of people have read and made comments on early drafts of chapters including Tristan, Dave, Loeske, Tim Coulson, Marg Mackinnon and Bill Hill. Thus, any typos are their fault.

Playing football with Sporting ICAP has been a welcome distraction over the last few years. They really do deserve to win a game at some point and perhaps it will happen - one day. While on the subject of football I acknowledge the work of Gérard Houllier in revitalising Liverpool FC. His efforts have certainly made me a calmer person during the writing-up stage. At last the era I gloomily think of as “The Gary Ablett Years” appears to be behind us.

I have had superb supervision during the course of this project. Peter Visscher wrote software for estimating power to detect QTL and also the variance components software used in Chapter 7. His insightful comments and vast knowledge of QTL mapping techniques made much of this project possible. Josephine Pemberton has contributed hugely to this thesis. Not only did she conceive it, but she has offered an enormous amount of encouragement and advice. She patiently read and made invaluable comments on every chapter. I’m extremely grateful to Josephine for introducing me to Rum, and giving me the opportunity to work on some marvellous projects over some very happy years.

My parents have been wonderfully supportive over the years. Furthermore they have never questioned (aloud) why I didn’t get a ‘proper job’.

Above all though, I thank Ruth for her love, patience and help. This thesis is for her.

List of Abbreviations

ABS	Annual breeding success
AFLP	Amplified fragment length polymorphism
AI	Artificial insemination
DMSO	Dimethyl sulphoxide
F1	First generation cross
F2	Second generation cross
GAW	Genetic Analysis Workshop
GLM	Generalised linear model
IBD	Identical by descent
IBRP	International Bovine Reference Panel
IBS	Identical by state
IMH	International Mapping Herd
IRRF	Illinois Reference / Resource Families
LBS	Lifetime breeding success
LRS	Lifetime reproductive success
ML	Maximum likelihood
MLH	Multi-locus heterozygosity
MYA	Million years ago
PCR	Polymerase chain reaction
RAPD	Randomly amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RFLV	Restriction fragment length variant
sMLH	Standardised multi-locus heterozygosity
TMACIDE	Tetramethylammonium chloride / formamide

Table of contents

Preface	ii
Abstract	iii
Acknowledgements	iv
Abbreviations	vi
Table of contents	vii
1 Introduction	1
1.1 Fisher's fundamental theorem of natural selection	1
1.2 Is there additive genetic variation for fitness?	2
1.3 Understanding additive genetic variation for fitness	8
1.4 Quantitative trait loci	11
1.5 Aims of this thesis	20
2 Red deer on Rum	21
2.1 The natural history of red deer	21
2.2 Background to the Rum project	21
2.3 Ecological and behavioural work	25
2.4 Genetic studies	27
2.5 Which traits are suitable for QTL analysis?	34
2.6 Are descendants of MAXI born heavier?	36
3 Power to detect QTL in the study population	39
3.1 Introduction	39
3.2 Methods	42
3.3 Results	46
3.4 Discussion	55
4 Obtaining and screening microsatellite markers	60
4.1 Introduction	60
4.2 Testing ruminant primers in red deer and other species	62
4.3 Screening microsatellites in the MAXI pedigree	69
5 A reassessment of previously inferred paternities	74
5.1 Introduction	74
5.2 Methods	76
5.3 Results	80
5.4 Discussion	83

6	A red deer genetic map	86
6.1	Introduction	86
6.2	Adding microsatellites to the current deer genetic map	86
6.3	A deer genetic map from the MAXI pedigree	94
6.4	Conclusions	103
7	A search for birth weight QTL	104
7.1	Introduction	104
7.2	Detecting QTL in complex pedigrees	104
7.3	Methods	106
7.4	Results	116
7.5	Discussion	120
8	Microsatellites as a tool for measuring non-additive genetic variance	126
8.1	Introduction	126
8.2	Methods	130
8.3	Results	133
8.4	Discussion	143
9	Dominance variance for life history and morphometric traits	150
9.1	Introduction	150
9.2	Methods	151
9.3	Results	152
9.4	Discussion	158
10	Conclusions and future work	161
	References	163
	Appendices	185
A	Ecological and environmental terms used in this thesis	185
B	Laboratory methods	189
C	Conservation of cattle microsatellites donated by John Williams	192
D	Conservation of other microsatellites	199
E	Detecting parent-offspring mismatches	202
F	Microsatellites screened in the IMH	204
G	Two-point linkage analysis in the MAXI pedigree	208
H	Histograms of allele counts for loci screened in the MAXI pedigree	209

1 Introduction

1.1 Fisher's fundamental theorem of natural selection

Ronald Fisher's (1930) fundamental theorem of natural selection states:

"The rate of increase in fitness of an organism is equal to its genetic variance in fitness at that time".

or

"The rate of increase in fitness of any species is equal to its genetic variance in fitness".

Despite some confusion as to the actual meaning of the theorem it is one of the most commonly stated theories in evolutionary genetics (Frank and Slatkin 1992). Although not strictly speaking the intended definition¹ (Lynch and Walsh, in prep), the classical interpretation of 'the theorem' can be represented by the formula:

$$\Delta \bar{W} = \sigma^2(W) / \bar{W}$$

where $\Delta \bar{W}$ = total change of mean fitness $\bar{W}$

and $\sigma^2(W)$ is the additive genetic variance in fitness.

An implication of this formula is that when there are no sources of new additive genetic variation (mutation or migration), natural selection should remove all additive variation for fitness that is present (Charlesworth 1987). A further prediction is that there should be no heritability of fitness in a population at equilibrium, and that the more highly correlated (genetically) with fitness a trait is, the lower will be

¹ Frank and Slatkin (1992) give an excellent account of what Fisher really meant. They argue that Fisher partitioned the change in fitness into two terms. The first is the change in fitness caused by natural selection (proportional to the additive genetic variance in fitness), and the second is that caused by the change in the environment. This second term is very broadly defined and even includes changes in allele frequency. In other words, if there is a change in fitness arising from the average effect of an allele being altered due to a change in allele frequency, then this is an environmental effect. Essentially this means that the fundamental theorem makes no general prediction about mean fitness following selection. While natural selection might increase the fitness of a population, there may be a subsequent 'deterioration in the environment' leading to no perceptible change in overall mean fitness.

the heritability of that trait (Charlesworth 1987; Falconer 1989). Clearly all populations have some input of new additive variation due to mutation (and other sources), but it seems reasonable that the classical interpretation of Fisher is a good approximation of how selection acts on additive variation for fitness (Lynch and Walsh, in prep).

1.2 *Is there additive genetic variation for fitness?*

1.2.1 Heritability of fitness and fitness components

Surprisingly little empirical data are available to support the predictions outlined above, in particular from natural populations. The principal reason for this is that individual lifetime fitness is difficult to measure in the wild. Longitudinal studies, following individuals from birth through all breeding attempts to death, are rare (Clutton-Brock 1988). Cross-sectional studies may not accurately measure fitness due to either a failure to represent individuals' entire life histories (Endler 1986) or the confounding effects of environmental variation (Clutton-Brock 1988). The only published estimate of the heritability of fitness in a natural population comes from Gustafsson's (1986) five year study of collared flycatchers (*Ficedula albicollis*) on the Swedish island of Gotland. Gustafsson (1986) reasoned that because there was no apparent directional selection for any metric characters, it could be assumed that the population was close to equilibrium. The heritability of morphological characters, fitness components, and fitness itself was estimated by parent-offspring regression, a method that is relatively uninfluenced by dominance variance and less likely to be biased by shared environmental effects, compared with a full-sib method (Falconer 1989; Roff 1997). The data support (interpretations of) Fisher; overall fitness had a heritability not significantly different from zero (males, $h^2 = 0.083 \pm 0.128$ SE; females, $h^2 = -0.0142 \pm 0.158$ SE), and the greater the proportion of overall fitness that a trait explained, the lower was the heritability of that trait. However, the standard errors on estimates of heritability are often large, and the heritability of fitness reported by Gustafsson (1986) may not actually be zero, merely low.

The heritability of fitness, fitness components and morphometric traits has also been estimated for the intensively monitored red deer population on the Isle of Rum,

Scotland (Kruuk *et al.*, in press). This thesis concerns the Rum red deer and so a comprehensive discussion of Kruuk's study follows in Chapter 2. Briefly, the findings of Kruuk support those of Gustafsson; lifetime reproductive success has a heritability close to zero and fitness components have a lower heritability than morphometric traits.

Although there are few studies that have estimated the heritability of overall fitness, components of fitness (e.g. longevity, fecundity) have been investigated in both the laboratory and in the wild. Roff and Mousseau (1987) reviewed the literature on *Drosophila*, compiling heritability estimates from 130 studies. Traits were classified as life history, behavioural, physiological or morphological. As predicted, life history traits had lower heritabilities than physiological or morphological traits, while behavioural traits had values similar to life history traits. Life history traits occasionally had heritabilities as high as 0.2 but were also prone to greater error than other traits, with different studies arriving at a wide range of estimates for the same life history trait. Roff and Mousseau (1987) also examined the genetic correlations between the various types of trait. Morphological traits tend to positively covary with each other and with life history traits. However, life history traits do not necessarily positively covary with other life history traits, providing some evidence that antagonistic pleiotropy (Rose 1982) may play a role in the maintenance of additive genetic variance for life history traits. Mousseau and Roff (1987) subsequently collected estimates of heritability of the same four categories of trait, derived from wild outbred animal populations other than *Drosophila*. In 140 studies, performed on a total of 75 species, 1120 heritability values were estimated. The same general trend was observed. Life history traits were the least heritable, followed by behavioural traits, with morphological traits the most heritable. However the mean heritability of a life history trait in a wild outbred animal was 0.26 compared with 0.12 for laboratory *Drosophila*. In other words, although natural selection appears to deplete additive genetic variance for life history traits more than other traits, substantial additive genetic variance persists in the wild.

In summary, it is clear that more estimates of the heritability of overall fitness in the wild are required before any definitive conclusions can be drawn. The current consensus is that overall fitness has zero or very low heritability, but that fitness components do exhibit some, often substantial, additive genetic variation.

1.2.2 Other measures of additive genetic variance

Houle (1992) offers an alternative explanation (to those discussed in the previous section) as to why fitness and fitness components show reduced heritability compared to morphometric traits. Consider the definition of narrow sense heritability, namely the ratio of additive genetic variance to phenotypic variance (Falconer 1989):

$$\text{Heritability, } h^2 = V_A/V_P$$

Where V_A is the additive genetic variance

V_P is the phenotypic variance,

where $V_P = V_A + V_D + V_I + V_E$ (Falconer 1989).

V_D is the genetic variance due to dominance.

V_I is the genetic variance due to epistasis.

V_E is the environmental deviation.

If fitness (or a fitness component) has a low heritability, then it is usually assumed that this is because there is little additive genetic variance present. However, low heritabilities could also be explained by high environmental variance (Barton and Turelli, 1989). The environment is likely to influence fitness over an organism's entire life-span, and so substantial variation in environmental effects between individuals is to be expected. Consequently, the denominator of the heritability equation becomes large relative to the numerator. Houle (1992) suggested the use of the coefficient of additive genetic variance as an alternative measure to heritability.

The coefficient of additive genetic variance is given by the formula:

$$CV_A = \frac{100 \sqrt{V_A}}{\bar{X}}$$

where V_A is the additive genetic variance and $\bar{X}$ is the trait mean

Houle (1992) calculated heritability, CV_A and CV_R (see below) for a total of 842 traits obtained from the literature, many of which were studies included in the Mousseau and Roff (1987) and Roff and Mousseau (1987) analyses. CV_R is the coefficient of residual variation and is calculated using the formula:

$$CV_R = \frac{100 \sqrt{V_P - V_A}}{\bar{X}}$$

By using CV_A , a measure scaled by the trait mean, the problem of environmental variance masking any additive variance is reduced. However, this measurement is still not scale independent (Roff 1997) and is also sensitive to the distribution of the trait (Kruuk *et al.* in press). Houle (1992) found that traits associated with fitness had higher values of CV_A and CV_R than those less closely associated with fitness (i.e. morphometric traits). Therefore, although the heritability of fitness components may be low, this does not necessarily mean that there is no additive genetic variance for fitness.

When measuring heritability of fitness, fitness components and morphological traits in the Rum red deer, Kruuk *et al.* (in press) also examined CV_A and CV_R , and found only partial support for Houle (1992). As predicted CV_R was greater for traits closely associated with overall fitness, and fitness components tended to have greater CV_A 's than morphometric traits. However, total fitness was more strongly associated with longevity, a trait with low CV_A , than any other trait (see also Chapter 2). In this population additive genetic variation for overall fitness could be masked by environmental noise, or it could genuinely have been depleted by selection.

The extent to which sexually-selected traits are heritable is another contentious area of debate (Jones 1987; Andersson 1994). Pomiankowski and Møller (1995) compared coefficients of additive and residual variance between sexually and non-sexually selected traits and found that sexually selected traits had greater additive

genetic variation. Hence females derive a genetic benefit from mating with phenotypically ‘good’ males. It was claimed that this resolved the ‘lek paradox’ - the observation that in some species females have evolved strong preferences for particular males at display sites (leks) without receiving any apparent benefit. Pomiankowski and Møller (1995) argued that fitness increases at a greater than linear rate with trait value, and that sexual selection will increase the variance in the trait. This may come about by modifier loci that either increase the number of loci or increase the average contribution of each locus to the trait. A more straightforward explanation for the high CV_A of sexually selected traits is offered by Rowe and Houle (1996). They suggest that sexually selected traits are condition dependent and that the high values of CV_A arise because variation in condition depends on the effects of many genes. This argument is essentially the same as that used by Houle (1992) to explain why life history traits show greater values of CV_A and CV_R than morphometric traits. However in the data set compiled by Pomiankowski and Møller (1995) sexually selected traits had only marginally greater values of CV_R than non-sexually selected traits. Merilä and Sheldon (1999) suggest caution when drawing conclusions about the genetic architecture of sexually-selected traits, as many studies fail to consider the possibility of environmental correlation between parent and offspring (see Griffith *et al.* 1999), and there may be a bias towards highly heritable sexual traits being reported in the literature (see Alatalo *et al.* 1997).

The coefficients of additive and residual genetic variance have proved to be useful tools for understanding the genetic architecture of fitness and related traits. They provide reasonably strong evidence that there is additive genetic variation for fitness components in natural populations, possibly segregating at a great many loci, but that these traits are also subject to high environmental variance. Consequently, the observation of low heritabilities is not incompatible with high coefficients of additive genetic variance.

1.2.3 Locus-specific associations in the pre-mapping era

The previous sections have detailed quantitative genetic approaches for detecting additive genetic variation for fitness components. Associations between genotype

and life history traits have also been demonstrated in natural populations (reviewed in Endler 1986; Mitton 1997). The majority of these studies took place in the 1970's and 1980's when, inspired by the neutralist-selectionist debate a huge effort was made to detect variation at allozyme loci (Avisé 1994). Well known examples of associations between genotype and fitness include the *PGI* locus influencing mating success in *Colias* butterflies (Watt 1977), the *LDH* locus associated with metabolic activity in the killifish *Fundulus heteroclitus* (DiMichele *et al.* 1991) and the *LAP* locus exhibiting varying levels of fitness depending on ocean salinity in the blue mussel *Mytilus edulis* (Hilbish and Koehn 1985). A number of studies have been carried out in the red deer population on which this thesis focuses, and these specific examples are discussed in Chapter 2. Since the heyday of allozyme-fitness studies, many of the major goalposts in evolutionary biology have shifted (Hey 1999). Most studies using DNA polymorphisms in natural populations today assume markers are neutral and ask different questions. For example, highly variable DNA markers are regularly used to infer parentage within a population so that measures of reproductive success or estimates of heritability can be made.

Drawing any general conclusions from studies demonstrating associations between genotype and fitness is not straightforward. Whilst there is little doubt of the robustness of a few well-known associations, this does not mean that substantial genetic variation is the underlying cause of variation in fitness in the wild. A number of polymorphisms that have been studied intensively may have been chosen *a priori* because they were suspected to be associated with fitness (Avisé 1994). A substantial proportion of reported associations between genotype and fitness involve cases where heterozygous individuals outperform homozygotes. This is not evidence for *additive* genetic variance. Furthermore many thousands of electrophoretic polymorphisms have been described, yet reported associations with fitness are rare (Pemberton *et al.* 1988), one possible reason being that it is extremely difficult to obtain field measures of lifetime fitness (Endler 1986). Until the advent of DNA molecular markers it was often necessary to kill individuals to obtain the blood sample required for genetic screening, and so morphometric measures assumed to be indicators of fitness had to be used as a fitness surrogate. Environmental variation

may mask any genetic differences in individual performance, making it impossible to detect differences between genotypes. Environmental variance in fitness can also be a problem in structured populations if particular genotypes are non-randomly distributed between environments, leading to an erroneous interpretation of fitness differences between genotypes (Coulson *et al.* 1998). Finally, it is likely that many studies failed to find associations between genotype and fitness, and remained unreported. The degree to which any such reporting bias has shaped the literature on allozyme-fitness studies is unclear.

1.3 Understanding additive genetic variation for fitness

It can be concluded from the quantitative genetics literature, and to a lesser extent from allozyme studies, that there is good evidence for additive genetic variation for fitness and especially fitness components in natural populations. However, very little is known about this variation. Eighty years after the end of the Mendelian-Biometrician clash that dominated genetics in the early 1900s, a number of the issues most relevant in 1920 remain unresolved. Do major genes (oligogenes) for fitness segregate in the wild or is additive genetic variation mainly attributable to many polygenes of small effect (Barton and Turelli 1989; Orr and Coyne 1992)? How many loci are responsible for quantitative genetic variation? The first of these questions is of particular importance as the short term response to selection is dependent on the magnitude of allelic effects rather than the number of loci (Barton and Turelli 1989). Furthermore, many of the models used in evolutionary biology today assume that quantitative variation is caused by many genes of imperceptible effect (Barton and Turelli 1989; Mitchell-Olds 1995). The best known of these, the infinitesimal model, makes the assumption that a trait is described by a large number of loci each with a small additive and dominance component, and a normally distributed environmental component. It is assumed that there is no epistasis, and that the number of loci is large enough to make the trait normally distributed.

No firm consensus on whether major genes play a large role in evolution has yet to be reached. For example:

“Overall the data are consistent with the hypothesis of a large number of loci of small effect” (Roff 1997)

“The limited data also reveal that major gene effects are not rare” (Orr and Coyne 1992)

The observation that many continuous traits approximate a normal distribution reveals little about the genetic architecture of the trait (Falconer 1989; Roff 1997). I illustrate this with the following example. Imagine a trait determined by four additive biallelic genes of equal effect. For each gene one allele, p , has a value of 1 and the other, q , a value of 0. The frequency of p = frequency of q = 0.5. There is no environmental variance for the trait. A simulated frequency distribution for 1000 individuals is given in Figure 1.1. The expected standard deviation of the trait is $\sqrt{2}$ and the effect of an allelic substitution is 1. Therefore an allelic substitution is equivalent to $1/\sqrt{2} \approx 0.71$ of a phenotypic standard deviation. Falconer and Mackay (1996) define a ‘major gene’ as one that has an allelic substitution effect of 0.5 of a phenotypic standard deviation or greater. Clearly the expected distribution from this major gene model is difficult to distinguish from the normal distribution expected under a model of many genes of small effect. If a small amount of random environmental variation was built into the model then the distribution would be almost perfectly normal. Falconer (1989) describes a similar example, but with one allele completely dominant to the other. With six loci underlying the trait the distribution is negatively skewed, but with 24 loci most of this skew disappears.

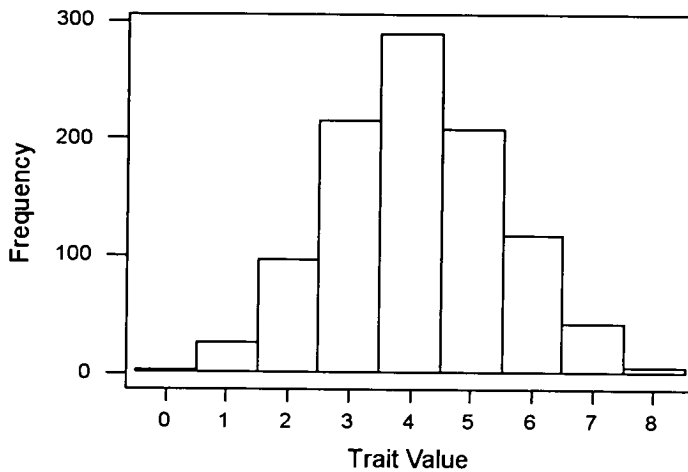


Fig 1.1. The distribution of a simulated trait determined exclusively by four additive biallelic loci of equal effect. 1000 individuals were simulated. Although the trait is determined by four genes of 'major effect' the phenotypic distribution is still close to a classical normal distribution. Note that Roff (1997) wrongly states that the expected frequencies for the phenotypic classes can be calculated from the coefficients of the binomial expansion $(p+q)^n$ where n is the number of loci underlying the trait. The correct frequencies are obtained from the coefficients of a binomial expansion $(p+q)^{2n}$.

In summary, it is clear that the distribution of a trait reveals little about the magnitude of allelic effects, the number of loci responsible, or the extent to which dominance plays a role in the genetic architecture of a trait. Roff (1997), however makes the point that if only a few loci were involved, strong directional selection should rapidly deplete variation, and the response to selection should cease. This is not usually observed in selection lines.

In recent years biologists have turned to molecular techniques, principally quantitative trait locus (QTL) mapping to try and understand the nature of quantitative trait variation (Mitchell-Olds 1995; Roff 1997; Lynch and Walsh 1998). In the following section I introduce the concept of QTL mapping and review the literature on QTL mapping studies to date.

1.4 Quantitative trait loci (QTL)

1.4.1 Background to QTL analysis

In the previous section it was demonstrated that a continuous trait distribution can result from many genes of small effect (polygenes or minor genes) or a few genes of much larger effect (oligogenes or major genes). Ultimately, to resolve the issue of effect size it would be desirable to search the genome for all genes influencing the trait, arriving at an accurate estimate of the number and magnitude of all genes involved. In principle, methods for detecting quantitative trait loci (QTL) have been known for most of this century (Lander and Botstein 1989; Lynch and Walsh 1998), although it is only in the last decade that it has become feasible to perform genome-wide searches. Early studies used visible markers that segregated in a Mendelian fashion to identify QTL. For example, Sax (1923) used a polymorphism in seed pigment between two inbred lines of bean to determine that a linked locus was associated with seed weight differences between the two lines. The underlying principle in QTL analysis is that if a marker and a QTL are linked, and if there is some form of linkage disequilibrium in the cross or population studied, then individuals segregating for one marker allele will have a different trait mean to those with another marker allele. For the most simple experimental design the presence of a QTL can be detected by a straightforward statistical test such as a t-test. The complexity of the experimental design depends on the organism being studied and the question being asked. Deriving crosses between lines of bean to find QTL responsible for seed weight differences between lines is relatively simple. Carrying out a search for genes underlying a complex trait in a human pedigree that cannot be manipulated is a far more demanding task. In this section I describe some of the methods and techniques that have been developed for QTL analysis. I also review what the studies to date reveal about the genetic architecture of fitness-related traits within populations, and discuss what features are desirable in a wild population to perform such a task.

In the last decade the number of QTL mapping programs has exploded. A number of earlier studies managed to locate QTL using visible polymorphisms or chromosomal rearrangements as markers (e.g. Sax 1923; Shrimpton and Robertson 1988), but it

was only with the advent of DNA polymorphisms such as restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA markers (RAPDs) and microsatellites that it became possible to carry out genome-wide scans for QTL in almost any organism (Lander and Botstein 1989). It has also been suggested that a 'catchy acronym', QTL, has aided the rapid uptake of such studies (Kearsey and Farquhar 1998). With DNA marker technology it became possible to construct whole genome maps using polymorphic, evenly spaced markers, permitting QTL analysis in almost any species (Lynch and Walsh 1998). Consequently QTL studies are now common-place in disciplines such as medicine, agriculture and evolutionary biology.

A bewildering number of different experimental designs and statistical tests have been developed for QTL analysis. To a large extent these depend on the organism studied and the question being asked. Most designs fall into one of two categories (Lynch and Walsh 1998). The first involves the crossing of (usually inbred) lines, so that genetically identical F1s are produced. From these parental lines it is usual to produce either an F2 generation or to perform a backcross. These methods are widely applied in crop plant and mouse studies. They are suitable if one is interested in determining the genetic basis of the difference *between* the two lines. Another application of inbred line crosses is in determining the genetic basis of reproductive isolation between two related species (Bradshaw Jr *et al.* 1995; Lin and Ritland 1997). The second category of design uses outbred populations. Examples include complex disease mapping in humans, and often the mapping of QTL for production traits in livestock. Two common features of this approach are that the organism being studied cannot be manipulated to create inbred lines, and that it is QTL *within* a population that are of interest (Lynch and Walsh 1998). At times the boundary between the two designs can be blurred, for example in livestock studies, but the majority of programs clearly follow one or the other design. Statistically, the inbred line design is a far more powerful approach. All F1 parents will be informative for marker and trait locus, and computation is relatively easy. Conversely it is highly unlikely that all parents in an outbred population will be informative for marker and trait locus. Furthermore different parents will be in different phase between marker and trait loci and so each family must be analysed separately. Despite these

drawbacks QTL detection is possible in outbred families of considerable complexity, and sophisticated statistical techniques have been developed to perform analyses in species such as man in which manipulating matings is impossible.

A number of refinements of the two basic QTL mapping techniques have been developed to increase power and reduce cost when carrying out a mapping program. These include selective genotyping (Darvasi and Soller 1992; Cardon and Fulker 1994), in which only individuals at the extremes of a phenotypic distribution are typed, hence reducing the amount of genotyping required. This labour-saving option can be taken even further by pooling DNA samples from selected individuals (Darvasi and Soller 1994), although such a design relies on accurate quantification of band patterns on gels and is rarely used in practice. Furthermore, only one trait (or several phenotypically highly correlated traits) can be analysed using this approach. The power to detect QTL can be increased if more than one marker is considered when carrying out the statistical analyses. Interval mapping uses each pair of adjacent markers to test for the presence of QTL (Lander and Botstein 1989; Haley and Knott 1992; Fulker and Cardon 1994) and leads to an increase in statistical power. Interval mapping also allows for more precise estimates of the position and magnitude of the QTL than single marker mapping (Lynch and Walsh 1998). A further extension is multipoint mapping which takes all marker data on a chromosome simultaneously. An increasingly common method used in human linkage analysis is to calculate an identity-by-descent (IBD) matrix between all pedigree members at regular intervals along a chromosome, and then to carry out a restricted maximum likelihood (REML) variance component analysis to detect QTL (Almasy and Blangero 1998; Lynch and Walsh 1998; Visscher *et al.* 1999). This method is suited to complex pedigrees and is extremely powerful, but is also computationally demanding. Variance component methods are discussed in greater detail in Chapter 7.

1.4.2 What do QTL studies reveal about the genetics underlying fitness?

The first point to make is that nobody has ever performed a QTL study for fitness or a fitness-related trait in an unmanipulated wild population. In this section I discuss

why this is so, and to what extent QTL studies in other types of population or on other types of trait might be pertinent to our understanding of the genetics of fitness. The principal reason that there has been no QTL study on fitness within a population is that fitness itself is so hard to measure in the wild (Endler 1986). Even if the QTL analysis is on a trait related to fitness, measurements of fitness or lifetime reproductive success have to be made to establish that link. For those field studies with the relevant data on fitness a number of further obstacles can hinder QTL analysis. First, individuals need to be sampled and a pedigree established before screening of markers can begin. Second, a sufficient number of markers need to be obtained and a linkage map constructed. Although this is possible for almost any species, developing and mapping a large panel of markers is time-consuming and expensive. Even if the above requirements are all met, analysis of a randomly-mating population is complicated and sample sizes need to be large.

A second consideration when reviewing the literature on QTL analysis is the problem of over-estimation of allelic effect (Roff 1997). In an often overlooked, yet important paper Beavis (1994) used empirical and simulated data to illustrate this phenomenon. First, by sub-dividing a mapping population of 400 individuals into four samples of 100, the dependency of estimated QTL magnitude on sample size was examined. Data were taken from a study that had mapped QTL for plant height in maize. When the QTL from the larger experiment could be detected in the sub-samples their effect was approximately double the original estimate. Beavis (1994) also carried out simulations to further investigate this effect. Two populations, one with 10 additive loci, and the other 40 additive loci contributing to the trait were simulated. Heritability was set at 0.30, 0.63 or 0.95 and the remaining variation sampled randomly across individuals. All QTL were on different chromosomes. Sample size was set to 100, 500 or 1000 individuals. The strength of this study is that the actual QTL effects was known and so the degree of over-estimate could be determined. When both sample size and the actual percentage variation explained by each QTL were small (100 individuals and 1.6%) the QTL effect could be overestimated by twenty-fold (Beavis 1994). Roff (1997) presents data from the literature, comparing the sample size used in mapping studies and the proportion of

phenotypic variation explained by QTL detected. There is a strong negative relationship between the two, suggesting that those studies that rely on a small sample size are prone to overestimate QTL magnitude. The reason for these observations is that the QTL actually detected are those with magnitude overestimated by chance sampling (Haley 1995). The smaller the sample, the more likely the occurrence of chance sampling. In the discussion of QTL studies that follows a number of large effects are reported. It must be remembered that a good deal of these are likely to be overestimates.

Given the lack of QTL studies on wild populations what can studies on model organisms or on artificial crosses tell us? Most of the discussion that follows is inevitably biased towards studies involving some kind of cross, as within population studies are still relatively rare. The relevance of a trait under stabilising selection in a laboratory organism, to a trait under directional selection in the wild is questionable. However, a review of the literature suggests that general patterns are beginning to emerge, in particular with respect to QTL magnitude. Unless otherwise stated, I will use the Falconer and Mackay (1996) definition of a major gene as one where an allelic substitution has an effect of at least 0.5 of a phenotypic SD.

The best understood quantitative traits are probably abdominal and sternoplural bristle number in *Drosophila melanogaster*. In a seminal paper Shrimpton and Robertson (1988) used visible markers to detect 17 loci on chromosome 3 that were associated with sternoplural bristle number. A number of these loci were of very large effect (as high as 2.8 phenotypic standard deviations), suggesting that additive genetic variation for this trait was due to a few loci of large effect and many more of smaller effect. It should be pointed out that the two lines being tested were derived from a high and a low selection line, so many of the QTL could be due to fixation of new mutations or rare alleles. However, Long *et al.* (1995) performed a similar analysis in which the lines were under weak selection (25%), in an attempt to maximise the probability that any QTL were due to pre-existing alleles rather than new mutants. No 'jumps' were observed in the response to selection, suggesting this was indeed the case, and again QTL of large effect were mapped. Mackay and

Langley (1990) showed that molecular variants at the achaete-scute complex in natural populations of *Drosophila* were associated with bristle number, suggesting that major genes segregate in the wild for this trait. A general picture from the *Drosophila* bristle number studies is that much of the genetic variation can be attributed to a few loci of large effect, and that these loci are often candidate loci (Mackay 1995). Furthermore, allelic effects often appear to be sex specific and may involve epistasis (Long *et al.* 1995; Mackay 1995). For this ‘classical quantitative trait’ at least, the infinitesimal model does not adequately explain the underlying genetic variation.

A number of studies have used QTL analysis to investigate the genetics of reproductive isolation in plants. Bradshaw Jr *et al.* (1995) created an F2 population from two sympatric species of monkeyflower, the insect pollinated *Mimulus lewisii* and the humming-bird pollinated *M. cardinalis*. The two species can be readily crossed in the laboratory but hybrids have never been observed in the wild. Eight floral traits associated with reproductive isolation were investigated and a major gene (classified here as being responsible for at least 25% of the phenotypic variation) was found for every one of them. Although the magnitude of these QTL appears impressive, only 93 F2 plants, a relatively small number, were used. To minimize the likelihood that allelic effects were overestimated Bradshaw Jr *et al.* (1998) repeated the experiment with a larger ($n = 465$) F2 population and also examined more traits ($n = 12$). Nine of the 12 traits had a QTL explaining 25% of the phenotypic variance and 11 of the 12 QTL found in the first experiment were detected in the second. However, of the nine major genes detected in experiment 1, only four were of similar magnitude in experiment 2. It seems that there was an upward bias in QTL magnitude in the first experiment, but that major genes were still present.

Lin and Ritland (1997) investigated the genetics of selfing rate by crossing two species of *Mimulus*; the inbreeding *M. platycalyx*, and the outbreeding *M. guttatus*. Six mating system characters were examined, of which QTL were found for five of them. In this study, the amount of overall phenotypic variation explained by individual QTL was lower than that observed in the Bradshaw Jr *et al.* (1995, 1998)

studies, and only one QTL explained more than 25% of the trait variation. The authors concluded that the majority of the phenotypic variation was due to polygenes of magnitude too small to detect, but that some genes of major effect may be segregating. Rieseberg *et al.* (1999) used an original approach to investigate the genetics of reproductive barriers in three natural hybrid zones between two sunflower species, *Helianthus petiolaris* and *H. annuus*. Maximum likelihood methods were used to detect regions with a significant underrepresentation of *H. petiolaris* ancestry. A conservative estimate of 16 'chromosomal segments' were associated with low pollen viability, suggesting that these regions contain genes responsible for reproductive isolation. It would appear that a large number of loci are responsible for reproductive barriers between the two species, perhaps suggesting that major genes are not segregating in these hybrid zones. In conclusion it appears that the number and magnitude of genes implicated in reproductive isolation may differ substantially between studies. It is possible that major genes are segregating, but care must be taken to distinguish major genes from upward-biased estimates of QTL magnitude. Those QTL that are found represent genes responsible for differences *between* species rather than genes underlying the variation observed *within* a species. Finally it is not clear whether some of the QTL observed are due to new mutations reaching fixation after speciation (Lin and Ritland 1997).

Kearsey and Farquhar (1998) reviewed 47 QTL studies (176 trial-trait combinations) in crop plants and their wild relatives, the brassicas and *Arabidopsis*. The majority of these experiments involved crosses between inbred lines. The mean number of QTL found was ≈ 4 (range = 0-16) and the percentage phenotypic variance explained by each had mean $\approx 15\%$ (range 1%-53%). A general observation is that most QTL are of magnitude 10% or less, but larger effects are not uncommon (perhaps 10-20% of QTL found). Again however, it must be remembered that some of these estimates will doubtless be inflated, and that QTL of large effect may be expected in experimental crosses between inbred lines.

It might be expected that QTL for production traits in livestock would be small as these traits have been under long term selection for decades or even centuries (Haley

1995). However, two large-scale searches for QTL associated with milk production in US Holstein cattle have revealed genes of large effect segregating (Georges *et al.* 1995; Zhang *et al.* 1998) despite the fact that milk production has increased almost 50% per cow in the last 20 years. A number of explanations are suggested for the continued segregation of major QTL, including negative pleiotropic effects, new mutations, or overestimation by chance sampling. The two studies used a granddaughter design (Weller *et al.* 1990, see Chapter 3.1) involving the same 14 paternal half-sib families and arrived at broadly similar conclusions. The study by Zhang *et al.* (1998) is notable in that it is the first time a variance components method has been used in an outbred population. A detailed review of additional QTL studies in livestock is not given here, particularly as many mapping programs involve crosses between breeds, but the occurrence of major genes is not uncommon (e.g. Andersson *et al.* 1994; Beh *et al.* 1998). At this stage it is worth noting that the intensity of selection observed in natural populations is often similar to that used in an artificial programme (Endler 1986). Natural populations have been subject to selection for far longer than artificial selection lines, but environmental heterogeneity or antagonistic pleiotropy may reduce the rate at which additive genetic variation is lost in the wild (Barton and Turelli 1989). Perhaps if major genes can segregate for traits such as milk yield, then they may also persist for fitness-related traits in the wild?

Few studies have attempted to map QTL for life history or fitness-related traits within a species (Mitchell-Olds 1996; Shook *et al.* 1996; Nuzhdin *et al.* 1997). None of these experiments has taken place in a natural population and all involved recombinant inbred lines. Nuzhdin *et al.* (1997) examined longevity in *Drosophila* and found 5 QTL of moderate to large effect. Shook *et al.* (1996) mapped QTL for both longevity and fertility in the nematode *Caenorhabditis elegans* and found that just three QTL explained $\approx 90\%$ of the genetic variance for each trait. Mitchell-Olds (1996) mapped QTL causing trade-offs between flowering time and growth rate in a cross between two lines of *Arabidopsis*. It remains to be seen whether these pleiotropic effects will be observed in a natural population. It is striking that four years after an influential review describing how QTL methodologies could be

applied to natural populations (Mitchell-Olds 1995), there are no studies that can truly claim to have mapped genes for a life history or fitness related trait in a natural population. On the face of it this may seem surprising, but very few research programmes have all of the necessary ingredients: a large collection of good life history data from the wild, a sufficient number of DNA samples, a known pedigree, and a genetic map with a large panel of suitably polymorphic markers.

Summarising the QTL literature it appears that major genes are detected for most types of trait regardless of experimental design. This is true for studies that look both within and between selected lines and species. Some caution must be taken as a good many major effects may be overestimates. However the idea that some of the additive genetic variation for fitness related traits in the wild is due to major genes is not unreasonable. Testing this hypothesis is currently impossible in all but a few natural populations.

1.5 Aims and outline of thesis

The principal goal of this study was to try and map QTL for birth weight, a fitness related morphometric measure, in a free-living population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland. The aims of this thesis are summarised below.

- To describe the Rum deer population and the long term study of its behaviour, ecology and genetics. To identify traits suitable for QTL analysis (Chapter 2).
- To determine the power to detect QTL in the study population by alternative mapping strategies (Chapter 3).
- To generate a large panel of polymorphic DNA markers for constructing a deer genetic map and screening in the Rum population (Chapter 4).
- To use this large panel of markers to reassess paternities previously assigned in the study population with the software package CERVUS (Chapter 5).
- To construct a deer genetic map (Chapter 6)
- To attempt to map birth weight QTL in the study population (Chapter 7)

- To investigate the usefulness of molecular markers as tools to estimate dominance variance (Chapter 8).
- To investigate the role of dominance variance in the genetic architecture of both life history and morphometric traits (Chapter 9).
- To identify future questions that deserve to be addressed as a consequence of this thesis (Chapter 10).

2 Red deer on Rum

2.1 *The natural history of red deer*

Red deer (*Cervus elaphus*) inhabit a wide range across Europe, Asia and parts of North Africa. There is some debate (Lowe 1989; Emerson and Tate 1993) as to whether they are conspecific with the larger North American wapiti (*Cervus elaphus canadensis*), since they readily hybridise with each other. Red deer show considerable sexual dimorphism – males (stags) weigh about 70% more than females (hinds) as adults (Clutton-Brock *et al.* 1982), and also have antlers. It is estimated that there are around 300,000 red deer in Scotland (Youngson 1998), the population having increased since the last century when stalking became fashionable and a source of income for estates (Clutton-Brock and Albon 1989).

Breeding is seasonal – the rut takes place in autumn, and following a gestation of around 235 days (Clutton-Brock *et al.* 1982) a single calf is born the following May/June. Females begin breeding from three years of age (or earlier in well-nourished populations) and produce a calf most (but not all) years until 12 years old, after which fecundity declines (Clutton-Brock *et al.* 1982). It is usual for a female to mate only once, and very rare for her to mate with more than one stag (Clutton-Brock *et al.* 1982), making paternity inference by behavioural methods possible. Males disperse from the natal group when aged 2 to 3 and reach reproductive age at around five. From August bachelor groups begin to break up as stags become less tolerant of each other, engaging in roaring contests, parallel walking and fighting. Dominant males establish and defend harems of females during the rut, although these are generally quite fluid, with younger stags opportunistically driving females away (Clutton-Brock *et al.* 1982).

2.2 *Background to the Rum project*

2.2.1 History of the population

The 10,600 ha island of Rum (57°0' N, 6°20' W) lies 19 km from mainland Scotland in the Inner Hebrides. The island is largely deforested and since 1957 has been owned by the Nature Conservancy Council (the NCC; now Scottish Natural Heritage, SNH) with a view to running it as an 'open air laboratory'. In addition to the deer project a programme to reintroduce the white-tailed eagle (*Haliaeetus albicilla*) to Britain has

been carried out on Rum, as well as attempts to regenerate native woodland from local seed stocks.

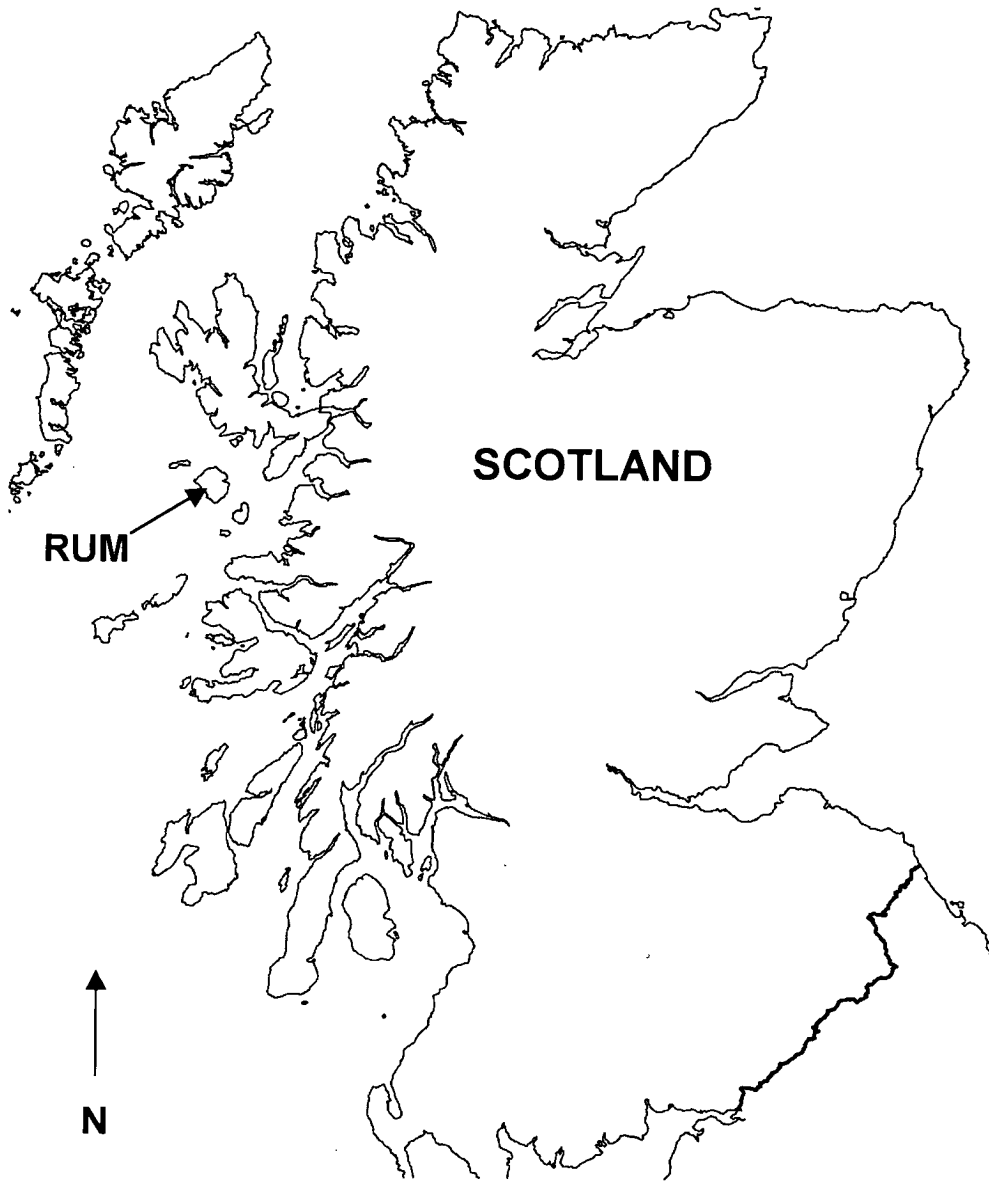


Figure 2.1: Map of Scotland, showing the location of Rum

It is not known whether red deer were brought to Rum by man or if they occurred naturally. The first record of their existence dates back to 1549 and they appear to have been abundant in the early 1700's (Clutton-Brock *et al.* 1982). Following the destruction of woodland, sheep farming became prevalent and deer numbers declined, until by 1787 they became extinct (Clutton-Brock *et al.* 1982). The human population also began to decline around this period, and most inhabitants were evicted in 1827

during the Highland Clearances (Rose 1995). In 1845 Rum was restocked with deer for stalking purposes, and following the purchase of the island by the Bullough family in 1886, further introductions were made. By 1939 the population had risen to between 1200 and 1700 (Clutton-Brock *et al.* 1982). A more detailed account of the introductions of deer to Rum from mainland Britain is given in Marshall (1998), from which it is apparent that the current population is derived from at least five British parks or estates. A culling regime has maintained the population between 1100 and 1900 for the last forty years (Marshall 1998).

The last introduction occurred unintentionally in 1972 and is of great relevance to this study. In the winter of 1969-1970 a hummel (antler-less stag) from Braemar, North-East Scotland was moved to an enclosure on Rum and crossed with a number of Rum hinds, as part of an experiment to investigate the inheritance of hummellism. The male offspring all had normal antlers (Lincoln *et al.* 1973), and following vasectomy operations all were released on Rum in 1972. However, one stag, MAXI, rutted in the study area in 1975 and went on to become a highly successful breeder, with many paternities confirmed by molecular methods (Pemberton *et al.* 1992; Marshall 1998), so his vasectomy was unsuccessful. From known mother-offspring relationships and using genetic techniques to infer paternity, it is estimated that at least 300 descendants of MAXI have been born into the study population. There is no evidence that the other vasectomised stags went on to breed. Chapter 3 of this thesis concerns the applicability in QTL analysis of the extended pedigree descending from MAXI.

2.2.2 The study area and field methods

The long term red deer project started in 1968 and is based around Kilmory Glen (see Figure 2.2) at the North of the island. Early studies on the reproductive biology of males required close observation, and so animals were fed concentrates until 1970 (Clutton-Brock *et al.* 1982). Attracted by this regime, a number of males immigrated into the study area during this period. In 1973 culling in the study area ceased and the population rose from 199 animals aged greater than one year old in 1973, to 284 in 1979. Since 1982 the population has remained relatively stable with a mean of 162 females and 109 males. Note also that the adult sex ratio has changed from being male to female biased. Five censuses of the study area are taken every month, and every animal is recognisable by either natural or artificial markings. Additionally, some

immigrant rutting stags are recognised annually. Animals born in the study area may be shot if they spend substantial time out of the study area, since culling continues on other parts of the island. During both the rut and calving censuses are carried out daily. Consequently, harem membership is recorded, enabling behavioural estimates of paternity.

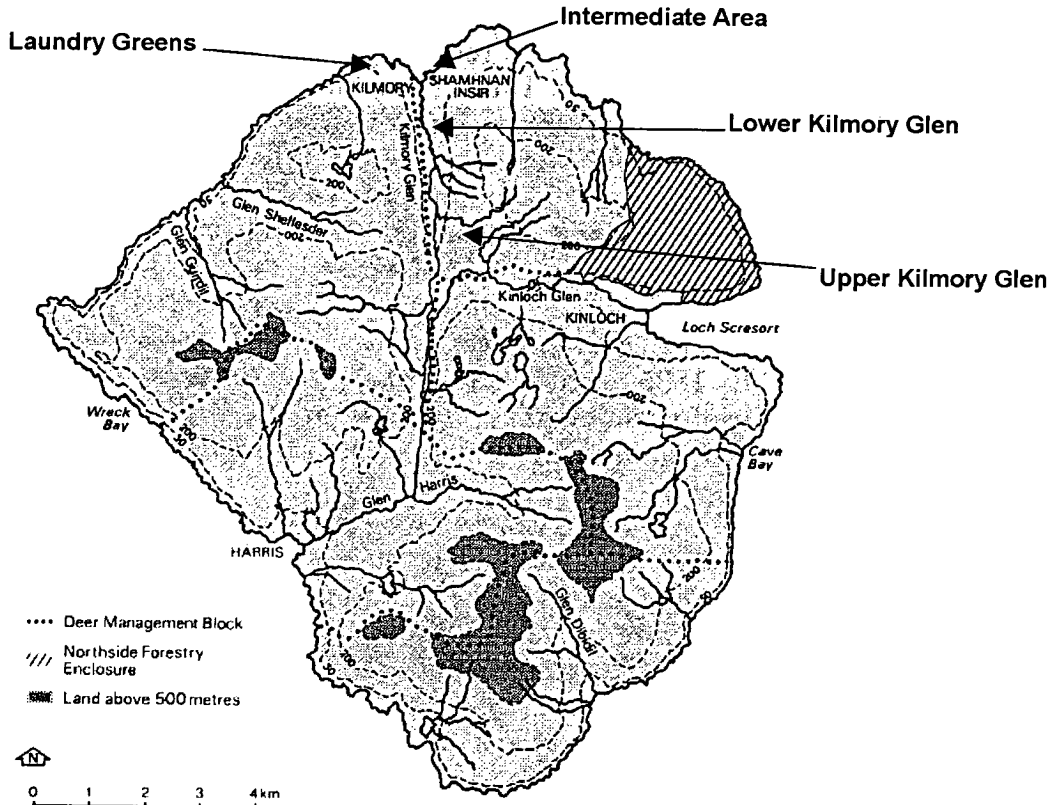


Figure 2.2. Map of Rum adapted from Clutton-Brock *et al.*, 1982.

The Kilmory study area is the management block in the NE section of the map. Note that the study area is below 500m and unforested, aiding identification of the deer.

Since 1970 around 60% (80% in recent years) of calves have been caught in the first three weeks after birth. On capture they are sexed, weighed and marked. Maternity of all calves is known from behavioural data. From 1982 onwards sampling for genetic purposes has also been performed (Pemberton *et al.* 1988). Markings include numbered ear tags, coloured flashes, ear punches and, for females, expandable coloured collars. A number of animals are captured as adults by darting them with the drug immobilon, after which they are marked (collars can be fitted to both sexes as adults), measured and

sampled. Additionally, most animals have natural markings and can be recognised by an experienced field worker on the basis of these alone. Animals lose many of the artificial markings over time, and so the ability to recognise unmarked animals is important to keep track of them over their entire lifespan. The carcasses of approximately 70% of animals that die in the study area are found, and can be measured and sampled. Since 1971 detailed life histories have been collected on well over 2,500 deer, of which approximately 2,000 have been sampled for genetic analysis.

2.3 Ecological and behavioural work

A detailed account of the first decade of the Rum study is given in the book *Red Deer - Behavior and Ecology of Two Sexes* (Clutton-Brock *et al.* 1982). The book focuses on hinds and stags separately and in particular on the factors that influence reproductive success. The book is regarded as a classic study of a species' ecology. I will outline the more important findings here, in particular those that describe how the environment influences fitness and fitness components, as they are of greatest relevance to this thesis.

Juvenile survival:

Approximately 18% of calves die as neonates (Guinness *et al.* 1978; Coulson *et al.* 1998b). Causes of death include desertion, drowning in burns and predation by eagles. Both birth weight and the mean temperature in June are positively associated with neonatal survival (Guinness *et al.* 1978; Coulson *et al.* 1998b). There is no evidence of a sex difference in neonatal mortality.

Of calves surviving their first summer a further 25% die during their first winter (Coulson *et al.* in 1999). Male calf survival is lower than that of females (Coulson *et al.* 1998a; Coulson *et al.* 1999). Survival is positively associated with birth weight (Clutton-Brock *et al.* 1987; Coulson *et al.* 1998a), although later analyses suggest this is true for males only (Coulson *et al.* 1998b; Coulson *et al.* 1999). First winter survival is negatively associated with density, local population density (see Appendix A), birth date (the number of days after May 1st on which the calf was born) and autumn rainfall (Guinness *et al.* 1978; Albon *et al.* 1987; Clutton-Brock *et al.* 1987; Coulson *et al.* 1998a; Coulson *et al.* 1999). Female first winter survival is also influenced by mother's reproductive status (whether or not she had given birth to a calf the previous year, see Appendix A) and is negatively associated with mother's age (Coulson *et al.* 1999).

Female lifetime reproductive success:

Lifetime reproductive success (LRS; see Appendix A) is defined as the total number of offspring born to an individual, that survive to age two. Female LRS can be considered in terms of its components (fecundity, longevity, lifetime breeding success and offspring survival), all of which are strongly and positively associated with overall fitness (Kruuk *et al.* 1999b). Mean fecundity (the proportion of years, after breeding age is reached, in which a female produces a calf) is approximately 0.70 (Clutton-Brock *et al.* 1983). Fecundity is positively associated with spring temperature in the year of birth (Kruuk *et al.* 1999b), and negatively associated with density both in the year of birth (Kruuk *et al.* 1999b) and the breeding year studied (Albon *et al.* 1983b). It is also influenced by reproductive status and body condition (Albon *et al.* 1983b; Albon *et al.* 1987).

In their analysis of female reproductive success on Rum, Kruuk *et al.* (1999b) found that both spring temperature and density in the year of birth influenced lifetime breeding success (LBS, see Appendix A) and LRS. Longevity was also positively associated with mean spring temperature in the year of birth, and offspring survival was associated with local density (see Appendix A) in the mother's year of birth.

Male lifetime reproductive success:

Amongst males that reach breeding age, LRS is principally determined by lifetime breeding success (Clutton-Brock *et al.* 1988; Rose 1995; Kruuk *et al.* 1999b). Birth weight is positively associated with LBS, offspring survival, maximum annual breeding success (ABS, see Appendix A) and overall LRS (Clutton-Brock *et al.* 1988; Rose 1995; Kruuk *et al.* 1999b). Lifetime reproductive success appears to be partly determined at an early age in both sexes. However, environmental variables such as density and mean spring temperature are the most important variables influencing female LRS, whereas birth weight, which is at least partially genetically determined (see next section), is more important in males (Kruuk *et al.* 1999b).

Sex ratio at birth: It has been suggested for mammals with greater variation in reproductive success among males than females, and where reproductive success is influenced by maternal investment, that mothers in good condition should produce male

offspring (Trivers and Willard 1973). This hypothesis was empirically demonstrated in the Rum deer when it was shown that dominant mothers produced a higher proportion of male calves than subordinates, and that maternal dominance rank influenced mating success of male offspring (Clutton-Brock *et al.* 1984). However this relationship is not straightforward, and is dependent on population density (Kruuk *et al.* 1999a). After culling in the study area ceased and the population density increased, the proportion of males born decreased. Furthermore, following winters of high rainfall the sex ratio is female biased, suggesting differential foetal mortality between the sexes. Thus, not only does the Rum study provide support for the Trivers-Willard hypothesis, but it also demonstrates that sex ratio may also be determined by environmental conditions when *in utero*. The influence of potentially confounding environmental effects may explain why a greater number of studies of mammalian populations have not provided similar support for Trivers-Willard (Kruuk *et al.* 1999a).

In this section I have described some of the environmental variables influencing various fitness components in the Rum red deer. An important point to make is that many of these effects would be impossible to detect in a shorter term or cross-sectional study. In the following section I describe some of the genetic studies that have been made in the population. The majority of these studies are dependent on the fact that much of the environmental variation can be partitioned out, enabling more subtle genetic effects to be detected. Without the ability to detect this environmental variation many of the genetic studies described would have been impossible. The ability to partition out environmental variation will also aid QTL analysis in the population.

2.4 Genetic studies

2.4.1 Associations between genotype and fitness components

In Chapter 1 I described how reported associations between allozyme variation and components of fitness provide tentative support for the existence of genetic variation for fitness. Since sampling of new-born calves began in 1982 a number of genetic analyses have been carried out on the Rum deer. In the first of these, Pemberton *et al.* (1988) found polymorphisms at 8 out of 33 allozyme systems, and three of these (*Mpi*, *Idh-2* and *Trf*) were screened for the 1982-1985 cohorts. In addition to the environmental factors described earlier, all three loci were associated with juvenile survival (to two years of age). Amongst studies reporting allozyme-fitness associations this one is

notable in that potentially confounding environmental effects are considered, the sample size is relatively large, and fitness data are accurately measured rather than inferred from morphometric measures. The most surprising finding from this study was that one *Mpi* allele (*f*) was negatively associated with juvenile survival yet present at high frequency in the population.

A follow-up study (Pemberton *et al.* 1991) examined variation at two of the same three loci, but with respect to fitness components associated with female breeding success. Again associations with genotype were reported, and strikingly those genotypes negatively associated with juvenile survival were generally positively associated with female reproductive success. Thus this study provides rare empirical evidence for countervailing selection (Rose 1982) operating to maintain genetic polymorphisms in a natural population. It has been suggested that at low population density *Mpi f* carriers did not experience reduced juvenile survival, yet still had enhanced reproductive success, explaining the high frequency of this allele in the early years of the study (Pemberton *et al.* 1991; Pemberton *et al.* 1996). As the population density increased, so did juvenile mortality (Clutton-Brock *et al.* 1987), particularly amongst *Mpi f* carriers and so the frequency of the *f* allele decreased (Pemberton *et al.* 1991).

Subsequently, Coulson *et al.* (1998a) investigated genotype and juvenile survival, but included a further nine cohorts of calves and genotypes at ten microsatellite loci (described in Marshall *et al.* 1998; Pemberton and Slate 1994). Of the 13 loci examined, associations with calf survival were found at seven, including both *Trf* and *Idh-2*. Five of these seven associations involved an interaction with an environmental term (usually population density) such that the detrimental effects of particular alleles were greater under poor conditions (e.g. high density, high autumn rainfall).

The study by Coulson *et al.* (1998a) raises a number of issues important to fitness-genotype association studies. By using a larger data set some of the associations found by Pemberton *et al.* (1988, 1991) no longer appeared significant, most notably those between juvenile survival and *Mpi* genotype. Other associations initially appeared significant, but following randomisation tests disappeared, presumably because there were too few individuals in some genotype classes (a problem more likely when highly variable microsatellite markers are used). In the statistical models employed by Coulson

et al. (1998a) environmental variables explained around one third of the variation in survival. Also most fitness-genotype associations were genotype by environment interaction terms rather than straightforward consistent fitness differences between alleles. Clearly, adequate measurements of environmental variables are required to satisfactorily perform genotype-fitness association studies in the wild. A point made by Pemberton *et al.* (1988) is that the allozymes themselves may be responsible for the observed differences in fitness between genotypic classes, or they may simply be markers detecting the influence of linked loci. The fact that associations between genotype and fitness were equally common for both functional allozymes and non-coding microsatellite loci in the study by Coulson *et al.* (1998a) provides weak support for the latter hypothesis.

Given that fitness components generally have low heritabilities in the wild (Mousseau and Roff 1987; Chapter 1) it is surprising that such a high proportion of the loci studied by Coulson *et al.* (1998a) were associated with calf survival. It is possible that each marker is representing a large proportion of the genome, resulting from linkage disequilibrium in the population due to recent introductions (Pemberton *et al.* 1996; Coulson *et al.* 1998a). Alternatively, a number of the reported associations could be spurious. At least three different mechanisms could be responsible for false associations.

First, the microsatellite markers used in the Coulson *et al.* (1998) study were initially selected for paternity analysis, and hence were multi-allelic (Marshall *et al.* 1998). Therefore, despite the large sample size, the number of individuals in certain genotype $\times$ environmental variable cells was small, leading to increased risk of Type I error. However the randomisation tests used by Coulson *et al.* (1998a) should detect any such false findings, although this is a general problem that should be considered in any genotype/fitness association study. Second, particular alleles may be non-randomly distributed across the study area such that differences in fitness due to environmental quality may appear to be due to genotype. Third, animals sharing particular alleles may be related, so that associations between that allele and a component of fitness may really be due to polygenic effects (Coulson *et al.* 1998a). By carrying out a QTL analysis within a pedigree, these problems common to association studies can be avoided.

2.4.2 Paternity Analysis:

Attempts to determine paternity in the Rum population have been motivated by the desire to estimate lifetime reproductive success amongst males, and to derive pedigrees so that quantitative genetic and inbreeding depression studies could be performed. In order to perform a QTL analysis in the population it is essential to have as detailed and accurate pedigree information as possible. In red deer, male lifetime reproductive success has a greater variance than female LRS (Clutton-Brock *et al.* 1982; Clutton-Brock *et al.* 1988), and so some stags are expected to have sired a large number of calves. Identifying and sampling the progeny of successful males may be essential to obtain sufficient power to perform QTL analysis (see Chapter 3). In this section I describe the methods that have been used to infer paternity in the population.

Initially, paternity was inferred using purely behavioural data (Clutton-Brock *et al.* 1982). From a sample of 70 hinds observed in oestrus, and for whom the birth date of the calf was known, it was estimated that male calves had a gestation of 236.1 ± 4.75 days and females a gestation of 234.2 ± 5.04 days (Clutton-Brock *et al.* 1982). Hence, by backdating from the estimated calving date the conception date could be estimated. Candidate fathers were stags observed holding the hind in their harems during the five days (1 SD) either side of the date, a period known as the ‘11-day window’. Two alternative approaches were used to infer paternity once candidate males were established. The *fractional* method awards 1/11 of a paternity to a male for every day he holds the hind in his harem. Hence multiple males may sire fractions of a paternity for a particular calf. The *longest hold* method awards paternity exclusively to the male that holds the hind in his harem for the most days during the 11-day window. In the event of a tie no paternity was awarded (although Kruuk *et al.* (1999b) randomly assigned paternity to one male in their analysis). Rose (1995) points out that although they claimed to use the fractional method in their analyses Clutton-Brock *et al.* (1982, 1988) actually used the longest hold approach.

Genetic methods to infer paternity in the Rum deer were developed by Pemberton *et al.* (1992), following the invention of DNA fingerprinting (Jeffreys *et al.* 1985) and the realisation it could be used for parentage in natural populations (Burke and Bruford 1987). DNA fingerprints of calf, mother and candidate fathers (from rut behavioural data) were run in adjacent lanes on gels for 107 calves, mainly from the 1982 and 1983

cohorts. Paternity was inferred for 80 calves, and in 74 the genetically assigned father was also observed holding the mother in his harem during the 11-day window. These genetic paternities were used by Pemberton *et al.* (1992) to investigate the accuracy of the behavioural methods used in earlier studies. They concluded that behavioural methods gave accurate estimates of relative mating success amongst males. However the variance in mating success was underestimated, and in particular the number of calves sired by successful stags was as much as three times greater than behavioural observations had suggested (Pemberton *et al.* 1992). Comparisons were also made between the 11-day fractional method of assigning paternity, and more sophisticated algorithms (for example, extending the 11-day window to a 2 SD 21-day window, and weighting probability of paternity according to a normal distribution around the estimated conception date). However, the simpler 11-day window method performed as well as any of the more complicated behavioural methods (Pemberton *et al.* 1992).

A problem with the fingerprinting method used by Pemberton *et al.* (1992) is that all candidate males must be run alongside the mother and calf on a gel. Hence identifying a true father from many candidates is laborious, and in the absence of candidate males is even more difficult. Ideally, large scale paternity analysis, in which there may be numerous candidate fathers, should use markers for which band patterns or sizes can be stored in a database, enabling comparison across gels (Pemberton *et al.* 1992). The advent of polymorphic microsatellite loci allowed such an approach (Queller *et al.* 1993, Chapter 4), and from 1993 microsatellites were routinely screened for paternity analysis in the Rum population (Pemberton and Slate 1994).

Tristan Marshall, a previous graduate student in the laboratory, developed a likelihood-based computer program, CERVUS for assigning paternity in natural populations when using codominant genetic markers such as microsatellites (Marshall 1998; Marshall *et al.* 1998; Chapter 5). Not only does CERVUS award paternity to the most likely male when there are several genetically compatible candidates, but it also uses simulation so that paternities can be inferred with any required statistical confidence. In Chapter 5 I discuss CERVUS in greater detail, and also perform an empirical investigation into the accuracy of the statistical confidence given by CERVUS when awarding paternities.

Using nine microsatellite markers and three protein loci, Marshall (1998) used CERVUS to infer paternity for over 500 calves. Around half of these paternities were assigned with 95% confidence and the remainder at 80% confidence. The principal goal of this analysis was to determine a pedigree so that inbreeding coefficients could be calculated, to investigate the extent and consequences of inbreeding in the Rum population. However, this study also enabled further investigation into the accuracy of awarding paternity by behavioural methods.

In his reassessment of previous paternity inference methods, Marshall (1998) showed that all of the fathers identified in the fingerprinting study by Pemberton *et al.* (1992) were correct. Therefore the high confidence expressed by Pemberton *et al.* (1992) in their genetically derived paternities appears justified. However there is one important discrepancy between the studies. Pemberton *et al.* (1992) concluded that if a stag was seen holding a hind for five or more days during the 11-day window there was a 90% probability he was the father of the subsequent calf, whereas Marshall (1998) gave a figure of around 75% correct identification. He argued that Pemberton *et al.* (1992) used a biased sample of calves in their analysis. Stags were only regarded as candidate males if they were observed holding a hind during the 11-day window. Therefore cases where the longest-hold stag was not the true father, and the true father was not observed during the 11-day window would be excluded from the analysis. This led to an exaggerated confidence in behaviourally determined paternities. A number of the analyses I perform in this thesis rely on inferred paternities being correct. In Chapter 5 I analyse paternities assigned at 80% and 95% confidence by CERVUS, and derive criteria for adjusting the confidence in genetically inferred paternities in this population given certain behavioural data.

2.4.3 Inbreeding Studies:

The detailed life history data and the existence of a documented pedigree make the Rum deer an ideal population in which to study the frequency and consequences of inbreeding in the wild (Marshall 1998). Close inbreeding (between full sibs or parent-offspring) in the study area appears to be rare (around 1.5% of matings) although inbreeding between moderately related individuals (particularly paternal half sibs) is more common (Marshall *et al.* submitted). It is estimated that 13.4% of calves are moderately or closely inbred (Marshall *et al.* submitted).

Although paternity has been assigned for an impressive number of calves, the pedigree is still sufficiently ‘gappy’ to make the use of inbreeding coefficients (Falconer 1989) inappropriate for investigating inbreeding depression (Marshall 1998). Alternative marker-based measures to detect inbreeding depression include multi-locus heterozygosity (David 1998; Marshall 1998) and a recently derived statistic *mean d^2* (Coulson *et al.* 1998b; Pemberton *et al.* 1999). In Chapter 8 I discuss these methods in greater detail, but for now it is sufficient to say that both methods have been used to detect the consequences of inbreeding in this population. There is good evidence that inbred deer in this population have reduced birth weight and neonatal survival (Coulson *et al.* 1998b), and that inbred female calves have reduced first winter survival (Coulson *et al.* 1999). There is weaker evidence that inbreeding influences lifetime mating success in both sexes (Marshall 1998). In Chapters 8 and 9 I use multi-locus heterozygosity to further analyse the effects of inbreeding in the population, and also to test the prediction that traits closely related to fitness exhibit more dominance variation than traits less strongly associated with fitness (Roff 1997; Merilä and Sheldon 1999).

2.4.4 Quantitative genetic studies:

Rose (1995) estimated the heritability of lifetime reproductive success (LRS) and various fitness components for males and females. I will not include a comprehensive discussion here, as Kruuk *et al.* (in press) used a more rigorous approach in performing a similar analysis. Rose (1995) used a paternal half-sib design to reduce the risk of obtaining inflated estimates of heritability through maternal effects, shared environment effects and dominance variance (Falconer 1989; Roff 1997). However, a number of traits had heritability estimates greater than 1, suggesting that environmental variation may be contributing to the paternal genetic effect. Rose (1995) suggested that a correlation between stag quality and rutting area quality may be responsible for these inflated estimates. In fact, paternal half-sibs are likely to share a common environment, causing overestimates of the paternal polygenic variance component, regardless of any correlation between stag quality and rutting area quality. Traits with heritability estimates >1 were those expressed early in life, so it seems probable that a shared environment between paternal half-sibs as neonates or *in utero* was the confounding factor in this analysis.

The approach used by Kruuk *et al.* (in press) to estimate heritabilities was more robust than that taken by Rose (1995) due to the method used and by virtue of having a larger sample size. In addition to providing a rare estimate of heritability of fitness in a wild population, this study is notable for a number of other reasons. First, a restricted maximum likelihood (REML) method was used, so that a number of the (frequently violated) assumptions of parent-offspring methods could be ignored (Falconer 1989). By using an ‘animal model’ (Lynch and Walsh 1998), the entire pedigree of 2,374 individuals was considered simultaneously, so that additional information to that used in a parent-offspring regression method was obtained. Second, variance components for each trait were estimated, so that comparisons between h^2 , CV_A and CV_R could be made. Third, the father-offspring relationships in this study were either genetically inferred, or were awarded to males holding a harem for 6 days or longer (i.e. 75% confidence could be attached to them). Thus the problem of wrongly-assigned paternities causing components of additive genetic variation to be underestimated was largely avoided.

As discussed in Chapter 1, Kruuk *et al.* (in press) found that overall fitness had a heritability close to zero, fitness components had lower heritability than morphometric traits, and that the coefficients of additive genetic variation and residual variation provided some support for Houle (1992). In other words, some traits closely related to fitness had high CV_A and CV_R , so that the observation of a low heritability was not incompatible with the notion that there was additive genetic variation for fitness components.

2.5. Which traits are suitable for QTL analysis?

In section 1.4.2 I discussed why a QTL study has never been performed in a wild outbred population. In this section I explain why the Rum deer provide a unique resource with which it may be possible to search for QTL for fitness components, and suggest which traits may be most suitable to study. First, consider the necessary requirements to perform this type of analysis: detailed life history data, a known pedigree, a large panel of mapped markers and genetic samples from a sufficiently large number of individuals. In this Chapter I have described how life history data was collected from over 2,500 deer, and how environmental factors are known to influence fitness components. I have described how maternity is known with near-certainty and how paternity can be inferred with statistical confidence, so that a sufficiently large

pedigree is available with which to perform a QTL analysis. Comprehensive details of how a large panel of markers were characterised and mapped are included in Chapters 4 and 6, but a brief background to the deer genetic map follows.

Since 1990 Mike Tate and co-workers (AgResearch, New Zealand) have been involved in a deer genetic mapping program. Red deer and Père David's deer (*Elaphurus davidianus*) have been hybridised and F1 males backcrossed to red deer hinds. A large panel of markers have been mapped by linkage analysis in the back-cross generation (see Tate *et al.* 1994; Tate *et al.* 1995; Tate 1997; Chapter 6) and QTL analyses for gestation length and live weight traits have also been performed (see Goosen 1997; Goosen *et al.* 1998; Chapter 3). For the purposes of this chapter the important point is that a deer genetic map was already developed at the outset of this study, making QTL mapping in the Rum population a possibility.

Given that QTL mapping is feasible in the Rum population, what traits might be most suitable for analysis? An ideal trait would be heritable (or have a high CV_A), be a component of fitness (so that it is under directional selection), and any potentially confounding environmental factors influencing the trait would be known. Traits most closely related to fitness (e.g. lifetime breeding success) are unsuitable as many animals do not live to breeding age, reducing the available sample size. In the analysis by Kruuk *et al.* (in press) the only life history traits with a heritability significantly different from zero were expressed in females only, once again causing problems of reduced sample size. A close inspection of quantitative genetic parameters would suggest that birth weight may be a suitable trait for QTL analysis. Heritability of birth weight is reasonably large (0.283 ± 0.073 for females; 0.149 ± 0.067 for males), CV_A is greater than for all other morphometric traits and a number of life history traits, and also birth weight is correlated with overall fitness ($r = 0.160$ for females; $r = 0.182$ for males). Birth weight is one of the most frequently and accurately measured traits in the population, making it further suitable for QTL analysis. Although not strictly speaking a fitness component, birth weight is associated with a number of fitness components, in particular lifetime reproductive success in males (Kruuk *et al.* 1999b; Rose 1995). Thus it is a suitable trait with which to examine whether or not alleles of large effect can persist in the face of directional selection.

2.6 Are descendants of MAXI born heavier?

In section 2.2.1 I described the recent introduction of the stag MAXI (Lincoln *et al.* 1973) to the study area. MAXI not only rutted successfully for over five years, but he also sired other successful stags. Given that MAXI's father was not from Rum, it is likely that novel genetic variation was introduced into the population. Consequently, new additive genetic variation could have been introduced, or if there is dominance variance for any traits, heterosis (Falconer 1989) could occur. This section describes an analysis I performed to determine whether MAXI descendants were born heavier than non-MAXI descendants. If MAXI's descendants were born heavier, it would imply that there has been an input of new genetic variation.

Generalized linear models (GLMs) were used to investigate factors associated with birth weight. Not only would this allow me to determine the effect of the MAXI introduction on birth weight, but it would also enable me to detect additional environmental factors that could be accounted for when performing a QTL analysis. A number of variables influencing birth weight in this population have been described previously (Albon *et al.* 1983a; Coulson *et al.* 1998b), but in this analysis I use a larger sample of 1,072 deer born between 1970 and 1996.

I used modelling techniques described in Crawley (1993), implemented in SPLUS 4.5 (MathSoft). I assumed a normal error structure and tested the significance of all terms (see below) as both main terms and interactions. Initially I constructed a model with all terms fitted. Comparison was made with alternative models in which each term was sequentially dropped. The difference in deviance between the full and reduced model is distributed as an F distribution, with the two degrees of freedom equal to the difference in d.f. between the models, and $n-k-1$ (where n is the number of individuals and k is the summed d.f. of the remaining terms in the reduced model). Terms were only retained if significant at $p < 0.05$. Main effects cannot be tested if they are also included as an interaction term, and so were tested by comparison with a model containing neither the main effect nor their interaction terms (Crawley 1993). No interaction terms were fitted if any cell size was lower than 30, as small cells can lead to Type 1 error.

Table 2.1 GLM for influence of being a 'MAXI' descendent on birth weight.

Null model deviance = 1732.1, full model deviance = 1415.4, deviance explained = 18.3 %.

Descendants of MAXI are born heavier than non-descendants. The model contained 1072 deer. For a full description of explanatory variables see Appendix A.

<i>Term</i>	<i>Term Type</i>	<i>d.f.</i>	<i>p</i>	<i>Deviance explained</i>	<i>Effect on birth weight</i>
Mother's reproductive status	Factor (5 levels)	4,1060	<0.0001	3.7%	Variable
Area	Factor (5 levels)	4,1056	<0.0001	3.6%	Variable
April-May mean temp	Continuous	1,1053	<0.0001	2.6%	+ ve
Sex	Factor (2 levels)	1,1053	<0.0001	1.7%	Males heavier
MAXI descendent	Factor (2 levels)	1,1053	<0.0001	1.2%	Heavier
Birth Date	Continuous	1,1057	0.0002	1.1 %	+ ve
Mother's age (Quadratic)	Continuous	2,1054	0.002	0.9%	+ ve
Rep. status × Birth Date	Interaction	4,1056	0.022	0.9%	Variable
Density	Continuous	1,1053	0.015	0.5%	- ve

Significant terms are shown in Figure 2.3. The only non-significant terms tested involved monthly rainfall in the winter/spring prior to birth. The two explanatory variables of greatest interest here are density and MAXI descendant. This is the first time that density has been shown to be negatively associated with birth weight in the study population, and may explain the apparently paradoxical result that birth weight is heritable and positively correlated with male LRS, and yet has not increased over the duration of the study (Rose 1995). A number of animals in the model were born before MAXI reached breeding age. However, if a reduced data set was used ($n = 970$) in which all animals were born after 1975, the model was almost identical (not shown). Thus, the effect of being a MAXI descendant on birth weight is not attributable to a greater proportion of non-descendants being born into earlier cohorts.

Descendants of MAXI were born on average 182g ($\pm$ SE 46g) heavier than non-descendants. The standard deviation of birth weight in the population is approximately 1.2 kg, thus the 'MAXI effect' is ~ 0.2 of a phenotypic standard deviation, a reasonably large effect. Obviously this is a crude measure as the analysis takes no account of how related any individual is to MAXI, and the 'MAXI effect' will be diluted for distantly related individuals. Furthermore, some animals may be descended from MAXI but classified as non-descendants due to gaps in the pedigree. These animals will tend to inflate the mean birth weight of the non-descendant group, giving a conservative estimate for the mean difference between the two groups. However, this analysis would suggest that MAXI has introduced novel genetic variation into the study population.

Summarising, in this Chapter I have described the history of the Rum red deer project. I have explained how it is a suitable population in which to perform a QTL mapping project and suggested that birth weight is a good trait for further study. Additionally, I have identified a recent founding event as a potential source of introduced genetic variation for birth weight. In Chapter 3 I describe a power analysis to determine the feasibility of detecting QTL segregating in the animals descended from MAXI.

3 Power to detect QTL in the study population

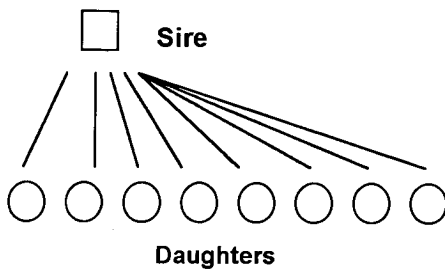
3.1 Introduction

In the first two chapters of this thesis I have explained how the Rum red deer might be a suitable natural population in which to attempt a QTL mapping experiment on a directionally selected trait. In this chapter I discuss different experimental designs that might be applied to detect QTL in the population, and present simulations that compare the statistical power of the various designs.

Chapter 1 discussed experimental designs for detecting QTL, and emphasised the distinction between designs that use inbred lines, and those that are performed in outbred populations. The former approach is more powerful (Lynch and Walsh 1998) but for many natural populations it is neither desirable nor possible to perform crosses. Clearly the Rum red deer are an outbred population in which designed crosses would be impractical or impossible. Thus, only experimental designs for outbred populations can be considered as options for this study. A number of mapping programmes have been performed in farmed ruminants (Georges *et al.* 1993a; Georges *et al.* 1993b; Montgomery *et al.* 1993; Georges *et al.* 1995; Zhang *et al.* 1998) and so this would appear an obvious point at which to start considering experimental design. Large families are common in farmed livestock breeds, with paternal half-sibships of several thousand not uncommon when artificial insemination (AI) is used (Weller *et al.* 1990).

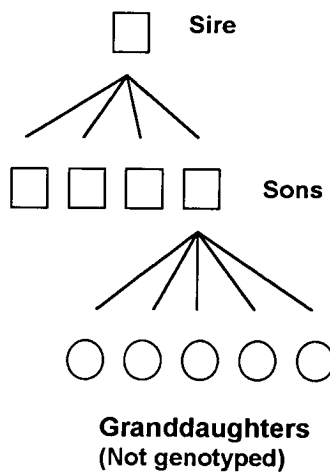
Considerable attention has been given in the animal breeding literature to the relative power of half-sib designs, and more complicated family structures, in detecting QTL (Weller *et al.* 1990; van der Beek *et al.* 1995). Weller *et al.* (1990) examined the power to detect QTL for the half-sib (or daughter) design and compared this to a three-generation granddaughter design (see Figure 3.1). In the daughter design each sire has a number of daughters by different dams. All sires and progeny are genotyped and progeny have recorded trait values. The alternative granddaughter design was developed with dairy cattle in mind: it requires sires to have a medium to large number of sons, who in turn sire a large number of daughters. The sons of the

sires are genotyped and trait values of their daughters are recorded. Breeding values of sons are accurately estimated by taking daughter phenotypic means. Weller *et al.* (1990) concluded that although not a particularly complex pedigree, the granddaughter design was a more powerful method than the half-sib design in detecting QTL. Additionally, less genotyping is required in obtaining this enhanced power. Georges *et al.* (1995) successfully used this design to detect milk production QTL in dairy cattle.



1) Daughter design.

Daughters are paternal half-sibs. Sire and daughters are genotyped, and an ANOVA is used to test for a mean trait difference between daughters inheriting alternative sire marker alleles.



2) Granddaughter design

Sires and sons are genotyped. Trait values only are recorded for granddaughters. The breeding value for each son is determined from the progeny trait mean. ANOVA is used to test for a difference in the breeding value between sons receiving alternative sire marker alleles.

Figure 3.1 Illustration of the daughter and granddaughter designs described in Weller *et al.* (1990).

Paternity analysis in the Rum study population has shown that there is considerable variation in male LRS (see Chapter 2) and that a number of successful stags have produced large paternal half-sibships. Although these sibships are not as large as those produced by AI in livestock, there are clearly similarities between the two breeding systems. Thus the daughter design must be considered as a plausible design for detecting QTL in the Rum deer. This method also has the advantage of being computationally simple (Weller *et al.* 1990). However, the analysis by Weller *et al.* (1990) suggests that more complex designs may have greater statistical power.

An alternative to a half-sib design is to use complex, extended pedigrees. Human geneticists have focused on the relative merits of extended pedigrees vs. nuclear families for detecting linkage. The recent Genetics Analysis Workshop 10 (GAW10) set a challenge to a number of groups to detect QTL for an oligogenic trait segregating in two simulated data sets, one containing nuclear families and the other an extended pedigree (Wijsman & Amos, 1997). The number of phenotypes and genotypes was equal for both data sets. Those analyses that used the extended data set were generally more successful in detecting the simulated disease loci, estimated QTL location more accurately and detected fewer false positives. Two properties of extended pedigrees lead to the increased power. First, there are more meioses in the extended pedigree than the nuclear family design for the same number of genotypes. Secondly, marker phase is estimated more accurately over several generations than one. Recent statistical advances have enabled geneticists to use information from extended pedigrees rather than their constituent nuclear families or sibships (Almasy & Blangero, 1998). Approaches such as these may enable evolutionary biologists to search for QTL without performing crosses within their study population. However, utilising whole pedigrees does have disadvantages. Analyses can be computationally demanding, and statistical techniques familiar to those using simple designs, such as least squares, cannot be performed.

In this Chapter simulation is used to address a number of questions, relating to power to detect QTL.

- Do small half-sibships contribute towards power in a half-sib design?
- Do least-squares and likelihood approaches give similar estimates of power for the half-sib design?
- What is the power of the Rum population to detect birth weight QTL compared with the Père David's / red deer pedigree successfully used by Goosen (1997) and Goosen *et al.* (1998)?
- Do a complex pedigree and the half-sib design give similar estimates of power?

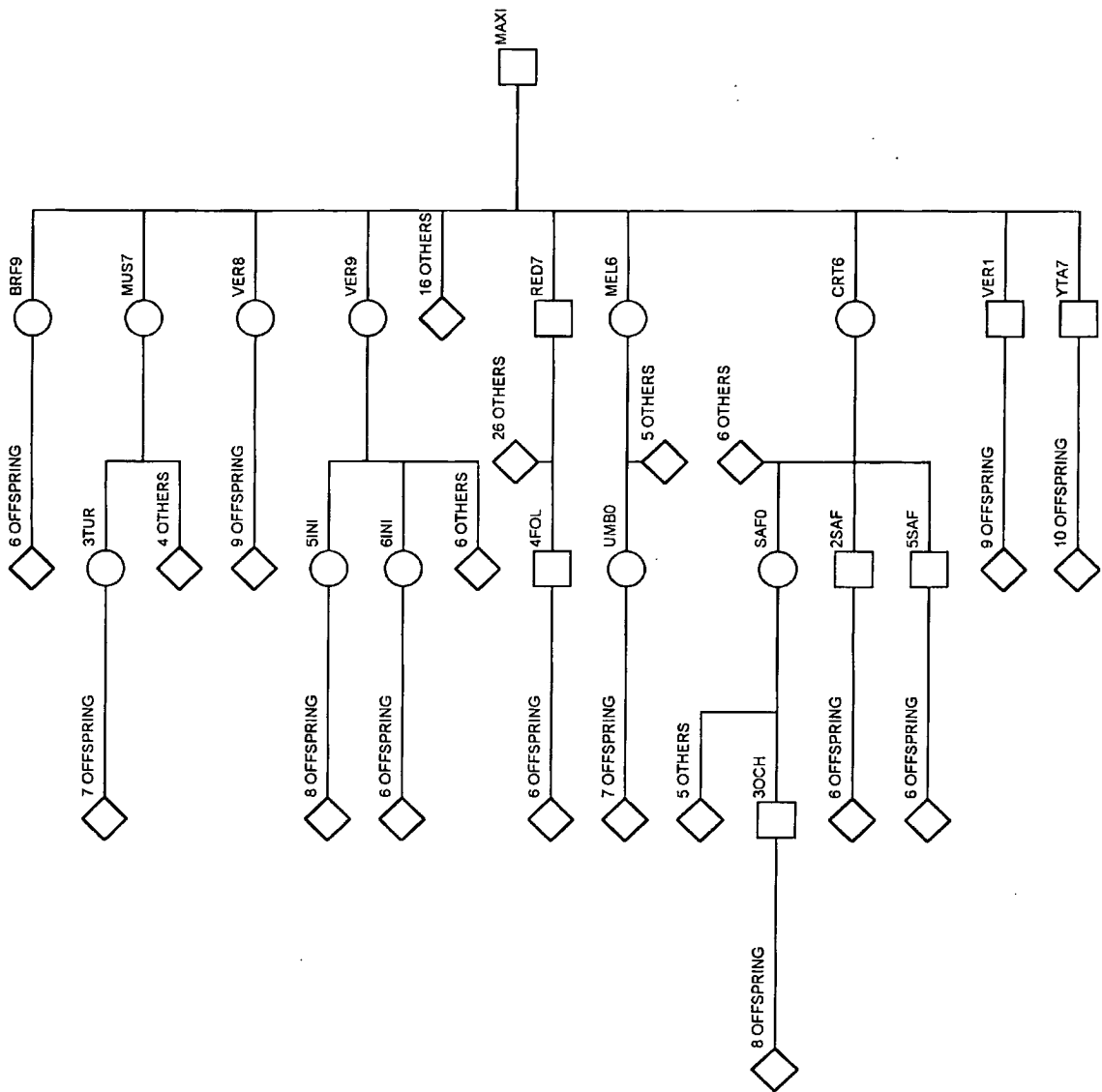
This last question is particularly important, as it should give a clear indication of which mapping strategy to use to detect QTL in this population.

3.2 Methods

3.2.1 Pedigrees used in the simulations

Previously I described how MAXI and a number of his offspring have bred successfully (Chapter 1), and I demonstrated that there is good evidence that MAXI introduced new genetic variation into the population (Chapter 2). It is also possible that marker heterozygosity will be higher within this specific pedigree than for the remainder of the population. Thus, for the purposes of the simulations I used the animals descending from MAXI (see Figure 3.2). The entire Rum pedigree was visualised using PEDVIEW (Kinghorn 1994) and 416 descendants of MAXI identified. These 416 individuals were spread over six generations and included 73 half-sibships ranging in size from two to 27. The power to detect QTL in this complex pedigree was compared with a simpler half-sib design by breaking the pedigree down into its composite half-sibships. Breaking down the complex pedigree in this way violates the assumption that the nuclear families are independent, but analysis is simplified so that least squares methods can be performed.

The pedigree used by Goosen, (1997) and Goosen *et al.* (1998) comprised five paternal half-sibships in which all sires were Père David's / red deer hybrids. Sibship sizes were 133, 44, 44, 22 and 16, giving a total of 259 backcross progeny.



All descendants with five or more offspring are shown. Squares represent males, circles females and diamonds five or more other individuals. Note particularly the success of MAXI's son RED7.

A quick method to estimate the power to detect QTL in the constituent half-sibships of the larger pedigree used a computer program written by Peter Visscher (University of Edinburgh). This analysis is an extension of that used by Weller *et al.* (1990) when evaluating a half-sib design (Weller uses the term daughter design), but allows for sibships of unequal size. I used this ‘Weller approach’ to investigate whether

small sibships contribute to power, and also to compare the power of a least squares approach with that from a likelihood approach for a half-sib design. In the balanced half-sib design used by Weller *et al.* (1990), the distribution of the test statistic under both the null hypothesis (no QTL segregating) and the alternative hypothesis can be approximated by a central and a noncentral χ^2 , respectively. With unequal family sizes, the test statistic under the alternative hypothesis is not obvious, so the following approach was taken.

For each replicated population the sum of squares attributable to the marker contrasts within sires was sampled by:

- (i) determining whether each sire was homozygous or heterozygous at the QTL, by sampling from a binomial distribution;
- (ii) binning each progeny into one or other marker allele class (all sires were assumed heterozygous for the marker), again by sampling from a binomial distribution;
- (iii) sampling the means of each marker class from a normal distribution with the mean difference between the marker classes equal to the QTL effect. The squared difference between the means, standardised by the variance of the difference between the two means, was drawn from a noncentral χ^2 distribution. It was thus assumed that the within-sire variance was known (or estimated without error).

Summing this marker contrast sum of squares across all sires gave the test statistic. Power was calculated by determining the probability of the test statistic exceeding a central χ^2 of Type I error α , with degrees of freedom equal to the number of sires which had progeny in both marker classes.

Unlike Weller *et al.* (1990) I investigated half-sibships of differing size, as occur in most natural populations. In addition, the method I used takes into account the finite sampling of progeny group sizes for each marker class. I assumed that all sires were heterozygous for the marker allele and that there was no recombination between marker and trait loci. The following parameter values were used for simulations.

- i) The QTL was assumed to be biallelic, with each allele at frequency 0.5.
- ii) QTL magnitude was varied in increments of 0.1 of a within QTL genotype phenotypic standard deviation, so that the difference between the heterozygote and either homozygote was 0.5 to 1.0 SD.
- iii) Polygenic heritability was 0.5.

I examined the effect of using all half-sibships (two sibs or more) or those containing greater than three, four, or five sibs on the power to detect linkage. Ten thousand replications were performed throughout.

3.2.3 Simlink program

Although the Weller approach gives a rapid and accurate estimate of power within a half-sib design, it cannot be used to analyse more complex pedigrees. To perform power comparisons between the extended pedigree and the half-sib design (the main purpose of this power analysis) I used the likelihood-based program Simlink (Boehnke, 1986; Ploughman & Boehnke, 1989). Simlink also enables investigation of the effects of marker heterozygosity and recombination on power. Simlink tests whether a segregating major gene can be detected when linked to a marker, compared to the null hypothesis of a major gene segregating but being unlinked. Note that Simlink and the Weller approach perform subtly different statistical tests. The Weller approach tests for a QTL segregating against the null hypothesis of no QTL, whereas Simlink tests for a QTL linked to a marker, compared with a QTL segregating but unlinked to the marker. The implications of this difference are discussed in section 3.4. Using Simlink I compared the power to detect linkage in the extended MAXI pedigree with that obtained from using only the constituent sibships, whilst also investigating the effect of QTL magnitude, marker heterozygosity and recombination between marker and QTL. One hundred replications were performed throughout.

Simlink parameters.

- i) QTL magnitude: as for the Weller approach.
- ii) Recombination fraction: the distance between marker and trait locus was set to 0, 10 or 20 cM.
- iii) Marker heterozygosity: the number of marker alleles was varied from two to nine with all alleles at equal frequency. This meant that marker heterozygosity varied from 0.5 to 0.89. Unlike the Weller approach, a marker heterozygosity of unity is impossible with Simlink. However, the parameter values used in Simlink are more realistic.

3.2.4 Significance thresholds

The relationship between the χ^2 - test of the Weller approach and the likelihood test of Simlink is complicated, but the same significance level for both is essential to make a comparison between the two approaches for a half-sib design. Baret *et al.* (1998) examined the relationship between the test statistics for the F -statistic (or its approximation by χ^2) and a likelihood ratio test, for the half-sib design. They showed for the null hypothesis of no QTL segregating, that a likelihood ratio test (or LOD score) follows an approximate distribution of a 50:50 mixture of a χ^2 with d.f. = 1 and a point mass at zero. This can be approximated by using a χ^2 distribution with 1 d.f. and doubling the probability value. So, if Simlink is used to test the power of obtaining a LOD of 3.0 (corresponding to a probability of 0.0002) then to perform the equivalent test with the Weller approach a p value of 0.0001 (half of 0.0002) should be used.

3.3 Results**3.3.1 Contribution of smaller sibships to power.**

I used the Weller approach to examine the effect of the smaller half-sibships on the power to detect linkage to a QTL (see Table 3.1). Of the 73 sibships within the MAXI pedigree, 31 of them only had two sibs and 17 just three sibs. Power was greatest when all half-sibships were used, but the contribution from the half-sibships with fewer than five progeny was small. In practical terms this means that the extra genotyping required in screening all sibships would produce only a small increase in

power, and would be inefficient in terms of cost and time. A further problem caused by small sibships was that all offspring often inherited the same sire marker allele. In consequence the average number of informative sires varied across replications of the simulation, and the Type 1 threshold was not constant. As the smaller families contributed little power, all subsequent Weller simulations were performed with the 18 half-sibships containing five or more offspring. This meant that a single Type 1 threshold could be used (assuming that all 18 sires were informative; see Table 3.1), and comparisons between Weller *et al.*'s (1990) least squares approach and the likelihood method of Simlink could be made.

Table 3.1: Effect of small half-sibships on power to detect QTL using a 'Weller approach' half-sib design. Heritability was set to 0.5, QTL effect was 1.0 phenotypic standard deviation, and a significance threshold of 0.05 was used. Ten thousand replications were performed. The number of informative sires was less than the actual number of sires because all offspring in small sibships can inherit the same sire marker allele.

Minimum sibship size	Number of sibships	Mean number of informative sires	Average Type 1 threshold (χ^2 units)	Average test statistic (χ^2 units)	Power
2	73	52.0	69.7	84.5	0.81
3	42	36.5	51.5	64.5	0.80
4	25	23.1	36.0	46.7	0.78
5	18	17.5	28.3	37.7	0.76

3.3.2 Comparison of the Weller and Simlink approaches for the half-sib design.

Both methods were used to investigate the power of detecting linkage to a QTL for the half-sib design. For a QTL of effect 0.5 – 1.0 phenotypic standard deviation, with no recombination between marker and trait loci, the methods yielded similar results (Table 3.2), although the Weller approach tended to give slightly higher power. This is perhaps unsurprising given that the Weller approach makes the assumption that all sires are heterozygous at the marker allele, whereas for the Simlink simulations a marker heterozygosity of 0.89 (see methods) was assumed. The maximum discrepancy in power between the two programs was 0.08.

Table 3.2: Comparison of the Weller approach and a LOD score approach (Simlink) for the half-sib design. Columns refer to the probability of obtaining LOD scores in the range 0.5–3.0 for the Weller (W) and Simlink (S) approaches. QTL effect ranged from 0.5 to 1.0 of a phenotypic standard deviation. Marker heterozygosity was 1.00 for the Weller approach and 0.89 for Simlink. Heritability was set to 0.5 in the Weller approach. Ten thousand replicates were performed for the Weller method and 100 for Simlink. The Weller approach used only sibships with five or more offspring.

QTL effect (SD)	0.5 (W)	0.5 (S)	1.0 (W)	1.0 (S)	2.0 (W)	2.0 (S)	3.0 (W)	3.0 (S)
0.5	0.22	0.22	0.10	0.02	0.01	0.00	0.00	0.00
0.6	0.33	0.30	0.15	0.14	0.03	0.01	0.00	0.00
0.7	0.44	0.38	0.25	0.23	0.06	0.02	0.01	0.01
0.8	0.56	0.49	0.35	0.31	0.10	0.10	0.03	0.01
0.9	0.68	0.62	0.49	0.42	0.18	0.18	0.07	0.03
1.0	0.78	0.72	0.60	0.52	0.30	0.27	0.14	0.10

3.3.3 Comparison of mean LOD score between the extended MAXI pedigree and its constituent sibships.

Table 3.3 compares the results obtained from Simlink using the extended pedigree and using the constituent half-sibships analysed separately. Mean LOD score and power were compared when recombination between marker and trait locus was set to 0, 10 or 20 cM. Figure 3.3 illustrates the effect of QTL magnitude on mean LOD for all six scenarios considered (extended and half-sib pedigrees at three different recombination fractions). The mean LOD score was always greater for the extended pedigree than for the equivalent half-sib design. The relative difference between the two designs was greater when recombination fraction was lower. At 0 cM the extended pedigree mean LOD was 2.5-3.0 times greater than for the half-sib design, whereas at 20 cM the ratio was only 1.5-2.0.

Table 3.3: Comparison between the extended MAXI pedigree and the MAXI descendant half-sib design using Simlink. Mean LOD score and probability of obtaining LOD score greater than 0.5 – 3.0 is shown for the two designs. Marker heterozygosity was set to 0.89, recombination fraction between marker and QTL was set to 0, 10 or 20 cM, and QTL effect was 0.5 – 1.0 phenotypic standard deviations. All available half-sibships were used for the half-sib design. One hundred replications performed throughout.

Recombination fraction	QTL effect (SD)	Extended pedigree					Half-sib design				
		Mean LOD (±SE)	Prob. > 0.5	Prob. > 1.0	Prob. > 2.0	Prob. > 3.0	Mean LOD (±SE)	Prob. > 0.5	Prob. > 1.0	Prob. > 2.0	Prob. > 3.0
0	0.5	0.676 (0.069)	0.49	0.24	0.06	0.02	0.246 (0.033)	0.22	0.02	0.00	0.00
	0.6	1.069 (0.093)	0.67	0.41	0.18	0.04	0.377 (0.047)	0.30	0.14	0.01	0.00
	0.7	1.557 (0.122)	0.78	0.56	0.32	0.17	0.551 (0.063)	0.38	0.23	0.02	0.01
	0.8	2.126 (0.150)	0.85	0.75	0.46	0.26	0.769 (0.080)	0.49	0.31	0.10	0.01
	0.9	2.769 (0.177)	0.92	0.82	0.60	0.40	1.033 (0.097)	0.62	0.42	0.18	0.03
	1.0	3.475 (0.203)	0.97	0.90	0.74	0.51	1.346 (0.115)	0.72	0.52	0.27	0.10
10	0.5	0.403 (0.049)	0.34	0.10	0.01	0.00	0.183 (0.028)	0.11	0.03	0.00	0.00
	0.6	0.608 (0.067)	0.43	0.22	0.06	0.01	0.266 (0.039)	0.22	0.05	0.00	0.00
	0.7	0.860 (0.085)	0.55	0.38	0.09	0.03	0.366 (0.050)	0.25	0.13	0.02	0.00
	0.8	1.152 (0.105)	0.63	0.44	0.18	0.07	0.485 (0.062)	0.31	0.17	0.03	0.00
	0.9	1.485 (0.124)	0.77	0.56	0.26	0.11	0.623 (0.073)	0.42	0.19	0.05	0.03
	1.0	1.850 (0.143)	0.85	0.71	0.39	0.19	0.779 (0.084)	0.53	0.27	0.10	0.04
20	0.5	0.239 (0.039)	0.19	0.08	0.00	0.00	0.141 (0.023)	0.05	0.02	0.00	0.00
	0.6	0.334 (0.050)	0.25	0.11	0.01	0.00	0.191 (0.030)	0.12	0.03	0.00	0.00
	0.7	0.448 (0.061)	0.26	0.19	0.05	0.00	0.244 (0.037)	0.20	0.04	0.00	0.00
	0.8	0.577 (0.072)	0.37	0.19	0.08	0.00	0.299 (0.044)	0.26	0.09	0.01	0.00
	0.9	0.729 (0.082)	0.43	0.25	0.08	0.03	0.355 (0.050)	0.27	0.10	0.02	0.00
	1.0	0.895 (0.092)	0.53	0.35	0.14	0.03	0.415 (0.056)	0.29	0.16	0.03	0.00

The relationship between pedigree design and power was similar to that of pedigree design and mean LOD score. Figure 3.4 (page 54) illustrates the probability of obtaining LOD scores in the range 0.5 to 3.0 for some of the models tested. When there was no recombination between the marker and the QTL, the extended pedigree had more than twice as likely as the half-sib design to obtain a LOD score of 2.0 and above, regardless of QTL effect. Power rapidly decreased with increasing recombination fraction between marker and QTL for both designs.

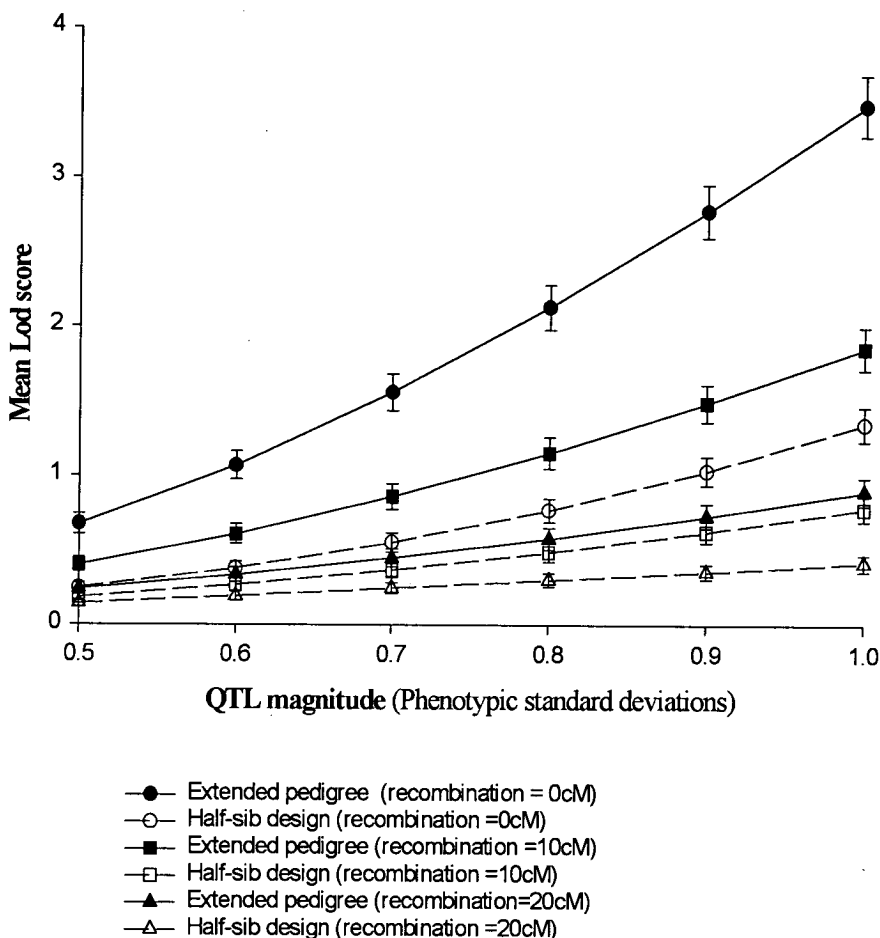


Figure 3.3. Plot showing mean LOD score obtained from using the extended MAXI pedigree, or the half-sibs within that pedigree, when a QTL of magnitude 0.5 – 1.0 phenotypic SD is segregating. Recombination fraction between QTL and marker is set at 0, 10 or 20 cM. All power calculations were performed with Simlink.

3.3.4 Effect of marker heterozygosity on mean LOD score and power.

I also examined the effect of marker heterozygosity on mean LOD score and power, for both extended and half-sib designs (Table 3.4). When marker heterozygosity was reduced from 0.89 to 0.50, mean LOD score for both designs was more than halved. However the extended pedigree still had a mean LOD score more than 2.5 times greater than that of the half-sib design. At the lower marker heterozygosity (0.50), the power to obtain a LOD score of 3.0 had decreased from the original value of 0.51 to just 0.10 for the extended pedigree.

3.3.5 Power to detect QTL in the Père David's / red deer backcross pedigree used by Goosen (1997).

The power (estimated with Simlink) to detect QTL in this series of five half-sibships is given in Table 3.5 and illustrated in Figure 3.4. Mean LOD score and power are slightly lower than that of the extended MAXI pedigree. Note however, that the marker heterozygosity is close to 1 for the hybrid sires in this design, as most of the markers used by Goosen (1997) showed a fixed allelic difference between Père David's deer and red deer. It should also be remembered that Simlink uses a likelihood approach where no polygenic component is fitted. In practice Goosen (1997) used an ANOVA based approach in which polygenic contribution from the dams was fitted. The consequences of not fitting a polygenic component in these simulations is discussed in section 3.4, although for now it is worth noting that by not fitting any polygenic effect the simulated power is unlikely to be upwardly biased, i.e. the tests are conservative.



Table 3.4: Effect of marker heterozygosity on mean LOD score and power.

Mean LOD score and probability of obtaining a LOD score greater than 0.5–3.0 for the extended MAXI pedigree and the half-sib design. QTL effect was 1.0 SD, recombination between marker and QTL was 0 cM and 100 replicates were performed. All calculations were performed using Simlink.

Marker alleles	Marker heterozygosity	Extended pedigree					Half-sib design				
		Mean LOD±SE	Prob. >0.5	Prob. >1.0	Prob. >2.0	Prob. >3.0	Mean LOD±SE	Prob. > 0.5	Prob. > 1.0	Prob. > 2.0	Prob. > 3.0
2	0.500	1.355 (0.110)	0.76	0.51	0.23	0.10	0.518 (0.065)	0.33	0.15	0.05	0.01
3	0.667	2.062 (0.150)	0.85	0.73	0.44	0.25	0.838 (0.081)	0.58	0.31	0.14	0.01
4	0.750	2.565 (0.172)	0.93	0.79	0.57	0.32	0.977 (0.088)	0.62	0.41	0.14	0.03
5	0.800	2.971 (0.181)	0.91	0.87	0.69	0.43	1.158 (0.106)	0.68	0.45	0.16	0.08
6	0.833	3.183 (0.186)	0.97	0.88	0.67	0.50	1.246 (0.107)	0.71	0.49	0.23	0.09
7	0.857	3.230 (0.186)	0.95	0.90	0.73	0.47	1.302 (0.113)	0.72	0.49	0.23	0.09
8	0.875	3.336 (0.195)	0.95	0.90	0.73	0.51	1.322 (0.113)	0.73	0.49	0.24	0.10
9	0.889	3.475 (0.203)	0.97	0.90	0.74	0.51	1.346 (0.115)	0.72	0.52	0.27	0.10

Table 3.5 LOD score and power for half-sib pedigrees used by Goosen (1997). Power is similar to that for the extended MAXI pedigree. QTL magnitude was 0.5-1.0 phenotypic standard deviations. Recombination fraction was set to 0, 10 or 20cM. Marker heterozygosity was 0.89. One hundred replications were performed throughout. All calculations were performed using Simlink.

Recombination fraction	QTL effect	Mean LOD score	SE	P>0.5	P>1.0	P>2.0	P>3.0
0	0.5	0.777	0.082	0.51	0.32	0.11	0.02
	0.6	1.180	0.115	0.65	0.45	0.25	0.09
	0.7	1.641	0.151	0.67	0.58	0.38	0.20
	0.8	2.155	0.188	0.73	0.61	0.44	0.34
	0.9	2.699	0.226	0.79	0.66	0.56	0.39
	1.0	3.253	0.264	0.84	0.71	0.60	0.46
10	0.5	0.409	0.058	0.26	0.10	0.04	0.00
	0.6	0.633	0.080	0.42	0.22	0.09	0.01
	0.7	0.902	0.104	0.51	0.35	0.12	0.08
	0.8	1.202	0.129	0.58	0.47	0.24	0.11
	0.9	1.525	0.154	0.66	0.52	0.31	0.16
	1.0	1.854	0.180	0.70	0.57	0.39	0.25
20	0.5	0.256	0.051	0.15	0.09	0.02	0.01
	0.6	0.371	0.067	0.24	0.11	0.04	0.02
	0.7	0.510	0.084	0.34	0.16	0.06	0.03
	0.8	0.665	0.100	0.40	0.21	0.10	0.05
	0.9	0.836	0.115	0.48	0.28	0.11	0.06
	1.0	1.011	0.129	0.56	0.35	0.17	0.08

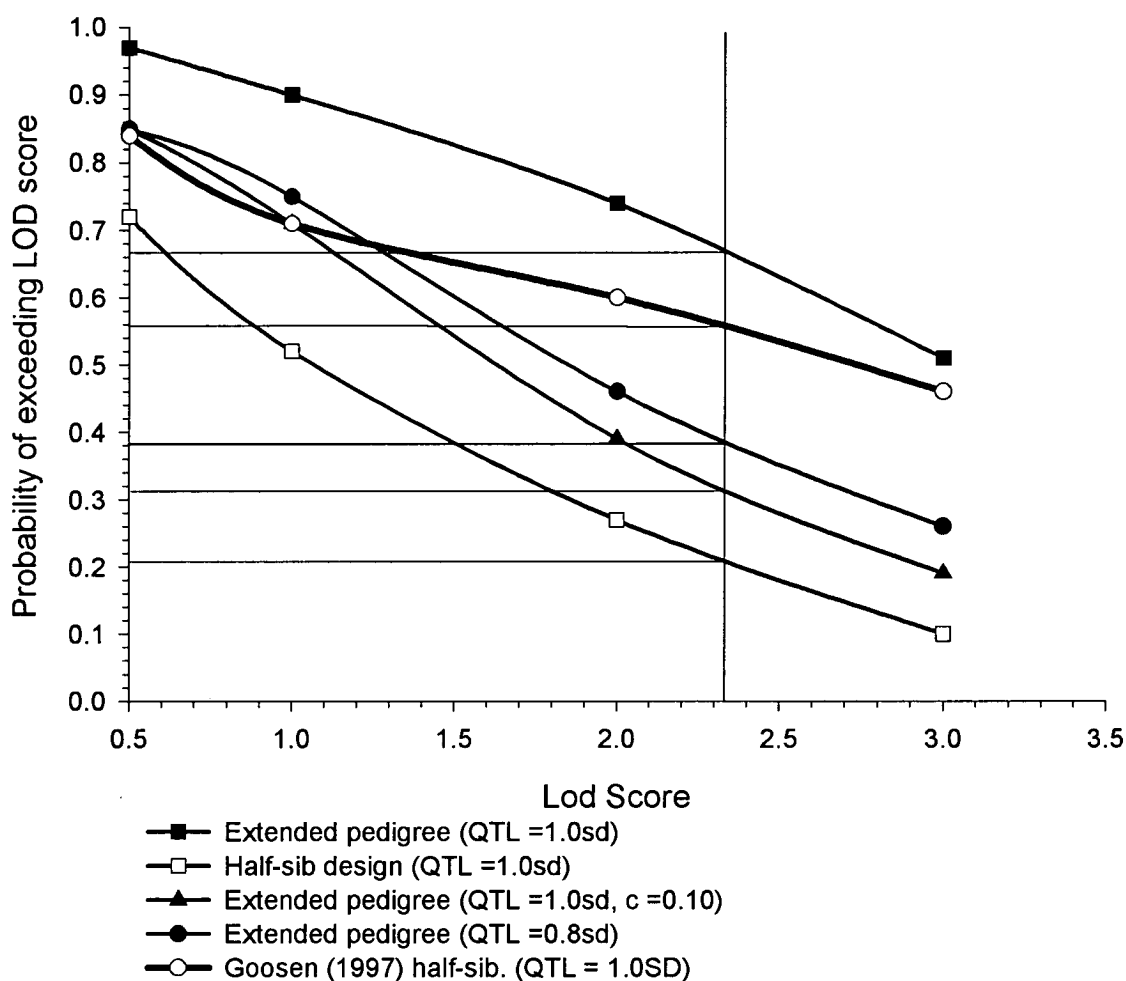


Figure 3.4 Power curves for extended MAXI pedigree and various half-sib designs. Curves show the probability of obtaining LOD scores in the range 0.5–3.0. The vertical line represents a LOD score of 2.34, equivalent to a genome-wide significance level of 0.05 when 100 markers are screened (see section 3.4). When a QTL of 1.0 SD is segregating and there is no recombination between the marker and QTL, the extended pedigree has a power of 0.67, whereas the half-sib design has a power of 0.21 and the half-sib design of Goosen (1997) has a power of 0.56. Power decreases if the QTL magnitude is reduced or if recombination is increased. All calculations were performed using Simlink.

3.4 Discussion

I have demonstrated that the probability of detecting QTL in the Rum study population is greatly increased if an extended pedigree rather than a simple half-sib design is used (see also Slate *et al.* 1999). This is the first time that complex pedigrees have been considered for mapping QTL in any wild population and the results are encouraging. A number of field studies could now produce pedigrees several generations deep, and similar to the one used in this analysis. However, it should be stressed that even using the extended pedigree only QTL of major effect can be detected in this population.

Why does the extended pedigree yield greater power than the half-sib design? The extended pedigree uses genotype and phenotype information for all individuals, and by tracing marker alleles down the pedigree, calculates the likelihood of a major gene segregating. In other words, both between and within family information is considered. In contrast, the half-sib design uses only within-family information.

With a real QTL mapping data set, one would probably perform a test that compares a model of QTL and background polygenic effect against a model which had only a polygenic effect. My comparison of a half-sib design and an extended pedigree used a model of QTL linked vs. QTL present but unlinked. Strictly speaking, to perform a power comparison between the half-sib and extended pedigree designs, the test statistic distribution under the null hypothesis of a QTL segregating but unlinked to the marker is required for both designs. For an extended pedigree it has been shown that this can be described by a 50:50 mixture of a χ^2 with d.f. = 1 and a point mass at zero (Lander and Krugylak 1995). For the half-sib design the test statistic under the subtly different null hypothesis of no QTL present follows the same distribution (Baret *et al.* 1998). It has been demonstrated for the half-sib design, that the test statistic for an unlinked segregating QTL has a similar distribution to the case of no QTL segregating (Le Roy and Elsen 1995). In fact the mean test statistic is slightly larger for the former case, suggesting that my comparison between the extended pedigree and the half-sib design is conservative. In my half-sib simulations the Weller approach gave marginally higher power than Simlink, although this may be

caused by the slightly higher marker heterozygosity used for the Weller approach. It should also be pointed out that my approach does not account for any polygenic variation that may be segregating. However, there is no evidence that ignoring a polygenic effect inflates the test statistic. Knott and Haley (1992) simulated a polygenic component in a full-sib design and showed that a QTL linked vs. QTL unlinked test gave a *lower* test statistic than one where polygenic effect was fitted (QTL linked + polygenic component vs. QTL unlinked + polygenic component). Again this suggests that the test used here may be conservative.

A contentious area of QTL mapping is that of significance thresholds. Lander and Kruglyak (1995) discussed the criteria that should be used for assigning linkage. A common problem is that when carrying out a genome-wide scan for QTL, multiple testing leads to false assignment of linkage because of Type I error. However, imposing too strict a value for significance may wrongly reject any suggestions of linkage that are found (Type II error). Traditionally a LOD score of 3 has been used to assign linkage. However Lander and Kruglyak (1995) suggest a different approach: the use of a genome-wide significance level to find what they term 'significant linkage'. This is defined as statistical evidence expected 0.05 times in a genome-wide scan. In Chapter 7 I describe how these thresholds can be calculated as a function of genome length and chromosome number (a LOD score of 3.0 was chosen with the length of the human genome in mind). As the deer map (described in Chapter 6) was of unknown length when the simulations were run these thresholds were not calculated at the time. However, if one were to carry out a genome-wide scan using 100 markers (a realistic number of markers for a natural population screening), an experiment-wide error rate of 0.05 could be approximated using a Bonferroni correction so that $\alpha' = 1 - (1 - 0.05)^{1/100} = 5.13 \times 10^{-4}$. Using the approach of Baret *et al.* (1998) this significance level corresponds to a LOD score of 2.34. In other words, rather than using a LOD score of 3.0 to assign linkage, a LOD score of 2.34 could be used for an experiment-wide significance level of 0.05. For the MAXI pedigree this equates to a power of about 0.67 (rather than 0.51) when a QTL of effect 1.0 SD is segregating, and there is no linkage between marker and

QTL (see Fig. 3.4). It should be stressed that the extended pedigree far out-performs the half-sib design regardless of significance threshold.

Although the power to detect QTL is high only in a somewhat idealised situation (highly polymorphic markers, QTL of very large effect, and low recombination fraction between marker and QTL), further considerations may increase this power. In Chapter 2 I described how a number of environmental factors were known to influence fitness components in the study population. Controlling for these variables will serve to increase the proportion of the residual variation explained by the QTL. In other words, the apparent heritability of the trait determined by the QTL will increase, as will the power to detect the QTL. Mapping projects in wild populations may also be able to draw on previous work carried out in closely related laboratory or farmed species where QTL for a trait of interest have been identified. If one knew the likely location of a QTL, then extra markers could be screened in that chromosomal region, hence reducing the recombination fraction between the QTL and marker(s). Alternatively only markers linked tightly to the candidate region could be typed and less stringent significance thresholds used. Again, this would increase the power to detect QTL. There is increasing evidence from the plant breeding literature that QTL affecting particular traits are found in syntenic genomic regions across species (Kearsey and Farquhar, 1998). To date there has only been one attempt to map QTL for birth weight in deer, and that involved a cross between two species (Goosen 1997; Goosen *et al.* 1998). For the purposes of this study a candidate region approach is not yet a viable option, although it may be justifiable to use a lower significance threshold in regions where birth weight QTL have been previously described.

For my simulations I only performed linkage analysis with single-point markers. Interval mapping and multipoint analysis (in which information from all markers on a chromosome are used simultaneously) would reduce much of the loss of power observed when recombination occurs between marker and QTL (Lander and Botstein, 1989; Knott *et al.* 1996). For example, when a QTL of effect 1.0 SD is segregating and there is no recombination, the power for a mean LOD > 3.0 is 0.51

in the extended pedigree. At 10 cM the power is only 0.19, and at 20 cM just 0.03. Multipoint mapping would partially alleviate this problem, and the effect would be most noticeable when markers are spaced at wide intervals (Fulker and Cardon, 1994). For the purposes of this study, a relatively small number of markers will be used, and so multipoint analysis will aid QTL detection. Multipoint mapping has the additional advantage over single-point mapping that other QTL parameters such as magnitude and location can be estimated (Lander and Botstein, 1989). Most multipoint mapping procedures developed thus far are applicable to sib-pair (Lander and Botstein, 1989; Fulker and Cardon, 1994) or half-sib (Haley and Knott, 1992; Knott *et al.*, 1996) families. However, a recent study (Almasy and Blangero, 1998) described a method in which large complex pedigrees can be analysed by multipoint linkage. The number of alleles shared identically-by-descent (IBD) at marker loci is used to estimate alleles IBD at various points along a chromosome for all relative pairs within a pedigree. Variance-component maximum likelihood linkage analysis is then performed on the overall pedigree to indicate the presence of a QTL. New statistical approaches like these make QTL mapping in the Rum population a possibility.

The Simlink software was used to make a comparison in power between the extended MAXI pedigree and the half-sib design used by Goosen (1997). The power of the two designs was similar, with that of the extended MAXI pedigree perhaps marginally higher, suggesting that if the QTL found by Goosen (1997) are also present in the Rum population they should be detected. However, it must be remembered that all five hybrid sires in the half-sib design were heterozygous at the majority of marker loci. Heterozygosity is expected to be lower within the Rum population. Furthermore, QTL are likely to be of larger effect in the pedigree used by Goosen (1997) as a cross between two species was involved. As expected, marker heterozygosity had a significant impact on ability to detect QTL. For both the extended pedigree and the constituent half-sibships, a heterozygosity of 0.8 gave about twice the LOD score of a heterozygosity of 0.5. Microsatellite loci typically have heterozygosity values in the range 0.5 – 0.8, although this varies from species

to species and population to population. Heterozygosity values for markers screened in the Rum population are given in Chapter 4.

In summary, I conclude that QTL detection in the Rum population is possible, but unlikely unless loci of very large effect are segregating. It may be that it is only possible to detect QTL because the study population has been pedigreed for several generations. As new statistical software is developed, QTL mapping studies in other natural populations may become commonplace, but the ability to perform such analyses will partly rest upon obtaining detailed pedigrees.

4 Obtaining and screening microsatellite markers

4.1 Introduction

Microsatellites are abundant, codominant Mendelian markers consisting of tandem repeat sequences of 1-5 base pair motifs. Since 1989 it has been realised that they are readily amplified by PCR (Litt and Luty 1989; Tautz, 1989; Weber and May 1989), and by virtue of their high mutability by slippage processes during replication, highly polymorphic. Consequently, over the last decade microsatellites have become the genetic marker of choice for population genetics, genetic mapping, paternity inference and conservation biology studies (Queller *et al.* 1993; Jarne and Lagoda 1996; Goldstein and Schlötterer 1999).

Microsatellites are amplified by PCR using primers that recognise unique sequences flanking the repeat motif. The resulting product is electrophoresed through a polyacrylamide gel to separate alleles of differing length. The alleles are then visualised using autoradiography or other detection systems, and scored by comparing the distance they have migrated with a known size standard. Hence, allele sizes can be stored in a database, and cross-gel comparisons made. Because the amplification process is PCR-based, only small amounts of tissue are required, and in principle 'difficult' template material such as hair, faeces and bone can be used (Queller *et al.* 1993). A major advantage of microsatellites in mapping studies is that as many as 15 loci per individual can be run on gels simultaneously, provided fluorescent primer technology and a judicious choice of markers are used.

Perhaps the major disadvantage of microsatellites as genetic markers is that the mutation process is not fully understood (Amos 1999), making inferences about genetic differentiation problematic. Additionally it is apparent that null alleles (whereby a deletion in the flanking sequence causes the non-amplification of particular alleles) are common (Callen *et al.* 1993; Pemberton *et al.* 1995), leading to an excess of individuals scored as homozygotes and cases of apparent non-Mendelian inheritance. A further drawback of microsatellites is that cloning them can be time-consuming and expensive (Jarne and Lagoda 1996). Finally, although

microsatellites are generally regarded as selectively neutral, there is limited evidence that they may play a functional role and could be associated with phenotypic variation (Kashi *et al.* 1997).

Broadly speaking, genetic maps are constructed with two distinct goals: to perform linkage analysis, and to study the evolution and organisation of genomes. Unfortunately, separate classes of marker are required to address each of these areas. To study genome evolution, the most informative markers are conserved across distantly related species, and are typically gene-encoding. Thus the linkage order of functionally important homologous loci can be compared across genomes. These loci were formally termed Type I loci by O'Brien *et al.* (1993). Allozyme loci and gene-probed RFLPs fall into this category of marker. Unfortunately Type I loci are generally fixed within a species, making them unsuitable for linkage analysis. Suitable markers for linkage analysis are highly polymorphic within a species, and need not be gene-encoding or conserved across distant taxa. These markers are termed Type II loci (O'Brien *et al.* 1993), and include microsatellites, minisatellites and amplified fragment length polymorphisms (AFLPs). One of the principal motivations for constructing the deer genetic map was to study the evolution of ruminant chromosomes (Tate 1997) and so evolutionarily conserved Type I loci were initially mapped in deer. At the outset of my study only 18 microsatellites were mapped (Tate *et al.* 1994), clearly an insufficient number to perform a genome wide search for QTL. This chapter describes how I developed a large panel of microsatellite markers suitable for screening in the Rum population.

Although Type I loci are better conserved across mammalian orders, it has been demonstrated that primers for some microsatellite loci can amplify products across species (Moore *et al.* 1991; Crawford *et al.* 1994; FitzSimmons *et al.* 1995; de Gortari *et al.* 1997) and even more distant taxa (Gemmell *et al.* 1997). In addition to saving time and effort in the laboratory, cross-species utilisation of microsatellite loci allows comparative maps between related species to be built. A number of studies that tested cattle microsatellite primers across species suggested that around 60% amplify a product in both sheep (Moore *et al.* 1991; Crawford *et al.* 1995; de Gortari

et al. 1997) and goats (Pepin *et al.* 1995). Indeed, the first sheep genetic map contained only 86 microsatellites cloned in sheep compared to 126 taken from the bovine map (Crawford *et al.* 1995). Earlier studies have also shown that cattle microsatellites are conserved in deer (Engel *et al.* 1996; Kuhn *et al.* 1996; Talbot *et al.* 1996), although a conservation rate of less than 60% is predicted, as deer are more diverged from cattle than are sheep (Modi *et al.* 1996). With this in mind, I collected microsatellite primer pairs isolated from cattle, sheep and other ruminants from as many sources as possible. In this chapter I describe how I used these primers to:

- Derive a panel of microsatellite primers that were conserved in deer, and polymorphic in the Rum population.
- Screen the polymorphic loci in the MAXI pedigree that was used in the simulations described in Chapter 3.

4.2 Testing ruminant primers in red deer and other species

Primers for a total of 354 microsatellite markers were tested in red deer. A subset of 174 primer pairs was also screened (with colleagues, see below) in sika deer (*Cervus nippon*) and Soay sheep (*Ovis aries*). Species of origin included cattle (*Bos domesticus*), domestic sheep (*Ovis aries*), red deer (*Cervus elaphus*), caribou (*Rangifer tarandus*) and gazelle (*Gazella gazella*). A list of the scientists who donated primers is given in Table 4.1.

4.2.1 Animals screened at each locus

The loci donated by Dr John Williams were tested in a more systematic way than the other loci in a collaborative project with Drs S Goodman and D Coltman (University of Edinburgh), who required amplifiable microsatellite loci in related ruminants (Slate *et al.* 1998). Simon Goodman required microsatellites with fixed allelic differences between red deer and sika deer, so that hybrids on the Kintyre peninsula, SW Scotland could be identified (Goodman *et al.* 1998). Dave Coltman required polymorphic markers to conduct paternity analysis in a free-living population of Soay sheep on St. Kilda, Scotland (Coltman *et al.* 1999a).

Table 4.1 Sources of microsatellite primer pairs donated for attempted amplification in red deer. Approximately half of the primer sets were obtained from Dr John Williams (Roslin Institute).

Donor	Academic Institution	No. primer pairs donated	Source Species
Josephine Pemberton (this laboratory)	University of Edinburgh	49	Cattle, sheep, red deer
John Williams	Roslin Institute, Edinburgh	174	Cattle
Allan Crawford	AgResearch, New Zealand	50	Cattle, sheep
David MacHugh	Trinity College, Dublin	36	Cattle, sheep
Ralph Kühn	University of Munich	12	Cattle
Greg Wilson	University of Alberta	9	Caribou
Mark Hughes	University of Liverpool	9	Cattle
Rob Hammond	Institute of Zoology, London	5	Gazelle

An initial screening panel consisted of DNA from two red deer, two sika deer, two Soay sheep, one Père David's deer and one cow. At this stage, the standard PCR conditions described in Appendix B were used, with a $MgCl_2$ concentration of 2.5 mM. Loci that gave a product in any of the non-source species were then evaluated over a range of PCR conditions for each species using the following larger sets of individuals.

- The deer International Mapping Herd (IMH) was screened (by the author) to investigate polymorphism in red deer, and to map the locus (see Chapter 6). The IMH consists of two pure Père David's deer, two Père David's × red deer F1 hybrid sires and a total of 89 progeny of the hybrids, produced from back-crossing with red deer hinds.
- A red deer / sika deer test panel was screened (by SG) to find informative markers for hybrids between the two species. The panel contained seven red deer from Cowal, Scotland, in addition to four sika deer from Kintyre, Scotland, and four sika deer from Dawyck, Scotland.

- The Soay sheep test panel (screened by DC) comprised six unrelated sheep from Hirta, St. Kilda, Scotland.

In order to identify the most suitable markers for each of the three studies, a number of PCR optimisation steps were performed in the species-specific test panels. Amplification was attempted across a range of alternative annealing temperatures, typically in 2°C increments over the range 46-58°C. Different MgCl₂ concentrations were tested, usually in 0.5 mM increments between 1.5mM and 3.5 mM. If amplification was still sub-optimal, varying quantities (between 5-10% of the overall reaction volume) of DMSO and TMACIDE (see Appendix B) were added to the PCR reaction mixture in an attempt to enhance amplification of products (Gemmell 1997). If a microsatellite was observed to be conserved in one deer species, a concerted effort was made to determine whether a product could be amplified in the other species.

I tested the remaining 180 loci (see Table 4.1) in red deer only, and in a less systematic manner. Typically around 20 Rum red deer were used, often with a source species control animal, and usually over a range of MgCl₂ and annealing temperature conditions.

4.2.2 Description of PCR products

If a PCR product was polymorphic with 'stutter' bands typical of a microsatellite, and of similar size to that observed or predicted in cattle, then it was regarded as the target bovine microsatellite locus, conserved across species. A monomorphic PCR product was only considered to be the conserved target microsatellite if the band showed a distinctive 'stutter' pattern, and was within 10 base pairs of the size range of the observed cattle product. If a monomorphic product was not within 10 bp of the observed size in cattle, or did not show a stutter pattern, then the PCR product was regarded as non-specific and the locus was considered not conserved in the test species. If no PCR product was amplified the same conclusion was reached.

4.2.3 Conservation of bovine microsatellites across species

In describing the results for the 354 loci tested in red deer, it is reasonable to distinguish those donated by John Williams (Roslin Institute) from the remainder. First, these loci were screened in three different species, whereas the others were largely only tested in red deer. Second, the Williams primers were tested in a systematic way, using a wider variety of PCR conditions. Details of the conservation and polymorphism of the Williams loci are given in Appendix C and in Slate *et al.* (1998). A brief summary is provided in Table 4.2. Results for the remaining loci, tested in red deer only are given in Appendix D.

Table 4.2. Summary of the conservation across other ruminants of the bovine microsatellite primer pairs donated by Dr John Williams (Roslin Institute). The number and proportion of loci amplifying a product, and of loci polymorphic in the three test species are described. The mean number of alleles per locus is derived from the species specific panels, rather than the initial test panel.

Species	Loci giving microsatellite product	Loci polymorphic	Mean no. of alleles (SD)
Red deer	129/174 (74.1%)	72/129 (55.8%)	3.4 ± 2.9
Sika deer	126/171 (73.7%)	47/126 (37.3%)	1.6 ± 1.0
Soay sheep	127/173 (73.4%)	54/127 (42.5%)	1.8 ± 1.2

Although tested in a less rigorous manner than the Williams loci, the remaining microsatellite loci had a higher conservation rate ($146/180 = 81.1\%$) in red deer. This is probably attributable to the fact that some of these loci were initially isolated from red deer and caribou, and so a higher amplification rate in red deer is expected for these loci. Additionally a number of the markers isolated from sheep and cow had previously been demonstrated to amplify a product in red deer (e.g. those donated by Ralph Kühn, University of Munich). Therefore, the following discussion on cross-species amplification of microsatellites refers only to the Williams loci.

In their study de Gortari *et al.* (1997) tested 1036 bovine primers in sheep and found that 605 (58%) amplified a locus. Here the proportion of bovine markers amplifying a microsatellite was significantly higher in all three species tested:

- Red deer (this study) vs. domestic sheep (de Gortari): $\chi^2 = 15.47$, d.f. =1, $p < 0.001$
- Sika deer (this study) vs. domestic sheep (de Gortari): $\chi^2 = 14.36$, d.f. =1, $p < 0.001$
- Soay sheep (this study) vs. dom. sheep (de Gortari): $\chi^2 = 13.99$, d.f. =1, $p < 0.001$.

Amongst other (smaller) studies testing bovine primers in deer, Kühn *et al.* (1996) reported a 52 % amplification rate (27 loci tested, 14 amplifying a product), while Engel *et al.* (1996) described a lower still amplification rate of 40%. The higher level of cross-species amplification reported here is probably attributable to the wide variety of PCR conditions used for each locus. Of the other studies mentioned above, only Kühn *et al.* (1996) optimised PCR conditions for all primers tested.

Another explanation for the high rate of cross-species amplification rate found in this study may be that there was a bias in the loci used (de Gortari *et al.* 1997), since they were initially selected for ease of use and high polymorphism content in cattle. However a detailed comparison with the data from de Gortari *et al.* (1997), in which loci were randomly chosen, suggests any such bias is unlikely. Seventy nine of the markers used in this study were also used by de Gortari *et al.* (1997). They found that 49 (62.0%) of these markers gave a product in domestic sheep. This is not significantly different from the remainder of markers giving a product in domestic sheep in that study (49/79 vs. 556/907: $\chi^2 = 0.463$, $p = 0.496$, ns). Furthermore, in the de Gortari *et al.* (1997) study, these 49 markers did not have a higher mean number of alleles in domestic sheep (2.9 ± 1.8 SD), than did the remainder of the 605 loci (3.2 ± 2.1 SD). On this basis there is no reason to suspect that the loci used in this study are biased in their degree of conservation across species, or have an upward bias in the number of alleles in the non-source species.

A third source of variation between this study and others could be that different criteria were used for designating amplification of monomorphic loci. Ideally, to

distinguish a monomorphic microsatellite from a spurious PCR band, one would sequence the product to check that it was the actual target microsatellite. I ran a cattle control alongside test species, and only declared a monomorphic product as the conserved target microsatellite if it was within 10 bp of the cattle size range and displayed a distinctive ‘stutter’ band typical of a microsatellite. De Gortari *et al.* (1997) did not describe their criteria for deciding which monomorphic products represented conserved microsatellites, but did not sequence PCR products. Thus, the criteria used in this study appear to be at least as conservative as similar studies.

Deer are thought to have shared a common ancestor with cattle 20–25 million years ago (MYA), while sheep and cattle are thought to have diverged more recently at 15 MYA (Modi *et al.* 1996). Hence, it is perhaps surprising that the two deer species showed a conservation rate as high as Soay sheep. This is probably explained by the extra effort employed in testing a marker in one deer species if a product was amplified in the other. Only two loci (HUJI177 and TGLA13) amplified a product in just one of the two deer species. It seems likely that an even greater proportion of bovine microsatellites might be useful in sheep if further effort is made to optimise PCR conditions.

Amongst the Williams loci, red deer had a greater mean number of alleles than either Soay sheep or sika deer. This may in part be due to the fact that more red deer (effectively $n = 52$ red deer, as each IMH backcross progeny receives a single red deer allele from its mother) were screened than either of the other species ($n = 8$ sika deer, $n = 6$ Soay sheep). However, it should also be noted that in contrast to red deer both of the other species have a history of small population sizes, and probably have depressed levels of genetic variation. The sika deer sampled in this study were the result of introductions of small numbers to Scotland from Asia (Abernethy 1994). Soay sheep are an ancient breed isolated on islands in the St Kilda archipelago, Scotland for many generations (Bancroft *et al.* 1995).

Père David’s deer are expected to have very little genetic variation, due to hunting to near extinction, culminating in a captive breeding population of under 20 individuals

(see Chapter 6). Two previous studies (Woodruff and Ryder 1986; Emerson and Tate 1993) have examined levels of allozyme variation in Père David's deer, and between them found polymorphism at only one of 41 loci tested, supporting this prediction. Twelve out of the 174 Williams markers tested gave a heterozygous product in at least one of the two Père David's deer. Thus, the limited genetic variation in Père David's deer is more readily detectable by microsatellites than allozymes. The 12 informative markers were BM1225, BM757, IDVGA46, ILSTS6, ILSTS93, INRA5, INRA37, INRA107, RM12, SPS113, TGLA226 and TGLA431.

There are two main conclusions that can be made from this cross-species amplification study. The first is of general interest to the ruminant genome mapping community and the second specific to this thesis.

It is apparent that primer sites flanking ruminant derived microsatellites and the repeat itself are more highly conserved across closely related species than previously reported. Fries (1993) first suggested that the bovine map can be used as a master map to establish genetic maps of other ruminants. This study provides further support that it should be possible to construct a relatively dense comparative map of this agriculturally important taxon using Type 2 (e.g. microsatellites) as well as Type 1 (e.g. RFLPs and allozymes) loci. In Chapter 6 I describe how I mapped a number of bovine derived microsatellites in red deer, and show that the majority of these markers have a conserved location and order between cattle and deer.

The primary motivation for testing microsatellite markers in deer was to develop a panel of highly variable microsatellites suitable for performing a genome-wide search for QTL in the Rum population. Of 354 primer sets tested, 269 (76.0%) amplified a microsatellite in red deer, of which 160 (59.4% of loci conserved, 45.2% of loci tested) were polymorphic. Clearly a large panel of suitable markers was characterised far more rapidly than if I had attempted to clone microsatellites from red deer genomic DNA. Additionally, most of the markers were already mapped in other ruminants making comparative mapping and candidate gene studies between deer and other ruminants a possibility.

4.3 Screening microsatellites in the MAXI pedigree.

A locus was regarded as suitable for screening in the MAXI pedigree if it was polymorphic, reliably amplified by PCR, and reasonably easy to score. Of the 354 primer pairs tested in red deer, a total of 160 loci gave polymorphic products. From this subset, 84 were regarded as sufficiently polymorphic and easily amplifiable to be screened in the Rum population. Of these, ten had previously been screened for around 1,200 individuals (by myself) for paternity analysis, and are described in Marshall *et al.* (1998). The remaining 74 loci were screened in a 'MAXI mapping panel' using methods described in Appendix B. This panel of animals included MAXI, all of his descendants for whom birth weight was recorded, and a number of 'married-ins' (i.e. unrelated individuals with whom MAXI or his descendants had mated). The identity and details of each of the 84 loci are given in Table 4.3.

Note that the final mapping panel contained only 365 individuals whereas the MAXI pedigree used in the simulations described in Chapter 3 contained 416 individuals. The extra animals, exclusive to the simulation pedigree, were either unsampled or not weighed at birth. However, the simulations did incorporate the fact that these animals were relatively uninformative. Thus, the actual power to detect QTL in the slightly smaller genotyped MAXI pedigree is unlikely to be significantly lower than the estimated power obtained by simulation.

Although selected for both high polymorphism and ease of use, eight microsatellite loci were difficult to interpret on gels. Rather than risk mis-scoring these loci, I pooled adjacent alleles. This method inevitably reduces the number of alleles and heterozygosity at the locus, but I believe is a more robust method than attempting to score all alleles. The assumptions that the markers are in Hardy-Weinberg equilibrium, co-dominant and inherited in a Mendelian manner should not be violated when applying this scoring technique. The markers for which alleles were pooled are indicated in Table 4.3. A number of markers also appeared to have null

alleles (Pemberton *et al.* 1995) segregating. I discuss the detection and consequences of these null alleles in greater detail in Chapter 5.

Following the scoring of gels, genotypes were stored in a Microsoft Access database in which all life history, phenotypic and environmental data were also recorded. The summary statistics for each locus (see Table 4.3) were calculated using the ‘allele frequency analysis’ option of the paternity inference program CERVUS (Marshall *et al.* 1998). On average, 294.7 (SD 35.5) animals were typed at each locus. The mean number of alleles per locus was 7.0 (SD 2.9) and mean heterozygosity was 0.68 (SD 0.19). Interestingly, MAXI had a higher individual heterozygosity (0.81) than any other deer. Although this is unsurprising given the history of the MAXI introduction it is nonetheless an asset in detecting QTL, as MAXI is informative at the majority of loci screened. The mean number of animals screened per locus is slightly disappointing, and largely explained by the fact that template DNA was often fairly degraded. A number of animals were sampled over 20 years ago, or were only sampled *post mortem*. Thus it was inevitable that the quality of template DNA was variable across individual deer.

Table 4.3. Loci screened in MAXI pedigree

The table shows the number of genotypes per locus, the number of alleles, the expected heterozygosity under Hardy-Weinberg conditions, the exclusion probability ($PrEx_1$) when the other parent is not scored, exclusion probability ($PrEx_2$) when the other parent is scored and the estimated error rate e_i . Exclusion probabilities and error rates are described and applied in Chapter 5.

<i>Locus</i>	<i>Number scored</i>	<i>Number alleles</i>	<i>Het (Exp)</i>	<i>Pr (Ex₁)</i>	<i>Pr (Ex₂)</i>	<i>e_i*</i>	<i>Reference[†]</i>
965	287	7	0.661	0.267	0.453	0	Kuhn <i>et al.</i> (1996)
ABS12	325	8	0.827	0.487	0.660	0.007	Slate <i>et al.</i> (1998)
AGLA293	316	3	0.086	0.004	0.043	0.073	Slate <i>et al.</i> (1998)
BM1258	293	8	0.776	0.392	0.571	0	Bishop <i>et al.</i> (1994)
BM1329	291	4	0.689	0.265	0.435	0	Slate <i>et al.</i> (1998)
BM1706	281	8	0.717	0.300	0.472	0.065	Slate <i>et al.</i> (1998)
BM1815	270	8	0.730	0.328	0.508	0	Bishop <i>et al.</i> (1994) [‡]
BM1818	310	8 ⁿ	0.766	0.366	0.544	0.151	Bishop <i>et al.</i> (1994)
BM203	260	7	0.685	0.282	0.462	0.192	Slate <i>et al.</i> (1998)
BM2934 ^p	321	5	0.681	0.261	0.424	0.056	Bishop <i>et al.</i> (1994) [‡]
BM4208	314	11	0.857	0.548	0.711	0	Talbot <i>et al.</i> (1996)
BM4513	283	10 ⁿ	0.834	0.501	0.672	0	Slate <i>et al.</i> (1998)
BM6506	211	6 ⁿ	0.649	0.227	0.385	0	Bishop <i>et al.</i> (1994) [‡]
BM757	326	7	0.607	0.208	0.380	0.078	Slate <i>et al.</i> (1998)
BM888	332	11	0.839	0.510	0.679	0.004	Slate <i>et al.</i> (1998)
BR3510	276	5	0.695	0.288	0.468	0	Slate <i>et al.</i> (1998)
CP26	333	9	0.691	0.285	0.454	0	Marshall <i>et al.</i> (1998)
CSRM60	311	12	0.832	0.500	0.670	0	Kuhn <i>et al.</i> (1996)
CSSM16	281	4	0.705	0.277	0.445	0	Kuhn <i>et al.</i> (1996)
CSSM19	321	8	0.684	0.275	0.445	0	Kuhn <i>et al.</i> (1996)
CSSM39	289	5	0.792	0.403	0.582	0.008	Slate <i>et al.</i> (1998)
CSSM41	292	9	0.732	0.329	0.508	0	Slate <i>et al.</i> (1998)
CSSM43 ^p	300	6	0.796	0.410	0.589	0	Slate <i>et al.</i> (1998)
CSSM66	277	8 ⁿ	0.834	0.492	0.664	0.008	Kuhn <i>et al.</i> (1996)
ETH225	288	6	0.652	0.231	0.390	0.114	Kuhn <i>et al.</i> (1996)
FCB193	321	11	0.791	0.423	0.600	0.004	Marshall <i>et al.</i> (1998)
FCB304	336	9	0.792	0.415	0.594	0	Marshall <i>et al.</i> (1998)
FCB5	220	7	0.701	0.296	0.476	0.128	Buchanan <i>et al.</i> (1994) [‡]
FSHB	296	9	0.754	0.356	0.533	0.084	Slate <i>et al.</i> (1998)
HIS-H1	314	2	0.246	0.030	0.108	0.038	Moore and Byrne (1993) [‡]

<i>Locus</i>	<i>Number scored</i>	<i>Number alleles</i>	<i>Het (Exp)</i>	<i>Pr (Ex₁)</i>	<i>Pr (Ex₂)</i>	<i>e_i[*]</i>	<i>Reference[†]</i>
HUJ175	286	3 ⁿ	0.637	0.202	0.348	0	Slate <i>et al.</i> (1998)
HUJ177 ^p	281	6 ⁿ	0.694	0.275	0.441	0	Slate <i>et al.</i> (1998)
IDVGA37	243	7	0.756	0.350	0.526	0.022	Slate <i>et al.</i> (1998)
IDVGA46	287	2	0.118	0.007	0.055	0.045	Slate <i>et al.</i> (1998)
IDVGA55	306	6	0.792	0.406	0.585	0.015	Slate <i>et al.</i> (1998)
IDVGA8	298	3	0.317	0.050	0.157	0.137	Slate <i>et al.</i> (1998)
ILSTS12	276	10 ⁿ	0.836	0.502	0.673	0	Kemp <i>et al.</i> (1995) [‡]
ILSTS33 ^p	249	6	0.687	0.264	0.431	0.185	Kemp <i>et al.</i> (1995) [‡]
ILSTS6 ^p	279	8	0.770	0.381	0.560	0.159	Slate <i>et al.</i> (1998)
ILSTS87	207	2	0.494	0.122	0.186	0	Kemp <i>et al.</i> (1995) [‡]
ILSTS93	300	7 ⁿ	0.781	0.395	0.575	0.010	Slate <i>et al.</i> (1998)
INRA11	279	11	0.683	0.298	0.486	0.107	Vaiman <i>et al.</i> (1992) [‡]
INRA121	323	18	0.871	0.590	0.743	0	Slate <i>et al.</i> (1998)
INRA131	318	6	0.641	0.227	0.384	0.068	Slate <i>et al.</i> (1998)
INRA35	316	8	0.848	0.526	0.693	0.037	Vaiman <i>et al.</i> (1994) [‡]
INRA40 ^p	236	5	0.670	0.239	0.398	0.043	Slate <i>et al.</i> (1998)
INRA6	330	5	0.321	0.052	0.159	0	Slate <i>et al.</i> (1998)
JAB8	178	3	0.327	0.053	0.162	0	Slate <i>et al.</i> (1998)
JP14	263	6 ⁿ	0.716	0.295	0.464	0.074	J. Pemberton
JP15	339	9	0.835	0.497	0.668	0.017	Marshall <i>et al.</i> (1998)
JP231	307	6 ⁿ	0.763	0.369	0.550	0.069	J. Pemberton
JP27	324	6	0.692	0.275	0.443	0	Marshall <i>et al.</i> (1998)
JP38	314	8	0.789	0.413	0.592	0	Marshall <i>et al.</i> (1998)
MAF109	321	6	0.738	0.324	0.500	0	Marshall <i>et al.</i> (1998)
MAF18	257	8	0.812	0.460	0.636	0.018	Crawford <i>et al.</i> (1990) [‡]
MAF35	308	6	0.669	0.257	0.428	0	Marshall <i>et al.</i> (1998)
MCM527 ^p	282	4	0.743	0.319	0.493	0.110	Hulme <i>et al.</i> (1994) [‡]
MGTG4B	301	7	0.749	0.349	0.527	0.047	Slate <i>et al.</i> (1998)
MM12	324	2	0.231	0.026	0.102	0.249	Kuhn <i>et al.</i> (1996)
PGAZAC2	328	5	0.372	0.072	0.203	0.099	R. Hammond (pers. comm.) [‡]
PRL	343	3	0.186	0.017	0.095	0.103	Fries <i>et al.</i> (1993) [‡]
RBP3	343	6 ⁿ	0.747	0.337	0.514	0	Slate <i>et al.</i> (1998)
RM12	322	10	0.787	0.416	0.593	0.034	Slate <i>et al.</i> (1998)
RM188	294	10	0.810	0.453	0.630	0.044	Slate <i>et al.</i> (1998)
RM4	356	2	0.140	0.010	0.065	0	Kossarek <i>et al.</i> (1993) [‡]
RM90	317	5	0.688	0.257	0.418	0	McGraw <i>et al.</i> (1997) [‡]

<i>Locus</i>	<i>Number scored</i>	<i>Number alleles</i>	<i>Het (Exp)</i>	<i>Pr (Ex₁)</i>	<i>Pr (Ex₂)</i>	<i>e_i*</i>	<i>Reference[†]</i>
RM95	299	8	0.708	0.298	0.469	0.092	Kossarek <i>et al.</i> (1994) [‡]
RT1	290	8	0.835	0.494	0.665	0.001	Wilson <i>et al.</i> (1997)
RT25	306	5	0.695	0.270	0.440	0.013	Wilson <i>et al.</i> (1997)
RT5	266	8	0.767	0.364	0.542	0.012	Wilson <i>et al.</i> (1997)
RT6	328	10 ⁿ	0.722	0.324	0.500	0.046	Wilson <i>et al.</i> (1997)
SPS113	317	10	0.762	0.372	0.550	0.015	Slate <i>et al.</i> (1998)
TGLA10	314	8	0.754	0.351	0.527	0.040	Slate <i>et al.</i> (1998)
TGLA127	324	10	0.841	0.523	0.690	0.007	Slate <i>et al.</i> (1998)
TGLA226 ^p	281	4	0.343	0.060	0.181	0.254	Slate <i>et al.</i> (1998)
TGLA322	277	14	0.881	0.605	0.756	0	Georges and Massey (1992) [‡]
TGLA337	230	8 ⁿ	0.750	0.347	0.526	0.039	Slate <i>et al.</i> (1998)
TGLA378	342	9	0.514	0.147	0.312	0	Slate <i>et al.</i> (1998)
TGLA40	311	5	0.668	0.245	0.411	0.050	Slate <i>et al.</i> (1998)
TGLA48	255	3	0.285	0.040	0.124	0	Fries <i>et al.</i> (1993) [‡]
TGLA86	317	5	0.737	0.328	0.507	0.240	Slate <i>et al.</i> (1998)
TGLA94	326	8	0.802	0.445	0.621	0	Marshall <i>et al.</i> (1998)
VH54	185	10	0.797	0.431	0.608	0.111	Pierson <i>et al.</i> (1994) [‡]
VH98	277	7	0.532	0.154	0.317	0.142	Slate <i>et al.</i> (1998)

Table Footnote

^p A locus that was difficult to score and so alleles were pooled to minimise typing error.

ⁿ Null allele segregating at this locus.

* Where estimated error rate was less than 0.01 it was regarded as 0.01 in the paternity likelihood calculations (see Chapter 5).

[†] First reference in which locus was described amplifying a product in red deer.

[‡] Locus not previously known to amplify a product in red deer, in which case first reference in source species is quoted.

In summary, a large panel of microsatellite markers was typed in members of the MAXI pedigree described in Chapter 3. The majority of markers were highly polymorphic, so that it should be possible to detect closely linked QTL of large effect on birth weight segregating in the pedigree. In Chapters 6 and 7 I describe how I mapped these markers and performed a QTL analysis. However, prior to performing these analyses it was essential to guarantee that inferred father-offspring relationships in the pedigree were correct. In Chapter 5 I describe how I confirmed or rejected previously awarded paternities.

5 A reassessment of previously inferred paternities

5.1 Introduction

All of the mother–offspring relationships in the MAXI pedigree are known to be correct. Detailed field observation of mother and calf at or soon after birth makes mis-assignment unlikely. Furthermore, in the large-scale paternity analysis described in Marshall (1998), no genetic incompatibilities were discovered between mother and calf after ten highly polymorphic markers were screened. Conversely a proportion of the inferred father–offspring relationships in the MAXI pedigree are certain to be wrong. As discussed in Chapter 2, behavioural data has severe limitations for assigning paternity, even in the closely monitored Rum population. Thus the 172 inferred paternities in the genotyped MAXI pedigree were all awarded using microsatellite data using the 80% or 95% confidence criteria generated by the paternity inference program CERVUS (Marshall *et al.* 1998). In this chapter I describe how I developed a likelihood approach, using data from all 84 microsatellite loci screened, to distinguish correctly inferred CERVUS paternities from those that were mis-assigned. Correcting the MAXI pedigree in this way was essential prior to attempting to map QTL. Additionally, this analysis allowed a retrospective assessment of the accuracy of the confidence assigned by CERVUS (Slate *et al.* in press).

I briefly introduced the program CERVUS in Chapter 2. Here I describe the program in greater detail and explain the advantages of the likelihood approach it uses over alternative methods of inferring paternity. Until recently, the majority of paternity analyses in the wild using molecular techniques relied upon the method of exclusion (e.g. Morin *et al.* 1994; Brotherton *et al.* 1997; Hogg and Forbes 1997). In exclusion-based paternity analysis any male that has a genotype incompatible with the offspring is rejected as the father until one male remains as the only possible father. This method is problematic as several males may remain non-excluded, or the correct father may be excluded due to typing error or mutation (San Christobel and Chevalet 1997; Marshall *et al.* 1998). If microsatellite data are used the correct male can also be excluded if null alleles are segregating (Pemberton *et al.* 1995). One way in which

these problems can be avoided is to use a likelihood-based approach (Thompson 1975). Such techniques allow for typing error (Marshall *et al.* 1998) and can assign paternity to the most likely male if several males are non-excluded (Meagher 1986).

Briefly, likelihood approaches use the available data to test alternative hypotheses (Edwards 1992). The probability of obtaining data D under a hypothesis H can be written $P(D|H)$. The ratio of the probability of observing the data under one hypothesis (H_1) compared with another hypothesis (H_2) is the likelihood ratio and is written:

$$L(H_1, H_2 | D) = \frac{P(D|H_1)}{P(D|H_2)}$$

The natural logarithm of the likelihood ratio is termed the LOD score. Note that the genetic mapping community take the common ($\log_{10}$) logarithm of the likelihood ratio to calculate LOD scores. I adhere to this distinction throughout the thesis. The likelihood method of Marshall *et al.* (1998) estimates the LOD score for each candidate male where the two hypotheses H_1 and H_2 are that the candidate male either is or is not the true father. The statistic Δ is the LOD of the highest ranking male minus the LOD of the next most likely male. By simulation, values of Δ are derived at which the most-likely male can be declared the father with relaxed (say 80%) or strict (95%) confidence. The simulations incorporate a number of parameters including typing error, the number of candidate fathers, the proportion of candidate fathers actually sampled and missing genotypes in the data set.

For the purposes of the paternity study performed by Marshall (1998), from which the MAXI pedigree was derived, the following simulation parameters were used:

Number of candidate males	75
Proportion of males sampled	0.65
Proportion of loci typed	0.854
Typing error rate	0.01

The 172 inferred paternities in the MAXI pedigree were assigned with at least 80% confidence, so it is expected that around one fifth (34) of them will be incorrect. Having screened all of the animals concerned at up to 84 loci it was now possible to determine unequivocally which of the inferred paternities were incorrect, and to adjust the MAXI pedigree accordingly.

5.2 Methods

5.2.1 Identifying incorrectly inferred paternities

The first stage in identifying incorrect paternities is to detect genetic mismatches between the CERVUS father and the calf. This was done using the relational database software Microsoft Access, and writing a structured query language (SQL) query for each locus (see Appendix E). Once all of the parent-offspring mismatches were determined, autoradiographs were checked for cases where the mismatch was attributable to scoring error. Any errors were corrected in the database and the query re-run for that locus. Four mother-offspring pairs showed frequent mismatches and it was apparent that these were attributable to template DNA from four wrong animals being erroneously placed in the deep well trays. These animals (AVO2, CLC9, COLC and TRT5) were removed from all subsequent analyses.

A number of loci were known to have null alleles segregating (see Table 4.3) and so homozygote-homozygote mismatches between parent and offspring were ignored at these loci. An alternative strategy would have excluded all data for these loci from the analysis, although such an approach would have failed to utilise all of the available information.

5.2.2 A likelihood method for reassessing paternities

The original paternity assignment method of Marshall *et al.* (1998) tested a number of candidate males as possible fathers of each calf. In contrast I am testing a single male, in an attempt to confirm whether he really is the father of a given calf. The simplest method with which to do this would be to use exclusion. However, it is certain, given the large number of loci I used, that a number of true fathers would

mismatch their offspring at some loci due to typing error or mutation. Hence, true fathers would be rejected. Here, I develop a new likelihood-based approach, taking into account locus-specific estimates of error rate, to reassess paternity and investigate the accuracy of CERVUS.

For the purposes of the likelihood equations two hypotheses are compared. The first is that the CERVUS assigned father *is* the true father, and the second is that the CERVUS assigned father *is not* the true father. The data used to test each hypothesis is either a genetic match or mismatch at each locus. A LOD score is calculated for each locus and then LODs are summed across all loci. A LOD score of zero implies that the CERVUS father is equally likely to be the true father as not the true father. A LOD of 3.0 means that the CERVUS father was 20 times more likely than not to be the true father. I used a LOD of 3.0 or more to confirm paternity and a LOD of less than -3.0 to reject paternity. Any LOD between -3.0 and 3.0 was regarded as inconclusive.

Four scenarios must be considered when deriving the likelihood equations:

- 1) Male and calf matching at a locus when the mother is unsampled.
- 2) Male and calf mismatching when mother is unsampled.
- 3) Male and calf matching when mother is sampled.
- 4) Male and calf mismatching when mother is sampled.

To calculate the likelihood ratio under any of these scenarios two locus-specific parameters, the error rate and the exclusion probability, are required.

Exclusion probability

This was calculated for each locus using the allele frequency option of CERVUS. For each locus two exclusion probabilities are calculated. One, $\text{Pr}(\text{Ex}_1)$ is the probability of excluding a random male from paternity when the offspring is sampled but the mother is not. The second, $\text{Pr}(\text{Ex}_2)$ is the probability of excluding a random male from paternity when both the offspring and mother are sampled. Formulae used to calculate these exclusion probabilities are given in Appendix 2 of Marshall *et al.* (1998). Exclusion probabilities for each locus are included in Table 4.3.

Error Rate

An initial estimate of error rate at each locus was calculated using the formula:

$$e_l \approx \frac{1}{2\text{Pr}(\text{Ex}_1)_l} \cdot \frac{m_l}{M_l}$$

(from Marshall *et al.* 1998)

where e_l = error rate at locus l .

m_l is no. of mismatching mother-offspring pairs.

M_l is no. of mother-offspring pairs compared.

$\text{Pr}(\text{Ex}_1)_l$ is the exclusion probability at that locus (see above).

This method assumes that each mother is only examined once, and that no single animal is analysed as both a calf and a mother. A number of animals in the MAXI pedigree appear in many mother-offspring comparisons, in which case a single comparison was randomly chosen so that no animal is included more than once. A total of 109 independent pairs was examined. Mother-offspring mismatches were detected using the SQL query given in Appendix E.

As mother-offspring pairs are known with certainty from field data, and have been confirmed with genetic data (Marshall *et al.* 1998), a number of mismatching genotypes can be corrected at each locus by retrospectively checking autoradiograms. The proportion of genotypes corrected at a locus is simply the number of genotypes changed, divided by the total number of genotypes.

The final error rate at each locus is the initial estimate of error rate minus the percentage of genotypes corrected. The final error rate at each locus is given in Table 4.3. An error rate of less than 1.0% was considered unlikely in a large-scale genome screen. Thus, to be conservative, any locus with an estimated final error rate of less than 1.0% was set to 1.0%. Note that only four correctable mother-offspring mismatches were discovered for the nine microsatellite loci used in the analysis by Marshall *et al.* (1998).

Derivation of likelihood equations

I first consider the two scenarios where the mother is not sampled.

For a true father and calf, the probability of both father and calf being correctly typed is $(1-e_l)^2$ where e_l is the error rate at locus l .

It follows that the probability of one or both being incorrectly typed is $1 - (1-e_l)^2$.

If there has been a typing error, the probability of it showing up as a mismatch is $\Pr(\text{Ex}_1)$, and so the probability of a mismatch between a true father and his offspring is $(1 - (1-e_l)^2) \Pr(\text{Ex}_1)$.

Therefore the probability of a match is $1 - (1 - (1-e_l)^2) \Pr(\text{Ex}_1)$.

If the CERVUS father is not the true father then the probability of a mismatch is by definition, $\Pr(\text{Ex}_1)$, hence the probability of a match is $1 - \Pr(\text{Ex}_1)$.

So for the case of father and calf matching, when mother is untyped the likelihood ratio is:

$$\frac{1 - (1 - (1-e_l)^2) \cdot \Pr(\text{Ex}_1)}{1 - \Pr(\text{Ex}_1)} \quad \text{Equation 1}$$

And for the case of a mismatch between father and calf when the mother is untyped the likelihood ratio is:

$$\frac{(1 - (1-e_l)^2) \cdot \Pr(\text{Ex}_1)}{\Pr(\text{Ex}_1)}$$

which simplifies to: $1 - (1-e_l)^2$ *Equation 2*

When deriving the likelihood equations for the two scenarios in which mother was sampled I made the assumption that the error rate in genotyping the mother was zero. There are two reasons for this. First, although I used only independent mother-

offspring pairs when estimating error rate, most mothers used in the analysis had more than one offspring. Many of them were also recorded *as offspring* of other hinds. Therefore the probability of a mother being wrongly typed is lower than for fathers or calves. Second, the majority of occasions on which a mother is mis-typed do not result in exclusion of the true father. For example, for a locus with 3 alleles at equal frequency (heterozygosity = 0.667) then only about 15% of maternal typing errors will result in a paternal exclusion.

Ignoring maternal typing errors gives the same likelihood equations as when father and offspring only are typed, except $\Pr(\text{Ex}_2)$ replaces $\Pr(\text{Ex}_1)$. Note that the likelihood equation is the same for the case of a mismatch between calf and CERVUS father regardless of whether or not the mother is genotyped.

5.2.3 Combining behavioural data with genetic data

In Chapter 2 I described how a daily census of the study area is made during the rut. Consequently the harem membership of all females is recorded. I used this data to test whether CERVUS assigned males who were also observed with the mother during the 11-day window (see Chapter 2) were more likely to be the true father than CERVUS males who were not behavioural candidates for paternity.

5.3 Results

The distribution of mismatches using a simple exclusion approach is given in Figure 5.1. Only 56 (32.6%) of the 172 paternities assigned using CERVUS, mismatched their putative offspring at zero loci. However the distribution of mismatching loci per calf tested is clearly bimodal, suggesting that a number of CERVUS fathers are the true father, but mismatch with their offspring at just a few loci.

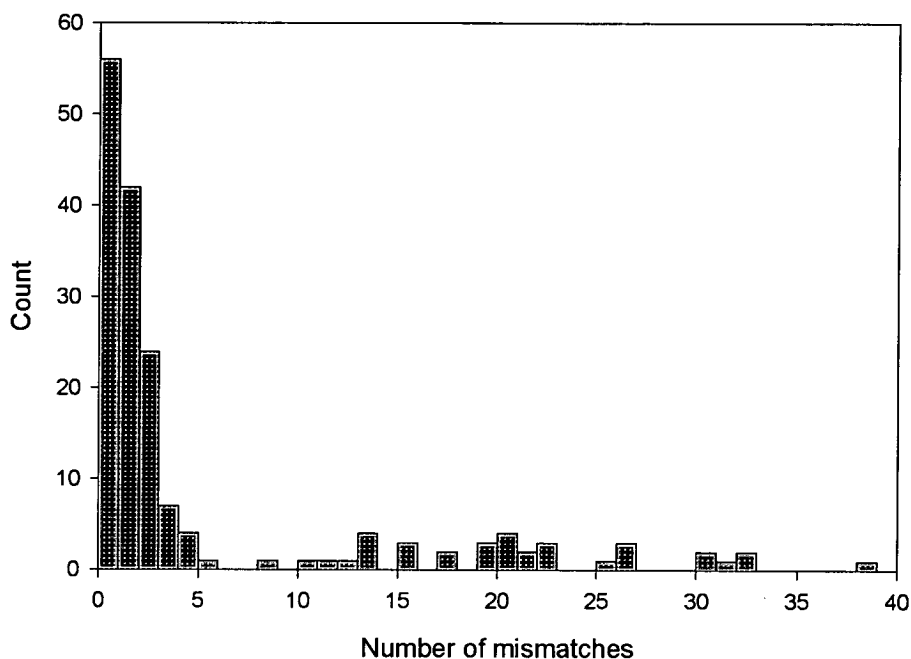


Figure 5.1 Plot showing number of mismatches per CERVUS father–offspring pair. Note the bi-modality of the distribution.

The distributions of LOD scores for the 80% and 95% paternities are shown in Figures 5.2 and 5.3 respectively. The LODs ranged from -100.5 to 48.7 and only three calves had a LOD score between -3.0 and 3.0 . These three calves had father – offspring comparisons at around only 20 loci (the mean number of comparisons per calf was 58) and so the inability to determine conclusively whether the CERVUS father was the true father is unsurprising. The remaining 169 paternities could be unequivocally confirmed or rejected, and are summarised in Table 5.1. The number of correct paternities assigned with both 80% and 95% confidence was slightly, but not significantly, lower than expected (80% paternities: $G = 1.14$, d.f. = 1, n.s; 95% paternities: $G = 0.616$, d.f. = 1, n.s).

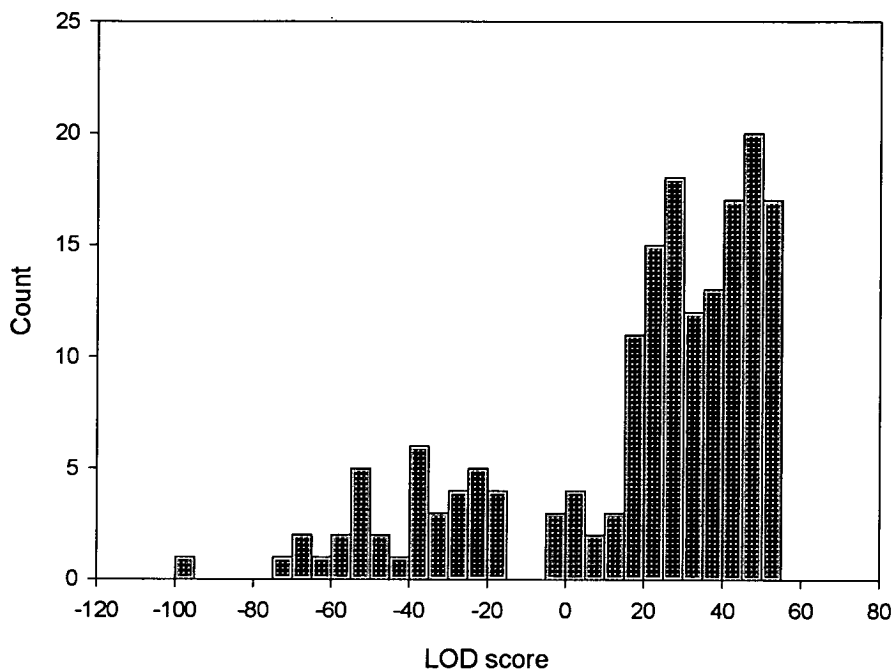


Figure 5.2 (upper) and Figure 5.3 (lower).

Distribution of LOD scores for CERVUS-assigned 80% paternities (Fig 5.2) and CERVUS assigned 95% paternities (Fig 5.3). Note that the distributions are bi-modal and that few individuals have a LOD score between -3.0 and 3.0 .

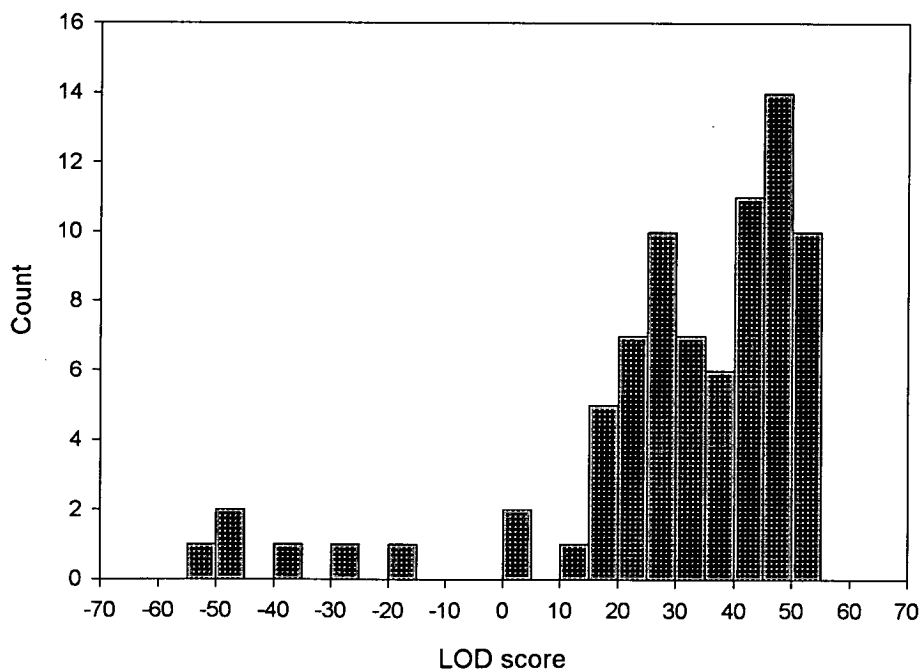


Table 5.1: True paternity of CERVUS-assigned males.

The table shows the number and proportion of 80% and 95% confident CERVUS fathers that were the true father. CERVUS fathers are separated into those which were or were not observed with the mother around the time of oestrus. Six mothers were not observed at all over the relevant rut interval. Three CERVUS-assigned fathers are excluded from the table as paternity could not be retrospectively confirmed or rejected (see text).

	CERVUS 80% confident	CERVUS 95% confident
Harem male and true father	96 / 96 (100%)	54 / 54 (100%)
Not observed as harem male, true father	25 / 67 (37%)	14 / 20 (70%)
No harem data available, true father	6 / 6 (100%)	0
Total true father	127 / 169 (75.1%)	68 / 74 (91.9%)

Maternal harem membership data were available for 163 of the 169 calves for whom the CERVUS assigned male could be confirmed or rejected as the father. Every candidate male assigned by CERVUS *and* observed with the mother in his harem was the true father. In contrast a male assigned at 80% confidence by CERVUS, but not observed holding the mother in his harem during the 11-day window was only 37% likely to be the true father. Two of the three males with a LOD score between -3.0 and 3.0 were not observed with the mother of their putative calf. In these cases the male was not regarded as the true father. In total, 44 (25.6%) of the 172 paternities initially included in the MAXI pedigree were rejected.

5.4 Discussion

To my knowledge this is the first time that paternities assigned in a natural population using molecular techniques have been retrospectively scrutinised. Clearly the statistical confidence assigned by CERVUS is accurate. To detect a small, yet significant, departure from the predicted confidence a large number of father-offspring pairs would be required. However the sample size (172 pairs) presented here is sufficient to recognise any marked difference between the predicted and actual confidence in CERVUS assigned paternities. Since its launch in 1998, the CERVUS software has been rapidly adopted by biologists using molecular markers to infer paternity in the wild, and already several papers which use the software have been published (e.g. Coltman *et al.* 1999a; Gerloff *et al.* 1999). This analysis

suggests that users of CERVUS can be confident in the accuracy of inferred paternities, provided that their simulation parameters are reasonably well estimated.

In the study population, behavioural data can be combined with genetic data retrospectively to adjust confidence in paternity. It was striking that every CERVUS-assigned male who was also observed as a harem male was the true father of the calf in question. In the analysis of Marshall (1998) paternity was assigned for 462 calves with 80% confidence. A highly accurate pedigree can now be drawn using a combination of paternities assigned with 95% confidence, and those assigned with 80% confidence that have additional behavioural support. Adjusting the pedigree in this way will improve the accuracy of analyses that require accurate assignment of paternity (e.g. QTL mapping, estimating heritability, estimating inbreeding coefficients), but without causing a large reduction in pedigree size. It should be noted that I am not advocating the use of behavioural data as a reliable predictor of paternity in the absence of genetic support.

This analysis has also demonstrated the value of measuring typing error rates when employing microsatellites. If a strict exclusionary approach had been used, around half of the true paternities would have been rejected. Recent attention in the literature has focused on high typing error rates when amplifying microsatellites from 'difficult' template material such as hair or faeces (Goossens *et al.* 1998; Taberlet and Luikart 1999). Although typing error is likely to be higher when small quantities of non-invasively sampled DNA are used, the possibility of error should always be considered, regardless of the type and quantity of tissue sampled. The loci with the highest error rates in this study tended to have particularly intense stutter-bands, an observation also made by Taberlet and Luikart (1999). Mother-offspring relationships, inferred with reasonable confidence, are a good resource for estimating error rates.

It is possible that there are small inaccuracies in the LOD scores presented in this chapter. I have assumed that all maternal typing errors were detected, and the exclusion probabilities I used did not account for segregating null alleles. However,

the majority of calculated LOD scores are so unequivocal in determining whether paternity is true or false, that any slight error is unlikely to change the outcome of the decision reached. The likelihood equations that account for these extra variables would be considerably more complex than those presented here, and the final outcome is unlikely to be substantially different.

In conclusion, information from the 84 loci that were screened to detect QTL was used to adjust the MAXI pedigree so that all inferred relationships were correct. The power to detect QTL in the revised pedigree will be slightly lower than that presented in Chapter 3. However the data set is now suitable for constructing a genetic map (Chapter 6) and performing a QTL analysis (Chapter 7).

6 A deer genetic map

6.1 Introduction

In order to perform QTL analysis a genetic map is required. Genetic maps give the order and position of loci along each chromosome. Several methods of QTL analysis use algorithms that require knowledge of marker positions relative to one another, e.g. interval mapping and multipoint mapping approaches. Even when single point mapping is performed (an approach that tests each marker independently), marker location is required to distinguish between a scenario in which one QTL is associated with several tightly linked markers from the alternative interpretation, in which several unlinked markers are each associated with a different QTL. In this Chapter I describe how I mapped a large panel of microsatellite markers, including those that were screened in the MAXI pedigree. Two different strategies were employed. Initially I screened each marker in the International Mapping Herd (IMH) used by Mike Tate and co-workers to produce a deer genetic map (Tate *et al.* 1994; Tate *et al.* 1995; Tate 1997). Thus, I attempted to place these markers on the pre-existing genetic map. However, for reasons I discuss later, I also attempted to construct a genetic map using only the genotypic data that was obtained from screening the markers within the MAXI pedigree.

6.2 Adding microsatellites to the current deer genetic map

6.2.1 Interspecies hybrids as a gene mapping resource

In Chapter 4 I described how evolutionarily conserved markers, such as allozyme loci and RFLPs obtained from gene probes, are known as Type 1 loci (O'Brien *et al.* 1993). In contrast, Type 2 loci are relatively poorly conserved, anonymous markers (e.g. microsatellites, RAPDs, AFLPs). The majority of linkage maps are constructed using Type 2 loci, as they tend to be highly polymorphic within the species of interest. Examples include the genetic maps of rats (Steen *et al.* 1999), cattle (Ma *et al.* 1996; Barendse *et al.* 1997), sheep (Crawford *et al.* 1995) and many crop plants (e.g. McCouch *et al.* 1997). A notable exception is in mice, where a large number of both Type 1 and Type 2 loci have been mapped. Interspecific backcross lines

between laboratory mice (*Mus musculus domesticus*) and *Mus spretus*, a wild species found on the Iberian peninsula, have provided an ideal mapping resource for mouse geneticists (Avner *et al.* 1988). As a consequence of the large genetic divergence between the two strains, hybrid mice tend to show two species-specific variants when probes to known genes are assayed using restriction fragment length polymorphisms (RFLPs). Thus, Type 1 loci can be mapped by performing linkage analysis in backcross mapping panels. Attempts to produce maps of Type 1 loci in other mammals have proved difficult, as hybrids between divergent strains, breeds or species tend to be sterile (Tate 1997). However fertile hybrids have been produced in deer by crossing red deer with Père David's deer (*Elaphurus davidianus*).

6.2.2. Red deer x Père David's deer hybrids as a gene mapping resource

Hybrids between deer species are not uncommon (Whitehead 1993), particularly in deer parks, but those between red deer and Père David's deer are perhaps the most remarkable. Père David's deer originate from China and are one of the rarest deer species in the world. Although they have the same number of chromosomes as red deer ($n = 33$), Père David's deer are very different in morphology, in particular with respect to antler, tail and foot shape. The two species are classified in different genera, and the genetic distance between them (Nei's $D = 0.48$; Tate 1997) is comparable to that between the *Mus* species described above. Despite being the heterogametic sex, hybrid male deer are nonetheless fertile, an important consideration when trying to produce backcross mapping herds rapidly.

The colourful history of Père David's deer also plays a role in their suitability as a genetic mapping resource. Père David's deer became extinct in the wild, possibly around 220 AD (Goosen 1997), and the only surviving animals were kept in an imperial hunting park in China. The species became known to the Western World after their discovery by the French explorer-naturalist Père Armand David in 1865, when the park population was estimated at 120. Following several unsuccessful attempts to import the species to Europe, a pair eventually bred at Woburn Abbey, England in 1898, and additional introductions were subsequently made. Around this time the Chinese population was killed, due largely to poaching by troops during the

Boxer rebellion. Hence, the entire current world population is descended from 18 animals at Woburn. In fact, the breeding population may have been even lower, and was perhaps between three and 13 individuals (Goosen 1997). Consequently, it is unusual to detect genetic variation at either allozyme or microsatellite loci in Père David's deer (Woodruff and Ryder 1986; Emerson and Tate 1993; Chapter 5). The lack of polymorphism enhances the probability that hybrid sires will show species specific alleles at a marker locus, and hence be informative in backcrossed mapping herds (Tate *et al.* 1995).

Although natural matings have occurred between red and Père David deer in at least one deer park, the F1 hybrids produced in New Zealand were created using artificial insemination (AI). Since the late 1980's over 300 $\frac{3}{4}$ red / $\frac{1}{4}$ Père David's deer have been produced (mainly by AI) by backcrossing F1 sires to red deer hinds at AgResearch, Invermay Agricultural Centre, New Zealand. By virtue of the large genetic distance between the two species, F1 sires show species specific restriction fragments at almost all genes when assayed by heterologous DNA probes. Subsequently it has been possible to produce a map of Type 1 loci in deer by linkage analysis in the backcross pedigrees derived from the F1 males. To date 16 protein polymorphisms and 148 restriction fragment length variants (RFLVs) for known genes have been mapped (Tate 1997). In contrast, only 19 microsatellites were mapped in deer at the beginning of this study, despite their potential in ruminant comparative mapping (Chapter 4).

6.2.3 Mapping new microsatellites

In this section I describe how I attempted to add microsatellite loci to the existing deer genetic map.

6.2.3.1 The mapping panel

At the beginning of the study I obtained DNA from all individuals in the deer International Mapping Herd (IMH). The mapping panel consisted of two hybrid sires (ID's 899 and 903) and a number ($n = 40$ and $n = 49$ respectively) of their backcross progeny. Aliquots of dilute DNA (100 μ g/ml) from the entire mapping pedigree were

stored in a 96 well microtitre plate, so that each locus could be rapidly screened by PCR using a multi-channel pipette. Duplicate DNA aliquots from each hybrid sire were included, as it was essential that genotypes were obtained for these two individuals. Additionally, DNA from a pure Père David's deer was included, so that the allele inherited from the Père David's deer sire could be distinguished in the F1 hybrids.

6.2.3.2 Screening microsatellites in the IMH

A total of 120 microsatellite markers were screened in the IMH (locus details are given in Appendix F). This panel of microsatellites included 68 of the 84 markers that were screened in the MAXI pedigree (Chapter 4). Of the remaining 16 loci, 15 were previously typed in the IMH by Mike Tate and colleagues (AgResearch, New Zealand). The only marker that was typed in the MAXI pedigree but not the IMH was the sheep microsatellite locus McM527. The standard PCR conditions described in Appendix B were used for all loci, unless indicated otherwise in Appendix C. A locus was mappable only if one or both of the hybrid sires were heterozygous. Loci that were monomorphic in red deer were still considered suitable for mapping for two reasons. First, they are useful as comparative map markers if they are conserved between other ruminants and deer. Second, they may act as anchor loci, making it easier to map informative linked loci that were screened in the IMH.

6.2.3.3 Scoring and coding microsatellite autoradiograms

Rather than code alleles by size, I adopted the system used by Tate (1997). Thus, the coding system was consistent with all loci previously screened in the IMH, regardless of the type of marker. The main feature of the coding system was that the F1 sires were coded 'PA' at informative loci, where a 'P' represented the allele inherited from the Père David's deer sire and an 'A' represented the allele inherited from the red deer dam. The paternally inherited 'P' allele was unambiguously identified, by comparing the F1's banding pattern with that of the pure Père David's deer. Distinguishing between the two F1 alleles in this way increased the power to map markers as the marker phase was known. In the backcross progeny the maternally inherited allele was coded as 'A' or 'C'. If coded 'A', then the maternally

inherited allele was the same size as the red deer allele in the hybrid sire. A 'C' allele indicated that the maternally received allele was a different size to both of the hybrid sire alleles. Hence, progeny could have one of the following genotypes: AA, PA, AC, PC or PP. PP genotypes were rare, and could only occur when the Père David's deer 'P' allele is also present in some of the red deer dams. A detailed discussion of the allele coding system is given in Tate (1997). An example of a locus scored using this system is given in Figure 6.1.

For some loci it was unclear from which of the parent species the F1 hybrid alleles had been inherited. In such cases, the two alleles were coded 'A' and 'B', while any other allele, inherited from the red deer dams, was coded 'C'.

Finally, it is theoretically possible to map a marker if the hybrid sires appear homozygous, but are in fact heterozygous with a null allele received from the Père David's deer. In such cases, the hybrid sires were coded as genotype AA, while any additional red deer alleles inherited from the dams were coded 'B'. Hence progeny could have the following genotypes: AA, AB or BB. AA genotypes are ambiguous (they could represent a genuine homozygote or an animal with a paternally inherited null allele). However AB progeny can only have inherited the red deer 'A' allele from the F1 sire, while BB progeny can only have inherited the Père David's deer null allele from the F1 sire.

All genotypes were entered into a Microsoft Excel (Microsoft Corporation) spreadsheet and sent by electronic mail to Mike Tate (AgResearch, New Zealand). The microsatellite data were then incorporated into a database containing genotypes from mapped protein and RFLV polymorphisms.

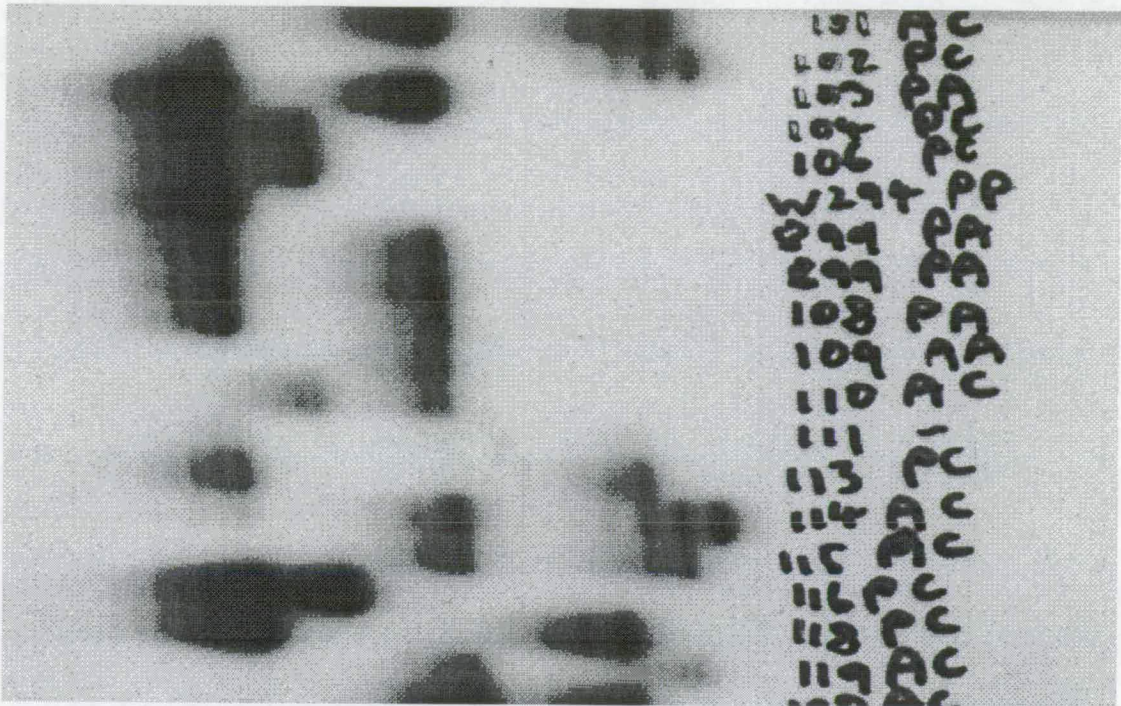


Figure 6.1. Autoradiogram of microsatellite locus SPS113 screened in the International Mapping Herd (IMH). Animal 899 is one of the hybrid sires, and any animal with code 1XX is one of his backcross progeny. Animal W294 is a pure Père David's deer stag, and is homozygous for the smaller of the two 899 alleles. Hence this allele can be labelled 'P' in sire 899 and the other allele labelled 'A'. (See text for a detailed description of how alleles were assigned).

6.2.3.4 The mapping software

The map location of each marker was determined by Mike Tate using the Mapmaker (Lander *et al.* 1987) and Animap (Nielsen *et al.* 1995) software packages. Mapmaker is the more-user friendly program, and analysis is rapid. However, there was one major drawback to using Mapmaker. At some loci, occasional backcross progeny had the genotype PP. Thus, the 'P' allele is present, albeit at low frequency, amongst the red deer dams. Consequently, Mapmaker cannot determine which allele was paternally inherited in progeny coded PA. Mapmaker treated PA progeny as 'unknown', and the power to map the locus was compromised. In contrast, analysis with Animap was slower as a full maximum likelihood approach was used. However, fewer assumptions about the origin of each sire allele were made. Animap estimates the allele frequencies in the red deer dams from the progeny genotypes. Hence, the frequency of PA genotypes where the 'P' allele was maternally inherited can be

estimated, and PA progeny need not be discarded from the linkage analysis. Although analysis is slower using Animap, a greater number of markers can be mapped and the final map positions are expected to be more accurate.

Microsatellite markers were added to the existing genetic map provided linkage to another marker was supported by a LOD score of at least 3.0. Once a group of linked markers was established, the most likely order was estimated using maximum likelihood. Next an analysis was performed (by Mike Tate) in which any double recombinant haplotypes were identified. I then re-examined the relevant autoradiograms to exclude the possibility of genotyping error. If any errors were identified, the linkage group in question was reconstructed using Mapmaker/Animap.

6.2.3.5 Summary of microsatellite mapping in the IMH

Of the 120 markers screened, 105 of them were theoretically mappable (by virtue of being heterozygous in at least one hybrid sire). However, only 31 of these markers are currently mapped in deer. The latest version of the deer genetic map can be viewed at a webpage maintained by the Roslin Institute, Midlothian, Scotland (URL: <http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb>). Currently there are 230 loci mapped in deer of which 148 are known genes, 66 are microsatellites and 16 are allozyme loci. There are three reasons for the discrepancy between the number of microsatellites screened, and the number actually mapped.

- 1) The more accurate linkage analysis using Animap has not been completed for a number of microsatellite markers. Loci with any progeny with genotype PP are relatively uninformative when using Mapmaker, as the progeny with genotype PA are treated as unknown.
- 2) A number of microsatellites were not typed for all individuals in the IMH. I obtained a limited amount of some microsatellite primers, and it was not possible to make repeated attempts to amplify 'problematic' loci. The statistical power to map partially typed loci was sufficiently low to make chromosomal assignment impossible.

3) The majority of the previously mapped RFLV and allozyme loci were informative in both F1 sires (Tate 1997). However, it was reasonably common to screen a microsatellite locus at which only one F1 sire was a heterozygote. Thus the power to map the locus was relatively low.

The attempt to map microsatellites in the IMH can be regarded as a success, in that a large panel of mappable markers was identified and screened. However, further analysis with Animap is required to actually assign a map position for the majority of these markers. Only 35 of the 84 microsatellites screened in the MAXI pedigree are currently assigned a map position, an insufficient number to perform a QTL analysis. With this in mind, I also attempted to map loci using the genotypes generated in the MAXI pedigree.

In addition to providing a map location for the remaining markers there are other reasons for independently constructing a deer map. It should be remembered that the map generated from the IMH is actually a map of the red deer / Père David's deer hybrid genome. It is likely, but not certain, that the locus order and locations in hybrids are the same as for red deer, particularly as the two species have similar karyotypes (Tate, 1997). However translocations may have occurred in the hybrids, giving a different locus order to that of red deer. Constructing a deer genetic map from the MAXI pedigree will prove useful if the same linkage groups and order of loci are found as those on the pre-existing deer map. Independently verifying the original map in this way will lend further support to its accuracy. Finally, some markers screened in the MAXI pedigree were homozygous in the two IMH hybrid sires, and thus could not be placed on the original map. In the next section I describe how I constructed a deer genetic map from the MAXI pedigree.

6.3 A deer genetic map from the MAXI pedigree

Previously I described how I screened markers (Chapter 4) and corrected wrongly inferred paternities (Chapter 5) within the MAXI pedigree. With this information it was possible to perform a linkage analysis in the pedigree, and map microsatellites of previously unknown location in deer. Note that more individuals were screened per locus than in the IMH, but the power to map loci in complex outbred pedigrees is generally lower than that for the backcross design used in the IMH (where markers are informative in all sires). Unlike the IMH, there was no pre-existing map for the MAXI pedigree, to which microsatellites could be linked. However, given the large number of animals in the MAXI pedigree and the informativeness of the majority of the microsatellites, it should nonetheless be possible to build a simple map.

6.3.1 Linkage analysis using CRI-MAP

For the linkage analysis I used the CRI-MAP V2.4 software (Green *et al.* 1990), run on the University of Edinburgh UNIX server, Holyrood. CRI-MAP allows construction of linkage maps from general pedigrees, even with some missing data. Where possible, CRI-MAP deduces missing genotypes from the genotypes of ancestors and descendants. Although highly flexible, CRI-MAP does not use a full likelihood algorithm (in the way that Animap does) for two reasons. 1) If an inferred genotype can plausibly be a homozygote, this genotype is scored as 'missing' and, 2) if an individual at the top of the pedigree is unscored, allele frequencies are not used to infer possible genotypes. However, the loss of power from these two assumptions seems to be minimal (Green *et al.* 1990). Furthermore, the most important animal at the top of pedigree, MAXI, is scored at nearly all loci, minimising any possible loss of power.

I used a LOD score of 3.0 to infer linkage between two loci. Markers were also assumed to be linked if they were supported by a LOD of greater than 1.0 *and* there was an *a priori* reason for expecting linkage: i.e. they were linked in another ruminant. With the exception of six loci (four isolated from caribou, one from gazelle, and one from red deer) a previously derived map location in the species of origin was available for all of the 84 microsatellites screened in the MAXI pedigree.

In addition to the 84 microsatellites, genotypes at the three protein loci screened by Pemberton *et al.* (1988, 1991) were included in the CRI-MAP analysis. All three of the proteins had a previously assigned chromosomal location in cattle. The source species map location of each locus was ascertained using one of the following published papers or websites:

Cattle genetic maps:

The map generated by the International Bovine Reference Panel (IBRP) described in Barendse *et al.* (1997). URL: <http://spinal.tag.csiro.au/cginfo.html>.

The Illinois Reference/Resource Families (IRRF) map described by Ma *et al.* (1996). URL: <http://cagst.animal.uiuc.edu/genemap/map.html>.

The ARK database maintained by the Roslin Institute (Midlothian, Scotland) was also used. This database contains all loci on both of the aforementioned cattle maps, in addition to other markers. Genetic maps of other livestock species including sheep and deer are also viewable at this website.

URL: <http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=cattle>

Sheep genetic maps:

The map generated by AgResearch, New Zealand and described in Crawford *et al.* (1995) and de Gortari *et al.* (1998). URL: <http://www.ri.bbsrc.ac.uk/sheepmap/>

Deer genetic maps:

The map described in section 6.2 and reported in Tate (1997).

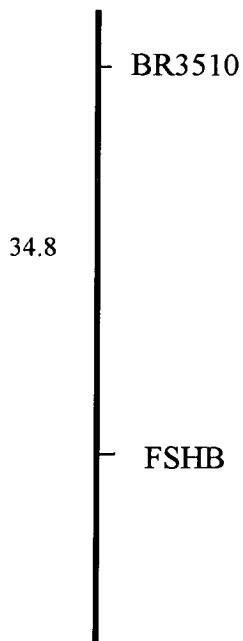
URL: <http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=deer>

Having established linkage groups, locus order was determined using the BUILD option of CRI-MAP. The BUILD option uses the Kosambi mapping function to estimate map distances between loci. A mapping function is a simple formula that predicts distance between two loci, given the number of observed recombinations between them (Lynch and Walsh 1998). Any mapping function takes into account

that an even number of cross-overs between two loci are undetectable, thus the observed number of recombination events is always a minimum estimate of the true number. The Kosambi function also incorporates a degree of interference (the phenomenon in which a cross-over event reduces the probability of a second cross-over occurring in an adjacent region) in its estimate of map distance (Lynch and Walsh 1998). Alternative locus orders were compared using the FLIPS option of CRI-MAP, and the order with the highest likelihood was used in the final map.

6.3.2 Summary of microsatellite and protein mapping in the MAXI pedigree

A summary of linked markers (supported by a $\text{LOD} > 3.0$) is given in Appendix G. Of the 87 (84 microsatellites and 3 allozymes) loci screened in the pedigree, 45 of them were linked to another locus with support of $\text{LOD} > 3.0$. A further 10 loci could be added to linkage groups with support of $\text{LOD} > 1.0$ *and* prior evidence of linkage from another map. The final map was 508.5 cM long and is illustrated in Figure 6.2.

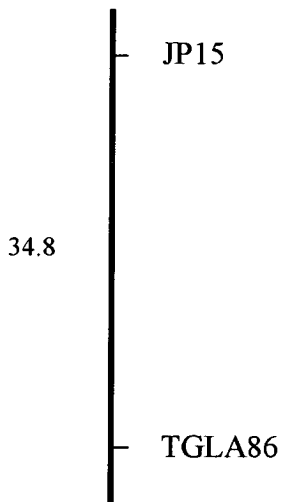


JAB8
RM4

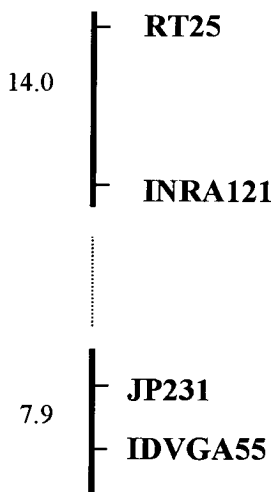
Linkage Group 1
= *cattle 15, sheep15*

AGLA293
FCB5

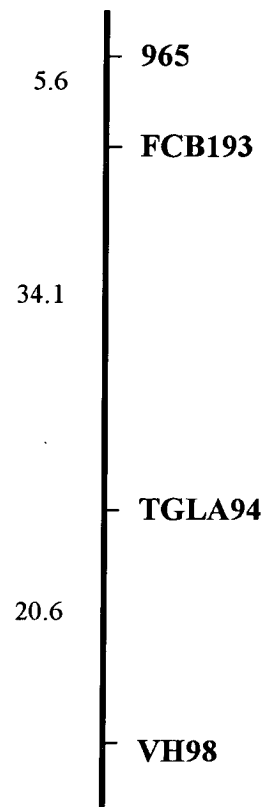
Linkage Group 3 (or 22)
= *cattle5, sheep3*



Linkage Group 2
= *cattle 29, sheep 21*

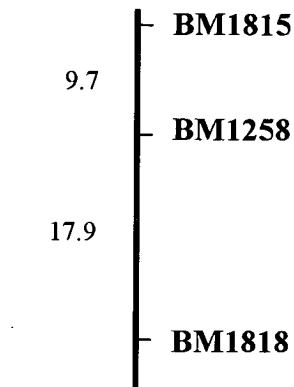


Linkage Group 4
= *cattle 18, sheep14*



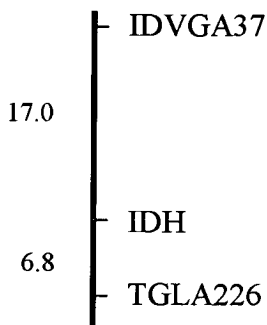
IDVGA46
TGLA322

Linkage Group 5
= *cattle 17&19, sheep 11&17*



PRL

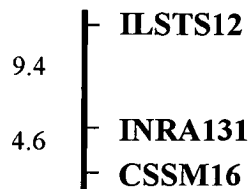
Linkage Group 7
= cattle 23, sheep 20



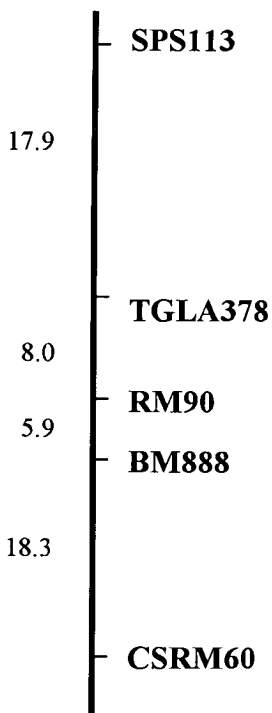
Linkage Group 8
= cattle 2, sheep 2

TGLA48
RM12
ILSTS6

Linkage Group 9
= cattle 7, sheep 5

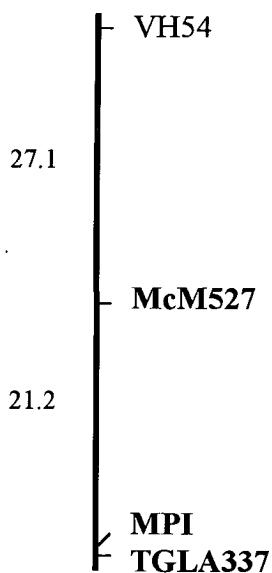


Linkage Group 11
= cattle 11, sheep 3

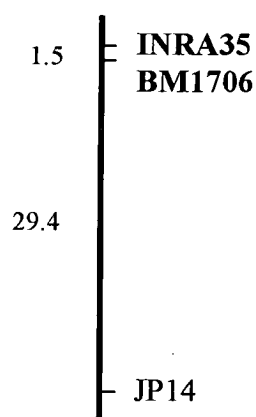


CSSM39

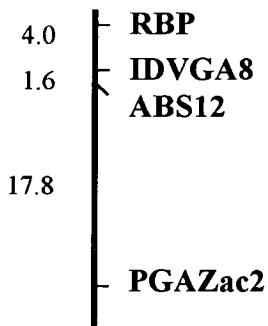
Linkage Group 12
= cattle 10, sheep 7



Linkage Group 13
= cattle 21, sheep 18 (&5?)



Linkage Group 14
= cattle 16, sheep 12



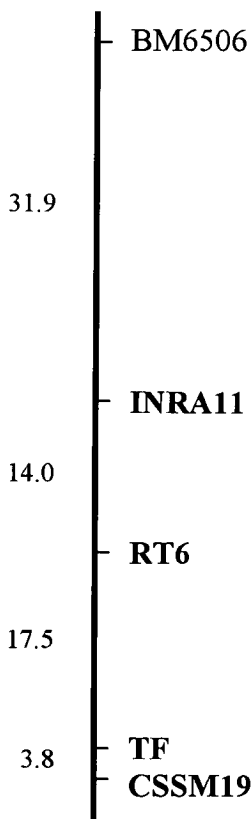
Linkage Group 15
= cattle 26 & 28,
sheep 22 & 25



Linkage Group 17
= cattle6, sheep6

RM188
CP26
MGTG4B

Linkage Group 18
= cattle4, sheep4



Linkage Group 19
= cattle 1, sheep 1

INRA6
HUJI177
TGLA127

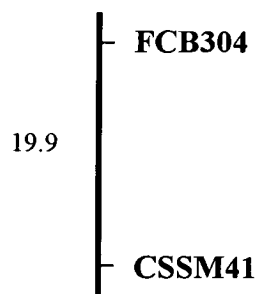
Linkage Group 20
= cattle 3, sheep 1



Linkage Group 21
= cattle 14, sheep 9

FCB5
AGLA293

Linkage Group 22 (or 3)
= cattle 5, sheep 3



Linkage Group 24
= cattle 22, sheep 19

MAF109

HIS-H1
HUJ175

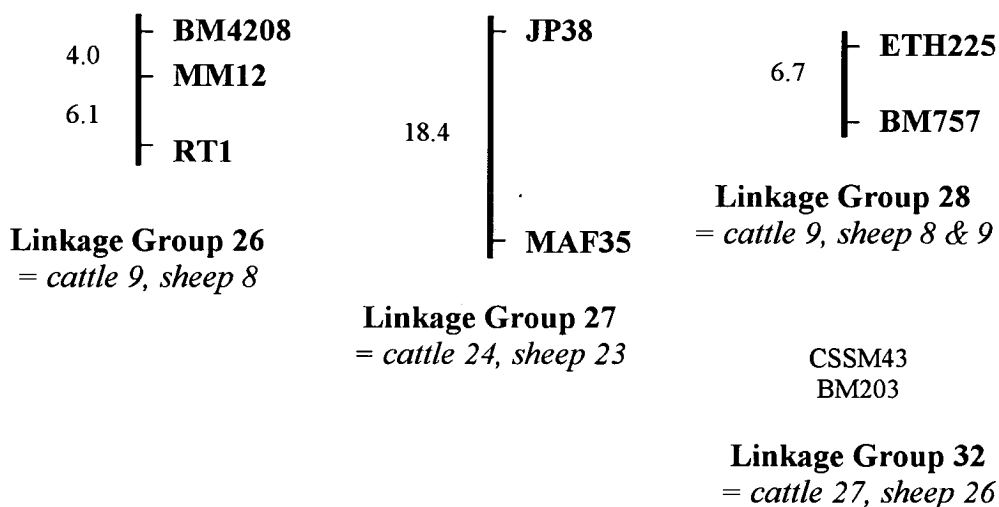


Figure 6.2: A deer genetic map derived from the MAXI pedigree (see methods in section 6.3.1).

Linkage group number is that used by Tate (1997).

Distances between markers are given in Centimorgans. Total map distance is 508.5 cM.

Markers mapped with a LOD score of at least 3.0 are represented in bold.

Markers mapped with a LOD score of 1.0 - 3.0 *and* previously shown to be on that deer linkage group or the bovine/ovine equivalent are represented in plain type face.

Markers with a positive LOD score of 0.0 - 1.0 *and* previously shown to be on that deer linkage group or the bovine/ovine equivalent are placed below the linkage group.

Each linkage group is placed in the most likely marker order, and is supported by the AgResearch deer genetic map order or a conserved order with cattle and/or sheep maps (see section 6.3.3).

Linkage Groups 6, 10, 16, 23, 25, 29, 30, 31 and 33 are not represented in the figure. From homology with cattle/sheep genetic maps it is expected that all but two of these linkage groups are represented by a single microsatellite used in the current study.

No microsatellites from the cattle/sheep homologues of the remaining two linkage groups (16 and 25) were screened, hence no markers were expected to map to these locations.

Marker RT5, a microsatellite originally characterised in caribou, did not map to any linkage group and is not mapped in any other species.

6.3.3. Discussion of the map derived from the MAXI pedigree

Comparison with other published maps suggests that the majority of the 32 loci that could not be assigned a linkage group, were not expected to be closely linked to any other genotyped locus. As six loci were not mapped in any other species it was impossible to predict exactly how many loci might be unlinked to any other. It is notable that the markers that were assigned to a linkage group did not have a significantly higher heterozygosity than markers that were not (linked markers, mean heterozygosity = $0.685 \pm \text{SE } 0.025$ vs. unlinked markers, mean heterozygosity = $0.633 \pm \text{SE } 0.034$: two tailed t-test, $t=1.21$, d.f. = 85, $p = 0.21$, NS). This would suggest that the failure to assign all markers to a linkage group is principally attributable to the relatively small number of markers screened, rather than any shortcoming in statistical power of the MAXI pedigree.

The deer genetic map generated using CRI-MAP and the MAXI pedigree is in remarkably good agreement with both the AgResearch deer map (Tate 1997) and other ruminant maps. Amongst the markers that were assigned with a LOD > 3.0, only one mapped to a different linkage group than expected. The sheep derived microsatellite McM527 was placed on deer linkage group 13 (equivalent to sheep chromosome 18), yet is mapped to chromosome 5 in sheep (Crawford *et al.* 1995). McM527 was assigned on the basis of a LOD score of 3.50 supporting linkage to the locus TGLA337. Although some loci were assigned to linkage groups partially on the basis of their known location on other maps, the order of markers was nonetheless identical between homologous chromosomes for all but two linkage groups, lending further support to the accuracy of the MAXI pedigree map.

The order of loci on two linkage groups was retrospectively adjusted, after comparison with other ruminant maps. Figure 6.2 shows the final adjusted map. In both cases the likelihoods of the original and adjusted locus orders were very similar. On linkage group 15 the original order (markers ABS12 and IDVGA8 juxtaposed) gave a slightly higher LOD score than that illustrated. However linkage group 15 is homologous to a fusion of cattle chromosomes 26 and 28, and an additional inversion would have to be invoked for the original order to be correct. On linkage

group 7 an alternative order (BM1258-BM1815-BM1818) gave a slightly higher LOD score ($\Delta\text{LOD} = 0.19$) to that shown in Figure 6.2, but the illustrated order is consistent with both the cattle map and the previous deer genetic map. The locus order of one other linkage group is of particular interest on this map. Tate (1997) found a different locus order between deer linkage group 19 and its bovine and ovine homologues. The current analysis is in agreement with the order suggested by Tate (1997), and supports his claim that there has been an inversion on this chromosome since the divergence of the cervidae from other artiodactyls.

The LOD scores used for assigning linkage in this analysis were lower than that used for other ruminant maps. For example, Barendse *et al.* (1997) used a LOD of 6 for assigning linkage and a LOD of 3 for assigning order, while Ma *et al.* (1996) used LODs of 4.5 and 2.0 for the same categories. However, the present analysis did take into account locus position from both the 'official' deer map and maps generated for cattle and sheep. The use of prior information can be considered similar to a Bayesian approach (Shoemaker *et al.* 1999), although no actual values for prior probabilities of linkage between two loci were estimated.

The overall map length was only 508.5 cM. This compares poorly with the IMH map which covers 1270 cM of the deer genome. Furthermore, the complete deer genome is expected to be at least 2000 cM long (Tate *et al.* 1994), suggesting that a genome-wide search for QTL is impossible using the current map. However, it should be remembered that the estimated map distance does not include unlinked markers, or the regions beyond the end-most markers on each linkage group. It is possible to detect QTL that are linked to these markers, yet not included in the regions from which the map length was estimated. Thus, rather more than 508.5 cM of the genome will be covered in the subsequent QTL analysis. It appears probable that markers were screened in the MAXI pedigree on all but two of the deer autosomes. While it would be wrong to claim that a complete genome-wide search for QTL is possible with the available data, it would also be erroneous to suggest that only $\frac{1}{4}$ of the genome could be analysed.

6.4 Conclusions

Two independent efforts were made to map microsatellite loci in deer. The first was an attempt to add loci to the existing deer genetic map (Tate 1997). However, a number of loci remained unassigned, and so an alternative approach was taken. A linkage analysis was performed using the genotypes derived from screening the MAXI pedigree. To my knowledge this is the first time that a linkage map has been constructed from a wild outbred population. Although not all loci could be assigned a linkage group, this was usually because they were not closely linked to another locus, rather than due to any lack of statistical power. It follows that a multipoint QTL analysis of these sparsely covered chromosomes would produce similar results to a method that tested each marker individually. Conversely, those chromosomes with several linked markers (e.g. linkage groups 5, 7, 8, 11-15, 19) are particularly suited to multipoint linkage analysis. Using this approach for the study population will increase the power to detect QTL, and provide estimates of QTL position and magnitude (Lynch and Walsh 1998). In Chapter 7 I describe a multipoint QTL analysis of birth weight using these data.

7 A search for birth weight QTL

7.1 Introduction

The main purpose of this thesis is to make the first attempt at mapping QTL for a fitness-related trait in an unmanipulated wild population. Birth weight in the Rum red deer population is an appropriate trait for such an analysis as it is related to fitness and has a heritable component (see Chapter 2). In Chapter 3 I described a power analysis to detect QTL in the study population and concluded that it was theoretically possible to detect QTL provided they were of large effect. The power to detect QTL was greatest when an approach that used information from a complex pedigree was used. In this Chapter I describe a QTL analysis for birth weight, using the genotypes screened in the six generation pedigree descending from the stag MAXI. I first review methodologies for detecting QTL in complex outbred pedigrees. I then describe the approach I took for this analysis, before presenting the results from the QTL analysis. Finally I discuss whether the data presented here provide good evidence that genes of large effect may segregate for fitness-related traits in a wild population.

7.2 Detecting QTL in complex pedigrees

The various approaches used by geneticists to detect QTL were reviewed in Chapter 1. By far the most demanding methods, computationally and statistically, are those developed for complex outbred pedigrees. In this section I describe the underlying principles behind these techniques. I focus on methods that estimate the expected number of alleles identical by descent (IBD) for all relatives in a pedigree, and then use variance components methods to infer the presence of a QTL (i.e. the method described by Almasy and Blangero, 1998).

An appropriate point at which to begin a description of these methodologies is with the Haseman-Elston regression (1972). This approach was developed to detect QTL when data is collected from many pairs of sibs. It is assumed that sib-pairs who share QTL alleles are, on average, more similar for the underlying trait than those who do not. The Haseman-Elston approach uses marker data and maximum likelihood to estimate the number of alleles likely to be IBD at a putative linked QTL. For every

sib pair, the squared difference in trait value is then regressed on the number of estimated alleles IBD. A significantly negative slope indicates that a QTL is present at that location. Although this approach for detecting QTL is elegant and relatively simple, it does have a number of drawbacks (Lynch and Walsh 1998). Only one type of relative pair can be considered at a time, parent-offspring pairs cannot be considered as they all share one allele IBD, and the method only tests for the presence of a QTL, rather than estimating position and magnitude. Finally there is debate as to whether all possible combinations of sib-pair in a family can be regarded as independent (Lynch and Walsh 1998).

Recently, interval mapping (Fulker and Cardon 1994) and multipoint mapping (Fulker *et al.* 1995; Krugylak and Lander 1995) approaches for sib-pair designs have been developed. In these methods, the information from several linked markers is used to estimate the number of alleles IBD more accurately and at any position on a chromosome. Consequently, these methods are preferable to the original Haseman-Elston method, as they provide greater statistical power and an estimate of QTL magnitude and position.

The use of variance components methods in QTL mapping was first suggested by Goldgar (1990). The major advantage of this approach over other methods is that different types of relative pair can be analysed simultaneously. Furthermore, a specific genetic model (e.g. additivity, dominance, epistatic interactions) need not be specified. Each chromosomal region is examined to see whether it explains a significant amount of the overall trait variance (Visscher *et al.* 1999). Any such regions are assumed to contain a QTL, and the proportion of the variance explained by the region gives an estimate of the QTL magnitude. A variance component method applicable to complex pedigrees was described by Almasy and Blangero (1998). A probability matrix for the IBD relationships between all pairs of individuals at any specified chromosomal location is estimated, using Monte Carlo techniques and information from all linked markers. The IBD probability matrix for each location is incorporated into a variance components model, and maximum likelihood is used to test for the presence of a QTL. At the recent 10th Genetics

Analysis Workshop (GAW10), where different QTL detection methods were employed to address the same simulated problem, variance components procedures applied to general pedigrees were shown to have high power, a low rate of false positives, and produced unbiased estimates of QTL magnitude and position (Wijsman and Amos 1997; Williams *et al.* 1997). Thus far, only a handful of studies (Almasy *et al.* 1999; Visscher *et al.* 1999; Williams *et al.* 1999) have actually used variance components methods to detect QTL in complex pedigrees, largely because analysis is computationally demanding and the technique is relatively new. However, this approach is likely to become widespread and will prove particularly useful in human pedigrees and natural populations. In the next section, I outline the multipoint, variance components based approach I used to attempt to detect birth weight QTL in the complex MAXI pedigree.

7.3 Methods

The QTL analysis took place in two stages. I first calculated IBD coefficients between all pairs of animals in the corrected MAXI pedigree. I then used the estimated IBD matrix to test each chromosomal region for the presence of a QTL, using a variance components analysis. This method is analogous to the Haley-Knott regression method, frequently used for QTL mapping in inbred lines. Under this method genotypes at putative QTL locations are estimated as a function of marker genotype and location, and then a least-squares regression approach is used to estimate QTL magnitude and location (Haley and Knott 1992).

7.3.1 Estimating IBD matrices.

Calculating the number of alleles IBD between all relative pairs in the pedigree was the most time-consuming and computationally demanding part of the analysis. To do so I used an adapted version of the LOKI V2.1 software (Heath 1997). I calculated IBD coefficients at 5cM intervals along each chromosome, using map distances from the deer map constructed from the MAXI pedigree (Chapter 6). IBD matrices were also calculated at actual marker positions, and for an additional 5 cM and 10cM beyond the most distal markers on each linkage group. Where a marker was unlinked to any other, the IBD matrix was calculated at that marker, and at positions 5cM and

10cM away. Calculating IBD coefficients at actual markers is relatively easy as the majority of markers were highly informative (Chapter 4). However, estimating the IBD matrix at other locations is more difficult and a function of the distance from the linked markers and their informativeness. LOKI uses a Markov Chain Monte Carlo method to estimate IBD coefficients at all locations (Heath and Thompson 1997). Initially I ran 10,000 iterations of the IBD program for each chromosomal location but this proved time-consuming. Consequently, I compared the IBD matrix derived after 10,000 iterations with that from 1,000 iterations. As the two outputs were almost identical (see Figure 7.1), I used 1,000 iterations for subsequent analyses. However, if a region provided evidence for a QTL the IBD matrix was re-calculated using 10,000 iterations at each location on that linkage group.

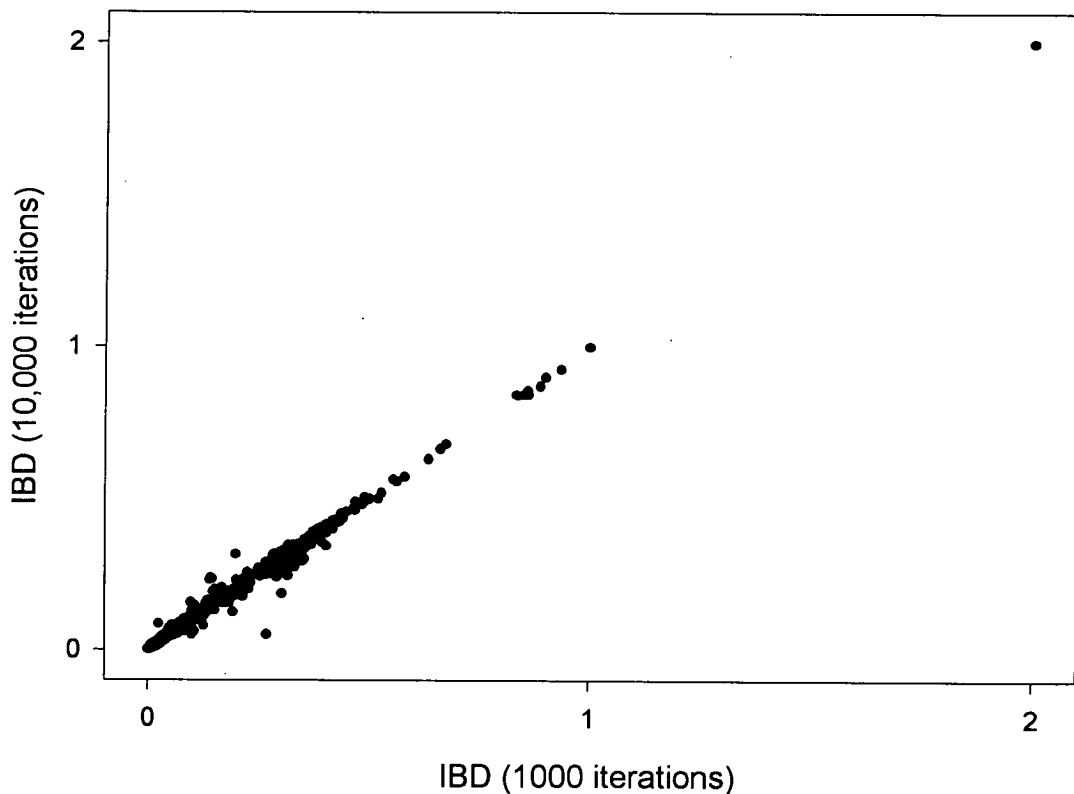


Figure 7.1: The relationship between IBD coefficients between pairs of individuals obtained from 1000 or 10000 iterations of LOKI.

The regression equation is $IBD_{10000} = -0.00104 + 0.996IBD_{1000}$. The R-squared value is 99.7%, indicating that the values obtained after 1000 iterations provide a satisfactory estimate of IBD coefficient.

To help distinguish alleles that are IBD from those that are identical by state (IBS), allele frequencies are used. In a similar analysis to the current one, Visscher *et al.* (1999) estimated IBD coefficients by two subtly different methods. The first approach used the marker allele frequencies in founder individuals to distinguish between IBD and IBS alleles, while an alternative approach assumed that all founder alleles were at equal frequency. Visscher *et al.* (1999) argued that the former approach may overestimate the number of alleles that are IBS rather than IBD, leading to a decrease in statistical power. Visscher *et al.* (1999) found that the latter (equal allele frequency) approach tended to yield higher LOD scores for a QTL responsible for uni- and bipolar disorder in humans. Thus, I used the approach in which allele frequencies were assumed equal in founder individuals.

LOKI failed to calculate IBD coefficients for a small number of deer markers. In general, the markers concerned were relatively uninformative and/or contained many missing genotypes, and would have been the least informative in QTL analysis. Provided other markers were linked to the problematic markers, it was still possible to calculate IBD coefficients along the relevant chromosome.

7.3.2. Using variance components to detect QTL

Having calculated IBD coefficients at all locations it was possible to perform a variance components analysis to detect QTL. For this purpose, I used a Fortran 77 program written by Peter Visscher (University of Edinburgh) which uses a restricted maximum likelihood (REML) method to estimate variance components. REML is preferable to other maximum likelihood (ML) approaches, in that fixed effects are not assumed to be known without error when estimating variance components (Lynch and Walsh 1998), and the sum of squares is divided by $n-k$ rather than n (where n is the sample size and k is degrees of freedom). This avoids the downwardly biased estimates of variance components that conventional ML techniques can sometimes yield. When the number of fixed effects is relatively small (as is the case here), the difference in variance estimates between the two methods is small (Visscher *et al.* 1999).

Three different models must be constructed in order to detect QTL by variance components analysis. The first is referred to as the *polygenic model*. In this model it is assumed that the trait is influenced by a large number of independent loci of small effect, and the heritability of the trait is estimated. In a second model, marker information is used to estimate whether any genetic variation can be attributable to a QTL in the region around the marker(s). I refer to this model as the *QTL model*. Finally, a *polygenic + QTL model* was constructed in which it is assumed that the trait variation is attributable to a QTL in the region under investigation, in addition to any background genetic effect.

The polygenic model

This is essentially the ‘animal model’ widely used in animal breeding to estimate breeding values of each individual in the pedigree. Kruuk *et al.* (in press) used an animal model to estimate heritabilities and coefficients of variation for a number of traits in the Rum population, including birth weight (see Chapter 2). Although the principal purpose of this analysis was not to estimate the heritability of birth weight, it was still necessary to build this model, because to detect QTL, it is important to determine that the region under investigation explains additional variation over that explained by the polygenic model. Otherwise, the QTL or QTL + polygenic model could be detecting underlying polygenic variation when in reality no QTL is present in the region under investigation. This problem is particularly acute for regions with uninformative markers, when the estimated number of alleles IBD in that region will be similar to the mean number of alleles expected IBD between two individuals at any location in the genome (i.e. the relatedness or twice the coefficient of coancestry between the individuals).

Using matrix notation the polygenic model can be written:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \text{ (Lynch and Walsh 1998)}$$

Where $\mathbf{y}$ is the vector of trait values

$\mathbf{b}$ is the vector of fixed effects (e.g. subdivision of study area, spring temperature)

X is an incidence matrix linking actual observations to fixed effects.

Z is an incidence matrix linking actual observations to polygenic effects.

a is the vector of random polygenic effects.

e is the vector of random environmental effects.

It is assumed that the mean and variance of **y** are:

$$E(\mathbf{y}) = \mathbf{Xb}$$

$$\sigma^2(\mathbf{y}) = \mathbf{V} = \mathbf{ZAZ}^T \sigma_a^2 + \mathbf{I} \sigma_e^2 \text{ (Lynch and Walsh 1998, Visscher *et al.* 1999)}$$

Where **A** is the numerator relationship matrix

Z^T is the transpose of **Z**

V is the variance-covariance matrix

I is the identity matrix.

Each element of matrix **A** simply represents the relatedness between a pair of individuals (or the proportion of alleles expected to be IBD between a pair of individuals across the entire genome). So, for a parent-offspring pair the matrix element would be 0.5 and for half-sibs it would be 0.25. Note that the elements of **A** are inferred from the pedigree only and that no marker information is used at this stage.

Under this model an estimate of heritability of the trait in the pedigree is obtained. A maximum likelihood value for the estimate is also obtained, and this is later used to compare the various models.

I also estimated the polygenic component of birth weight using the PEST VCE software (Groeneveld 1993). This REML package does not allow a QTL model to be fitted, but was used by Kruuk *et al.* (in press) to estimate the heritability of fitness components and morphometric traits in the study population. This independent analysis enabled me to check that the software I used obtained realistic estimates of heritability, and allowed further comparison of my estimate of heritability of birth weight with that described by Kruuk *et al.* (in press).

QTL model

This model is similar to the polygenic model, except that marker information is used to determine whether specific regions explain trait variation. The model can be expressed in matrix notation as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zq} + \mathbf{e}$$

where $\mathbf{q}$ is the vector of random QTL effects (Visscher *et al.* 1999) with expected variance $\sigma^2(\mathbf{q})$.

$\mathbf{Z}$ is an incidence matrix linking actual observations to QTL effects.

The expected variance is given by $\sigma^2(\mathbf{y}) = \mathbf{V} = \mathbf{ZQ_iZ}^T \sigma_q^2 + \mathbf{I} \sigma_e^2$

where $\mathbf{Q_i}$ is the IBD relationship matrix at a particular chromosomal location.

It is this relationship matrix that is estimated by LOKI, using the information from all markers on that chromosome. When marker data are incomplete or uninformative the elements in this IBD relationship matrix will be similar to those in the numerator relationship matrix specified in the polygenic model, and unlinked polygenic information may be mistaken for a QTL. An estimate of the amount of trait variation attributable to the QTL and a corresponding maximum likelihood value are obtained.

Polygenic + QTL model

Under this model variance components attributable to both a polygenic effect and the chromosomal region being tested are estimated. The model may be written as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Zq} + \mathbf{e}$$

An estimate of the polygenic heritability (h^2) and of the variance explained by the QTL (q^2) are obtained. An overall maximum likelihood value is again obtained.

7.3.3 Fitting fixed effects

The variance components method that I used allows fixed effects to be included in all of the models. In Chapter 2 I described a generalised linear model (GLM) in which various terms influencing birth weight were fitted. A number of the fixed effects that

were demonstrated to be associated with birth weight were categorical, e.g. mother's reproductive status, subdivision of study area, sex (see Chapter 2; Appendix A). The model described in Chapter 2 had a large sample size ($n = 1072$) so that all levels of any categorical explanatory variable contained at least 30 cases. However, this may not be true for the smaller sample used in the QTL analysis ($n = 365$) and so any categorical variables where cell sizes were less than 30 were susceptible to Type 1 error (Crawley 1993). Applying this rule, only the following covariates and fixed effects were fitted: April-May mean temperature, birth date, mother's reproductive status, and sub-division of study area. Models that used this data set are referred to as using a *fixed effects* input file.

Although the fixed effects data set was conservative in that categorical variables were not fitted if cell sizes were small, a number of environmental variables known to influence birth weight could not be included. The ability to detect QTL may depend on removing as much environmental variation as possible (Chapter 3) and so an alternative input file to that used in the fixed effects data set was also used. Rather than use raw values of birth weight and include fixed effects in the model, the residual values from a GLM similar to the one described in chapter 2 were used. The only explanatory variable not fitted in this *GLM residuals* data set was 'MAXI descendant'. This model is expected to have slightly greater power to detect QTL than the fixed effects model as more environmental variation has been accounted for.

Finally, I ran a model that fitted standardised multi-locus heterozygosity (MLH) as a covariate, in addition to the GLM residual values. In Chapter 8 I describe how standardised MLH is positively associated with birth weight. However, there is a good reason for performing a QTL analysis both with and without MLH included as a covariate. The variance components method used does not specify a particular genetic model. In theory it is not possible to determine whether a QTL acts in an additive or a dominant fashion. Thus, if there was overdominance acting at a QTL, and animals heterozygous at this locus had a higher MLH, then the effect of the QTL may be underestimated when MLH is fitted as a covariate. The data file that included MLH as a covariate was termed the *GLM residuals + heterozygosity* input file.

7.3.4 Obtaining test statistics

Two different test statistics were obtained from the variance components analysis. One tested for polygenic variation (heritability), and the other for the presence of a QTL in a specific region. Performing this second test across the entire genome was the ultimate goal of this thesis.

1) Polygenic variation:

By comparing the maximum likelihood value obtained from the polygenic model, with that obtained from a model in which heritability is assumed to be zero (the null model) a likelihood ratio test (LRT) statistic can be calculated using the formula:

$$\text{LRT} = -2 [\text{maximum likelihood (ML) of reduced model} - \text{ML of full model}].$$

(Lynch and Walsh 1998)

The LRT statistic is distributed as a half times a χ^2 with one degree of freedom, and a half times a point mass of zero (Almasy and Blangero 1998). To obtain a significance value a χ^2 distribution with 1 d.f can be used, and the p -value halved. In other words LRT statistics of 2.71 and 5.41 correspond to p -values of 0.10 and 0.02 for a χ^2 with 1 d.f., but should be halved, giving p -values of 0.05 and 0.01. An LRT statistic can be converted to a LOD score (base 10) by dividing by $2\ln(10) = 4.6$ (Lynch and Walsh 1998).

2) Detecting QTL

For this test the LRT statistic was obtained by comparing the maximum likelihood values for the polygenic + QTL model with the polygenic model. In other words, the region under investigation had to be describing trait variation over and above that attributable to the polygenic effects of unlinked loci. The LRT statistic and p -value are obtained in the same way as described for the polygenic model. By estimating the LRT statistic and LOD score for different points along a chromosome a profile for the entire chromosome can be plotted (see Figure 7.2).

In theory, a LRT statistic could be obtained by comparing the QTL model with the null model. However, this comparison may include a polygenic component, hence inflating the amount of trait variation attributable to loci in the region under test. To be conservative I do not report the LRT statistics obtained from the QTL model versus null model comparison.

7.3.5 Significance thresholds

A highly contentious issue in QTL mapping is that of statistical significance (Lander and Kruglyak 1995). When carrying out a genome-wide search for QTL, multiple tests for linkage are performed, and it is probable that a number of these will exceed a p value of 0.05 by chance alone (Type 1 error). A solution may be to use a Bonferroni correction for multiple tests, whereby an overall experiment-wide significance level can be obtained by dividing the desired p value by the number of tests (Sokal and Rohlf 1995). However, a Bonferroni correction assumes that all tests are independent, and clearly this is not the case when several closely linked regions are being tested for the presence of a QTL. One solution may be to treat each chromosome as an independent test, and obtain an experiment wide significance level by dividing the desired p value by the number of chromosomes tested. However, red deer have a relatively large number of autosomes ($n = 33$), and an experiment-wide significance threshold of $p = 0.05/33 = 0.0015$ may be too stringent, resulting in real QTL being missed (Type 2 error).

An alternative and more robust approach to obtaining experiment wide significance results is to use permutation testing (Churchill and Doerge 1994; Doerge and Churchill 1996). By this method trait values are randomly shuffled between individuals but the marker data are retained. The test statistic is calculated and the procedure repeated many times generating an empirical distribution of the test statistic under the null hypothesis of no QTL segregating. From this distribution significance thresholds can be calculated that are robust to the distribution of effects (Lynch and Walsh 1998). However, a permutation test is not applicable to the models tested here, because swapping the phenotypes with respect to genotypes will not only change the estimate of QTL magnitude, but will also change the amount of polygenic

variance. Hence, an empirical distribution for the case of no QTL segregating over and above the background polygenic variation cannot be obtained.

Guidelines to what significance levels should be used to assign linkage were suggested by Lander and Krugylak (1995). They recommended the use of different criteria for evidence that a QTL is segregating. *Suggestive linkage* corresponds to a test statistic that is expected to be exceeded at least once by chance in a genome-wide scan. *Significant linkage* corresponds to a test statistic that is expected to be exceeded 0.05 times by chance in a genome-wide scan. Lander and Krugylak (1995) derived a formula for calculating these thresholds as a function of genome length and number of chromosomes. For an outbred pedigree:

$$\mu(T) = [C + 2\rho GX] \alpha(T)$$

where $\mu(T)$ is the mean number of times that a LOD score T is expected to be exceeded in a genome-wide scan.

C is the number of chromosomal pairs ($n=33$ for red deer)

ρ is a parameter related to the crossing-over rate and $=1$ for general pedigrees

G is the total map length (in Morgans)

X is the LRT statistic described above.

$\alpha(T)$ is the significance of exceeding threshold T assuming that X is distributed as half times a χ^2 with d.f. $=1$ and half times zero.

I obtained estimates of thresholds for suggestive linkage and significant linkage by solving this formula for $\mu(T) = 1$ and 0.05 respectively.

The total length of the map I constructed from the MAXI pedigree was 508.5 cM. However, in the QTL analysis IBD coefficients were calculated for positions 10 cM beyond the markers at either end of each linkage group. Additionally, markers that were unlinked to any other were used in the QTL analysis, and I regarded these as representing a 10cM segment of chromosome. Thus, when calculating thresholds for suggestive and significant linkage I used an overall map length of 937.0 cM (9.37M).

Solving the Lander and Krugylak (1995) formula yields LOD scores of 1.4 (LRT = 6.44) and 2.8 (LRT = 12.88) for suggestive and significant linkage respectively. It could be argued that these thresholds should be reduced as the markers were spaced a relatively large distance from each other, and the formula assumes an infinitely dense map.

Once thresholds for suggestive and significant linkage are determined there still remains the problem of actually deciding whether a QTL is really segregating. Lander and Krugylak (1995) suggest that every test yielding a p value of 0.05 or less (before allowing for multiple comparisons) should be reported, but that a QTL can only be considered as confirmed if exceeding an experiment-wide significance of 0.05 in two independent studies. With this in mind I report any tests that are significant at $p < 0.05$ (corresponding to an LRT of 2.71 or a LOD of 0.59).

7.4 Results

7.4.1 Polygenic Variation

All three input files (fixed effects, GLM residuals and GLM residuals + heterozygosity) gave significant and similar estimates for the amount of polygenic variation for birth weight segregating in the pedigree (see Table 7.1). Additionally, the PEST VCE software gave an almost identical estimate for the fixed effects data set. These values are all similar to those obtained by Kruuk *et al.* (in press), who estimated birth weight separately for males and females, obtaining values of 0.112 ± 0.067 and 0.250 ± 0.071 respectively. Note that only the PEST VCE software provides a standard error for the estimated heritability.

Clearly there is substantial additive genetic variation for birth weight segregating in the MAXI pedigree. As expected, the residuals data set gave a higher estimate of heritability than the fixed effects model, as more of the environmental variation had been removed.

Table 7.1: Estimates of polygenic component for birth weight obtained from the three different input files described in section 7.3.3. An estimate for the fixed effects data set was also obtained using the PEST VCE software.

Input File	ML of null model	ML of polygenic model	LRT	LOD	<i>P</i>	Heritability
Fixed effects	-195.0166	-192.0595	5.91	1.29	0.004	0.176
GLM Residuals	-180.1100	-176.5535	7.11	1.55	0.002	0.195
GLM Residuals + heterozygosity	-176.4962	-173.7386	5.52	1.20	0.005	0.176
PEST VCE (using fixed effects)	-	-	-	-	-	0.178 ± 0.067

One other fitness related trait for which there were complete data in the MAXI pedigree, and for which a QTL analysis might be possible, was juvenile survival (to age two). However, there was no evidence for any heritable variation for this trait (data not shown) and so no further analyses were undertaken.

7.4.2 Variation in birth weight due to QTL

Weak evidence for birth weight QTL was found on three different chromosomes (see Table 7.2). The thresholds for suggestive linkage (LOD = 1.4) and significant linkage (LOD = 2.8) were never exceeded, although all three regions were significant at $p < 0.05$. Plots showing the LRT statistic and LOD score at intervals along linkage groups 14 and 21 are given in Figure 7.2. The other significant result was for the marker MAF18 which is believed to be on linkage group 23, but was unlinked to any other marker, and so only analysed as a single point marker. The three different input files gave very similar results across the entire genome, and no single input file yielded a significant result at a location where the other two did not.

Table 7.2: Evidence for birth weight QTL on 3 linkage groups.

The table shows the proportion of trait variation attributable to the QTL (q^2), the remaining polygenic variation (h^2), the nearest marker to the point giving the highest test statistic, the LOD score, the likelihood ratio test statistic (LRT) and the probability of obtaining that LRT by chance (p) in a single test. The fixed effects input file included four continuous explanatory variables (see 7.3.3), the GLM residuals file used the residual values obtained from a GLM containing extra categorical terms and a larger sample size (see 7.3.3), while the GLM residuals + heterozygosity file additionally included multilocus heterozygosity estimated from 71 markers, as a continuous fixed effect (7.3.3). The three input files give similar results.

Linkage group	Input File	q^2	h^2	Nearest marker	LOD	LRT	p
14	Fixed effects	0.245	0	JP14	0.87	3.75	0.026
	GLM residuals	0.245	0	JP14	1.17	5.40	0.010
	GLM residuals + heterozygosity	0.236	0	JP14	0.87	3.98	0.023
21	Fixed effects	0.243	0	BM2934	1.17	5.40	0.010
	GLM residuals	0.239	0	BM2934	0.85	3.90	0.024
	GLM residuals + heterozygosity	0.183	0	BM2934	0.53	2.43	0.060
23	Fixed effects	0.273	0.087	MAF18	1.31	6.02	0.007
	GLM residuals	0.194	0.077	MAF18	0.86	3.94	0.024
	GLM residuals + heterozygosity	0.197	0	MAF18	0.72	3.29	0.035

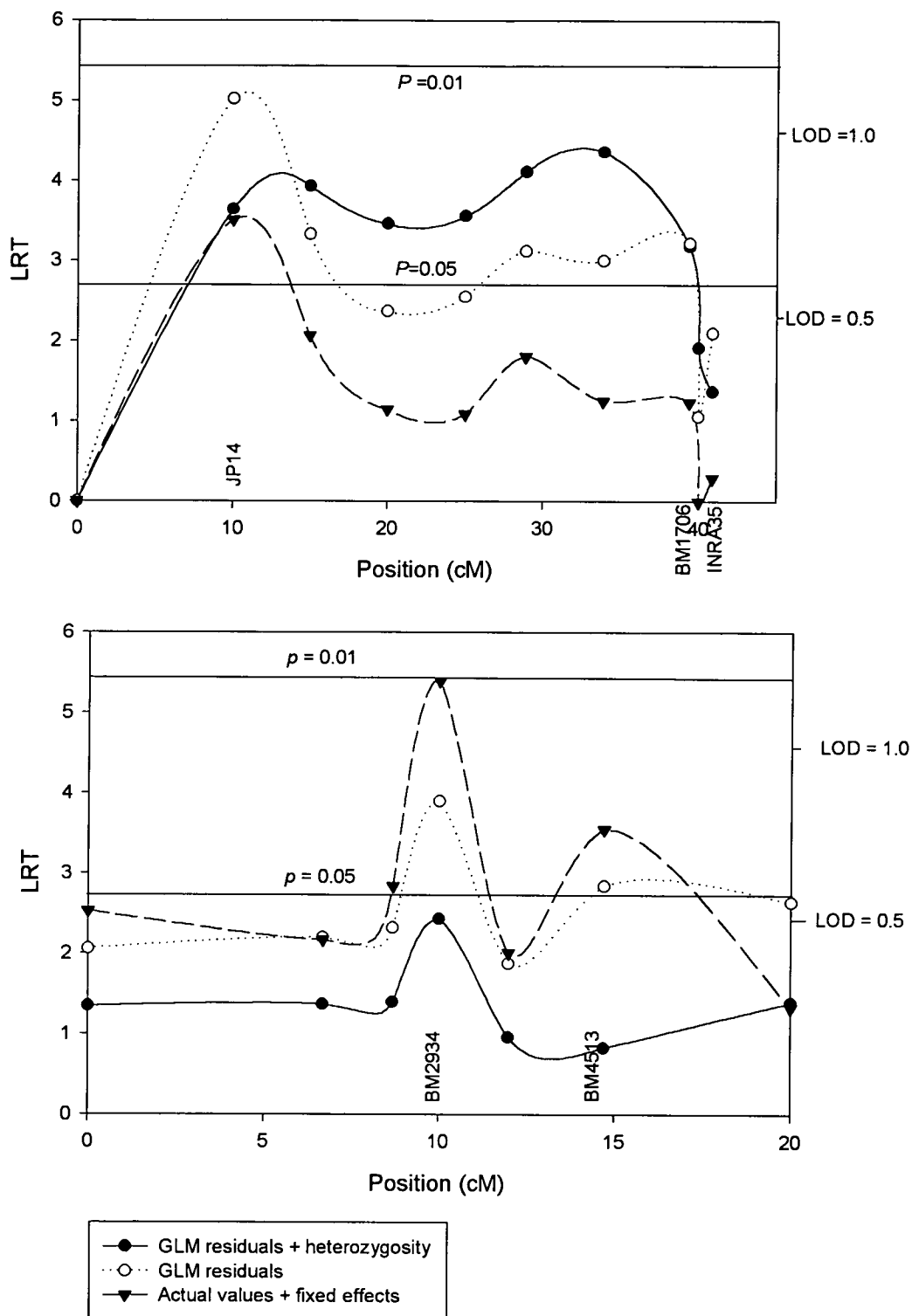


Figure 7.2: Profiles of likelihood ratio test (LRT) statistics along linkage groups 14 (upper plot) and 21 (lower plot). Locations of markers are indicated, in addition to the thresholds for $p < 0.05$ and $p < 0.01$.

7.5 Discussion

The results of the QTL analysis are somewhat tantalising. No region of the genome exceeds the suggestive or significant linkage thresholds proposed by Lander and Krugylak (1995). However, three unlinked regions all have an LRT statistic significant at the $p < 0.05$ level. From the Lander and Krugylak (1995) formula it is estimated that an LRT of 3.90 (the value for the least significant of the 3 putative QTL) would be exceeded about 2-3 times by chance alone in a genome-wide scan. It could be argued that my estimated values for suggestive and significant linkage are conservative for two reasons. First, I used an estimate of total genome length that was greater than the length of the map presented in Chapter 6 (to account for the fact that unlinked markers were included in the QTL analysis). Second, I assumed that the map used was infinitely dense. Although the multipoint mapping method employed can be used to calculate IBD coefficients at any location, it is also the case that the markers were not densely mapped. Hence the ability to accurately estimate IBD coefficients was severely compromised at many locations, reducing the power to detect QTL. Lander and Krugylak (1995) suggest reducing their significance thresholds for single point mapping by 20% when markers are spaced at 10cM, and if a similar adjustment was made here all three regions would have a LOD score in excess of the suggestive linkage threshold. It is clearly too early to say whether these significant regions are true QTL or merely represent statistical artefacts.

If one or more of the significant regions really does represent a QTL segregating then they are probably of large effect. The maximum likelihood method used for the simulations presented in Chapter 3 has similar power to the variance components method that was used in the actual QTL analysis (Wijsman and Amos 1997). The simulations clearly show that only QTL exceeding the Falconer and Mackay (1996) threshold for a major gene (in which an allelic substitution is equivalent to at least 0.5 of a phenotypic SD) are likely to give a LOD score of 1.0 or greater (see Chapter 3). Even under an idealised scenario in which a QTL of magnitude 0.5 phenotypic SD is completely linked to a marker with heterozygosity 0.89, a LOD of 1.0 will only be exceeded with probability 0.24 (Table 3.3). The estimates of QTL magnitude derived from the variance components analysis also suggest that QTL of major effect

are segregating. For all three significant regions it was estimated that the QTL accounted for around 25% of the phenotypic variation. In fact, at two locations it was estimated that the entire additive genetic variation was attributable to the QTL. Clearly independent regions cannot each account for all of the additive variation, and it is almost certain that if any of these regions represent true QTL their magnitude has been overestimated. Interestingly, Visscher *et al.* (1999) found that QTL detected for unipolar and bipolar disorder in humans explained all of the additive variation when using exactly the same technique as the one presented here. Although uni- and bipolar disorder may well be explained by one or a few major genes, it also seems probable that variance components methods are not exempt from the problem that QTL magnitude tends to be overestimated in most studies (Beavis 1994; Chapter 1).

In Chapter 3 I showed that the power to detect QTL in the MAXI pedigree was similar to that for the Père David's / red deer backcross pedigree used by Goosen (1997) to detect birth weight QTL on linkage group 4. There was no evidence for any QTL on linkage group 4 in this analysis, suggesting that the effect observed by Goosen (1997) is due to a fixed difference between the two species used in that cross. However Goosen (1997) also found a region on linkage group 23 that gave a LOD score of 2.37, exceeding a suggestive linkage threshold of 1.90. This possible QTL was assigned to the interval flanked by MAF18 and another marker 20cM away. Clearly this result lends further support to the significant LRT statistic found at MAF18 in this study. Although caution must be employed in interpreting this result, it is certainly feasible that a QTL for birth weight is segregating in this region, and is conserved across both mapping populations. There is increasingly good evidence from the plant breeding literature that QTL are conserved across species (Kearsey and Farquhar 1998). Mapping studies in livestock have also provided evidence that QTL are conserved across breeds of pig (Walling *et al.* 1998) and across cattle breeds (Velmalala *et al.* 1999).

The LRT statistic profile for linkage group 21 is also of some interest. The maximum value is obtained at the marker BM2934 and is significant at $p = 0.010$. In Chapter 8 I present an analysis in which I examine the effects of both multi-locus and locus

specific heterozygosity on birth weight. A more detailed discussion of this analysis is given in Chapter 8, but two loci, one of which was BM2934, showed an association between genotype and birth weight. Interestingly, the LRT profile for linkage group 21 was not significant when the residuals + multi-locus heterozygosity input file was used. This might suggest that if a QTL is segregating at this location it includes a dominance component, and that the effect is dampened by fitting MLH as a fixed effect, which may be aliased with heterozygosity at the QTL.

Given the degree of uncertainty over these results, how can it be established whether QTL really have been detected? In their summarising of the 10th Genetics Analysis Workshop (GAW10), Wijman and Amos (1997) pinpointed five factors that enhanced the ability to detect QTL.

“These factors are: allowance for covariates, use of extended pedigrees, analysis with multipoint methods, use of a continuous as opposed to a discrete phenotype, and selective sampling of pedigrees” (Wijman and Amos 1997).

I have certainly adhered to the first four of these criteria, and it could be argued that in selecting the MAXI pedigree, in which it is thought that novel genetic variation is segregating (Chapter 2), I have also complied with the fifth. This leaves two possible methods by which power could be increased. The first is to screen more markers in the regions of interest, increasing the accuracy of the estimated IBD relationship matrix. However, even with near perfect marker data, the simulations presented in Chapter 3 suggest that power would only be high to detect QTL of very large effect. An alternative (or additional) approach would be to screen more animals for those markers that gave a significant LRT statistic. Over one thousand animals were included in the birth weight GLM described in Chapter 2, and the majority have been sampled for genetic purposes. Therefore the remaining animals could be genotyped for any loci of interest, and the QTL analysis repeated. One possible drawback of this approach is that the additional animals are probably not descended from MAXI (although not all paternal links in the pedigree are known). Thus, if any QTL

segregating in the smaller pedigree were introduced by MAXI, they may still be impossible to detect, even if using the much larger pedigree.

Of the three regions with putative QTL, linkage group 14 may benefit the most from screening additional markers. The LRT statistic profile for this region shows significant ($p < 0.05$) peaks at markers JP14 and BM1706, which are spaced about 30cM apart. These peaks could represent 0,1 or 2 regions that genuinely explain variation in birth weight. However, it appears possible that a single QTL may be segregating at a location between these two markers, and that a larger central peak was not observed due to a lack of marker information. It is already known that microsatellites can be reciprocally assigned linkage across homologous ruminant chromosomes (Crawford *et al.* 1995; de Gortari *et al.* 1998; Chapter 6). Thus it would be worthwhile testing microsatellites mapped on cattle chromosome 16 and sheep chromosome 12, the homologues of deer linkage group 14, to see if they amplify a product in red deer. Any polymorphic markers could then mapped and those in the region of interest screened in the MAXI pedigree, increasing the power to detect QTL in this region.

I have made no attempt to determine a 95% confidence interval for the location of any putative QTL in this study. As a limited number of markers were used on each linkage group, it is inevitable that test statistics will be highest near these markers and that determining the position of QTL without a degree of bias will be impossible. It is generally regarded that a one-LOD support interval gives a reasonably good 95% confidence interval for the position of a QTL (Paterson *et al.* 1988; Lander and Botstein 1989; Lynch and Walsh 1998), although Visscher *et al.* (1996) showed that a bootstrap approach is more accurate. Under the former method, the 95% confidence interval incorporates the region where the LOD score falls within a LOD of 1 of the maximum value. However, the greatest LOD score that I obtained on both linkage groups 14 and 21 was $\text{LOD} = 1.17$ (see Table 7.2) and the 95% confidence region would include the entire linkage group. Screening more markers and more animals for these linkage groups would increase the precision with which QTL could

be located, although 95% confidence intervals are rarely less than 20cM (Lynch and Walsh 1998).

The estimate of heritability for birth weight in this study is marginally lower than expected. Taking the mean from the male and female estimates obtained by Kruuk *et al.* (in press) gives an estimated heritability of 0.182, almost exactly the estimate that I obtained. However it was anticipated that this analysis would yield a higher estimate of heritability than that of Kruuk *et al.* (in press) for three reasons. First, in this study the estimate was made after the removal of fixed effects explaining around 18% of the trait variation (see Chapter 2). Second, it was expected that heritability would be higher in the MAXI pedigree than for the general population, due to the input of novel genetic variation from this recent introduction. Third, all paternities used in the corrected MAXI pedigree can be assumed to be true (Chapter 5), whereas up to 20% of those used by Kruuk *et al.* (in press) are likely to be wrong. False assignment of paternity is likely to cause slight underestimates of heritability, although Kruuk *et al.* (in press) argue that any such bias will be small in their study. There is no obvious reason why a higher estimate of heritability was not obtained in this study, although it may be a consequence of using a smaller sample size than that used by Kruuk *et al.* (in press). It should also be pointed out that the 95% confidence intervals from both studies are approximately 0.05 - 0.30, and that it may not be possible to detect any differences between estimates of heritability. Finally, it is possible that the MAXI introduction led to an input of novel dominance variance rather than additive genetic variation, in which case heritability might not be higher in the MAXI pedigree.

In summary, this chapter represents the first ever attempt to map QTL in an unmanipulated natural population. Additionally this is the first QTL study to use a variance components approach in a complex pedigree for any species other than humans. Although the results are open to several interpretations, it is possible that QTL of major effect on birth weight are segregating. Birth weight is associated with most fitness components in this population, especially those related to lifetime reproductive success in males (Kruuk *et al.* 1999), and the existence of major genes

segregating in the population is in direct contrast to inferences commonly made from Fisher's fundamental theorem. If major genes for birth weight genuinely are segregating it remains to be seen whether this is a consequence of the recent introduction, and if so whether selection will drive any favourable alleles to fixation in future generations.

8 Microsatellites as a tool for measuring non-additive genetic variance

8.1 Introduction

So far, this thesis has focused on additive genetic variation for fitness related traits. In Chapter 1 I reviewed the literature on heritability and other measures of additive variance for both fitness and fitness related traits. The current consensus is that fitness traits tend to be less heritable than morphometric traits, but that this low heritability is attributable to a high residual variance rather than a low additive genetic variance (Price and Schluter 1991; Houle 1992; Roff 1997; Merilä and Sheldon 1999; Kruuk *et al.* in press). Until recently, quantitative geneticists paid little attention to residual variance in natural populations, and a common assumption was that high residual variance is caused by high environmental variance. Consequently, the role of non-additive genetic effects (dominance and epistasis) in the wild have been largely ignored. This chapter focuses on microsatellite-based measures that may be useful as tools to measure dominance variance.

Given the limited number of studies that have attempted to estimate the heritability and additive genetic variance of fitness related traits in wild populations (Chapter 1), it is unsurprising that dominance variance has been largely ignored. Measuring dominance variance in the wild is not trivial, the most straightforward method being to compare the covariances between full-sibs with those between half-sibs. Dominance variance only contributes to the covariance between full-sibs and can be estimated by solving the following equation:

$$\text{Covariance between full sibs} - 2 (\text{Covariance between half sibs}) = \frac{1}{4} V_D$$

(Falconer 1989)

However this method is likely to be problematic for several reasons. First, it is assumed that there is no environmental contribution to the phenotypic covariances. This assumption seems unrealistic, especially for full-sibs. Second, the standard errors of the estimated covariances are likely to be fairly large, leading to an estimate

of dominance variance with a large standard error. Third, it is often difficult to distinguish between full and half-sibs in wild populations. Finally, even if they can be distinguished, there may be insufficient numbers of one or other type of sibship to estimate dominance variance.

Dominance variance can also be estimated using variance components procedures, and extending the ‘animal model’ so that a dominance genetic relationship matrix can be fitted as a random effect (Lynch and Walsh 1998). However, this approach is prohibitive for most natural populations as detailed, accurate pedigree information is required. Furthermore, the problems encountered when estimating dominance variance from half-sib and full-sib covariances are also likely to apply to these alternative procedures.

Recently derived methods use a large panel of molecular markers to infer relatedness between individuals, so that quantitative genetic parameters can be estimated (Ritland 1996; Lynch and Ritland 1999). By comparing covariances between the different classes of inferred relative, dominance variance components can be estimated. These methods are attractive, as detailed multigenerational pedigrees are not required. However, the standard errors of estimated relatedness between individuals tend to be large, unless many highly informative markers are used (Lynch and Ritland 1999). Consequently, distinguishing between the different categories of relative (e.g. between full and half-sibs) is likely to be problematic, making estimates of dominance variance difficult to obtain. It remains to be seen whether these methods become widely applied in natural populations, although current progress is encouraging.

Given the difficulty of estimating dominance variance in the wild directly, another approach might be to measure variables that are associated with dominance variance. Multi-locus heterozygosity (hereafter referred to as MLH) is often positively correlated with quantitative traits (Allendorf and Leary 1986; Mitton 1994; Roff 1997; Lynch and Walsh 1998). A number of alternative, and not necessarily exclusive mechanisms have been proposed for these associations including

overdominance, associative overdominance and the inbreeding hypotheses (Mitton 1994; David 1998; Lynch and Walsh 1998). An important feature of all of these mechanisms is that there must be dominance variance for the trait in order for an association with MLH to be observed. In other words, the strength of the relationship between MLH and trait value should be related to the amount of dominance variance.

8.1.1 Earlier work using MLH and related measures in the study population

Earlier work on the consequences of inbreeding in the study population (Coulson *et al.* 1998b; Marshall 1998; Coulson *et al.* 1999) was introduced in Chapter 2. Two different molecular measures have been employed to detect the effect of inbreeding on fitness – MLH and a recently derived measure, mean d^2 (Coulson *et al.* 1998b; Coltman *et al.* 1998). Mean d^2 for an individual is calculated by summing the squared difference in repeat units of the two alleles at each microsatellite locus and dividing by the number of loci screened. It has been suggested that mean d^2 detects the degree of divergence between the gametes which formed an individual (Coulson *et al.* 1998b; Coltman *et al.* 1998). Thus, in addition to detecting the degree of inbreeding (which is also measured by MLH), mean d^2 also has a degree of outbreeding component.

The first investigation of inbreeding depression in the study population (Coulson *et al.* 1998b) showed that birth weight was positively associated with high mean d^2 and that there was also an interaction term with mean April temperature, such that in years with low temperature, calves with low mean d^2 had particularly low birth weights. In an analysis of neonatal survival high mean d^2 calves were more likely to survive, although this effect was only significant when birth weight was excluded from the model. Note however, that MLH never explained significant variation in either birth weight or neonatal survival, despite the large sample size ($n = 670$ calves). It was argued that mean d^2 was measuring heterosis, caused by the Rum population being founded from different sources (Coulson *et al.* 1998b).

A subsequent analysis investigated associations between MLH or mean d^2 and first-year over-winter survival (Coulson *et al.* 1999). Male and female calves were

analysed in separate models as non-genetic factors appeared to act differently on the two sexes. For females, mean d^2 but not MLH was positively associated with calf survival. However the relationship was in the opposite direction for males, with high mean d^2 calves experiencing *reduced* over-winter survival. Coulson *et al.* (1999) speculated that male calves with high mean d^2 grow muscle rather than fat deposits, with the consequence that they experience reduced survival in adverse winters. However, calves who survive are relatively large and have increased lifetime reproductive success. In males birth weight was a significant term, and again there was no association between survival and MLH. Note that in both studies of the associations between inbreeding measures and juvenile traits, mean d^2 appeared to be a more reliable measure than MLH.

Mean d^2 and MLH have also been used to investigate the existence of inbreeding depression and heterosis for adult life history traits (Marshall 1998). However these analyses only included calves born between 1982 (when routine sampling began) and 1986 (the inclusion of later years would have been inappropriate as some members of each cohort were still living and had incomplete life history data), so the sample sizes were considerably smaller than for the studies of juvenile traits. The earlier work on adult life history traits is discussed in greater detail in Chapter 9, but it is worth mentioning that Marshall (1998) concluded that mean d^2 was at least as good as MLH as a tool for measuring the consequences of inbreeding.

8.1.2 Aims of this chapter

A panel of 350 animals with well-documented life histories and genotypes at up to 84 loci (Chapter 4) represents a good resource for reassessing the earlier work on inbreeding depression / heterosis. In particular the data were used to address three related questions:

- How are the two genetic variables MLH and mean d^2 related to each other, and are they correlated across loci?
- Do the previous analyses that used MLH / mean d^2 to detect the consequences of inbreeding appear to be robust when a greater number of loci are screened?

- How many loci and individuals are required to reliably measure dominance variance of a trait?

In this chapter, particular emphasis is placed on birth weight. Previous chapters have investigated additive genetic variance for birth weight, and in order to further understand the genetic architecture of this trait, the role of dominance variance must be considered. Furthermore, birth weight is the most extensively recorded trait in the population, and can be used to investigate the power to detect associations between a trait and the genetic variables considered.

8.2 Methods

8.2.1 Deriving genetic variables

Four different genetic variables were calculated from the microsatellite data: MLH, standardised MLH, mean d^2 and restricted mean d^2 . All 71 loci for which there was no evidence of null alleles were used, although an animal had to be genotyped for at least 20 loci in order to be included in the data set. For comparison the same genetic variables were also calculated for the 1,168 animals that were typed (by myself) at nine microsatellite loci in an earlier large scale paternity analysis (Marshall 1998, Marshall *et al.* 1998). Animals were only included in the larger data set if they were genotyped for at least six loci. These nine loci were used in the previous investigations of inbreeding depression in the study population (Coulson *et al.* 1998b, Marshall 1998, Coulson *et al.* 1999). The genetic variables were calculated as follows:

Multilocus heterozygosity (MLH): The proportion of typed microsatellite loci at which an individual was heterozygous. MLH_{71} refers to MLH when calculated in individuals typed at up to 71 loci, and MLH_9 is calculated from the larger set of individuals scored at up to nine loci.

Standardised MLH: The ratio of the MLH of an individual to the mean MLH at the loci at which the individual is typed. This measure avoids any bias that may be introduced by individuals being typed at different loci (due to missing genotypes),

and was used by Coltman *et al.* (1999b) in a study of parasite resistance in Soay sheep (*Ovis aries*). Standardised MLH is hereafter referred to as $sMLH$

Mean d^2 : A derivation of this statistic is given in Appendix A.

Restricted mean d^2 : A number of loci had a distribution that did not appear compatible with a step-wise mutation process (Valdes *et al.* 1993), and so mean d^2 was re-calculated without these loci. A locus was regarded as non-stepwise if there was an interval of 20 bp or more between any two alleles. Excluded loci were BM1815, BM757, CSRM60, INRA11 and INRA121 (see Appendix H). Note that all of the nine loci described by Marshall (1998), and used by Coulson *et al.* (1998b) in their mean d^2 analysis appeared compatible with a stepwise model, and so restricted mean d^2 was not calculated for the nine-locus data set.

8.2.2 Model Construction

I used generalised linear modelling (GLM) techniques implemented with the software package SPLUS 4.5 (MathSoft) to test for associations between MLH / mean d^2 and either birth weight or juvenile survival. A description of predictor and response variables is given in Appendix A. Birth weight was normally distributed (see Chapter 2) and was modelled assuming a normal error structure. Components of juvenile survival to age two were modelled assuming a binomial error distribution. As with the GLM presented in Chapter 2, deletion testing was used to investigate the significance of each term. Terms were retained in a model if significant at $p < 0.05$, and categorical variables were only tested if the smallest cell size was at least 30 individuals. Unless stated otherwise, a maximum of one genetic term was included in any model. The predictor variables tested are presented with the actual models.

8.2.3 Power to detect the $sMLH$ -birth weight association:

Since a positive association was discovered between $sMLH_{71}$ and birth weight (see results), various power analyses were performed. Three factors influencing power were investigated: the number of loci typed, the number of animals analysed, and the heterozygosity of the loci typed.

Effect of number of loci on power:

$sMLH$ was calculated for 100 replicates of 10, 20 and 40 randomly chosen loci. Each locus was drawn from the original 71 loci and sampled without replacement. $sMLH$ was tested in a GLM for birth weight in which the following predictor variables were included: mother's reproductive status, subdivision of study area, spring temperature and birth date. Power was determined by scoring the number of replicates (out of 100) for which $sMLH$ explained significant variation ($p < 0.05$) in birth weight when fitted in the GLM. The sample size for all replicates was 281, as birth weight or particular predictor variables were unrecorded for a number of individuals.

Effect of sample size on power:

100 replicates of datasets containing 25, 50, 100 or 200 randomly chosen animals were created. For each replicate a linear regression was performed, in which the relationship between $sMLH_{71}$ and residual birth weight (calculated from the GLM described above) was tested. The effect of the non-genetic terms described in the previous section were removed prior to the regression because a GLM fitting the categorical terms could not have been performed for the smaller samples. Power was determined by scoring the number of replicates for which standardised $sMLH_{71}$ explained significant ($p < 0.05$) variation in birth weight.

Effect of marker heterozygosity on power:

The 71 loci were ranked in order of decreasing heterozygosity and divided into seven groups (six of ten loci and one of 11 loci). MLH and $sMLH$ were calculated for each group, and these variables were tested as predictor terms in a GLM for birth weight ($n = 281$). The same non-genetic terms as described in the other power analyses were also fitted.

8.3 Results

8.3.1 Summary statistics for genetic variables

Histograms of the genetic variables (calculated from the 71 loci and nine loci datasets) are given in Figures 8.1 and 8.2. Note that $sMLH$ (at nine or 71 loci) and MLH_{71} are approximately normally distributed. In contrast MLH_9 and all of the mean d^2 variables are positively skewed. As with previous studies (Coulson *et al.* 1998; Coulson *et al.* 1999) these data were not log transformed for subsequent analyses, as the GLM methods used for further analysis are relatively robust to non-normal data.

When genetic variables were measured using the nine original loci, the summary statistics were almost identical for the MAXI pedigree (MLH_9 : mean = 0.76, SD = 0.16; mean d^2 : mean = 36.8, SD = 20.2) and the overall population (MLH_9 : mean = 0.75, SD = 0.16; mean d^2 : mean = 36.4, SD = 20.6). It appears that the MAXI pedigree is representative of the overall population with respect to genetic variables.

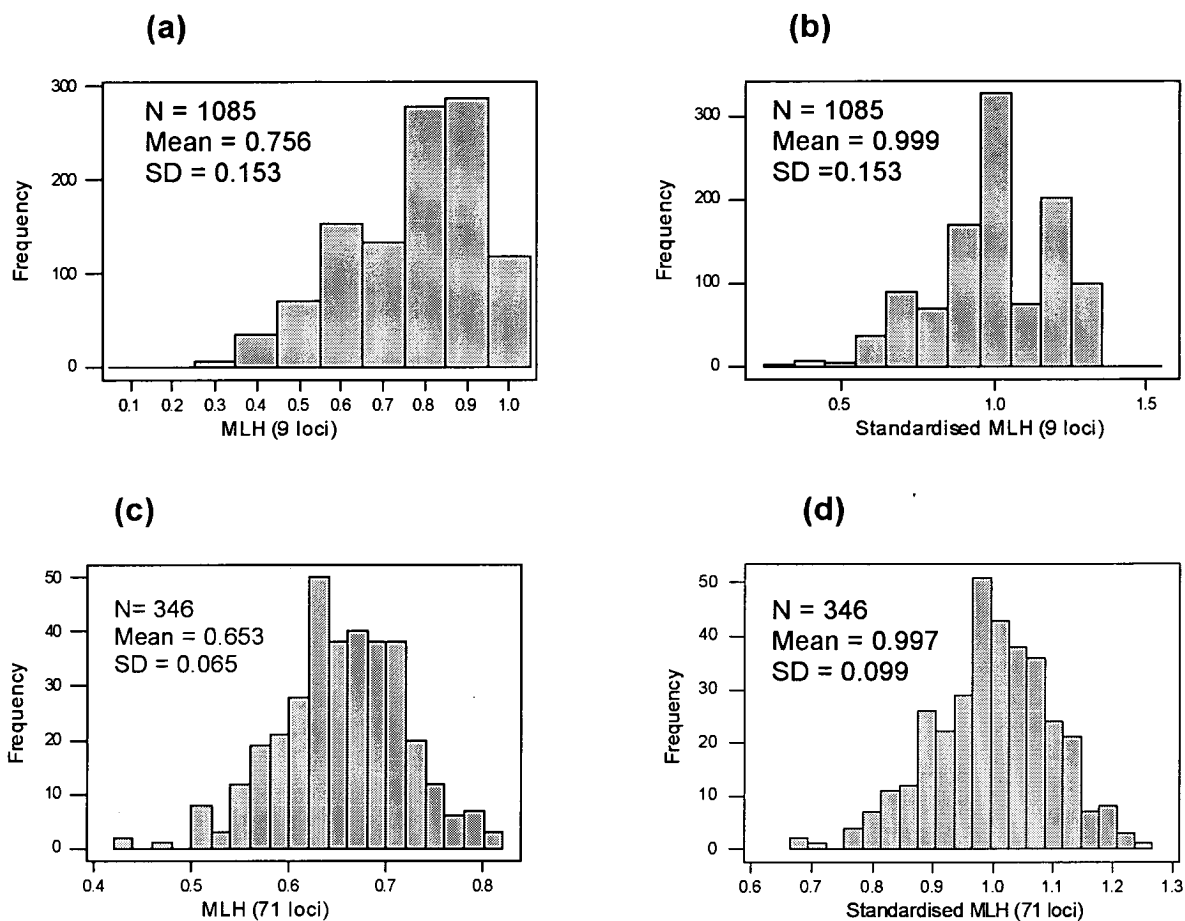


Figure 8.1 (a-d): The distribution of MLH and standardised MLH for calves typed at 9 loci ($n = 1085$) and 71 loci ($n = 346$).

The sample statistics and histogram (Figure 8.2b) for unrestricted mean d^2 (71 loci) were of particular interest. When mean d^2 was estimated with the inclusion of the five non-stepwise loci, the distribution appeared bi-modal. Furthermore, the mean and the variance were large compared with the restricted estimate (Fig. 8.2c) or the nine locus estimate (Fig. 8.2a). It appeared that these particular loci contributed disproportionately to the unrestricted measure and so it was not used in any subsequent analyses.

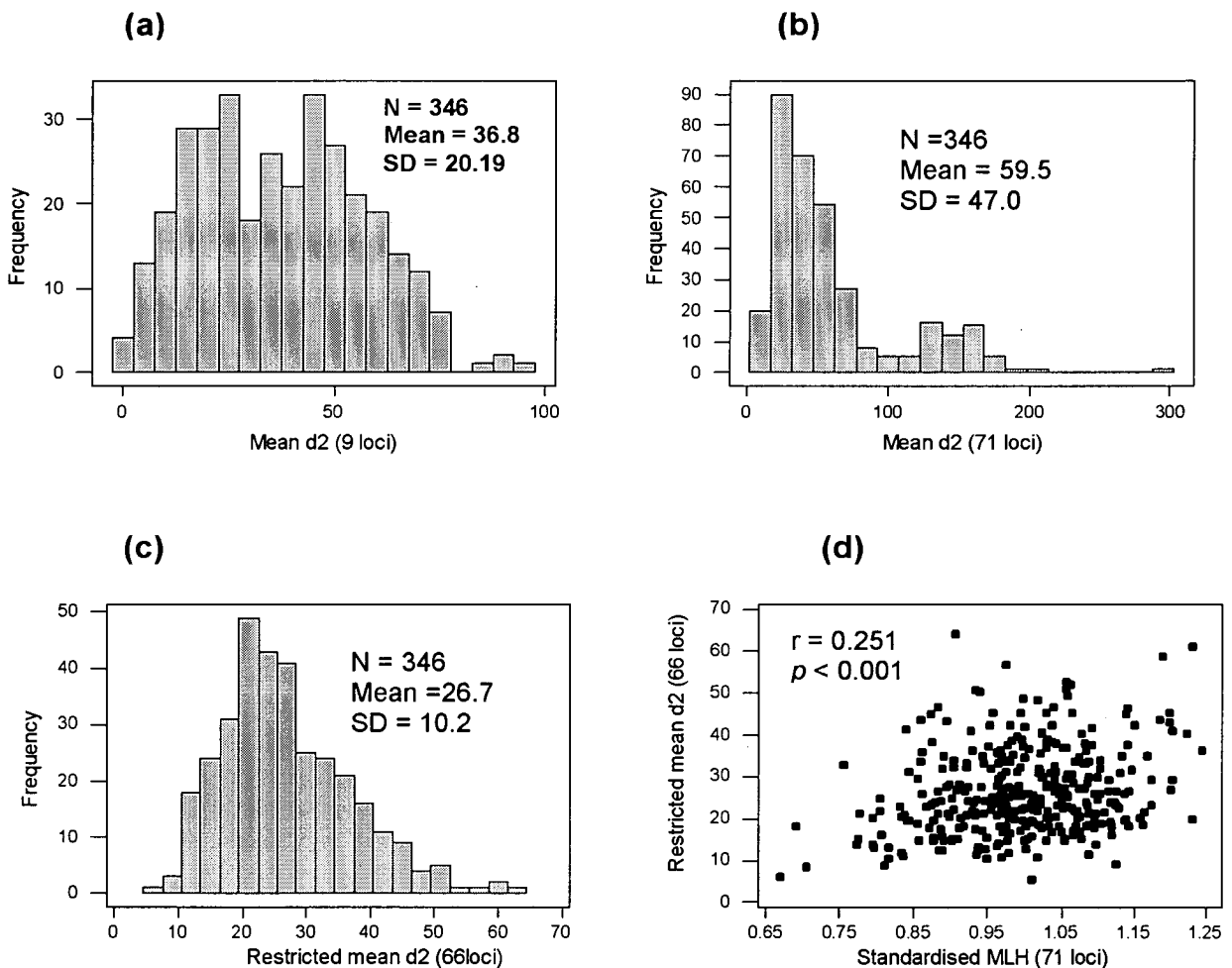


Figure 8.2 (a-d): Histograms of (a) mean d^2 scored at the 9 loci used by Coulson *et al.* (1998b). (b) mean d^2 scored at 71 loci and (c) restricted mean d^2 (see text). Figure 8.2 (d) shows the relationship between standardised heterozygosity (71 loci) and restricted mean d^2 . $n=346$ individuals for all 4 plots.

A summary of the correlation coefficients between the various genetic variables is given in Table 8.1. $sMLH_{71}$ was highly correlated with MLH_{71} ($r = 0.985$), was normally distributed, and is unbiased by animals being scored at different combinations of loci. Hence, the standardised measure was used in all subsequent analyses unless stated otherwise. Although they are clearly related genetic variables, mean d^2 was only weakly correlated with MLH . The correlation coefficient between $sMLH_{71}$ (which is presumably a good indicator of true genome-wide MLH) and mean d^2 estimated from nine loci (the variable used in the earlier analyses) was only 0.138 (see Table 8.2). $sMLH_{71}$ was only weakly correlated ($r = 0.332$) with $sMLH_9$, suggesting that nine loci were insufficient to accurately measure genome-wide heterozygosity.

Table 8.1: Correlation coefficients between restricted mean d^2 , mean d^2 estimated from 9 loci, MLH_{71} , standardised MLH_{71} and standardised MLH_9 . The above diagonal shows the product-moment correlation coefficient r , and the below diagonal is the corresponding probability value ($n = 346$ individuals).

	Restricted mean d^2 (66 loci)	Mean d^2 (9 loci)	MLH_{71}	Standardised MLH_{71}	Standardised MLH_9
Restricted mean d^2 (66 loci)	-	0.428	0.252	0.251	0.233
Mean d^2 (9 loci)	< 0.001	-	0.141	0.138	0.399
MLH_{71}	< 0.001	0.009	-	0.985	0.332
Standardised MLH_{71}	< 0.001	0.011	< 0.001	-	0.332
Standardised MLH_9	< 0.001	< 0.001	< 0.001	< 0.001	-

An earlier analysis (Coulson *et al.* 1999) measured individual d^2 scores at each of the original nine loci, and then examined correlations across loci. A significant proportion (25/36, $p < 0.05$) were positive. However, in this study, individual d^2 scores at each locus were not positively correlated (Spearman's ranked) across the 71 loci more often than expected by chance (1257 positive vs. 1228 negative correlations, $G = 0.26$, ns). More correlations were significant at $p < 0.05$ than expected by chance (174 significant, $G = 18.62$, $p < 0.01$), but amongst this subset

there was no evidence that individual d^2 scores were positively correlated across loci (96 positive vs. 78 negative, $G = 1.88$, ns). Overall, there was no evidence that d^2 was correlated across loci.

A similar analysis of the (Spearman's ranked) correlation between heterozygosity at each locus was performed. There were significantly more positive correlations than expected by chance (1292 positive vs. 1193 negative, $G = 3.95$, $p = 0.047$), but there were no more significant (at $p < 0.05$) correlations than expected by chance (144 significant correlations, $G = 2.75$, ns). This subset also contained significantly more positive than negative correlations (88 positive vs. 56 negative, $G = 7.17$, $p < 0.01$). Thus, unlike d^2 , heterozygosity is weakly correlated across loci, indicating that MLH can distinguish between relatively inbred and outbred animals in this population. Note that for both d^2 and heterozygosity, Spearman rank correlations were performed as both variables were non-normally distributed when measured at individual loci.

8.3.2 Associations between MLH or mean d^2 and juvenile traits

8.3.2.1 Birth weight

$sMLH$ was positively associated with birth weight in a number of different models. For brevity, not every model is described in detail. In contrast mean d^2 was never associated with birth weight. In a joint sex model (Table 8.2) which included subdivision of study area, mother's reproductive status, spring temperature and birth date as significant terms, $sMLH_{71}$ was positively associated with birth weight ($p < 0.01$). The sex of the calf was not a significant term in this model, and there was no significant interaction term between sex and $sMLH_{71}$. This model, minus $sMLH_{71}$, was used as the non-genetic model in the subsequent power analyses.

Table 8.2: GLM for birth weight for MAXI pedigree animals typed at up to 71 loci

Null model deviance = 387.8. Full model deviance =288.9.

Deviance explained = 25.5%. $n = 281$

Term	Term Type	d.f.	F	P	Deviance explained
Mother's reproductive status	Factor (5 levels)	4, 273	10.24	< 0.0001	44.0 (11.3%)
Area	Factor (5 levels)	4, 273	4.45	< 0.005	19.1 (4.9%)
Birth date	Continuous	1, 270	13.71	< 0.001	14.7 (3.8%)
Mean spring temp	Continuous	1, 270	12.85	< 0.001	13.8 (3.6%)
$sMLH_{71}$	Continuous	1, 270	7.77	< 0.01	8.4 (2.2%)

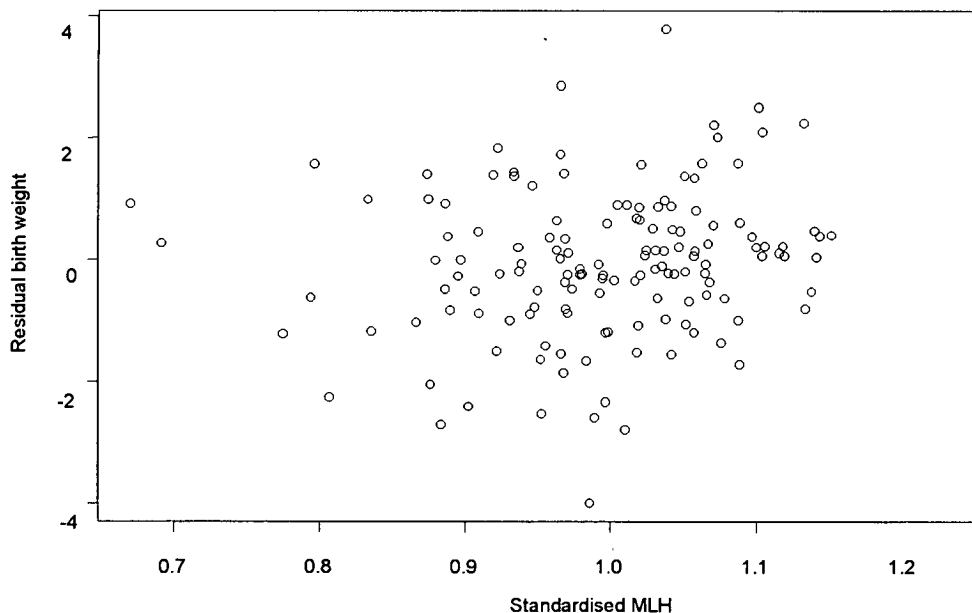
In addition to using $sMLH_{71}$ as a predictor variable, each locus was tested separately. Animals were coded as 0 if homozygous and 1 if heterozygous at a locus. Locus specific heterozygosity was fitted as a two-level categorical variable in a GLM containing the non-genetic terms listed in Table 8.2. Heterozygotes were significantly heavier than homozygotes at two loci (SPS113 and BM2934). As a large number (71) of loci were tested in the locus-specific models, problems of multiple testing arise. However, because heterozygosity was correlated across loci (8.3.1) the tests were non-independent, and a Bonferroni correction would have been inappropriate. Note that SPS113 was highly significant at $p < 0.005$, while BM2934 was one of the loci on linkage group 21 with a LRT statistic significant at $p < 0.05$ in the QTL analysis (Chapter 7). When fitted in a GLM that contained $sMLH_{71}$, these two loci still remained as significant terms, as did $sMLH_{71}$ (Table 8.3). Although only two loci showed significant associations with birth weight, heterozygotes were heavier than homozygotes at significantly more than half of the 71 loci (heterozygotes heaviest at 45 loci vs. homozygotes heaviest at 26 loci; $G = 5.15$, $p < 0.05$).

Table 8.3: GLM for birth weight with locus-specific and multi-locus terms fitted.

Significant genetic terms include $sMLH_{71}$ and heterozygosity at two unlinked loci (SPS113 and BM2934). Null model deviance = 347.6, full model deviance = 229.9. Total deviance explained = 33.9%.

TERM	TERM TYPE	d.f.	F	p	Deviance explained
Mother's reproductive status	Factor (5 levels)	4,234	11.50	< 0.0001	45.98 (13.3%)
Area	Factor (5 levels)	4,234	5.11	< 0.001	20.41 (5.9%)
Birth Date	Continuous	1,231	13.80	< 0.0005	13.80 (4.0%)
Mean spring temperature	Continuous	1,231	10.41	< 0.005	10.41 (3.0%)
H_{SPS113}	Factor (2 levels)	1,231	9.72	< 0.005	9.71 (2.8%)
H_{BM2934}	Factor (2 levels)	1,231	6.84	< 0.01	6.84 (2.0%)
$sMLH_{71}$	Continuous	1,231	6.84	< 0.01	6.84 (2.0%)

Separate sex models were also constructed for birth weight. Both spring temperature ($p < 0.001$) and MLH_{71} ($p < 0.01$) explained significant variation in male birth weight (see Figure 8.3). However neither term explained variation in female birth weight. The two five-level categorical terms were not fitted in these models, as cell sizes would have been small.

**Figure 8.3: Relationship between $sMLH_{71}$ and male birth weight**

The y-axis shows residual birth weight (measured in standard deviates), having fitted the effect of spring temperature in the year of birth ($p < 0.001$). MLH_{71} is significant at $p < 0.01$ and explains 5.3% of the variance.

8.3.2.2 Neonatal survival:

Neonatal survival was modelled by logistic regression, assuming a binomial error structure. No terms were significant in a sample size of 302 deer (157 females, 145 males) when joint-sex or single sex models were constructed.

8.3.2.3 Juvenile survival:

Like neonatal survival, variables associated with juvenile survival (to two years old) were tested in a GLM assuming a binomial error structure. When fitted as a single predictor variable $sMLH_{71}$ was significant at $p < 0.01$. Calves with a higher $sMLH_{71}$ had a greater probability of survival. In a joint sex model the following terms were significant at $p < 0.05$: density and spring temperature in the year of birth, birth weight, birth date, sex and mother's age. However, in the full model (not shown) $sMLH_{71}$ was no longer significant ($p = 0.061$). There was no significant interaction between $sMLH_{71}$ and any other term. Mean d^2 did not explain significant variation in juvenile survival, whether tested on its own, or in a GLM with non-genetic variables. Surprisingly, calves born in warm springs had relatively poor juvenile survival.

Single sex models were constructed, and when tested as a single predictor variable $sMLH_{71}$ explained significant variation in male juvenile survival ($p < 0.01$, $n = 145$) but not female juvenile survival ($p = 0.36$, $n = 157$). In a more detailed model, $sMLH_{71}$ remained associated with male juvenile survival (see Table 8.4). Again mean d^2 was not a significant term in any model.

Table 8.4: GLM for male juvenile survival.

$n = 145$, null model deviance = 198.5, full model deviance = 160.8, total deviance explained = 19.0%. d.f. = 1 for all terms shown.

Variable	χ^2	p	% Deviance explained	Direction of effect
Density	10.53	< 0.01	5.3%	Survival low for calves born at high density
Spring temperature	4.65	< 0.05	2.3%	Survival low for calves born in warm springs
Birth weight	4.29	< 0.05	2.2%	Heavier calves survive better
Birth Date	4.24	< 0.05	2.1%	Late born calves survive worse
$sMLH$	3.86	< 0.05	1.9%	High $sMLH$ calves survive better

8.3.3 Power to detect MLH - birth weight associations

8.3.3.1 Effect of number of loci on power

The power to detect the birth weight- $sMLH$ association in the joint-sex model shown in Table 8.2 increased with the number of loci typed (Table 8.5). When 10 loci were used to calculate $sMLH$, power was very low (0.18). The greater the number of loci typed (x), the stronger was the correlation (Pearson) between $sMLH_x$ and $sMLH_{71}$. Some caution must be taken when interpreting these data, as there is a degree of non-independence between each replicate (as some replicates were estimated from a subset of the same loci). The most probable consequence of this is that power will be overestimated. Thus the power to detect an association between 10-locus $sMLH$ and birth weight may be even lower than 0.18.

Table 8.5: The influence of the number of loci typed on the power to detect associations between $sMLH$ and birth weight.
100 replicates were performed throughout. $n=281$ in each model.

Number of loci typed	Power	Mean (SD) correlation coefficient (r) Between $sMLH_x$ and $sMLH_{71}$
10	0.18	0.425 (0.053)
20	0.31	0.572 (0.055)
40	0.63	0.784 (0.042)

8.3.3.2 Effect of sample size on power

The power to detect the $sMLH_{71}$ -birth weight association (see Table 8.2) was also dependent on sample size (Table 8.6). For a sample size of 100 individuals, power was only 0.42.

Table 8.6: Effect of sample size on power to detect the birth weight- $sMLH$ association.
Note that the F statistic required for significance at $p < 0.05$ decreases with increasing sample size. The mean F statistic and power increase with increasing sample size.

Sample size	Required F for significance at $p < 0.05$	Mean F (SD)	Power
25	4.28	1.77 (2.85)	0.12
50	4.04	2.48 (2.54)	0.21
100	3.93	3.85 (3.00)	0.42
200	3.88	5.43 (2.00)	0.71

8.3.3.3 Effect of variability of loci on power

A summary of the effect of heterozygosity on power to detect an association (see 8.3.3.1) is given in Table 8.7. The association between MLH and birth weight was only detected by one subset of ten loci (the group containing the 21st to the 30th most heterozygous loci). There was no evidence that the most variable loci had the greatest power to detect the association. It is possible that the most heterozygous loci are so variable that there is insufficient variation in MLH between individuals to detect any associations with birth weight. If this were the case, groups of loci with the greatest between individual variation would have the greatest power. There is no support for this theory either (Table 8.7). An alternative explanation for the observed results is that the low power obtained with 10 loci (see 8.3.3.1), masks any effect of marker heterozygosity. Note that the group of loci that was associated with birth weight included SPS113, a locus independently associated with birth weight (see 8.3.2.1).

Table 8.7: Effect of locus variability on power to detect the MLH-birth weight association. Loci are sorted by decreasing heterozygosity and divided into groups of 10 (group 1 being the most variable, group 7 the least variable). Mean heterozygosity is the average of the observed heterozygosity measured at each locus in that group. MLH was fitted as a predictor variable in the birth weight GLM shown in Table 8.2. Only group 3 is significant at $p < 0.05$, whether measured as standardised or unstandardised MLH. There is no evidence that power is greater for groups with the most variable loci, or for groups with the greatest between individual variation in MLH.

Locus group	Mean (SD) heterozygosity	Variance in between individual MLH		Standardised MLH		Unstandardised MLH	
		Standardised	Unstandardised	F	p	F	p
1	0.868 (0.015)	0.022	0.017	0.46	0.50	0.45	0.50
2	0.812 (0.021)	0.026	0.017	0.82	0.37	0.84	0.36
3	0.766 (0.016)	0.046	0.027	4.61	< 0.05	4.60	< 0.05
4	0.710 (0.017)	0.057	0.029	0.58	0.45	0.58	0.45
5	0.666 (0.011)	0.072	0.032	1.35	0.25	1.42	0.23
6	0.572 (0.089)	0.084	0.028	1.56	0.21	1.42	0.24
7	0.233 (0.090)	0.404	0.021	1.00	0.32	0.80	0.37

8.4 Discussion

8.4.1 Do the MAXI pedigree data support previous studies of inbreeding depression in the study population?

Previous studies of inbreeding depression in the study population used the genetic variables MLH and mean d^2 estimated from nine microsatellite loci (Coulson *et al.* 1998b; Marshall 1998; Coulson *et al.* 1999). In this chapter the earlier analyses were repeated, but based on a much larger set of 71 loci. There was only limited support for the earlier findings. It was notable that most of the previously reported evidence for inbreeding depression or heterosis in the population came from associations with mean d^2 rather than MLH. It was argued that the component of mean d^2 that measures ancestral information is particularly sensitive in detecting the effects of inbreeding depression or heterosis (Marshall 1998). However, in current analysis *all* significant associations with a trait involved MLH₇₁ rather than mean d^2 .

In their analysis of birth weight, Coulson *et al.* (1998b), found a positive association with mean d^2 . Although the current analysis did not reveal any associations with mean d^2 , there was an association with sMLH₇₁ in the same direction. In both studies there was no interaction with sex. However, in single sex models the association between birth weight and sMLH₇₁ was only detected in males. The observation that relatively outbred calves are born heavier does seem to be robust. It seems reasonable to conclude that birth weight, in males at least, includes a dominance variance component. Locus-specific and multi-locus terms between them explained around 7% of the variance in male birth weight (see Table 8.3). In comparison Kruuk *et al.* (in press) estimated heritability of birth weight in males to be 0.11. Thus, dominance variance may play almost as important a role as additive genetic variance in the genetic architecture of male birth weight.

Coulson *et al.* (1998b) also found evidence for a positive association between neonatal survival and mean d^2 , although mean d^2 was not significant in a model that included birth weight. In this analysis, variation in neonatal survival was not explained by either birth weight or any genetic variable. The sample size in this analysis was smaller ($n = 302$ compared to $n = 670$), but it seems probable that any

genuine effect of inbreeding on neonatal survival is relatively weak and mediated through birth weight.

The most serious discrepancies between this and earlier studies concern male juvenile survival. Coulson *et al.* (1999) showed that male calves born with high mean d^2 had reduced first winter survival. Conversely, this study shows that males with high $sMLH$ (which is positively correlated with mean d^2) have *increased* juvenile survival. Although the latter analysis was performed on survival to age two, it is nonetheless difficult to reconcile these two contrasting results. Both Coulson *et al.* (1999) and Marshall (1998) also showed positive associations between genetic variables (mean d^2 and MLH respectively) and female juvenile survival. However, there was no evidence for associations between genetic variables and female survival in this study.

Although the 71-locus genetic variables lend only partial support to the previous studies of inbreeding depression and heterosis in the study population, one important fact must be taken into consideration. The earlier analyses were conducted across the whole study population, whereas the 71-locus analyses were performed within the MAXI pedigree. In terms of the genetic variables measured across nine loci the MAXI animals appear representative (see 8.3.1). However, in terms of trait measures, an analysis in Chapter 2 showed that descendants of MAXI were born heavier, and it is possible that the genetic architecture of other traits differs between the MAXI pedigree and the overall population. For example, a number of animals in the MAXI pedigree were born before 1982 (when routine genetic sampling began), so there is probably a bias in favour of animals with increased juvenile survival. These considerations, and the smaller sample size of the MAXI pedigree could explain the different results between this and earlier analyses, and possibly one or two other unusual observations (e.g. no sex difference in birth weight, Table 8.2)

8.4.2 How informative are MLH and mean d^2 ?

This study raises some doubt over the efficacy of mean d^2 to detect inbreeding depression or heterosis. There was no evidence that d^2 at a particular locus was

correlated with d^2 at any other locus. Thus a very large number of loci may be required to accurately reflect mean coalescence times between parental alleles. In contrast heterozygosity was correlated across loci, albeit weakly, suggesting that it may be the preferred variable for measuring the consequences of inbreeding. Furthermore, when 71 loci were typed, $sMLH$ detected associations and mean d^2 did not, suggesting that a prohibitively large number of loci may be required in order to use mean d^2 reliably. It is also worth noting that the correlation coefficient between MLH and mean d^2 ($r = 0.25$ for 71 loci) was lower than that observed in previous studies (reviewed in Pemberton *et al.* 1999). This is in comparison to $r = 0.38$ for the original nine loci (Coulson *et al.* 1998b), and $r = 0.30$ for six loci typed in harbour seals, *Phoca vitulina* (Coltman *et al.* 1998).

An additional problem with mean d^2 arises when certain loci contribute disproportionately to the overall score. This raises the question of whether each locus should be scaled by its variance when the overall mean d^2 statistic is being calculated. In their analysis of calf winter survival Coulson *et al.* (1999) also calculated a scaled mean d^2 statistic, but did not find any association with survival. This would suggest that important information regarding the ancestry of the two gametes was lost by the scaling process (or alternatively, the unscaled measure detected Type I errors, which were corrected by the scaling process). Problematically, there is no obvious way in which to distinguish between conflicting causes of high variance in locus specific d^2 measures. For example, a large variance could represent information about coalescence times between the two parental alleles or could be an artefact of either a non-stepwise mutation process or high mutation rate (Coulson *et al.* 1999). Such conflicting processes mean it is difficult to make hard and fast rules about whether to scale mean d^2 , and whether or not loci with high variance should be included in the calculation.

8.4.3 How many loci are required to detect MLH -fitness associations?

Although correlations between MLH and fitness have been reported for many organisms and traits, there are a number of notable exceptions for which no association was found. For example, Whitlock (1993) failed to find any association

between MLH (measured at five allozyme loci) and a number of traits in the forked fungus beetle (*Bolitotherus cornutus*), despite a sample size of over 900. Furthermore, it is almost certain that there is a bias in the literature, so that studies that fail to show any association remain unreported. A meta-analysis by Britten (1996) suggested that the mean correlation coefficient between MLH and growth rates was weak (mean $r = 0.181$, 95% C.I. = $0.158 - 0.207$). Hence it is unsurprising that a number of studies have failed to detect associations. Some attention has been given to the fact that large sample sizes are required to confirm the absence of any association (i.e. to avoid Type II error). In contrast, the effect of the number of loci typed on power has been largely ignored.

I performed a power analysis, testing the effect of both sample size and the number of loci typed, on the ability to detect the association between birth weight and MLH. A plot of the number of genotypes against power suggests that sample size and the number of loci are equally important in detecting the association (see Figure 8.4).

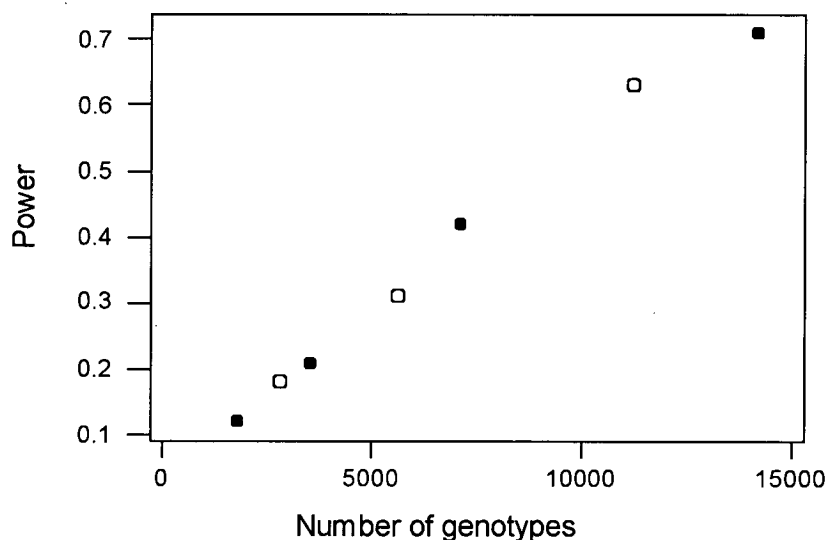


Figure 8.4: Effect of the number of genotypes on power.

Open circles represent power calculations where the number of loci typed was varied, and filled circles represent power calculations where the number of individuals typed was varied. There is no apparent advantage to typing additional loci at the expense of fewer individuals or vice-versa. The relationship is linear for at least the first 10,000 genotypes.

The full model (see Table 8.2) suggested that MLH explained around 2.2 % of the variation in birth weight, which is fairly typical for other estimates of MLH-fitness association (Britten 1996). Although, the association was sometimes detected with a relatively small number of markers, power with ten or 20 loci was poor. Most MLH-fitness studies to date typically used between five and ten loci. Furthermore, the majority of previous studies have relied on allozyme markers which tend to be less variable than microsatellites, and possibly therefore have lower resolution to detect any associations. Clearly, future studies that fail to detect MLH-fitness associations must consider the number of loci typed before rejecting the possibility of Type II error. For example, although Whitlock (1993) screened 900 beetles, the total number of genotypes was under 5,000. Thus, the power to detect a MLH-trait association of similar magnitude to that presented here was only 0.25-0.30 (see Figure 8.4). The major determinant of power in MLH-fitness association studies is the number of genotypes, not sample size.

8.4.4: What mechanisms cause MLH-fitness associations?

At least three alternative mechanisms have been proposed to explain associations between MLH and fitness (Mitton 1994; Roff 1997; David 1998; Lynch and Walsh 1998): the *true overdominance* hypothesis, the *associative overdominance* hypothesis, and the *inbreeding depression* hypothesis (sometimes referred to as the *dominance* hypothesis).

True overdominance hypothesis:

As the majority of MLH-fitness associations have been detected using allozyme markers, it is possible that these functional loci have a direct effect on fitness. If true overdominance is the causal mechanism then it is expected that MLH measured with DNA markers will be less strongly associated with fitness traits. Some support for this hypothesis was given by Pogson and Zouros (1994) who failed to find any association between RFLP heterozygosity and growth rate in a scallop (*Placopecten magellanicus*) population, even though a previous association between allozyme MLH and growth rate had been reported. However a number of recent studies have reported associations between DNA marker MLH and fitness traits (Pogson and

Fevolden 1998; Coltman *et al.* 1999b; this study). Assuming that the microsatellite markers used in the present study are not functional, then true dominance cannot be the mechanism behind the observed results.

Associative overdominance hypothesis:

Under this model the apparent advantage of heterozygous individuals is attributable to the effects of linked loci. These linked loci may exhibit overdominance or carry deleterious recessive alleles. In a population with no inbreeding, associative overdominance should still be detectable if present.

Inbreeding depression hypothesis:

Heterozygosity at the typed loci reflects the overall extent of genome-wide heterozygosity. Thus, for a population in which there is a degree of inbreeding, individuals with high MLH should have high scores for any trait for which there is dominance variance. Overdominance, deleterious recessives, or directional dominance could all be acting on the loci underlying the trait. Unlike the associative overdominance hypothesis, loci influencing the trait need not be physically linked to the markers used to measure MLH.

The latter two mechanisms are difficult to distinguish, particularly in structured populations such as the Rum red deer. However, it appears probable that both the associative overdominance and the inbreeding depression hypotheses could explain the observed association between MLH and birth weight. Associative overdominance appears to be acting at the two loci SPS113 and BM2934. Heterozygotes at these loci were significantly heavier than homozygotes, even when MLH at all other loci was fitted in a GLM. Between them, the three terms (overall MLH, and heterozygosity at each of the 2 loci) explained about 7% of the variation in birth weight - substantially more than when MLH was fitted alone. In principle it may be possible to determine whether linked loci are overdominant or have deleterious recessive alleles. If there were deleterious recessives segregating at a linked locus, then animals homozygous for the marker allele in linkage disequilibrium with the recessive allele should be substantially lighter at birth. However, both markers have a large number of alleles,

making this analysis impossible with the current sample size. Finally, the two markers appear to be acting independently of each other. They are unlinked and both are significant when fitted together in a GLM. Additionally, heterozygosity at each marker is non-significantly but negatively correlated ($r = -0.08$, $p = 0.17$), providing further evidence that the associations are independent of each other.

The inbreeding depression hypothesis also seems applicable to the observed MLH-birth weight association. First, it is known that some animals in the population are inbred ($\sim 13\%$ of calves are inbred, Marshall *et al.* submitted.). Second, the association was detected when different subsets of loci were used (in the power analysis), so the relationship is not attributable to specific loci (associative overdominance). Third, heterozygous individuals were born heavier than homozygotes at significantly more than half of the loci. This would suggest that the relationship between heterozygosity and birth weight is genome-wide. However, the present data are insufficient to distinguish whether partial dominance or overdominance is the cause of associations with MLH found in this study.

In summary, the results presented in this chapter suggest that MLH was a useful indicator of dominance variance for male birth weight. However there is doubt over the suitability of mean d^2 as a tool for detecting inbreeding depression or heterosis. In the next chapter MLH is used as a tool to measure dominance variance across a suite of morphometric and life history traits.

9 Dominance variance for life history and morphometric traits

9.1 Introduction

If selection acts to erode additive genetic variation faster for fitness related traits than for morphometric traits, then a greater proportion of the remaining genetic variation for fitness traits should be due to dominance (Roff 1997; Merilä and Sheldon 1999). Recently, some evidence for the relative importance of dominance variance in fitness traits compared to non-fitness traits has emerged. A meta-analysis by Crnokrak and Roff (1995) compared life-history and morphometric traits in both domesticated and non-domesticated species. The amount of dominance variance was measured in two ways – as a proportion of the overall phenotypic variation, $D_P = V_D / V_P$, and as a proportion of the additive and dominance variance, $D_G = V_D / (V_D + V_A)$. Using these measures, Crnokrak and Roff (1995) concluded that morphometric traits have less dominance variance than life history traits in non-domestic species. Furthermore, there was greater dominance variance for morphometric traits in domestic species than in non-domestic species. This was predicted, as morphometric traits are likely to have been under directional selection in domestic species, but stabilising selection in the wild.

Comparisons amongst both laboratory (Lynch and Walsh 1998) and non-domestic (DeRose and Roff 1999) animals suggest that fitness related traits experience greater inbreeding depression than morphometric traits. Given that inbreeding depression can only occur in traits with a dominance variance component (see Chapter 8), it can be concluded that studies of inbreeding depression also provide evidence that dominance variance is important for fitness traits.

Despite the mounting evidence that life history traits exhibit relatively high levels of dominance variance, there are perhaps no studies that have measured dominance variance across a suite of morphometric and life history traits in a wild population. This is probably because dominance variance is difficult to measure in the wild (see Chapter 8). However, in the previous chapter it was demonstrated that dominance variance representing as little as 2% of the overall phenotypic variation can be

detected using multi-locus heterozygosity (MLH). A meta-analysis by Roff (1997) suggested that morphological traits were less likely than life history traits to show an association with MLH. However, this result was not significant as only eight morphometric traits were considered. Comparisons of this nature may also be confounded by a reporting bias in favour of significant associations. In this chapter MLH is used to estimate levels of dominance variance across a suite of morphometric and life history traits in the Rum population.

9.2 Methods

Standardised MLH (see Chapter 8) was used as the genetic variable to detect dominance variance for each trait. For two reasons the nine-loci measures were used rather than the 71-loci alternative. First, the sample sizes would have been prohibitively small for adult life history traits if the 71-loci data set was used. Second, it would have been an unfair comparison if the 71-loci data set was used for some traits and the nine-loci data set for others. Statistical power to detect associations would have been far greater for traits analysed with 71 loci (see Chapter 8). Also, the only animals typed at 71 loci were members of the MAXI pedigree. If the MAXI pedigree and the overall study population have different trait measures or genetic architectures (see Chapter 2; Chapter 8) then predictions about the relative amount of dominance variance across traits would have been harder to make.

As with earlier chapters generalized linear models (GLMs) were used to test for sMLH-trait associations. A summary of each response variable tested is given in Table 9.1, and a description of every response and predictor variable is given in Appendix A. Deletion testing was used in the model construction, and terms were retained if significant at $p < 0.05$. Main effects and first order interactions were tested. Categorical variables were not tested if any cell sizes were less than thirty. Separate sex models were constructed as environmental factors and selection pressures influence the various traits differently between the sexes (Kruuk *et al.* in press). Additionally some life history traits are only applicable to one sex (e.g. female fecundity). Life history traits generally had lower sample sizes than

morphometric traits, and so the power to detect $sMLH$ associations was higher for morphometric traits.

Table 9.1: Response variables for which associations with $sMLH$ were tested.

Note that sample sizes are generally larger for morphometric traits than life history traits

<i>Morphometric traits</i>	<i>Sample size</i>	<i>Error structure</i>
Females		
Birth weight	391	Normal
Hind leg length	112	Normal
Jaw length	154	Normal
Males		
Birth weight	404	Normal
Hind leg length	112	Normal
Jaw length	173	Normal
<i>Life history traits</i>		
Females		
Juvenile survival	347	Binomial
Longevity	105	Normal
Female fecundity	99	Normal
Female lifetime breeding success	99	Normal
Males		
Juvenile Survival	348	Binomial
Longevity	91	Normal
Male lifetime breeding success	87	Negative binomial

9.3 Results

9.3.1 Morphometric traits

Birth weight:

$sMLH$ was not associated with female birth weight. In males $sMLH$ did not explain significant variation in birth weight when fitted as a main term ($p = 0.07$), but was significant when fitted as an interaction term with subdivision of study area ($p < 0.05$). The full model is shown in Table 9.2.

Table 9.2 GLM for male birth weight.

Null model deviance = 617.6, full model deviance = 489.9. Deviance explained = 20.8 %.
 $n = 404$. s_{MLH} is not significantly associated with birth weight as a main term, but does appear as an interaction term with subdivision of study area. Between them the two terms explain 3.4 % of the trait variance.

Term	d.f	F	p	Deviance explained
Mother's reproductive status	4, 387	9.70	< 0.0001	49.2 (8.0 %)
Subdivision of study area	4, 391	7.45	< 0.0001	38.7 (6.3 %)
s_{MLH} * Area	4, 387	3.24	0.012	16.5 (2.7 %)
Mean April-May temperature	1, 387	11.98	< 0.0001	15.2 (2.5 %)
Mother's age	1, 387	5.91	0.015	7.5 (1.2 %)
Birth Date	1, 387	3.88	0.050	4.92 (0.8 %)
s_{MLH}	1, 391	3.23	0.07	4.19 (0.7 %)

Jaw length:

In both males and females jaw length was positively associated with age at death and birth weight. Both predictor variables remained significant, whether fitted in models that included animals of all ages, or adults only (models not shown). However, s_{MLH} did not explain significant variation in jaw length for either sex.

Hind leg length:

As with jaw length, age at death and birth weight explained significant variation in hind leg length of both sexes. s_{MLH} was not significant in any model tested (not shown).

9.3.2 Life history traits**Juvenile survival**

Variables associated with juvenile survival (to two years old) were tested in a logistic regression model assuming a binomial error structure. When fitted as a single predictor variable s_{MLH} explained significant variation in female survival ($n = 347$, $p = 0.03$), but not male survival ($n = 348$, $p = 0.16$). Female calves with a higher

$sMLH$ had a greater probability of survival. In a more detailed model (Table 9.3) $sMLH$ remained as a significant explanatory variable for female juvenile survival.

Table 9.3 GLM for female juvenile survival

Null model deviance = 461.0, full model deviance = 385.0, deviance explained = 16.5 %.
 $n = 347$. $sMLH$ explained 1% of the overall deviance. The deviance explained by each term is the percentage of the null model deviance.

Term	d.f.	χ^2	p	Deviance explained
Density	1	31.76	< 0.0001	6.9 %
Birth weight	1	16.60	< 0.001	3.6 %
Mean April-May temperature	1	12.14	< 0.001	2.6 %
Birth Date	1	9.20	< 0.005	2.0 %
$sMLH$	1	4.62	. 0.03	1.0 %

$sMLH$ was not a significant explanatory variable ($p = 0.46$) in a more detailed model (not shown) for male juvenile survival. Note however, that $sMLH_{71}$ was positively associated with male juvenile survival in the analysis presented in Chapter 8.

Female longevity:

An earlier analysis found that spring temperature in the year of birth explained variation in female longevity (Kruuk *et al.* 1999b). Here the effects of $sMLH$, spring temperature and density on longevity were tested using GLMs with normal error structures. Only females born before 1985 were considered, as many later born females are still alive. Females that were shot were not included. In the full model (not shown) density ($p < 0.01$) but not spring temperature ($p = 0.06$) or $sMLH$ ($p = 0.35$) explained significant variation in longevity. It was not clear whether the density in the year of birth *per se* explained variation in longevity, or whether density in the year of birth was reflecting the average population density over an individual's entire life time. During the 1970s the population was steadily increasing (see Chapter 2), and consequently an individual born in a low density year would spend most of its life at low density.

Female fecundity:

Fecundity (the proportion of years in which a female produced a calf, once she had survived to three years old) was modelled using a normal error structure. When fitted alone in a simple linear regression $sMLH$ ($n = 99$, $p = 0.12$) failed to explain significant variation in fecundity. In a more complex GLM the only term that explained significant variation in fecundity was spring temperature in the year of birth (full model not shown). Non-significant terms were birth weight, density and birth date.

Female lifetime breeding success:

Female LBS showed a similar pattern to fecundity. It was also modelled assuming a normal error structure. When fitted alone $sMLH$ ($n = 99$, $p = 0.13$) did not explain significant variation in female LBS. In the full model (not shown) spring temperature and density in the year of birth were both significant terms. Birth weight did not influence female LBS. The same significant non-genetic explanatory variables were identified in an earlier analysis by Kruuk *et al.* (1999b).

Male longevity:

Longevity is strongly correlated with overall LBS in males ($r = 0.614$; Kruuk *et al.*, in press). The effects on male longevity of $sMLH$, birth weight, density and spring temperature in the year of birth were examined. Animals were only included in the analysis if they were born prior to 1987 (so that no animals were still living), had survived to at least age 2, and were not shot. There was no evidence that birth weight, density or spring temperature in the year of birth explained significant variation in longevity. When fitted as a single explanatory variable in a linear regression $sMLH$ did explain variation in longevity ($n = 91$, $p = 0.021$, variance explained = 5.8%). However, a plot of $sMLH$ and longevity suggested that the relationship may be attributable to a few animals that died at an early age and had a low $sMLH$ (see Figure 9.1). If the data set was restricted to animals that survived to breeding age (five years old), the relationship was no longer significant ($n = 87$, $p = 0.064$).

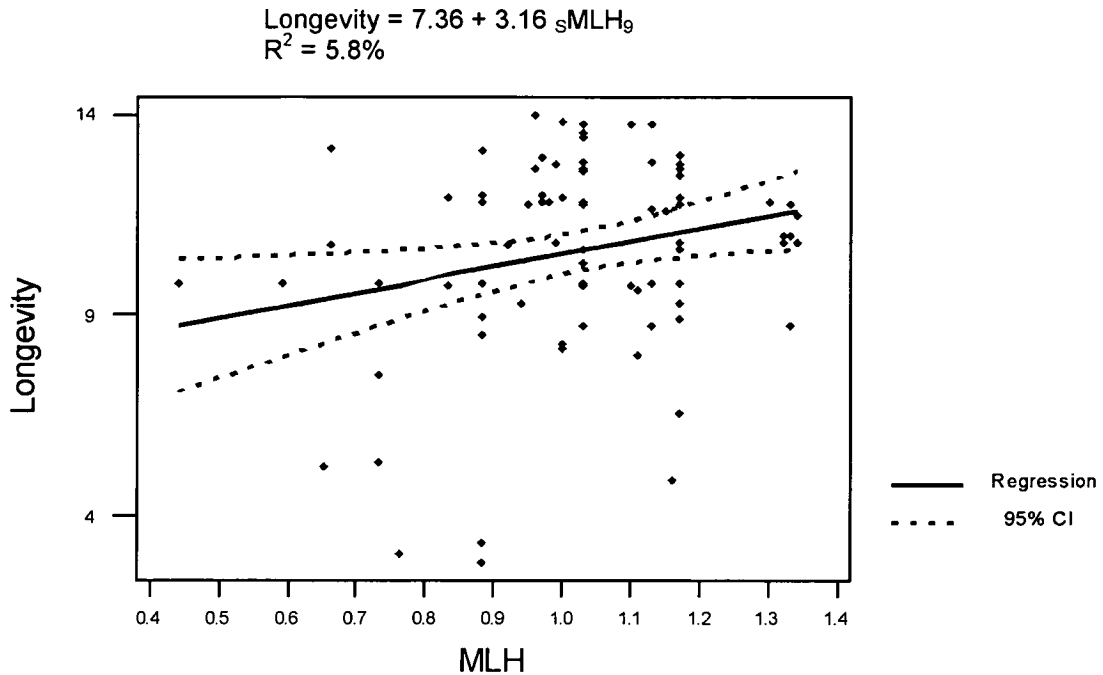


Figure 9.1: The relationship between $sMLH_9$ and male longevity

All animals were born prior to 1987, survived to at least two years old, and were not shot. $sMLH_9$ explains 5.8% of the variance in longevity and the regression slope is significant ($p = 0.021$). $n = 91$.

Although the relationship between $sMLH$ and longevity is statistically significant, it does appear to be largely dependent on a few data points. It is unclear whether $sMLH$ really does explain variation in male longevity.

Male lifetime breeding success:

Previous analyses have suggested that male LBS is influenced by birth weight (Kruuk *et al.* 1999b) and $sMLH$ (Marshall 1998). However no model has tested both terms together. It is unclear whether the putative association between $sMLH$ and LBS is independent of the effect of $sMLH$ on birth weight. Furthermore, in an earlier analysis of $sMLH$ and LBS, Marshall (1998) only included adult stags that were born between 1982 and 1986, giving a sample size of 28.

Male LBS is highly skewed, and so following the approach of Kruuk *et al.* (1999b), models were constructed using a negative binomial error structure. Like Kruuk *et al.* (1999), I used behaviourally assigned paternities to estimate individual lifetime breeding success. A male was considered to be the father of a calf if the mother was

observed to be in oestrus while in his harem, or if she was in his harem for longer than that of any other male. In the event of a tie between two males, paternity was assigned at random to one male. Although this method is less accurate than using genetic data, it allows LBS to be estimated for a greater number of males, and is highly correlated with genetically determined LBS ($r = 0.86$, Marshall 1998).

In a data set ($n = 44$) that included males who were typed for at least six loci and were weighed at birth, $sMLH$ explained significant variation in LBS ($\chi^2 = 4.22$, $p = 0.04$), but birth weight did not ($\chi^2 = 0.70$, $p = 0.40$). A model was also constructed in which birth weight was fitted first, to see if $sMLH$ remained a significant explanatory variable. In this model neither birth weight ($\chi^2 = 1.94$, $p = 0.16$) nor $sMLH$ ($\chi^2 = 3.06$, $p = 0.08$) were significant. However the fact that $sMLH$ approached significance for this small sample, even after fitting birth weight, suggested that the association with LBS was genuine. A model was also constructed using males that were typed at 6 or more loci, but were not necessarily weighed at birth. In this model ($n = 88$), $sMLH$ was significantly associated with LBS ($\chi^2 = 5.62$, $p = 0.018$, see Figure 9.2).

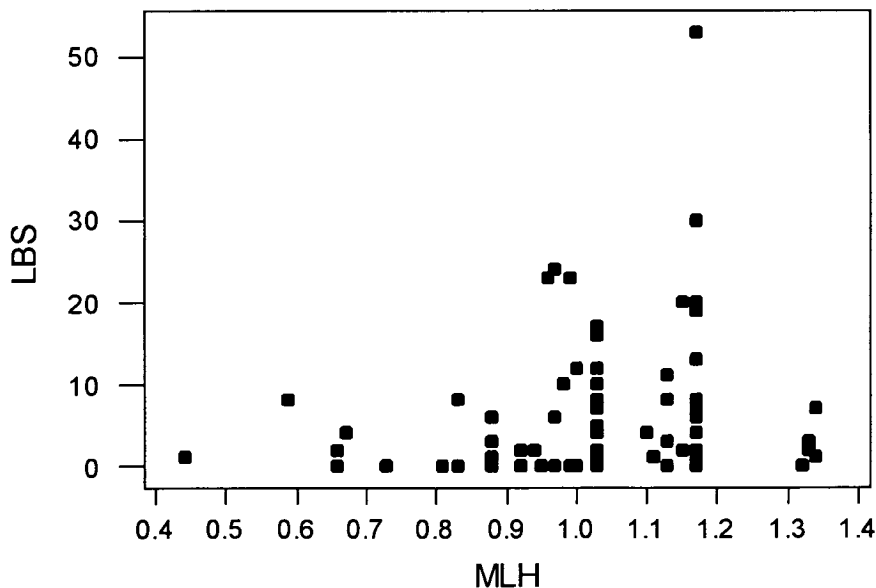


Figure 9.2: The relationship between $sMLH$, and male LBS.

MLH explains 5.8% of the variation in male LBS and is significant at $p < 0.05$ when tested in a GLM with a negative binomial error structure. $n = 88$

9.4 Discussion

The principal aim of this chapter was to test the hypothesis that life history traits were more likely to show an association with MLH than morphometric traits (Roff 1997). This prediction can be extended between sex comparisons within specific traits. For example leg length and birth weight are both associated with male LRS (Rose 1995; Kruuk *et al.* 1999b), and these traits are less heritable in males than females (Kruuk *et al.* in press). Thus, it was predicted that s_{MLH} would be associated with birth weight and leg length in males only. Associations between s_{MLH} and jaw length were not expected for either sex. This is the first study to investigate associations between s_{MLH} and a suite of traits for which heritability and the correlation with total fitness are known. A summary of each trait's heritability, correlation with total fitness, and whether it was associated with s_{MLH} is given in Table 9.4.

Three of the four traits that showed an association with s_{MLH} were life history traits, and the other (male birth weight) was a morphometric trait. Although it is difficult to reach any firm conclusions regarding the relative importance of dominance variance on the different types of trait, this study does provide tentative evidence that traits closely related to fitness are the most likely to be associated with s_{MLH} . The mean heritability of the traits associated with s_{MLH} was lower than that of the remaining traits (s_{MLH} associated traits: mean $h^2 = 0.05$, SE = 0.03, $n = 4$; other traits: mean $h^2 = 0.25$, SE = 0.08, $n = 9$). However, this difference is not significant (Mann-Whitney $U = 11$, ns).

Table 9.4: Summary of $sMLH$ – trait associations.

The heritability and correlation with total fitness of each trait was estimated by Kruuk *et al.* (in press).

Trait	Heritability	Correlation with total fitness	Association with $sMLH$	% variance explained by $sMLH$
<i>Morphometric traits</i>				
FEMALES				
Birth weight	0.250 (0.071)	0.16	No	-
Leg length	0.372 (0.168)	0.21	No	-
Jaw length	0.524 (0.218)	0.02	No	-
MALES				
Birth weight	0.112 (0.067)	0.18	Yes	3.4 %
Leg length	0.072 (0.144)	0.43	No	-
Jaw length	0.601 (0.117)	0.03	No	-
<i>Life history traits</i>				
FEMALES				
Juvenile survival	0 *	-	Yes	1.0 %
Longevity	0.000 (0.001)	0.95	No	-
Fecundity	0.461 (0.150)	0.23	No	-
LBS	0.000 (0.056)	1.00	No	-
MALES				
Juvenile survival	0 *	-	No	-
Longevity	0.000 (0.001)	0.61	Yes	5.8 %
LBS	0.084 (0.076)	1.00	Yes	5.6 %

* Heritability for these traits was not estimated in the study by Kruuk *et al.* (in press), but estimated as 0 in the MAXI pedigree (see Chapter 7).

The measure of total fitness used by Kruuk *et al.* (in press) was LBS, the number of offspring an individual produced in its lifetime.

There was a number of traits closely related to total fitness for which no association with $sMLH$ was found (e.g. female longevity, female lifetime breeding success). Several explanations can be given for this lack of association. The sample sizes available were generally larger for morphometric traits than life history traits. Thus, the power to detect associations was lower for life history traits. In particular, sample sizes for adult life history traits were very low (approximately $n = 100$). The power

analysis presented in Chapter 8 suggested that the probability of detecting an association of similar magnitude to that observed between $sMLH_{71}$ and birth weight was approximately 0.10 (see Figure 8.4) with 900 genotypes. It was also notable that the life history traits for which associations with $sMLH$ were not found tended to have a high residual variance (Kruuk *et al.* in press). It is probable that environmental variation contributes largely to the residual variance. Thus, the traits in which it was particularly important to control for environmental effects were the ones with the smallest sample sizes, and so categorical variables such as subdivision of study area could not be fitted. The fact that any associations were found between $sMLH$ and life history traits suggests that the amount of variance explained by $sMLH$ is relatively high, and that dominance variance plays an important role in the genetic architecture of these traits.

It is striking that most of the significant associations with $sMLH$ were observed in males. In addition to the $sMLH$ -trait associations presented here, it was also shown previously (see Chapter 8) that $sMLH_{71}$ was associated with male juvenile survival. Relatively little is known about the genetic architecture of sexually-selected traits (Merilä and Sheldon 1999), and some of the available data are controversial. For example Pomiankowski and Møller (1995) conducted a meta-analysis that suggested sexually selected traits were more heritable than equivalent non-sexually selected traits. However, a number of these estimates may be confounded by environmental correlations between relatives (Griffith *et al.* 1999). Additionally the possibility of a reporting bias in the literature towards the more heritable sexually selected traits cannot be discounted (Alatalo *et al.* 1997). Intuitively, one would expect sexually selected traits to have relatively low heritabilities, as directional selection will fix favourable alleles. In this population there is much greater variance in LRS in males than females (Kruuk *et al.* in press) and so directional selection should be strongest in male traits related to overall fitness. The fact that the majority of associations with $sMLH$ were in males suggests that dominance variance plays an important role in the genetic architecture of sexually selected traits in the study population.

10 Conclusions and future work

The major aim of this thesis was to map QTL for birth weight in a wild population of red deer. This is the first study to attempt a QTL mapping exercise in an unmanipulated wild population, and as such, highlights a number of problems likely to be encountered in similar endeavours. Biologists planning to map QTL in wild populations must first address the question, “Do I have sufficient power to detect QTL, given the sample sizes and data available?” The power analysis presented in Chapter 3 would suggest that the more conventional experimental designs, for example using half or full sibships, lack sufficient statistical power in most unmanipulated populations. Even with large paternal half-sibships generated by successful red deer males, power was low. In this study, two developments helped to circumvent this problem. The first was the observation that power was greater for an extended multi-generational pedigree than for its composite sibships. The second was the use of variance components methods to detect QTL.

Deriving pedigrees from natural populations is not trivial. However populations for which detailed life history data are available are generally those that have been studied over a substantial number of years, and therefore most likely to have the best-documented pedigrees. Species with short generation times and a relatively large number of offspring per breeding adult will offer the greatest power in mapping experiments. Provided maternal and environmental effects are accounted for, many of the long term bird studies are well suited to QTL mapping – for example the flycatchers on Gotland (described in Chapter 1; Gustafsson 1986)

Variance components methods offer a real breakthrough for QTL mapping in the wild. These approaches can be used in pedigrees of great complexity, and can allow for unknown parents or unsampled individuals. They offer the additional advantage that more traditional quantitative genetic parameters can be estimated simultaneously. This is one of the first studies to use variance components QTL mapping in a non-human population. The biologist wishing to map QTL in a wild population will probably benefit more from adopting this approach, usually

associated with human disease mapping, than if they follow a design used in a laboratory or domestic organism closely related to their own study species.

Does this study provide evidence for genes of major effect segregating in a directionally selected trait? Three regions were identified in the QTL analysis, each of which *may* represent a QTL. Each region was estimated to explain around 25% of the variance in birth weight. If all three QTL were real and of this magnitude they would explain 75% of the variation in birth weight, which is obviously impossible given heritability was only 0.18. If any of these regions represent true QTL, then their magnitude has been overestimated, a problem common to most QTL studies (see Chapter 1). Determining whether the QTL are 'real' is certainly possible in the study population, as there are over one thousand archived samples of deer with recorded birth weight. A failure to detect QTL in such large samples would establish that these regions did not include major genes. Of particular interest is the putative QTL on linkage group 23, where a birth weight QTL was also identified in a hybrid deer pedigree in New Zealand. The notion that QTL are conserved across breeds and species is of particular relevance to animal breeding, where huge investments of time and money into QTL mapping are under way. Conversely, the discovery of candidate genes in related domestic species will help evolutionary biologists to detect QTL in wild populations.

Recently, methods have been described in which molecular markers are used to estimate relatedness between randomly sampled individuals in wild populations (Ritland 1996; Lynch and Ritland 1999). Thus quantitative genetic parameters can be estimated without any field data on familial relationships. Using the MAXI pedigree, it should be possible to assess the accuracy of these exciting, yet relatively untested methodologies. Not only is the MAXI pedigree typed for 84 loci, it is known to be correct (Chapter 5), and life history data are comprehensive. It would be interesting to compare estimates of relatedness and heritability derived from marker-based approaches with estimates derived from more traditional techniques. If the marker-based methods are shown to be accurate, it may even be possible to extend the approach to QTL mapping. A relationship matrix could first be estimated using a

large panel of markers, and then a linkage analysis performed using a variance components method similar to the one described in Chapter 7.

This thesis has highlighted the potential importance of dominance variance in fitness-related traits. Chapter 9 provided some evidence that dominance variance is greater in life history traits than morphometric traits. However, the power to detect dominance variance in adult life history traits was severely compromised by a lack of available individuals. This problem could be partly addressed by typing the relevant individuals for extra markers, so that multi-locus heterozygosity was more accurately estimated. However, in the long term the preferred method for estimating dominance variance might be to use variance components methods in the overall Rum pedigree.

As an increasing number of wild populations are screened for large panels of markers, documented large pedigrees will become more commonplace. Biologists will be able to measure additive and non-additive genetic variation with greater resolution. It will at last be possible to use data from wild populations to test basic theoretical predictions that were made in the early twentieth century.

References

- Abernethy, K., (1994) The establishment of a hybrid zone between red and sika deer (genus *Cervus*). *Molecular Ecology* **3**, 551-562.
- Alatalo, R. V., J. Mappes and M. Elgar, (1997) Heritabilities and paradigm shifts. *Nature* **385**, 402-403.
- Albon, S. D., F. E. Guinness and T. H. Clutton-Brock, (1983a) The influence of climatic variation on the birth weights of red deer (*Cervus elaphus*). *Journal of Zoology* **200**, 295-298.
- Albon, S. D., B. Mitchell and B. W. Staines, (1983b) Fertility and body weight in female red deer: a density-dependent relationship. *Journal of Animal Ecology* **52**, 969-980.
- Albon, S. D., T. H. Clutton-Brock and F. E. Guinness, (1987) Early development and population dynamics in red deer. II. Density-independent effects and cohort variation. *Journal of Animal Ecology* **56**, 69-81.
- Allendorf, F. W., and R. F. Leary, (1986) Heterozygosity and fitness in natural populations of animals. In: *Conservation Biology*, edited by M. E. Soulé., pp57-76. Sinauer, Sunderland, Ma.
- Almasy, L., and J. Blangero, (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. *American Journal of Human Genetics* **62**, 1198-1211.
- Almasy, L., J. E. Hixson, D. L. Rainwater, S. Cole, J. T. Williams *et al.*, (1999) Human Pedigree-Based Quantitative-Trait Locus Mapping: Localization of Two Genes Influencing HDL-Cholesterol Metabolism. *American Journal of Human Genetics* **64**, 1686-1693.
- Amos, W., (1999) A comparative approach to the study of microsatellite evolution. In: *Microsatellites. Evolution and applications*, edited by D. B. Goldstein, and C. Schlötterer. Oxford University Press, Oxford.
- Andersson, L., C. S. Haley, H. Ellegren, S. A. Knott, M. Johansson *et al.* (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science*. **263**, 1771-1774.
- Andersson, M., (1994) *Sexual Selection*. Princeton University Press, Princeton.

- Avise, J. C., (1994) *Molecular Markers. Natural History and Evolution*. Chapman and Hall, New York.
- Avner, P., L. Amar, L. Dandolo and J. L. Guenet, (1988) Genetic analysis of the mouse using interspecific crosses. *Trends in Genetics* **4**, 18-23.
- Bahri-Darwich, I., D. Vaiman, I. Olsaker, A. Oustry and E. P. Cribiu, (1994) Assignment of bovine synteny group -U27 and group -U8 to R-banded chromosome -12 and chromosome -27, respectively. *Hereditas* **120**, 261-265.
- Bancroft, D. R., J. M. Pemberton and P. King, (1995) Extensive protein and microsatellite variability in an isolated, cyclic ungulate population. *Heredity* **74**, 326-336.
- Barendse, W., S. M. Armitage, L. M. Kossarek, A. Shalom, B. W. Kirkpatrick *et al.*, (1994) A genetic linkage map of the bovine genome. *Nature Genetics* **6**, 227-235.
- Barendse, W., D. Vaiman, S. J. Kemp, Y. Sugimoto, S. M. Armitage *et al.*, (1997) A medium-density genetic linkage map of the bovine genome. *Mammalian Genome* **8**, 21-28.
- Baret, P. V., S. A. Knott and P. M. Visscher, (1998) On the use of linear regression and maximum likelihood for QTL mapping in half-sib designs. *Genetical Research* **72**, 149-158.
- Barton, N. H., and M. Turelli, (1989) Evolutionary quantitative genetics: how little do we know? *Annual Review of Genetics* **23**, 337-370.
- Beavis, W. D., (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In: *Proceedings of the forty-ninth annual corn and sorghum industry research conference*, pp 250-266.
- Beh, K.J., M. J. Callaghan, D. J. Hulme, Z. Leish, K. Diianno and I. Lenane (1998) A search for genes affecting gastrointestinal parasite resistance in sheep. *Animal Genetics* **29**. Supplement 1, 67.
- Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden *et al.*, (1994) A genetic linkage map of cattle. *Genetics* **136**, 619-639.
- Boehnke, M., (1986) Estimating the power of a proposed linkage study: a practical computer simulation approach. *American Journal of Human Genetics* **39**, 513-527.

- Bradshaw Jr, H. D., K. G. Otto, B. E. Frewen, J. K. McKay and D. W. Schemske, (1998) Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*mimulus*). *Genetics* **149**, 367-382.
- Bradshaw Jr, H. D., S. M. Wilbert, K. G. Otto and D. W. Schemske, (1995) Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers(*Mimulus*). *Nature* **376**, 762-765.
- Brezinsky, L., S. J. Kemp and A. J. Teale, (1993) ILSTS6 - a polymorphic bovine microsatellite. *Animal Genetics* **24**, 73.
- Britten, H. B., (1996) Meta-analyses of the association between multilocus heterozygosity and fitness. *Evolution* **1996**, 2158-2164.
- Brotherton, P. N. M., J. M. Pemberton, P. Komers and G. Malarky, (1997) Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (*Madoqua kirkii*). *Proceedings of the Royal Society of London, Series B* **264**, 675-681.
- Buchanan, F. C., S. M. Galloway and A. M. Crawford, (1994) Ovine microsatellites at the OarFCB5, OarFCB19, OarFCB20, OarFCB48, OarFCB129 and OarFCB266 loci. *Animal Genetics* **25**, 60.
- Burke, T., and M. W. Bruford, (1987) DNA fingerprinting in birds. *Nature* **327**, 149-152.
- Callen, D. F., A. D. Thompson, Y. Shen, H. A. Phillips, R. I. Richards *et al.*, (1993) Incidence and origin of "null" alleles in the (AC)_n microsatellite markers. *American Journal of Human Genetics* **52**, 922-927.
- Cardon, L., and D. W. Fulker, (1994) The power of interval mapping of quantitative trait loci , using selected sib pairs. *American Journal of Human Genetics* **55**, 825-833.
- Charlesworth, B., (1987) The heritability of fitness. In: *Sexual Selection: Testing the Alternatives*, edited by J. W. Bradbury, and M. B. Andersson, pp 21-40. John Wiley and Sons Limited, Chicago.
- Churchill, G. A., and R. W. Doerge, (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963-971.
- Clutton-Brock, T.H., (1988) In: *Reproductive Success.*, edited by T.H. Clutton-Brock. The University of Chicago Press, Chicago.

- Clutton-Brock, T. H., and S. D. Albon, (1989) *Red deer in the Highlands*. Blackwell, Oxford.
- Clutton-Brock, T. H., S. D. Albon and F. E. Guinness, (1984) Maternal dominance, breeding success and birth sex ratios in red deer. *Nature* **308**, 358-360.
- Clutton-Brock, T. H., S. D. Albon and F. E. Guinness, (1988) Reproductive success in male and female red deer. In: *Reproductive Success*, edited by T. H. Clutton-Brock. The University of Chicago Press, Chicago and London.
- Clutton-Brock, T. H., F. E. Guinness and S. D. Albon, (1982) *Red deer - behavior and ecology of two sexes*. Edinburgh University Press, Edinburgh.
- Clutton-Brock, T. H., F. E. Guinness and S. D. Albon, (1983) The costs of reproduction to red deer hinds. *Journal of Animal Ecology* **52**, 367-383.
- Clutton-Brock, T. H., M. Major, S. D. Albon and F. E. Guinness, (1987) Early development and population dynamics in red deer. I. Density-dependent effects on juvenile survival. *Journal of Animal Ecology* **56**, 53-67.
- Coltman, D. W., D. R. Bancroft, A. Robertson, J. A. Smith, T. H. Clutton-Brock *et al.*, (1999a) Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Molecular Ecology* **8**, 1199-1209.
- Coltman, D. W., W. D. Bowen and J. M. Wright, (1998) Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measure by microsatellites. *Proceedings of the Royal Society of London, Series B* **262**, 803-809.
- Coltman, D. W., J. G. Pilkington, J. A. Smith and J. M. Pemberton, (1999b) Parasite mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* **53**, 1259-1267.
- Coulson, T. N., S. D. Albon, F. E. Guinness, J. M. Pemberton and T. H. Clutton-Brock, (1997) Population substructure, local density, and calf winter survival in red deer (*Cervus elaphus*). *Ecology* **78**, 852-863.
- Coulson, T. N., S. D. Albon, J. M. Pemberton, T. H. Clutton-Brock, J. Slate *et al.*, (1998a) Genotype by environment interactions in winter survival in red deer. *Journal of Animal Ecology* **67**, 434-445.

- Coulson, T., S. Albon, J. Slate and J. M. Pemberton, (1999) Microsatellite loci reveal sex dependent responses to inbreeding and outbreeding in red deer calves. *Evolution* **53**, 1951-1960.
- Coulson, T. N., J. M. Pemberton, S. D. Albon, M. A. Beaumont, T. C. Marshall *et al.*, (1998b) Microsatellites measure inbreeding depression and heterosis in red deer. *Proceedings of the Royal Society of London: Series B* **265**, 489-495.
- Crawford, A. M., F. C. Buchanan and P. A. Swarbrick, (1990) Ovine dinucleotide repeat polymorphism at the MAF18 locus. *Animal Genetics* **21**, 433-434.
- Crawford, A. M., K. G. Dodds, A. J. Ede, C. A. Pierson, G. W. Montgomery *et al.*, (1995) An autosomal genetic linkage map of the sheep genome. *Genetics* **140**, 703-724.
- Crawford, A. M., G. W. Montgomery, C. A. Pierson, T. Brown, K. G. Dodds *et al.*, (1994) Sheep linkage mapping: nineteen linkage groups derived from the analysis of paternal half-sib families. *Genetics* **137**, 573-579.
- Crawley, M. J., 1993 *GLIM for ecologists*. Blackwell Scientific, Oxford.
- Cronkrak, P., and D. A. Roff, (1995) Dominance variance: associations with selection and fitness. *Heredity* **75**, 530-540.
- Darvasi, A., and M. Soller, (1992) Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theoretical Applied Genetics* **85**, 353-359.
- Darvasi, A., and M. Soller, (1994) Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait locus. *Genetics* **138**, 1365-1373.
- David, P., (1998) Heterozygosity-fitness correlations: new perspectives on old problems. *Heredity* **80**, 531-537.
- de Gortari, M. J., B. A. Freking, R. P. Cuthbertson, S. M. Kappes, J. W. Keele *et al.*, (1998) A second-generation linkage map of the sheep genome. *Mammalian Genome* **9**, 204-209.
- de Gortari, M. J., B. A. Freking, S. M. Kappes, K. A. Leymaster, A. M. Crawford *et al.*, (1997) Extensive genomic conservation of cattle microsatellite heterozygosity in sheep. *Animal Genetics* **28**, 274-290.

- DeRose, M. A., and D. A. Roff, (1999) A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution* **53**, 1288-1292.
- DiMichele, L., K. Paynter and D. A. Powers, (1991) Lactate dehydrogenase-B allozymes directly affect development of *Fundulus heteroclitus*. *Science* **23**, 898-900.
- Doerge, R. W., and C. G. A., (1996) Permutation tests for multiple loci affecting a quantitative character. *Genetics* **142**, 285-294.
- Edwards, A. W. F., 1992 *Likelihood*. John Hopkins University Press, Baltimore and London.
- Emerson, B. C., and M. L. Tate, (1993) Genetic analysis of evolutionary relationships among deer (subfamily cervinae). *Journal of Heredity* **84**, 266-273.
- Endler, J. A., (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton.
- Engel, S. R., R. A. Linn, J. F. Taylor and S. K. Davis, (1996) Conservation of microsatellite loci across species of artiodactyls: implications for population studies. *Journal of Mammology* **77**, 504-518.
- Falconer, D. S., (1989) *Introduction to Quantitative Genetics*. (Third edition) Longman, New York.
- Falconer, D. S., and T. F. C. Mackay, (1996) *Introduction to Quantitative Genetics*. (Fourth edition) Longman.
- Fisher, R. A., (1930) *The Genetical Theory of Natural Selection*. Dover Publications Inc, New York.
- FitzSimmons, N. N., C. Moritz and S. S. Moore, (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution* **12**, 432-440.
- Frank, S. A., and M. Slatkin, (1992) Fisher's Fundamental Theorem of Natural Selection. *Trends in Ecology and Evolution* **7**, 92-95.
- Fries, R., (1993) Mapping the bovine genome; methodological aspects and strategy. *Animal Genetics* **24**, 111-116.
- Fries, R., A. Eggen and J. E. Womack, (1993) The bovine genome map. *Mammalian Genome* **4**, 405-428.

- Fulker, D. W., and L. R. Cardon, (1994) A sib-pair approach to interval mapping of quantitative trait loci. *American Journal of Human Genetics* **54**, 1092-1103.
- Fulker, D. W., S. S. Cherry and L. R. Cardon, (1995) Multipoint interval mapping of quantitative trait loci, using sib pairs. *American Journal of Human Genetics* **56**, 1224-1233.
- Gemmell, N. J., (1997) Enhancement of microsatellite amplification using tetramethylammonium chloride and formamide (TMACIDE). Technical Tips Online (<http://www.elsevier.nl/locate/tto>) **T40029**.
- Gemmell, N. J., P. J. Allen, S. J. Goodman and J. Z. Reed, (1997) Interspecific microsatellite markers for the study of pinniped populations. *Molecular Ecology* **6**, 661-666.
- Georges, M. S., A. B. Dietz, A. Mishra, D. Nielsen, L. S. Sargeant *et al.*, (1993b) Microsatellite mapping of the gene causing weaver disease in cattle will allow the study of an associated quantitative trait locus. *Proceedings of the National Academy of Science USA* **90**, 1058-1062.
- Georges, M., R. Drinkwater, T. King, A. Mishra, S. S. Moore *et al.*, (1993a) Microsatellite mapping of a gene affecting horn development in *Bos taurus*. *Nature Genetics* **4**, 206-210.
- Georges, M., and J. M. Massey, (1992) Polymorphic DNA markers in *Bovidae*. Patent W092/13102.
- Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto *et al.*, (1995) Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* **139**, 907-920.
- Gerloff, U., B. Hartung, B. Fruth, G. Hohmann and D. Tautz, (1999) Intracommunity relationships, dispersal pattern and paternity success in a wild living community of bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. *Proceedings of the Royal Society of London, Series B* **266**, 1189-1195.
- Goldgar, D. E., (1990) Multipoint analysis of human quantitative genetic variation. *American Journal of Human Genetics* **47**, 957-967.
- Goldstein, D. B., and C. Schlötterer. (1999) *Microsatellites. Evolution and applications*. Oxford University Press, Oxford.

- Goodman, S. J., N. H. Barton, G. Swanson, K. Abernethy and J. M. Pemberton, (1999) Introgression through rare hybridization: a genetic study of a hybrid zone between red and sika deer (genus *Cervus*) in Argyll, Scotland. *Genetics* **152**, 355-371.
- Goosen, G. J. C., (1997) An interspecies hybrid in deer. PhD thesis. University of New England, Armidale.
- Goosen, G. J., M. L. Tate, A. J. Pearse, K. G. Dodds and P. F. Fennessy, (1998) Genetic analysis of birth weight and weaning weight in an interspecies hybrid in deer. In: *6th World Congress on Genetics Applied to Livestock Production*, Armidale, Australia.
- Goossens, B., L. P. Waits and P. Taberlet, (1998) Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. *Molecular Ecology* **7**, 1237-1241.
- Griffith, S. C., I. P. F. Owens and T. Burke, (1999) Environmental determination of a sexually selected trait. *Nature* **400**, 358-360.
- Groeneveld, E., (1993) REML VCE- a multivariate multimodal restricted maximum likelihood (co)variance component estimation package. In: *Symposium on application of mixed linear models in the prediction of genetic merit in pigs*, Institute of Animal husbandry and Ethology, Federal Agricultural Research Centre, Germany.
- Guinness, F. E., T. H. Clutton-Brock and S. D. Albon, (1978) Factors affecting calf mortality in red deer (*Cervus elaphus*). *Journal of Animal Ecology* **47**, 817-832.
- Gustafsson, L., (1986) Lifetime reproductive success and heritability: empirical support for Fisher's fundamental theorem. *The American Naturalist* **128**, 761-764.
- Haley, C. S., (1995) Livestock QTLs - bringing home the bacon? *Trends in Genetics* **11**, 488-492.
- Haley, C. S., and S. A. Knott, (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315-324.

- Hanrahan, V., A. J. Ede, C. A. Pierson and D. F. Hill, (1993) Ovine microsatellites at the *OarVH98*, *Oar VH110*, *OarVH116*, *OarVH117* and *OarVH130* loci. *Animal Genetics* **24**, 223.
- Haseman, J. K., and R. C. Elston, (1972) The investigation of linkage between a quantitative trait and a marker locus. *Behavioural Genetics* **2**, 3-19.
- Heath, S. C., (1997) A package for multipoint linkage analysis on large pedigrees using Reversible Jump Markov Chain Monte Carlo. Rockefeller University, New York.
- Heath, S. C., and E. A. Thompson, (1997) MCMC samplers for multilocus analyses on complex traits. *American Journal of Human Genetics* **61**, 1623.
- Hey, J., (1999) The neutralist, the fly and the selectionist. *Trends in Ecology and Evolution* **14**, 35-38.
- Hilbish, T. J., and R. K. Koehn, (1985) The physiological basis of natural selection at the LAP locus. *Evolution* **39**, 1302-1317.
- Hogg, J. T., and S. H. Forbes, (1997) Mating in bighorn sheep: frequent male reproduction via a high-risk 'unconventional' tactic. *Behavioural Ecology and Sociobiology* **41**, 33-48.
- Houle, D., (1992) Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195-204.
- Hulme, D. J., J. P. Silk, J. M. Redwin and W. Barendse, (1994) 10 polymorphic ovine microsatellites. *Animal Genetics* **25**, 434-435.
- Inoue, M., T. Hirano, S. Nakane, T. Watanabe, H. Takeda *et al.*, (1995) Five bovine polymorphic dinucleotide loci (DIK008, DIK010, DIK015, DIK016, DIK020). *Animal Genetics* **26**, 447.
- Jarne, P., and P. J. L. Lagoda, (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11**, 424-429.
- Jeffreys, A. J., V. Wilson and S. L. Thein, (1985) Individual specific 'fingerprints' of human DNA. *Nature* **316**, 76-79.
- Jones, J.S., (1987) The heritability of fitness: bad news for 'good genes'? *Trends in Ecology and Evolution* **37**, 35-38.

- Jorgenson, C., B. A. Konfortov, W. Barendse and J. R. Miller, (1995) Two polymorphic bovine microsatellite loci (AFR227 and AFR2215). *Animal Genetics* **26**, 132.
- Kashi, Y., D. King and M. Soller, (1997) Simple sequence repeats as a source of quantitative genetic variation. *Trends in Genetics* **13**, 74-78.
- Kaukinen, J., and S. L. Varvio, (1993) Eight polymorphic bovine microsatellites. *Animal Genetics* **24**, 148.
- Kearsey, M. J., and A. G. L. Farquhar, (1998) QTL analysis in plants; where are we now? *Heredity* **80**, 137-142.
- Kemp, S. J., O. Hishida, J. Wamburger, A. Rink, M. L. Longeri *et al.*, (1995) A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Animal Genetics* **26**, 299-306.
- Kinghorn, B. P., (1994) Pedigree Viewer - a graphical utility for browsing pedigreed data sets. In: *5th World Congress on Genetics applied to livestock production*, pp85-86. Guelph, Canada.
- Knott, S. A., J. M. Elsen and C. S. Haley, (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theoretical and Applied Genetics* **93**, 71-80.
- Konfortov, B. A., C. B. Jorgensen, W. Barendse and J. R. Miller, (1996) Five bovine polymorphic markers (AF1-AF5). *Animal Genetics* **27**, 220.
- Kossarek, L. M., O. Finlay, W. M. Grosse, X. Su, S. M. Armitage *et al.*, (1995) Five bovine dinucleotide repeat polymorphisms: RM040, RM044, RM096, RM153 and RM179. *Animal Genetics* **26**, 57-58.
- Kossarek, L. M., O. Finlay, W. M. Grosse, X. Su and R. A. McGraw, (1996) Seven bovine dinucleotide repeat polymorphisms: RM042, RM128, RM134, RM154, RM162, RM181 and RM509. *Animal Genetics* **27**, 61.
- Kossarek, L. M., W. M. Grosse, J. E. Dietz, J. E. Womack and R. A. McGraw, (1993) Bovine dinucleotide repeat polymorphism RM004. *Journal of Animal Science* **71**, 3175.
- Kossarek, L. M., W. M. Grosse, O. Finlay, A. B. Dietz, J. E. Womack *et al.*, (1994) bovine dinucleotide repeat polymorphism RM095. *Journal of Animal Science* **72**, 254.

- Kruglyak, L., and E. S. Lander, (1995) Complete multi-point sib-pair analysis of qualitative and quantitative traits. *American Journal of Human Genetics* **57**, 439-454.
- Kruuk, L. E. B., T. H. Clutton-Brock, S. D. Albon, J. M. Pemberton and F. E. Guinness, (1999a) Population density affects sex ratio variation in red deer. *Nature* **399**, 459-461.
- Kruuk, L. E. B., T. H. Clutton-Brock, K. E. Rose and F. E. Guinness, (1999b) Early determinants of lifetime reproductive success differ between the sexes in red deer. *Proceedings of the Royal Society of London, Series B* **266**, 1655-1661.
- Kruuk, L. E. B., T. H. Clutton-Brock, J. Slate, J. M. Pemberton, S. Brotherstone and F. E. Guinness, (in press) Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences, USA*.
- Kühn, R., C. Anastassiadis and F. Pirchner, (1996) Transfer of bovine microsatellites to the cervine (*Cervus elaphus*). *Animal Genetics* **27**, 199-201.
- Lander, E. S., and D. Botstein, (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185-99.
- Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly *et al.*, (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**, 174-181.
- Lander, E., and L. Kruglyak, (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics* **11**, 241-247.
- Le Roy, P., and Elsen, J.M., (1995) Numerical comparisons between powers of maximum likelihood and analysis of variance methods for QTL detection in progeny test designs: the case of monogenic inheritance. *Theoretical and Applied Genetics* **90**, 65-72.
- Lin, J.-Z., and K. Ritland, (1997) Quantitative trait loci differentiating the outbreeding *Mimulus guttatus* from the inbreeding *M. platycalyx*. *Genetics* **146**, 1115-1121.
- Lincoln, G. A., F. E. Guinness and T. J. Fletcher, (1973) History of a hummel - Part 3. Sons with antlers. *Deer* **3**, 26-31.

- Litt, M., and J. A. Luty, (1989) A hypervariable microsatellite revealed by In Vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* **44**, 397-401.
- Long, A. D., S. L. Mullaney, L. A. Reid, J. D. Fry, C. H. Langley *et al.*, (1995) High resolution mapping of genetic factors affecting abdominal bristle number in *drosophila melanogaster*. *Genetics* **139**, 1273-1291.
- Lowe, V. P. W., and A. S. Gardiner, (1989) Are the New and Old World wapitis (*Cervus canadensis*) conspecific with red deer (*Cervus elaphus*)? *Journal of Zoology* **218**, 51-58.
- Lynch, M., and K. Ritland, (1999) Estimation of pairwise relatedness with molecular markers. *Genetics* **152**, 1753-1766.
- Lynch, M., and B. Walsh, (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, Massachusetts.
- Lynch, M., and B. Walsh, (in prep) *Evolution and Selection of Quantitative Traits*. Sinauer, Sunderland, Massachusetts.
- Ma, R. Z., J. E. Beever, Y. Da, C. A. Green, I. Russ *et al.*, (1996) A male linkage map of the cattle (*Bos taurus*) genome. *Journal of Heredity* **87**, 261-277.
- Mackay, T. F. C., (1995) The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system. *Trends in Genetics* **11**, 464-470.
- Mackay, T. F. C., and C. H. Langley, (1990) Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. *Nature* **348**, 64-66.
- Marshall, T. C., (1998) Inbreeding and fitness in wild ungulates. PhD Thesis. University of Edinburgh, Edinburgh.
- Marshall, T. C., D. W. Coltman, J. M. Pemberton, F. E. Guinness, J. A. Spalton *et al.*, (submitted) Estimating the prevalence of inbreeding from pedigrees.
- Marshall, T. C., J. Slate, L. E. B. Kruuk and J. M. Pemberton, (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**, 639-655.
- McCouch, S. R., X. L. Chen, O. Panaud, S. Temnykh, Y. B. Xu *et al.*, (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology* **35**, 89-99.

- McGraw, R. A., W. M. Grosse, S. M. Kappes, C. W. Beattie and R. T. Stone, (1997) Thirty-four bovine microsatellite markers. *Animal Genetics* **28**, 66-68.
- Meagher, T. R., (1986) Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely parents. *The American Naturalist* **128**, 199-215.
- Merilä, J., and B. C. Sheldon, (1999) Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity* **83**, 103-109.
- Mezzelani, A., Y. Zhang, L. Redaelli, B. Castiglioni, P. Leone *et al.*, (1995) Chromosomal localization and molecular characterization of 53 cosmid-derived bovine microsatellites. *Mammalian Genome* **6**, 629-635.
- Mitchell-Olds, T., (1995) The molecular basis of quantitative genetic variation in natural populations. *Trends in Ecology and Evolution* **10**, 324-328.
- Mitchell-Olds, T., (1996) Genetic constraints on life-history evolution: quantitative-trait loci influencing growth and flowering in *Arabidopsis thaliana*. *Evolution* **50**, 140-145.
- Mitton, J. B., (1994) Molecular approaches to population biology. *Annual Review of Ecology and Systematics* **25**, 45-69.
- Mitton, J. B., (1997) *Selection in Natural Populations*. Oxford University Press, Oxford.
- Modi, W. S., D. S. Gallagher and J. E. Womack, (1996) Evolutionary histories of highly repeated DNA families among the artiodactyla (mammalia). *Journal of Molecular Evolution* **42**, 337-349.
- Montgomery, G. W., A. M. Crawford, J. M. Penty, K. G. Dodds, A. E. Ede *et al.*, (1993) The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. *Nature Genetics* **4**, 410-414.
- Moore, S. S., and K. Byrne, (1993) Dinucleotide polymorphism at the bovine histamine H1 receptor locus. *Animal Genetics* **24**, 72.
- Moore, S. S., K. Byrne, K. T. Berger, W. Barendse, F. McCarthy *et al.*, (1994) Characterization of 65 bovine microsatellites. *Mammalian Genome* **5**, 84-90.
- Moore, S. S., L. L. Sargeant, T. J. King, J. S. Mattick, M. Georges *et al.*, (1991) The conservation of dinucleotide microsatellites among mammalian genomes

- allows the use of heterologous PCR primer pairs in closely related species. *Genomics* **10**, 654-660.
- Morin, P. A., J. Wallis, J. J. Moore and D. S. Woodruff, (1994) Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. *Molecular Ecology* **3**, 469-478.
- Mousseau, T. A., and D. A. Roff, (1987) Natural selection and the heritability of fitness components. *Heredity* **59**, 181-197.
- Nielsen, D., N. E. Cockett and M. Georges, (1995) Mapping markers and quantitative traits in large half-sib pedigrees. *Proceedings of the Western Section of the American Society of Animal Science* **46**, 205-208.
- Nuzhdin, S. V., E. G. Pasyukova, C. L. Dilda, Z.-B. Zeng and T. F. Mackay, (1997) Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **94**, 9734-9739.
- O'Brien, S. J., J. E. Womack, L. A. Lyons, K. J. Moore, N. A. Jenkins *et al.*, (1993) Anchored reference loci for comparative genome mapping in mammals. *Nature Genetics* **3**, 103-112.
- Orr, H. A., and J. A. Coyne, (1992) The genetics of adaption: a reassessment. *The American Naturalist* **140**, 725-742.
- Paterson, A. H., E. S. Lander, J. D. Hewitt, S. Peterson, S. E. Lincoln *et al.*, (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* **335**, 721-726.
- Pemberton, J. M., S. D. Albon, F. E. Guinness and T. H. Clutton-Brock, (1991) Countervailing selection in different fitness components in female red deer. *Evolution* **45**, 93-103.
- Pemberton, J. M., S. D. Albon, F. E. Guinness, T. H. Clutton-Brock and R. J. Berry, (1988) Genetic variation and juvenile survival in red deer. *Evolution* **42**, 921-934.
- Pemberton, J. M., S. D. Albon, F. E. Guinness, T. H. Clutton-Brock and G. A. Dover, (1992) Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioral Ecology* **3**, 66-75.

- Pemberton, J. M., D. W. Coltman, T. N. Coulson and J. Slate, (1999) Using microsatellites to measure the fitness consequences of inbreeding and outbreeding. In: *Microsatellites: Evolution and Applications*, edited by D. B. Goldstein and C. Schlotterer. OUP, Oxford.
- Pemberton, J. M., and J. Slate, (1994) Genetic studies of wild deer populations: a technical revolution. In: *Third International Congress on the biology of Deer*, edited by J. A. Milne, Edinburgh.
- Pemberton, J. M., J. Slate, D. R. Bancroft and J. A. Barrett, (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology* **4**, 249-252.
- Pemberton, J. M., J. A. Smith, T. N. Coulson, T. M. Marshall, J. Slate *et al.*, (1996) The maintenance of genetic polymorphism in small island populations: Large mammals in the Hebrides. *Philosophical Transactions of the Royal Society of London: Series B* **351**, 745-752.
- Pepin, L., Y. Amigues, A. Lepingle, J.-L. Berthier, A. Bensaid *et al.*, (1995) Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis. *Heredity* **74**, 53-61.
- Pfister-Genskow, M. A., G. A. Hawkins, L. I. Doud, G. L. Jurgella, B. T. Murkve *et al.*, (1995) Bovine dinucleotide repeat polymorphisms ABS010, ABS011, ABS012, ABS013 and ABS014. *Journal of Animal Science* **73**, 3189.
- Pierson, C. A., A. J. Ede and A. M. Crawford, (1994) Ovine microsatellites at the OarHH30, OarHH51, OarVH54, OarCP88, OarCP93 and OarCP134 loci. *Animal Genetics* **25**, 294-295.
- Ploughman, L. M., and M. Boehnke, (1989) Estimating the power of a proposed linkage study for a complex genetic trait. *American Journal of Human Genetics* **44**, 543-551.
- Pogson, G. H., and S. E. Fevoldon, (1998) DNA heterozygosity and growth rate in the Atlantic Cod *Gadus morhua* (L). *Evolution* **52**, 915-920.
- Pogson, G. H., and E. Zouros, (1994) Allozyme and RFLP heterozygosities as correlates of growth rate in the scallop *Placopecten magellanicus*: a test of the associative overdominance hypothesis. *Genetics* **137**, 221-231.

- Pomiankowski, A., and A. P. Moller, (1995) A resolution of the lek paradox. *Proceedings of the Royal Society of London: Series B* **260**, 21-29.
- Price, T., and D. Schluter, (1991) On the low heritability of life-history traits. *Evolution* **45**, 853-861.
- Queller, D. C., J. E. Strassman and C. R. Hughes, (1993) Microsatellites and kinship. *Trends in Ecology and Evolution* **8**, 285-288.
- Rieseberg, L. H., J. Whitton and K. Gardner, (1999) Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* **152**, 713-727.
- Ritland, K., (1996) A marker-based method for inferences about quantitative inheritance in natural populations. *Evolution* **50**, 1062-1073.
- Roff, D. A., (1997) *Evolutionary Quantitative Genetics*. ITP, New York.
- Roff, D. A., and T. A. Mousseau, (1987) Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity* **58**, 103-118.
- Rose, K. E., (1995) Factors affecting lifetime reproductive success in red deer stags (*Cervus elaphus*). PhD Thesis. University of Cambridge, Cambridge.
- Rose, M. R., (1982) Antagonistic pleiotropy, dominance, and genetic variation. *Heredity* **48**, 63-78.
- Rowe, L., and D. Houle, (1996) The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**, 1415-1421.
- Sambrook, J., E. F. Fritsch and T. Maniatis, (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory Press, N.Y.
- SanCristobel, M., and C. Chevalet, (1997) Error tolerant parent identification from a finite set of individuals. *Genetical Research* **70**, 53-62.
- Sax, K., (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* **8**, 552-560.
- Schlötterer, C. (1998) Microsatellites. In: *Molecular genetic analysis of populations*, pp237-261. Edited by A. R. Hoelzel. IRL Press, Oxford.
- Shalom, A., M. O. Mosig, W. Barendse, F. A. and M. Soller, (1994) Dinucleotide repeat polymorphism at the bovine HUI246, HUII77, HUI223, HUIV174 and HUI I75 loci. *Animal Genetics* **25**, 56.

- Shalom, A., M. O. Mosig, W. Barendse, M. Soller and A. Friedmann, (1995) Dinucleotide repeat polymorphisms at the bovine HUI673, HUI121, HUI174, HUI225, HUI113 and HUI129 loci. *Animal Genetics* **26**, 202-203.
- Shalom, A., M. Soller and A. Friedmann, (1993) Dinucleotide repeat polymorphism at the bovine HUI616 locus. *Animal Genetics* **24**, 327.
- Shook, D. R., A. Brooks and T. E. Johnson, (1996) Mapping quantitative trait loci affecting life history traits in the nematode *Caenorhabditis elegans*. *Genetics* **142**, 801-817.
- Shrimpton, A. E., and A. Robertson, (1988) The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. II. Distribution of third chromosome bristle effects within chromosome sections. *Genetics* **118**, 445-459.
- Slate, J., D. W. Coltman, S. J. Goodman, I. Maclean, J. M. Pemberton *et al.*, (1998) Microsatellite loci are highly conserved in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), and Soay sheep (*Ovis aries*). *Animal Genetics* **29**, 307-315.
- Slate, J., T. C. Marshall and J. M. Pemberton (in press) A retrospective assessment of the accuracy of paternity inference program CERVUS. *Molecular Ecology*.
- Slate, J., J. M. Pemberton, and P. M. Visscher, (1999) Power to detect QTL in a free-living polygynous population. *Heredity* **83**, 327-336.
- Sokal, R. R., and F. J. Rohlf, 1995 *Biometry. The Principles and Practice of Statistics in Biological Research*. Freeman, New York.
- Solinas-Toldo, S., R. Fries, P. Steffen, H. L. Neibergs, W. Barendse *et al.*, (1993) Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. *Mammalian Genome* **4**, 720-727.
- Steen, R. G., A. E. KwikteBlack, C. Glenn, J. GullingsHandley, W. VanEtten *et al.*, (1999) A high-density integrated genetic linkage and radiation hybrid map of the laboratory rat. *Genome Research* **9**, 793.
- Steffen, P., A. Eggen, A. B. Dietz, J. E. Womack, G. Sranzinger *et al.*, (1993) Isolation and mapping of polymorphic microsatellites in cattle. *Animal Genetics* **24**, 121-124.

- Sun, H. S., B. M. Huff, M. R. Dentine and B. W. Kirkpatrick, (1994) Polymorphic bovine microsatellites UWCA5, UWCA7 and UWCA9. *Animal Genetics* **25**, 59.
- Sun, H. S., S. Whallon, A. Ponce de Leon and B. W. Kirkpatrick, (1993) Development of polymorphic bovine microsatellites from a cosmid library. *Journal of Animal Science* **71 (Supplement)**, 100.
- Taberlet, P., and G. Luikart, (1999) Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society* **68**, 41-55.
- Talbot, J., J. Haigh and Y. Plante, (1996) A parentage evaluation test in North American Elk (Wapiti) using microsatellites of ovine and bovine origin. *Animal Genetics* **27**, 117-119.
- Tate, M. L., (1997) Evolution of ruminant chromosomes, PhD thesis. University of Otago, Dunedin.
- Tate, M. L., H. C. Mathias, P. F. Fennessy, K. G. Dodds, J. M. Penty *et al.*, (1995) A new gene mapping resource: interspecies hybrids between Pere David's deer (*Elaphurus davidianus*) and red deer (*Cervus elaphus*). *Genetics* **139**, 1383-1391.
- Tate, M. L., H. C. Mathias, D. F. Hill and P. F. Fennessy, (1994) Development and applications of a genetic linkage map of deer. In: *Proceedings of the Third International Deer Congress*, edited by J. A. Milne, Edinburgh.
- Tautz, D., (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17**, 6463-6471.
- Thieven, U., S. Solinas-Toldo, R. Friedl, J. Masabanda, R. Fries *et al.*, (1997) Polymorphic CA microsatellites for the integration of the bovine genetic and physical map. *Mammalian Genome* **8**, 52-55.
- Thompson, E. A., (1975) The estimation of pairwise relationships. *Annals of Human Genetics* **39**, 173-188.
- Trivers, R. L., and D. E. Willard, (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**, 90-92.
- Vaiman, D., M. Imam-Gahli, K. Moazami-Goudarzi, G. Guerin, C. Grohs *et al.*, (1994a) Conservation of a syntenic group of microsatellite loci between cattle and sheep. *Mammalian Genome* **5**, 310-314.

- Vaiman, D., D. Mercier, A. Eggen, I. Bahri-Darwich, C. Grohs *et al.*, (1994b) A genetic and physical map of bovine chromosome-11. *Mammalian Genome* **5**, 553-556.
- Vaiman, D., D. Mercier, K. Moazami-Goudarzi, A. Eggen, R. Ciampolini *et al.*, (1994c) A set of 99 cattle microsatellites- characterization, syntenic mapping and polymorphism. *Mammalian Genome* **5**, 288-297.
- Vaiman, D., R. Osta, D. Mercier, C. Grohs and H. Leveziel, (1992) Characterization of 5 new bovine dinucleotide repeats. *Animal Genetics* **23**, 537-541.
- Valdes, A. M., M. Slatkin and N. B. Freimer, (1993) Allele frequencies at microsatellite loci: the stepwise model revisited. *Genetics* **133**, 737-749.
- van der Beek, S., J. A. M. van Arendonk and A. F. Groen, (1995) Power of two- and three-generation QTL mapping experiments in an outbred population containing full-sib or half-sib families. *Theoretical and Applied Genetics* **91**, 1115-1124.
- Velmalä, R. J., H. J. Vilkkilä, K. T. Elo, H. J. de Koning and A. V. Mäki-Tanila, (1999) A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. *Animal Genetics* **30**, 136-143.
- Visscher, P. M., C. S. Haley, S. C. Heath, W. J. Muir and D. H. R. Blackwood, (1999) Detecting QTLs for uni- and bipolar disorder using a variance component method. *Psychiatric Genetics* **9**, 75-84.
- Visscher, P. M., R. Thompson, and C. S. Haley, (1996) confidence intervals in QTL mapping by bootstrapping. *Genetics* **143**, 1013-1020.
- Walling, G. A., A. L. Archibald, J. A. Cattermole, A. C. Downing, H. A. Finlayson *et al.*, (1998) Mapping of quantitative trait loci on porcine chromosome 4. *Animal Genetics* **29**, 415-424.
- Watt, W. B., (1977) Adaption at specific loci. 1. Natural selection in phosphoglucose isomerase of *Colias* butterflies. *Genetics* **87**, 177-194.
- Weber, J. L., and P. E. May, (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* **44**, 388-396.

- Weller, J. I., Y. Kashi and M. Soller, (1990) Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *Journal of Dairy Science* **73**, 2525-2537.
- Whitehead, G. K., 1993 *The Whitehead Encyclopedia of Deer*. Swan Hill, Shrewsbury, UK.
- Whitlock, M., (1993) Lack of correlation between heterozygosity and fitness in forked fungus beetles. *Heredity* **70**, 574-581.
- Wijmsman, E. M., and C. I. Amos, (1997) Genetic analysis of simulated oligogenic traits in nuclear and extended pedigrees: summary of GAW10 contributions. *Genetic Epidemiology* **14**, 719-735.
- Williams, J. L., A. L. G. Morgan, G. Guerin and B. G. D. Urquhart, (1996) Ten new cosmid-derived bovine microsatellite markers. *Animal Genetics* **27**, 380.
- Williams, J. L., B. G. Urquhart and B. Barendse, (1995) 3 bovine chromosome -15 microsatellite markers. *Animal Genetics* **26**, 124.
- Williams, J. T., B. Begleiter, B. Porjesz, H. J. Edenberg, T. Foroud *et al.*, (1999) Joint Multipoint Linkage Analysis of Multivariate Qualitative and Quantitative Traits. II. Alcoholism and Event-Related Potentials. *American Journal of Human Genetics* **65**, 1148-1160.
- Williams, J. T., R. Duggirala and J. Blangero, (1997) Statistical properties of a variance components method for quantitative trait linkage analysis in nuclear families and extended pedigrees. *Genetic Epidemiology* **14**, 1065-1070.
- Wilson, G. A., C. Strobeck, L. Wu and J. W. Coffin, (1997) Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology* **6**, 697-699.
- Woodruff, D. S., and O. A. Ryder, (1986) Genetic characterization and conservation of endangered species: Arabian Oryx and Pre David's deer. *Isozyme Bulletin* **19**, 33.
- Youngson, R., (1998) The distribution, status and management of red deer. In: *Population Ecology, Management and Welfare of Deer*, edited by E. R. Goldspink, S. King, And R. J. Putman. Manchester Metropolitan University, Manchester.

- Zhang, Q., D. Boichard, I. Hoeschele, C. Ernst, A. Eggen *et al.*, (1998) Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. *Genetics* **149**, 1959-1973.
- Zhang, Y., L. Redaelli, B. Castiglioni, A. Mezzelani and L. Ferretti, (1995) Five polymorphic bovine microsatellite loci (IDVGA62-A, IDVGA71, IDVGA82, IDVGA88, IDVGA90). *Animal Genetics* **26**, 365-366.

Appendix A: ecological and environmental terms used in this thesis

Listed below are response and explanatory variables modelled or discussed in this thesis. Terms are distributed either as continuous variables or as categorical factors, as stated.

Response Variables (usually fitness components or fitness related traits):

Birth weight: continuous. Estimated by regressing the weight at capture (measured in kg) on the age at capture (estimated number of hours old) provided calf was weighed at 14 days or younger. It was assumed that a calf gained 0.0148 kg h^{-1} , as measured by Guinness *et al.* (1978).

Fecundity: continuous. The proportion of years during her breeding lifespan that a female produces a calf. Only females aged 6 or greater are included so that at least 3 breeding attempts are incorporated.

First winter survival: factor. Whether or not a calf survived from October 1st in its year of birth to May 1st the following year.

Jaw length: continuous. Jaws are removed after death. Length in mm of the dried and measured the bone from the outer point of the fourth incisor socket to the posterior edge (See Kruuk *et al.*, in press)

Juvenile survival: factor. Calf survival from birth until May 1st of its second year.

Note that some earlier studies analysed juvenile survival to one year (e.g. Coulson *et al.* 1999). Where appropriate this distinction is made in the main text.

Leg length: continuous. Hind legs are removed after death. The length in mm of the metatarsus is measured once dried (see Kruuk *et al.*, in press).

Lifetime breeding success (LBS): continuous. The total number of offspring born to a hind or sired by a stag. Calves remain with their mother for at least a year and so maternity is awarded using behavioural data. Genetic data has confirmed this method is always correct (Marshall, 1998). Paternity is awarded by one of the following methods:

- i) Assigned with at least 80% statistical confidence using microsatellite markers and the computer program CERVUS (see Chapter 2).
- ii) Multilocus minisatellite DNA fingerprinting (see Chapter 2; Pemberton *et al.*, 1992).
- iii) The observation that a hind is in oestrus while in the male's harem (see Chapter 2; Kruuk *et al.*, in press).
- iv) The male held the hind in his harem for at least 6 days during the 11-day window (see Chapter 2; Kruuk *et al.*, in press).

Lifetime reproductive success (LRS): continuous. The total number of offspring born to a male or female that survived to 2 years old. See lifetime breeding success for guide to maternity / paternity assignment. In other words a generation is counted as being from adults aged two to their offspring surviving to age two (see Kruuk *et al.* 1999b)

Longevity: continuous. The age at which an individual died. Adult longevity only includes animals that survived to at least age 3.

Maximum annual breeding success (ABS): continuous. The number of offspring sired in a stag's most successful year. See lifetime breeding success (LBS) for criteria used to infer paternity.

Neonatal survival: factor. Whether or not a calf survived from birth until October 1st in the year of birth.

Environmental explanatory variables:

Density: continuous. The number of females over one year old using the study area.

This measure is highly correlated with measures that include males and calves. Density is estimated from the regular censuses (five per month) undertaken in the study area. An animal is considered resident in the study area if seen in at least 10% of censuses in at least four months per year (Clutton-Brock *et al.* 1982)

Local population density: continuous. A measure of the mean spatial proximity of other individuals to a focal individual, developed by Coulson *et al.* (1997). Not used in this thesis but referred to in Chapter 2.

Rainfall: continuous. Precipitation (in mm) for each month. Models were fitted using each month individually or sums of 2,3,4 etc consecutive month periods.

Subdivision of study area: factor. That part of the study area in which an animal was usually resident. The five areas (Shamhnán Inshir, Lower Kilmory Glen, Upper Kilmory Glen, Intermediate Area and Laundry Greens) are shown in Figure 2.2.

Temperature: continuous. Mean (in °C) for each month. Models were fitted containing each month individually or means of 2,3,4 etc month periods.

Phenotypic explanatory variables:

Birth date: continuous. The number of days after May 1st that a calf was born.

Mother's age: continuous. Measured in years. Fitted either as a linear or a quadratic term.

Mother's reproductive status: factor.

Naive – never previously bred.

True yield – did not breed in previous year.

Summer yield – bred in previous year, but calf died before October 1st.

Winter yield – as above, but calf died in winter.

Milk – calf successfully reared the previous year.

Genetic explanatory variables

Maxi descendant: factor. Whether or not an animal's ancestry can be traced back to MAXI. Paternal links are inferred by either DNA fingerprinting (see Pemberton *et al.*, 1992) or likelihood based analysis (CERVUS) using microsatellite markers (see Marshall, 1998).

Sex: Factor. Female or male.

Multilocus heterozygosity (MLH): Continuous. The proportion of typed loci (microsatellite and allozyme) for which an individual is heterozygous.

Standardised multilocus heterozygosity: Continuous. The ratio of the heterozygosity of an individual to the mean heterozygosity at those loci at which an individual is typed. This measure avoids any potential bias due to individuals being typed at different loci (see Coltman *et al.*, 1999b).

Mean d^2 : Continuous. A recently derived measure calculated from microsatellite data. The squared difference in repeat units between the two alleles at a locus, averaged over all loci at which an individual is scored (see Coulson *et al.* 1998; Pemberton *et al.* 1999).

$$\text{Mean } d^2 = \sum_{i=1}^n \frac{(i_a - i_b)^2}{n}$$

where i_a and i_b are the length in repeat units of alleles a and b at locus i and n is the number of typed loci.

Appendix B: laboratory methods

DNA extraction

DNA was extracted from skin (usually an ear punch) or white blood cells using standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation (see Sambrook *et al.* 1989)

A 2mm × 2mm piece of skin (or less than 0.1 ml volume of white blood) was transferred to a 1.5 ml eppendorf tube containing 0.55 ml of digestion solution and proteinase K. Following dicing with a scalpel blade, the sample was transferred to a 65°C water bath for 10-15 mins and then a 37°C water bath overnight. The next day 0.55ml of phenol was added, the tube was mixed vigorously, and then centrifuged at 13,000 rpm for 12 seconds. Then 0.5 ml of the aqueous phase was transferred to another 1.5ml eppendorf, to which 0.5ml of chloroform was added. Again the tube was mixed and spun at 13,000 rpm for 12 seconds. 0.45 ml of the aqueous phase was transferred to a new eppendorf tube, and 0.15 ml of 10M LiCl was added. After gentle mixing, the tube was moved to a -20°C freezer for 20 minutes. Subsequently the tube was removed from the freezer and spun at 13,000 rpm for five minutes. Next 0.5ml of the supernatant was transferred to another new eppendorf tube, and 1.0 ml of 100% EtOH was added to precipitate the DNA. If no DNA was visible at this stage the sample was returned to the freezer for an hour. Next the tube was spun for five minutes, the EtOH was removed and 1ml of 70% EtOH was added to the remaining pellet. After standing for 20 minutes at room temperature the sample was again spun at 13,000 rpm for five minutes, the 70% EtOH was removed and the DNA pellet dried using a vacuum drier. Once dried 0.25 - 1ml of TE buffer was added to dissolve the pellet, and the DNA sample was stored at -20°C.

DNA concentration was determined by running 5 µl of sample in a 0.8% agarose gel in TBE buffer at 100V for one hour. Ficoll / bromophenol blue was used as a loading buffer, and bands were visualised by staining with ethidium bromide (Sambrook *et al.* 1989). DNA was typically diluted 10 fold with TE buffer prior to PCR amplification.

PCR amplification

Both the MAXI pedigree (4 plates) and the International Mapping Herd (1 plate) were stored in Beckman 96 deep well storage plates. Initially the plates were covered in parafilm and stored at 4°C, but evaporation proved to be a problem, and so plates were subsequently stored at -20°C. Approximately 50 µg of genomic DNA was used as template DNA in a 10 µl reaction mix containing 0.1 mM dATP, dGTP and dTTP; 0.01 mM dCTP, 2 µmol each primer, 1× 'PARR' buffer containing 1.5mM MgCl₂ (Cambio); 0.25 U Taq polymerase (Advanced Biotechnologies) and <1 µCi [α -32P] dCTP. Microsatellite amplification was optimised (see Appendix C for optimised conditions) by adjusting the concentration of MgCl₂ (total MgCl₂ 1.5 –3.0 mM) and by the addition of DMSO (0-10%) and/or TMACIDE (60 mM tetramethylammonium chloride / 2.5% formamide), see Gemmell (1997)).

Reactions were overlaid with 1 drop of mineral oil and amplified in a Hybaid Omnigene Temperature Cycler. PCR was performed as follows:

2 minute denaturation step at 94 °C

7× 30 s at 94 °C denaturation
 1 minute at 50 °C annealing
 30 s at 72 °C extension

30× 30 s at 94 °C denaturation
 1 minute at 52 °C annealing
 30 s at 72 °C extension.

After PCR was completed 5µl of loading buffer (95% formamide, 20mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to the product (Schlötterer 1998). 2µl of the product/dye mixture was loaded on standard 6% polyacrylamide sequencing gels using a multichannel pipette and 96-well shark's tooth combs, and electrophoresed for approximately two hours. Some loci were double-loaded, with the second set of samples loaded 15 minutes after the first. The

gel was dried under a vacuum drier and exposed to X-ray film for between 1 hour and 1 week. A ^{35}S -labelled M13 sequencing reaction was also loaded on each gel as a size marker.

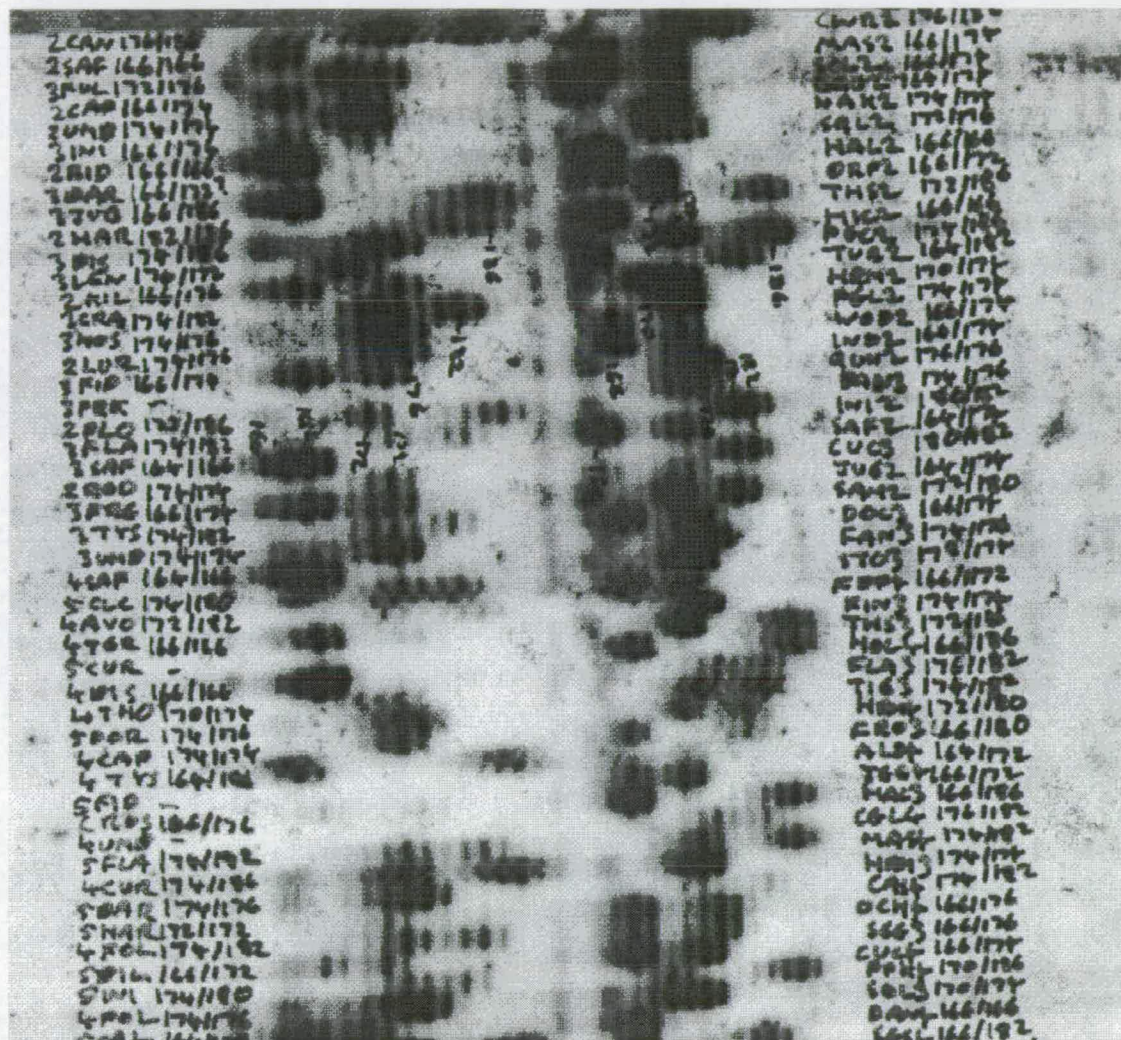


Figure B1: Autoradiogram of locus TGLA127. The gel was double-loaded with plates 2 and 4 of the MAXI pedigree. Animal IDs and genotypes are marked on the autorad prior to data entry in a Microsoft Access (Microsoft Corporation) database.

Appendix C

Details of the conservation of bovine microsatellite primers donated by Dr John Williams (Roslin Institute).

See Chapter 4 for details. Unless indicated otherwise a MgCl₂ concentration of 2.5 mM was used.

– denotes that no product was observed in the test species.

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
BM3205	1	204-221	3	1	1			Bishop <i>et al.</i> 1994
TGLA130	1	126-201	–	3	1	10% +TMAC	2.0mM	Georges and Massey 1992
TGLA415	1	106-126	1	1	1			Georges and Massey 1992
TGLA213	1	500	–	–	–			Georges and Massey 1992
TGLA49	1	120	–	–	–			Georges and Massey 1992
TGLA135	1	110	1	–	–			Georges and Massey 1992
TGLA57	1	80-104	–	1	1			Georges and Massey 1992
INRA128	1	182	3	2	4			Vaiman <i>et al.</i> 1994c
CSSM42	2	165-222	–	1	1			Moore <i>et al.</i> 1994
IDVGA37	2	210	2	7	3		2.0mM	Mezzelani <i>et al.</i> 1995
BM4440	2	121-147	1	4	2			Bishop <i>et al.</i> 1994
IDVGA2	2	140	1	–	–			Mezzelani <i>et al.</i> 1995
INRA40 (1)	2		Not tested	3	Not tested	5%	2.0mM	Additional locus amplified in red deer
INRA40 (2)	2	205-230	1	8	5	5%	2.0mM	Vaiman <i>et al.</i> 1994c
TGLA431	2	134-163	–	7	3		3.0mM	Georges and Massey 1992
TGLA226	2	145-151	–	6	2	10%	2.0mM	Barendse <i>et al.</i> 1994
INRA23	3	197-223	3	1	1			Vaiman <i>et al.</i> 1994a
INRA6	3	105	3	2	1		2.0mM	Vaiman <i>et al.</i> 1992
HUJ1177	3	210	–	7	–		2.0mM	Shalom <i>et al.</i> 1994
IDVGA35	3	230	1	–	–	10% + TMAC	2.0mM	Mezzelani <i>et al.</i> 1995
TGLA127	3	170	–	9	2	5%	2.0mM	Georges and Massey 1992

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
IDVGA27	3	136-140	1	2	1			Mezzelani <i>et al.</i> 1995
TGLA263	3	100-136	7	—	—			Georges and Massey 1992
IOBT250	3	105-117	1	1	1			Barendse <i>et al.</i> 1997
RM188	4	124-150	1	6	1	10% + TMAC	2.0mM	Barendse <i>et al.</i> 1994
INRA37	4	130	1	1	1	5%	2.0mM	Vaiman <i>et al.</i> 1994c
HEL25	4	139-149	—	—	—			Barendse <i>et al.</i> 1997
MGTG4B	4	134-164	2	6	1	5%	2.0mM	Barendse <i>et al.</i> 1997
AGLA293	5	196-260	2	3	1		2.0mM	Georges and Massey 1992
BM6026	5	148-168	1	2	1			Bishop <i>et al.</i> 1994
ETH152	5	196-204	1	1	1			Steffen <i>et al.</i> 1993
BM1819	5	114-128	1	1	1			Bishop <i>et al.</i> 1994
BM315	5	107-163	1	—	—			Bishop <i>et al.</i> 1994
BM2830	5	149-203	1	1	1			Bishop <i>et al.</i> 1994
IDVGA9	5	200	1	1	1			Mezzelani <i>et al.</i> 1995
ETH10	5	210-226	1	1	1			Solinas-Toldo <i>et al.</i> 1993
BM2320	6	128-152	—	6	2			Bishop <i>et al.</i> 1994
BM4528	6	238-276	—	1	1			Bishop <i>et al.</i> 1994
ILSTS90	6	150-190	—	—	—			Kemp <i>et al.</i> 1995
BM1329	6	145-161	1	6	1	10% + TMAC	2.5mM	Bishop <i>et al.</i> 1994
ILSTS93	6	160	3	5	2	10% + TMAC	3.0mM	Kemp <i>et al.</i> 1995
ILSTS97	6	225	1	1	1			Kemp <i>et al.</i> 1995
RM28	6	96-110	1	1	1			Barendse <i>et al.</i> 1994
AFR227	6	113	4	6	1	10% + TMAC	1.5mM	Jorgenson <i>et al.</i> 1995
BM741	7	172-184	1	1	1			Bishop <i>et al.</i> 1994
ILSTS6	7	283-295	—	9	2	10% + TMAC	3.0mM	Brezinsky <i>et al.</i> 1993
BP41	7	312-326	—	—	—			Bishop <i>et al.</i> 1994
INRA53	7	100	1	—	—			Vaiman <i>et al.</i> 1994c

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
CSSM29	7	230	1	2	5	10% + TMAC	3.0mM	Moore <i>et al.</i> 1994
RM12	7	107-111	1	8	1	10% + TMAC	2.0mM	Barendse <i>et al.</i> 1994
IDVGA11	8	230	1	1	1			Mezzelani <i>et al.</i> 1995
HEL9	8	143-165	1	1	1			Kaukinen and Varvio 1993
BM4006	8	97-119	1	3	1	10% + TMAC	2.0mM	Bishop <i>et al.</i> 1994
CSSM47	8	141-171	2	1	1			Moore <i>et al.</i> 1994
TGLA10	8	150	3	6	3	10% + TMAC	3.0mM	Georges and Massey 1992
UWCA47	8		3	1	1			Barendse <i>et al.</i> 1997
CSSM37	8	172	2	1	1			Moore <i>et al.</i> 1994
TGLA13	8	183-209	5	—	1			Georges and Massey 1992
CSSM56	9	230-240	1	—	—			Moore <i>et al.</i> 1994
UWCA9	9	100	1	—	—			Sun <i>et al.</i> 1994
BM757	9	182-220	3	7	2	10% + TMAC	2.0mM	Bishop <i>et al.</i> 1994
BM2504	9	130-146	3	1	1			Bishop <i>et al.</i> 1994
TGLA73	9	110-128	3	1	1			Georges and Massey 1992
SPS113	10	133-165	1	8	2	10% + TMAC	3.0mM	
CSSM38	10	180	—	—	—			Moore <i>et al.</i> 1994
BM888	10	173-183	1	11	1	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
CSSM39	10	195-208	1	5	2	10% + TMAC	3.0mM	Moore <i>et al.</i> 1994
JAB10	10	276?-288	1	1	2			Williams <i>et al.</i> 1996
TGLA378	10	100-120	6	6	4	10% + TMAC	3.0mM	Georges and Massey 1992
INRA107	10	166	2	10	2	10% + TMAC	3.0mM	Vaiman <i>et al.</i> 1994a
CSSM46	10	152	—	—	—			Moore <i>et al.</i> 1994
HEL13	11	177-197	1	—	—			Kaukinen and Varvio 1993
IDVGA3	11	150	—	4	1		2.0mM	Mezzelani <i>et al.</i> 1995
BM716	11	114-155	2	1	2			Bishop <i>et al.</i> 1994
INRA131	11	125	3	3	1		2.5mM	Vaiman <i>et al.</i> 1994c

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
JAB7	11	360	2	1	1		2.5mM	Williams <i>et al.</i> 1996
INRA169	11		1	5	1		2.5mM	Vaiman <i>et al.</i> 1994b
BM6108	12	116-144	2	1	1			Bishop <i>et al.</i> 1994
TGLA36	12	77-107	—	1	1			Georges and Massey 1992
RM178	12		—	3	1	10% + TMAC	3.0mM	Barendse <i>et al.</i> 1994
BM6404	12	127-149	3	1	1			Bishop <i>et al.</i> 1994
INRA5	12	119-123	5	1	1	10% + TMAC	3.0mM	Vaiman <i>et al.</i> 1992
INRA209	12	140-150	1	2	1			Bahri-Darwich <i>et al.</i> 1994
RM162	12	121-139	1	—	—			Kossarek <i>et al.</i> 1996
DIK16	12		2	1	1			Inoue <i>et al.</i> 1995
HUJ616	13	140	4	1	1	10% + TMAC	2.0mM	Shalom <i>et al.</i> 1993
JAB3	13	226-230	1	—	—			Williams <i>et al.</i> 1996
BM1222	13	272-303	—	5	1	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
ETH7	13	270	1	—	—			Solinas-Toldo <i>et al.</i> 1993
TGLA23	13	92-116	2	—	—			Georges and Massey 1992
L6003	13		1	—	—			
TGLA131	13	150	Not tested	Not tested	Not tested			Georges and Massey 1992
ABS10	13		1	5	1			Pfister-Genskow <i>et al.</i> 1995
CSSM36	14	163-185	1	1	1			Moore <i>et al.</i> 1994
BM4513	14	141-161	1	12	3	10% + TMAC	2.0mM	Bishop <i>et al.</i> 1994
BM4630	14	151-165	3	—	—			Bishop <i>et al.</i> 1994
BM6425	14	172-192	1	1	1			Bishop <i>et al.</i> 1994
INRA94	14	157	1	—	—			Vaiman <i>et al.</i> 1994c
BM2934	14	81-103	1	6	4	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
BR3510	15	90-114	3	5	1	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
JAB8	15	220	3	5	1	10% + TMAC	3.0mM	Williams <i>et al.</i> 1995
JAB1	15	224-234	2	1	1	10% + TMAC	3.0mM	Williams <i>et al.</i> 1995

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
JAB4	15	187-193	1	1	1		2.5mM	Williams <i>et al.</i> 1995
IDVGA10	15	190	3	1	1			Mezzelani <i>et al.</i> 1995
FSHB	15	200	2	6	2	10% + TMAC	3.0mM	Moore <i>et al.</i> 1992
SPS115	15		3	3	2		2.0mM	
BM6430	16	105-164	—	—	—			Bishop <i>et al.</i> 1994
BM1706	16	233-259	—	5	1		2.5 mM	Bishop <i>et al.</i> 1994
BR6504	16	125-145	—	1	1			Bishop <i>et al.</i> 1994
ETH11	16	214	—	1	1			Solinas-Toldo <i>et al.</i> 1993
BM719	16	147-155	3	—	—			Bishop <i>et al.</i> 1994
BM1862	17	191-224	2	—	—			Bishop <i>et al.</i> 1994
OarVH98	17	133-161	3	6	2	10% + TMAC	3.0mM	Hanrahan <i>et al.</i> 1993
CSSM33	17	179	1	2	1		2.0mM	Moore <i>et al.</i> 1994
INRA25	17	220	—	1	1			Vaiman <i>et al.</i> 1994c
IDVGA40	17	240	1	1	1			Mezzelani <i>et al.</i> 1995
INRA110	17	213	1	—	—			Vaiman <i>et al.</i> 1994c
INRA38	18	115	2	2	1			Vaiman <i>et al.</i> 1994c
IDVGA31	18	210-220	—	—	—			Mezzelani <i>et al.</i> 1995
INRA121	18	128-152	1	15	2	10% + TMAC	3.0mM	Vaiman <i>et al.</i> 1994c
IDVGA55	18	195	1	7	4		2.5mM	Mezzelani <i>et al.</i> 1995
BP20	19	219-233	1	1	1			Bishop <i>et al.</i> 1994
HEL10	19		3	2	Not tested			Kaukinen and Varvio 1993
IDVGA44	19		1	1	1			Mezzelani <i>et al.</i> 1995
IDVGA46	19	190	3	4	2	10% + TMAC	3.0mM	Mezzelani <i>et al.</i> 1995
AFL361	19		—	—	—			Barendse <i>et al.</i> 1997
BM713	20	100-112	1	—	—			Bishop <i>et al.</i> 1994
BM3517	20	104-124	—	1	1			Bishop <i>et al.</i> 1994
TGLA304	20	86-100	2	3	2		2.5mM	Georges and Massey 1992

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
TGLA126	20	109-127	2	1	2			Georges and Massey 1992
BM5004	20	120-154	1	5	4	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
AFR2215	20	96	1	—	—			Jorgenson <i>et al.</i> 1995
BM1225	20	227-253	1	6	2	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
TGLA337	21		1	6	2		2.5mM	Georges and Massey 1992
IDVGA39	21	185-196	1	4	1	10% + TMAC	3.0mM	Mezzelani <i>et al.</i> 1995
CSSM26	22	240-250	1	3	2		2.5mM	Moore <i>et al.</i> 1994
HRH1	22	133-141	1	—	—			Bishop <i>et al.</i> 1994
CSSM41	22	130-140	3	6	4	10% + TMAC	3.0mM	Moore <i>et al.</i> 1994
BM3628	22	243-263?	1	2	2	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
HUJ175	22	130-160?	3	5	3		2.0mM	Shalom <i>et al.</i> 1994
INRA130	22	100	3	1	1			Vaiman <i>et al.</i> 1994c
BM47	23	94-126	1	—	—			Bishop <i>et al.</i> 1994
UWCA1	23	102-130	1		—			Sun <i>et al.</i> 1993
BM1905	23	170-199	2	1	1			Bishop <i>et al.</i> 1994
DRB	23	121-137	Not tested	Not tested	Not tested			
IOBT528	23	182	—	—	Not tested			Barendse <i>et al.</i> 1997
BM226	24	128-164	—	1	—			Bishop <i>et al.</i> 1994
CSSM23	24	214	1	—	—			Moore <i>et al.</i> 1994
ILSTS101	24	171	1	1	1			Kemp <i>et al.</i> 1995
INRA90	24	168	1	2	2			Vaiman <i>et al.</i> 1994c
TGLA86	25	128-140?	2	6	3	10% + TMAC	3.0mM	Georges and Massey 1992
RM44	25	100	—	—	—			Kossarek <i>et al.</i> 1995
JAB5	25		—	—	—			Williams <i>et al.</i> 1996
HEL11	26	181-193	—	—	—			Kaukinen and Varvio 1993
BM4505	26	204-248	—	1	1			Bishop <i>et al.</i> 1994
BM804	26	144-152	—	—	—			Bishop <i>et al.</i> 1994

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc	Reference
ABS12	26		—	9	4		2.0mM	Pfister-Genskow <i>et al.</i> 1995
HAUT27	26		—	—	—			Thieven <i>et al.</i> 1997
BM203	27	203-244?	6	8	3		2.0mM	Bishop <i>et al.</i> 1994
BM3507	27	159-187	—	—	—			Bishop <i>et al.</i> 1994
RM209	27	115-145	1	1	1	10% + TMAC	3.0mM	Kossarek <i>et al.</i> 1994
HUJ113	27		—	1	1			Shalom <i>et al.</i> 1995
CSSM43	27	253-261	3	11	4	10% + TMAC	3.0mM	Moore <i>et al.</i> 1994
BP23	28	272-288	—	3	1	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
IDVGA29	28	144-154	1	2	3		2.0mM	Mezzelani <i>et al.</i> 1995
IDVGA8	28	212-226	—	4	2	10% + TMAC	3.0mM	Mezzelani <i>et al.</i> 1995
RBP3	28	132-144	3	7	2		2.5mM	Fries <i>et al.</i> 1993
IDVGA43	28	170-184	3	—	—			Mezzelani <i>et al.</i> 1995
AF4 (L6068)	29		3	1	1			Konfortov <i>et al.</i> 1996
BM4005	29	108-122	1	2	3	10% + TMAC	3.0mM	Bishop <i>et al.</i> 1994
AF5	29	130-164	—	6	3	10% + TMAC	3.0mM	Konfortov <i>et al.</i> 1996
IDVGA71	29	192-201?	1	1	1			Zhang <i>et al.</i> 1995
TGLA40	29	100-118?	1	4	2	10% + TMAC	3.0mM	Georges and Massey 1992
BM6017	X	112-139	—	—	—			Bishop <i>et al.</i> 1994
INRA30	X	156-166	—	1	1			Vaiman <i>et al.</i> 1994c
HEL26	X	180-193?	—	1	1			Barendse <i>et al.</i> 1997

Appendix D

Conservation of microsatellite markers other than those donated by John Williams (Roslin Institute).

Listed are markers for which primers were obtained from sources other than John Williams, and were not screened in the 'MAXI mapping panel' (Chapter 4). Non-Williams markers that were screened in the MAXI pedigree are described in Table 4.3. Some of the markers listed here were screened in the International Mapping Herd (Chapter 6).

<i>Locus</i>	<i>Source Species</i>	<i>Product in red deer</i>	<i>Alleles in red deer</i>
918	Cattle	✓	3+
BL41	Cattle	✓	1
BM103	Cattle	✓	2
BM1233	Cattle	✗	-
BM1314	Cattle	✓	1
BM1824	Sheep	✓	1
BM2113	Cattle	✓	1
BM305	Cattle	✓	2
BM3413	Cattle	?	-
BM4440	Cattle		
BM6438	Cattle	✓	2
BM6506	Cattle	✓	2
BM8125	Cattle	✗	-
BM864	Cattle	✓	1
BM9202	Cattle	✓	1
BMC1013	Cattle	✓	1
BMS1617	Cattle	?	-
BOVGAPR	Cattle	✓	1
BP20	Cattle	✓	2
BR2936	Cattle	✗	-
BT μ S	Cattle	✓	2
Cer14	Red Deer ?	✓	2
CSSM14	Cattle	✓	2
CSSM18	Cattle	✓	2
CSSM22	Cattle	✓	1
CSSM26	Cattle	✓	1
CSSM32	Cattle	✓	1
CSSM65	Cattle	✓	2
DB6	Sheep	✓	1
DRB3	Sheep	✓	7+
DRBP1	Sheep	✗	-
DRBP2	Sheep	✓	2
ETH131	Cattle	✗	-
ETH185	Cattle	✓	1
ETH3	Cattle	✓	1
Haut14	Cattle	✗	-
HBB	Cattle	✗	-
HEL1	Cattle	✓	2
HEL10	Cattle	✓	1
HEL5	Cattle	✓	2
HUJ223	Cattle	✓	1
HUJ616	Cattle	✓	1
ILSTS1	Cattle	✗	-

Locus	Source Species	Product in red deer	Alleles in red deer
ILSTS104	Cattle	✓	2
ILSTS13	Cattle	✓	1
ILSTS14	Cattle	✓	1
ILSTS2	Cattle	✓	1
ILSTS28	Cattle	✓	2
ILSTS30	Cattle	✓	2
ILSTS4	Cattle	✓	1
ILSTS44	Cattle	✓	1
ILSTS55	Cattle	✓	1
ILSTS56	Cattle	✗	-
ILSTS58	Cattle	✓	2
JP1	Red Deer	✗	-
JP14	Red Deer	✓	2
JP18	Red Deer	✓	2+
JP30	Red Deer	✗	-
JP31	Red Deer	✗	-
JP6	Red Deer	✓	1
MB087	Cattle	✓	5
(=MAP2C)			
McM210	Sheep	✗	-
McM357	Sheep	✓	1
McM42	Sheep	?	-
OarAE119	Sheep	✓	1
OarCP134	Sheep	✓	1
OarCP16	Sheep	✓	2
OarCP21	Sheep	?	-
OarCP43	Sheep	✗	-
OarCP49	Sheep	✓	2
OarCP79	Sheep	✓	1
OarFCB11	Sheep	✓	1
OarFCB128	Sheep	✓	2+
OarFCB20	Sheep	✓	1
OarFCB266	Sheep	✓	1
OarFCB48	Sheep	✓	2
OarHH41	Sheep	✗	-
OarHH47	Sheep	✓	1
OarHH56	Sheep	✗	-
OarHH64	Sheep	✓	2+
OarMAF209	Sheep	✓	1
OarMAF214	Sheep	✓	1
OarMAF23	Sheep	✓	2
OarMAF36	Sheep	✓	4+
OarMAF4	Sheep	?	-
OarMAF45	Sheep	✓	1
OarMAF46	Sheep	✓	1
OarMAF50	Sheep	✓	7+
OarMAF64	Sheep	✓	1
OarMAF65	Sheep	✓	2 (+ null)
OarVH116	Sheep	✓	4
OarVH34	Sheep	✓	1
OarVH4	Sheep	✗	-
OarVH58	Sheep	✗	2
OCAM	Cattle	✓	3+
OIFNG	Sheep	✓	2+
OMHC	Sheep	?	-
PgazAC1	Gazelle	✓	2

<i>Locus</i>	<i>Source Species</i>	<i>Product in red deer</i>	<i>Alleles in red deer</i>
PgazAC3	Gazelle	?	-
PgazAC4	Gazelle	?	-
PgazAC6	Gazelle	✓	2
PgazAGC2	Gazelle	✓	2
RASA	Cattle	✓	1
RM106	Cattle	✓	1
RM178	Cattle	✓	3
RM4	Cattle	✓	2
RM65	Cattle	✗	-
RM96	Cattle	✓	1
RME25	Cattle	✓	1
RT1	Caribou	✓	6
RT10	Caribou	✓	2
RT13	Caribou	✓	4+
RT20	Caribou	✓	1
RT23	Caribou	✓	1
RT24	Caribou	✓	1
RT25	Caribou	✓	6
RT27	Caribou	✓	2
RT30	Caribou	✓	2
RT7	Caribou	✓	1
RT9	Caribou	✓	1
Sμ0	Sheep	✗	-
Sμ2	Sheep	✓	2
Sμ3	Sheep	✓	1
Sμ6	Sheep	✗	-
SμS	Sheep	✓	1
SKFG	Sheep	?	-
SMHCC	Sheep	✓	6 +
TGLA116	Cattle	✓	1
TGLA122	Cattle	✓	1
TGLA131	Cattle	✓	1
TGLA170	Cattle	✓	1
TGLA272	Cattle	✓	1
TGLA51	Cattle	✗	-
TGLA53	Cattle	✗	-
UWCA4	Cattle	✓	2

Appendix E

Detecting parent–offspring mismatches

Parent off-spring mismatches were detected by running structured query language (SQL) statements for each locus in Microsoft Access. Mother-offspring mismatches were relatively easy to detect, while detection of father-offspring mismatches, in which it is assumed that the mother was correctly genotyped, was more complicated.

SQL query to detect mother–offspring mismatches

A simple query was written to detect any mother–offspring pairs where the offspring had neither maternal allele. The example shown is for the locus IDVGA55.

```
( [micjon].[IDVGA55a] <> [micjon_1].[IDVGA55a] And
  [micjon].[IDVGA55a] <> [micjon_1].[IDVGA55b] ) And
( [micjon].[IDVGA55b] <> [micjon_1].[IDVGA55a] And
  [micjon].[IDVGA55b] <> [micjon_1].[IDVGA55b] )
```

SQL query to detect father–offspring mismatches

Father–offspring incompatibilities are possible in two different ways. The most obvious way is when neither of the father’s alleles are present in the offspring (the first part of the query). The second way is when father and mother share an allele which is also present in the offspring. However, the other offspring allele is present in neither father or mother. The second and third parts of the query detect this type of mismatch.

```
((([micjon].[IDVGA55a] <> [micjon_2].[IDVGA55a]
and [micjon].[IDVGA55a] <> [micjon_2].[IDVGA55b] ) And
  ( [micjon].[IDVGA55b] <> [micjon_2].[IDVGA55a]
and [micjon].[IDVGA55b] <> [micjon_2].[IDVGA55b] ))
OR
  (( [micjon].[IDVGA55a] = [micjon_1].[IDVGA55a] or
    [micjon].[IDVGA55a] = [micjon_1].[IDVGA55b] ) and
    ( [micjon].[IDVGA55a] = [micjon_2].[IDVGA55a] or
      [micjon].[IDVGA55a] = [micjon_2].[IDVGA55b] )
and ( [micjon].[IDVGA55b] <> [micjon_1].[IDVGA55a]
and [micjon].[IDVGA55b] <> [micjon_1].[IDVGA55b]
and [micjon].[IDVGA55b] <> [micjon_2].[IDVGA55a]
and [micjon].[IDVGA55b] <> [micjon_2].[IDVGA55b] ))
```

OR

```
(( [micjon].[IDVGA55b] = [micjon_1].[IDVGA55a] or
  [micjon].[IDVGA55b] = [micjon_1].[IDVGA55b] ) and
  ( [micjon].[IDVGA55b] = [micjon_2].[IDVGA55a] or
    [micjon].[IDVGA55b] = [micjon_2].[IDVGA55b] )
and ( [micjon].[IDVGA55a] <> [micjon_1].[IDVGA55a]
and [micjon].[IDVGA55a] <> [micjon_1].[IDVGA55b]
and [micjon].[IDVGA55a] <> [micjon_2].[IDVGA55a]
and [micjon].[IDVGA55a] <> [micjon_2].[IDVGA55b] )))
```

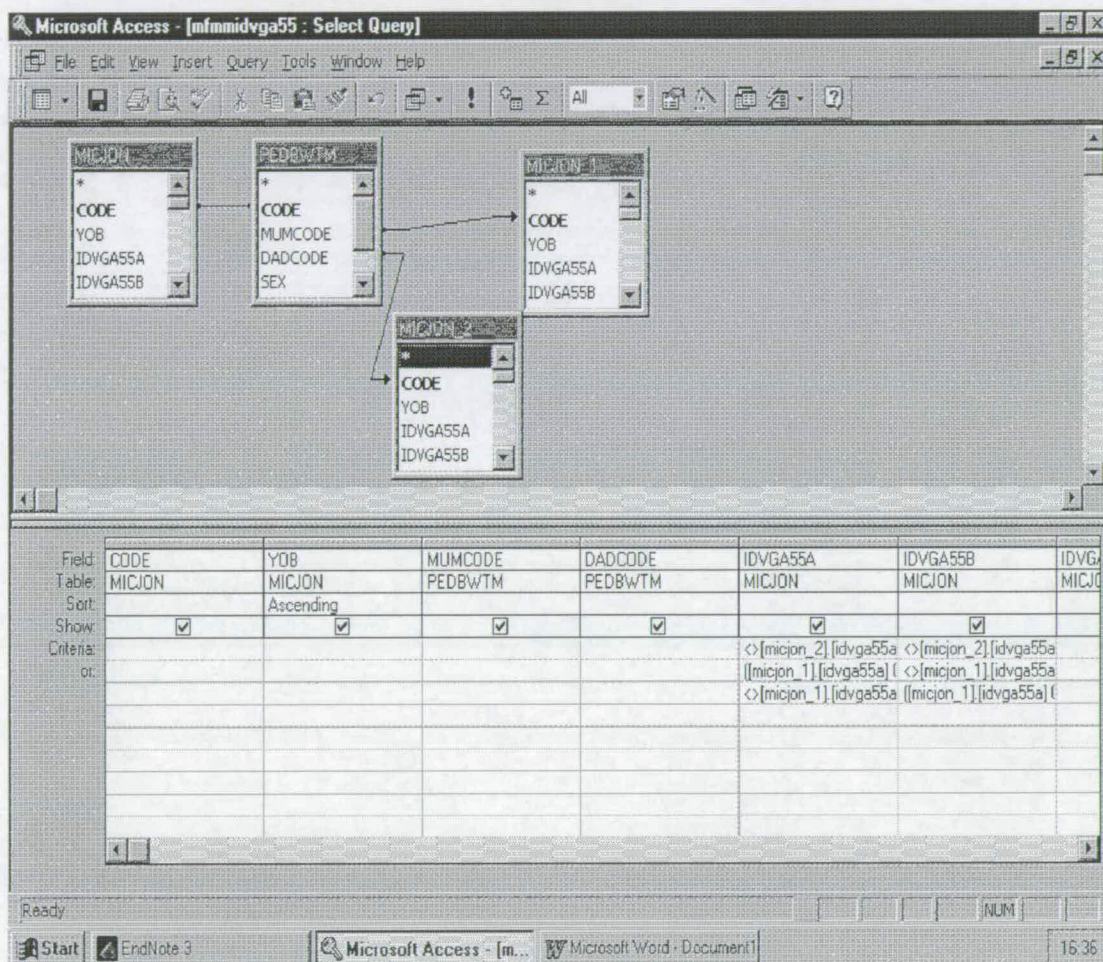


Figure E1: Diagram showing the relational database query for detecting father–offspring mismatches at the locus IDVGA55. Genetic data is stored in the table MICJON and parentage data in the table PEDBWTM. The tables are joined so that genotypes for calf, mother and CERVUS father are compared. When the query is run, only calves who have a genetic mismatch with the CERVUS father are displayed. The SQL command shown overleaf is required to detect these mismatching pairs.

Appendix F: Microsatellite markers screened in the deer International Mapping Herd (IMH) in this study

For each locus the number of alleles detected in red deer is included. It is assumed that Père David's deer are monomorphic unless stated otherwise in Chapter 4. Loci are mappable if one or both sires is heterozygous, or if the Père David's allele is a null allele. Mappable loci are indicated in the fourth column. Column 5 indicates those loci that have been designated a map location. A number of unassigned loci are likely to be mapped following further analysis using Animap. (The deer genetic map is viewable at <http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/>).

<i>Locus</i>	<i>No. alleles in red deer</i>	<i>Genotype of sires</i>	<i>Mappable ?</i>	<i>Currently on map ?</i>
965	3 +	Heterozygous	✓	✗
ABS12	9	Heterozygous	✓	✗
AFR227	6	Heterozygous	✓	✗
AGLA293	3	Heterozygous	✓	✗
BM1222	5	Heterozygous	✓	✗
BM1225	6 + null	Homozygous	✗	✗
BM1258	7	Heterozygous	✓	✓
BM1329	6	Heterozygous	✓	✗
BM1706	6	Heterozygous	✓	✗
BM1818	5	Heterozygous	✓	✓
BM203	8	Heterozygous	✓	✓
BM2934	6	Heterozygous	✓	✗
BM3628	3	Heterozygous	✓	✓
BM4005	2	Heterozygous	✓	✗
BM4006	3	Homozygous	✗	✗
BM4208	7	Heterozygous	✓	✗
BM4440	3 +	Heterozygous	Non- Mendelian	✗
BM4513	12 +null	Heterozygous		✗
BM5004	4	Homozygous	✗	✗
BM6438	4	Heterozygous	✓	✓
BM6506	6	Heterozygous	✓	✓
BM716	1	Homozygous	✓	✗
BM757	8	Heterozygous	✓	✗
BM888	11	Heterozygous	✓	✗
BR3510	5	Homozygous (PD null)	✓	✗

Locus	No. alleles in red deer	Genotype of sires	Mappable ?	Currently on map ?
CSRM60	11 +	Heterozygous	✓	✓
CSSM14	2	Heterozygous	✓	✓
CSSM16	3	Heterozygous	✓	✓
CSSM19	4	Heterozygous	✓	✓
CSSM22	3	Heterozygous	✓	✓
CSSM26	3	Heterozygous	✓	✗
CSSM29	2	Heterozygous	✓	✗
CSSM39	5	Heterozygous	✓	✗
CSSM41	6	Heterozygous	✓	✗
CSSM43	11	Heterozygous	✓	✓
CSSM65	3	Heterozygous	✓	✓
CSSM66	7	Heterozygous	✓	✓
DRB3	5	Heterozygous	✓	✗
DRBP2	2	Homozygous	✗	✗
ETH225	6	Heterozygous	✓	✓
HEL1	4	Heterozygous	✗	✗
HEL5	2	Homozygous	✗	✗
HIS-H1	2	Heterozygous	✓	✗
HUJ175	5	Heterozygous	✓	✗
HUJ177	7	Homozygous (+Null)	✓	✗
IDVGA37	7	Heterozygous	✓	✗
IDVGA46	4	Heterozygous	✓	✗
IDVGA55	7	Heterozygous	✓	✗
IDVGA8	4	Heterozygous	✓	✗
ILSTS12	5	Homozygous (PD null)	✓	✗
ILSTS14	1	Homozygous	✓	✗
ILSTS30	1	Heterozygous	✓	✓
ILSTS33	4	Heterozygous	✓	✓
ILSTS5	1	Heterozygous	✓	✓
ILSTS6	9	Heterozygous	✓	✗
ILSTS87	3	Heterozygous	✓	✓
ILSTS93	4	Heterozygous	✓	✗
INRA107	10	Heterozygous	✓	✗
INRA11	7 +	Heterozygous	✓	✓

Locus	No. alleles in red deer	Genotype of sires	Mappable ?	Currently on map ?
INRA121	15	Homozygous (PD null)	✓	✗
INRA130	1	Heterozygous	✓	✗
INRA131	3	Heterozygous	✓	✗
INRA169	5	Heterozygous	✓	✗
INRA35	7	Heterozygous	✓	✓
INRA37	1	Heterozygous	✓	✗
INRA38	3	Heterozygous	✓	✗
INRA40	9 + (or 2 loci?)	Heterozygous	✓	✗
INRA5	1	Heterozygous	✓	✗
INRA6	3	Heterozygous	✓	✗
JAB1	2	Heterozygous	✓	✗
JAB4	1	Heterozygous	✓	✗
JAB7	1	Heterozygous	✓	✗
JAB8	5	Heterozygous	✓	✗
JP14	3 +	Heterozygous	✓	✗
JP23	4 +	Homozygous (Null)	✓	✓
MAP2C	2	Homozygous	✗	✗
MGTG4B	6	Heterozygous	✓	✗
MM12	2	Heterozygous	✓	✓
OarCP134	1	Heterozygous	✓	✓
OarCP16	2	Homozygous	✗	✗
OarMAF18	9	Heterozygous	✓	✓
OarMAF46	1	Homozygous	✗	✗
OarMAF65	2 + null	Homozygous	✗	✗
OarVH54	6	Homozygous (PD null)	✓	✓
OarVH98	6	Heterozygous	✓	✗
PGazAC2	4	Heterozygous	✓	✗
PgazAGC2	2	Homozygous	✗	✗
PRL	3	Heterozygous	✓	✓
RBP3	7	Heterozygous	✓	✓
RM12	8	Heterozygous	✓	✗
RM178	3	Heterozygous	✓	✗
RM188	6	Heterozygous	✓	✗
RM4	2	Heterozygous	✓	✓

<i>Locus</i>	<i>No. alleles in red deer</i>	<i>Genotype of sires</i>	<i>Mappable ?</i>	<i>Currently on map ?</i>
RM96	1	Heterozygous	✓	✓
RME25	3	Heterozygous	✓	✓
RT1	6	Heterozygous	✓	✗
RT13	4 +	Homozygous	✗	✗
RT23	1	Heterozygous	✓	✗
RT24	1	Homozygous	✗	✗
RT25	6	Heterozygous	✓	✗
RT27	2	Heterozygous	✓	✗
RT30	2	Homozygous	✓	✗
RT5	6	Heterozygous	✓	✗
RT6	10	Heterozygous	✓	✗
S μ 2	6 +	Heterozygous	✓	✗
SMHCC	2 loci amplified?	-	-	✗
SPS113	8	Heterozygous	✓	✗
TGLA10	6 + null	Heterozygous	✓	✗
TGLA127	9	Homozygous (PD null)	✓	✗
TGLA130	3	Heterozygous	✓	✗
TGLA226	6 +	Heterozygous	✓	✗
TGLA304	2	Heterozygous	✓	✗
TGLA337	6	Heterozygous	✓	✗
TGLA378	6	Homozygous (PD null)	✓	✗
TGLA40	4	Heterozygous	✓	✗
TGLA431	7	Heterozygous	✓	✗
TGLA48	2	Heterozygous	✓	✗
TGLA86	6	Heterozygous	✓	✗
UWCA47	1	Homozygous	✗	✗

APPENDIX G

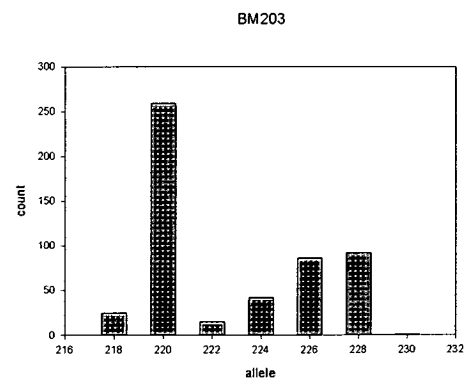
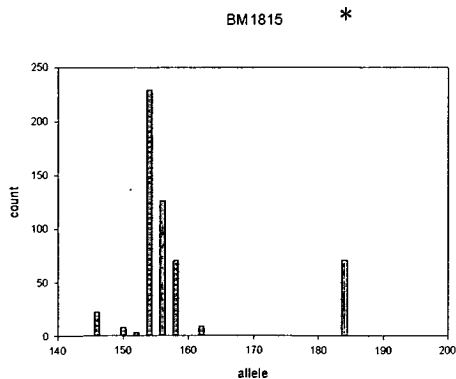
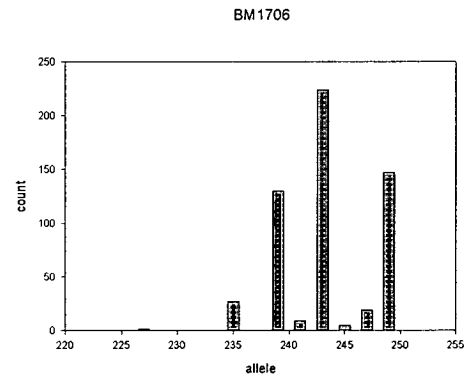
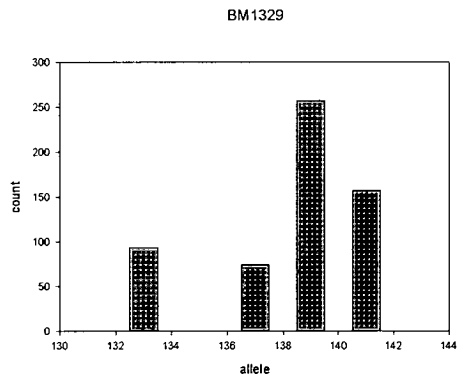
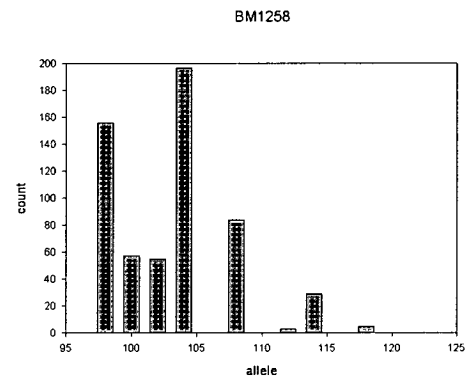
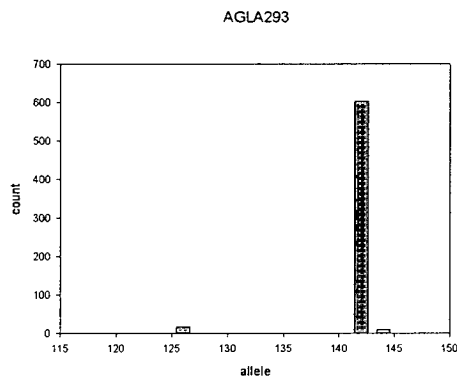
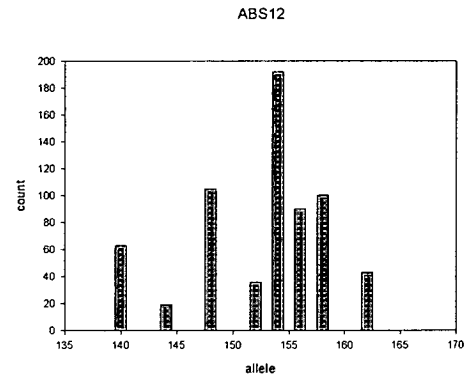
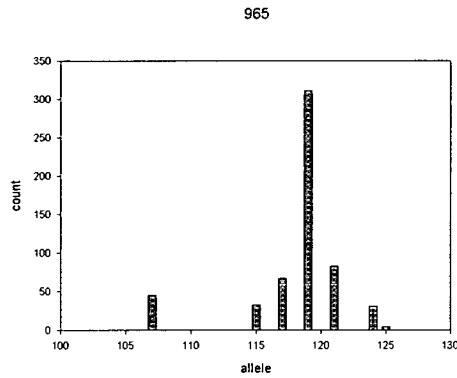
LOD scores supporting linkage between pairs of loci screened in the MAXI pedigree (see Chapter 6)

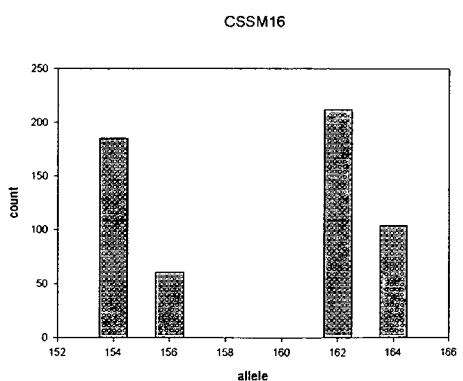
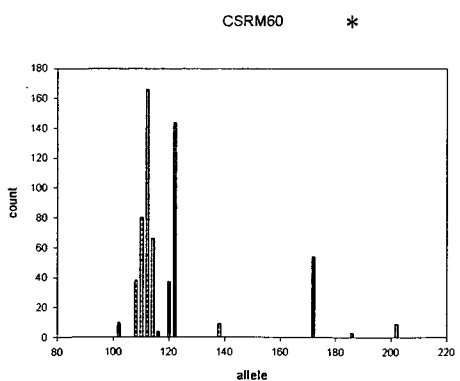
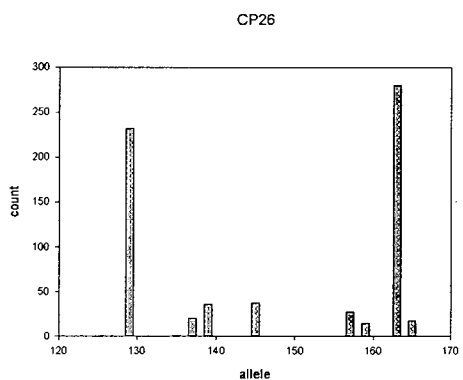
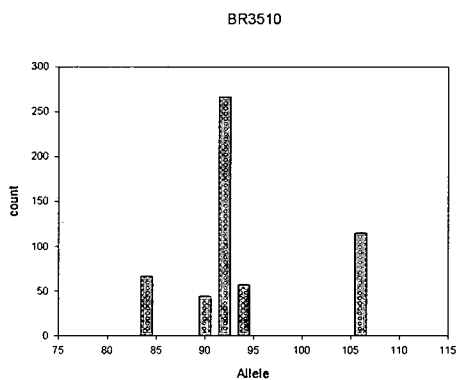
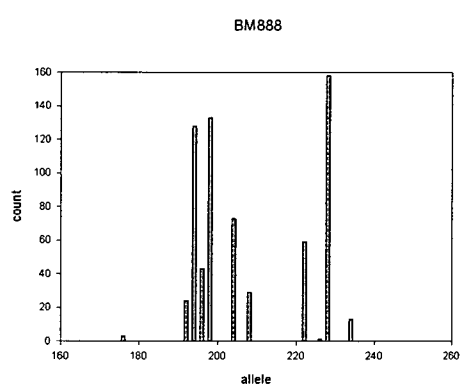
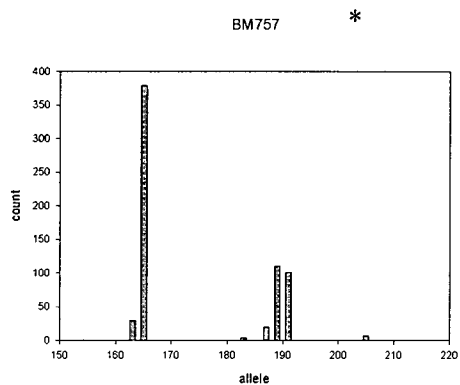
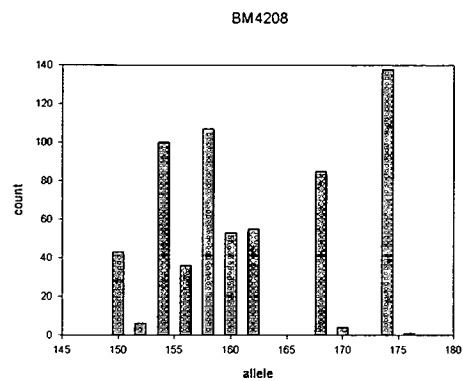
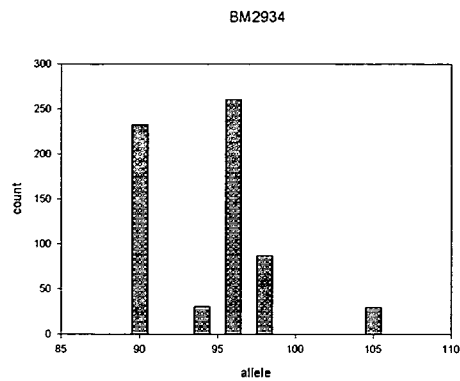
<i>LOCI</i>	<i>LINKAGE GROUP</i>	<i>RECOMBINATION FRACTION</i>	<i>LOD</i>
JP231-IDVGA55	4	0.08	11.62
INRA121-RT25	4	0.14	6.25
FCB193-965	5	0.05	15.29
TGLA94-VH98	5	0.19	3.61
BM1815-BM1258	7	0.10	9.33
BM1818-BM1258	7	0.19	5.19
BM1818-BM1815	7	0.16	4.96
IDH-TGLA226	8	0.05	2.96
INRA131-CSSM16	11	0.05	6.09
ILSTS12-CSSM16	11	0.15	6.11
ILSTS12-INRA131	11	0.10	6.89
TGLA378-RM90	12	0.06	6.75
BM888-RM90	12	0.04	13.22
BM888-TGLA378	12	0.12	7.04
SPS113-RM90	12	0.14	3.47
SPS113-TGLA378	12	0.15	3.44
CSRM60-RM90	12	0.20	4.34
CSRM60-BM888	12	0.15	8.02
MCM527-TGLA337	13	0.18	3.50
MPI-TGLA337	13	0.00	3.01
INRA35-BM1706	14	0.01	14.89
RBP-ABS12	15	0.04	21.52
RBP-PGAZAC2	15	0.17	4.12
ABS12-PGAZAC2	15	0.19	4.15
ABS12-IDVGA8	15	0.03	5.84
JP27-BM1329	17	0.12	3.26
RT6-INRA11	19	0.16	3.58
CSSM19-TF	19	0.03	5.57
BM4513-BM2934	21	0.05	10.67
FCB304-CSSM41	24	0.19	4.82
MM12-BM4208	26	0.04	3.00
RT1-BM4208	26	0.09	13.05
JP38-MAF35	27	0.18	5.68
ETH225-BM757	28	0.07	9.55

Appendix H: histograms of allele counts for all loci screened in the MAXI pedigree.

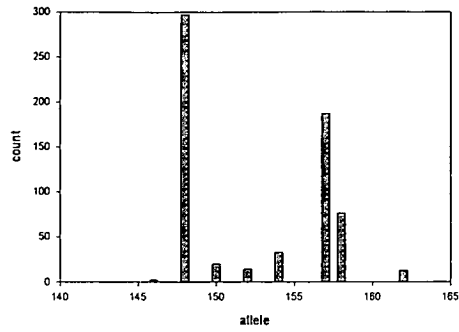
Note that loci for which null alleles were segregating (Ch 4) are not shown.

* Locus not included in the restricted mean d^2 measure (see Chapter 6).

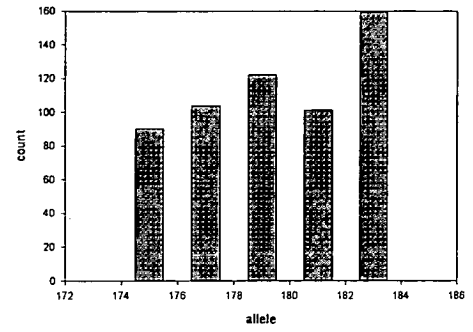




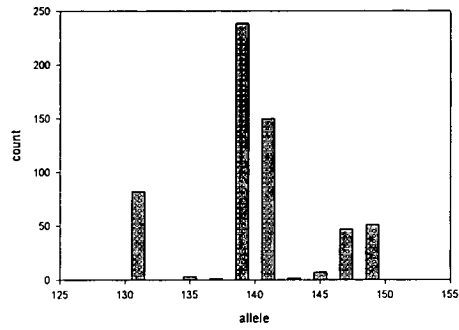
CSSM19



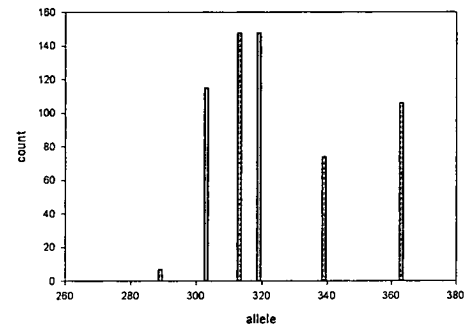
CSSM39



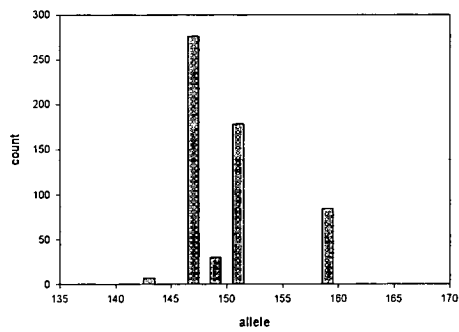
CSSM41



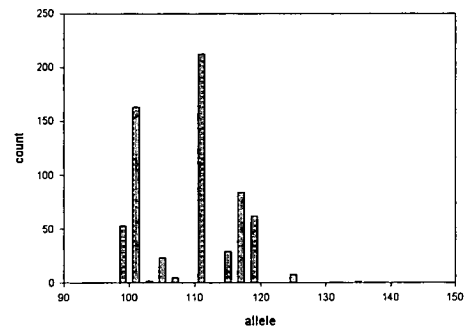
CSSM43



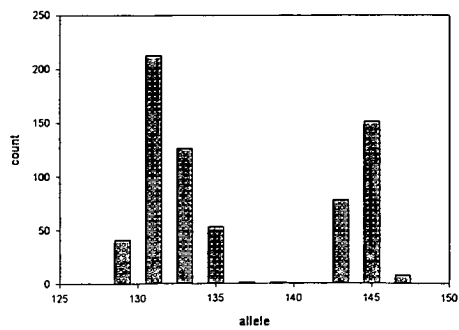
ETH225



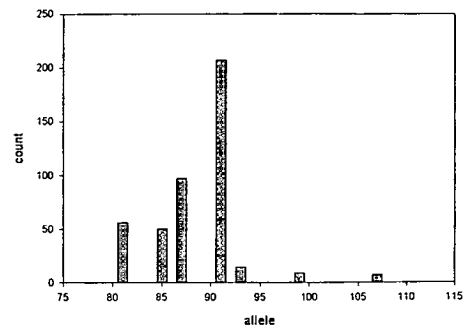
FCB193

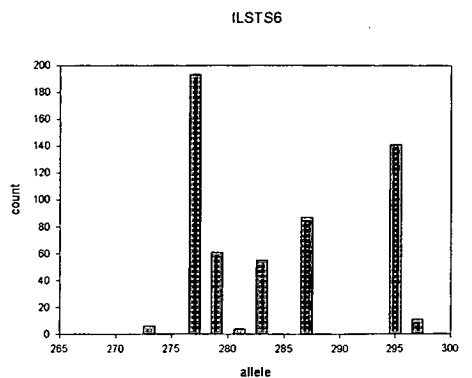
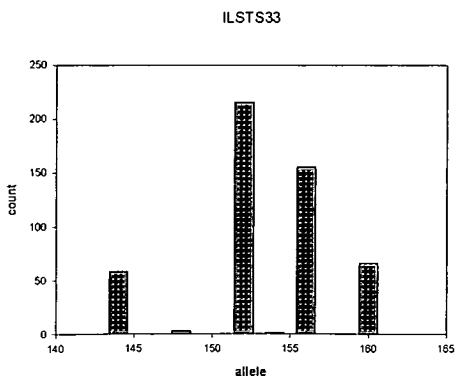
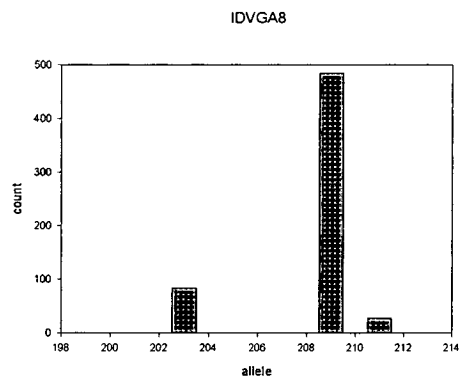
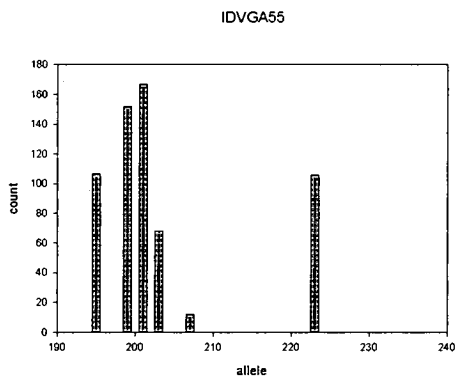
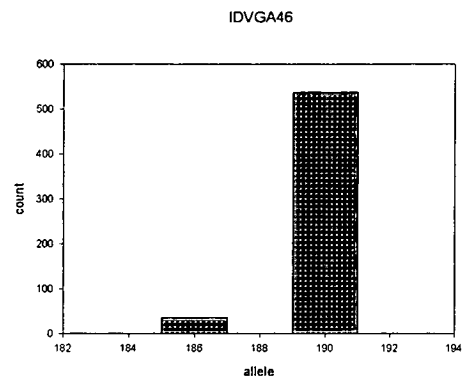
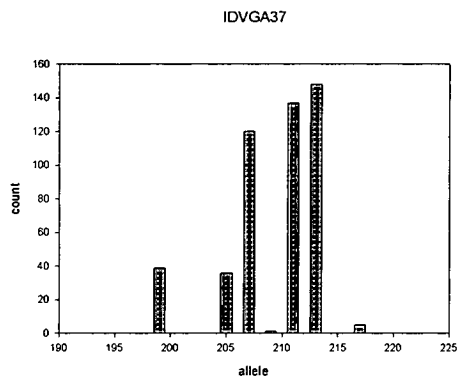
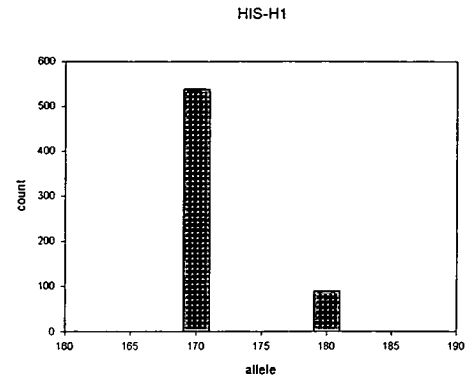
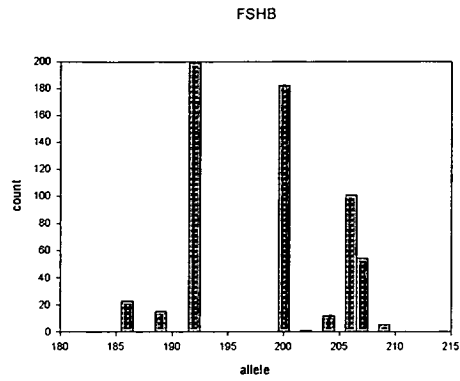


FCB304

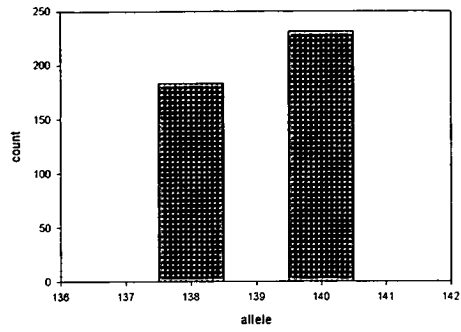


FCB5



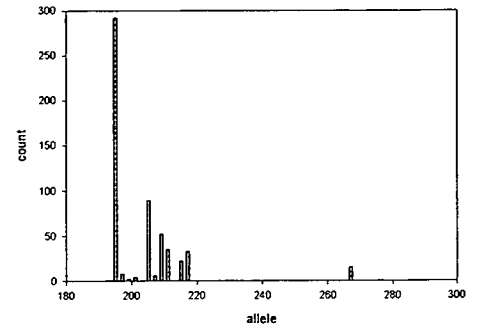


ILSTS87



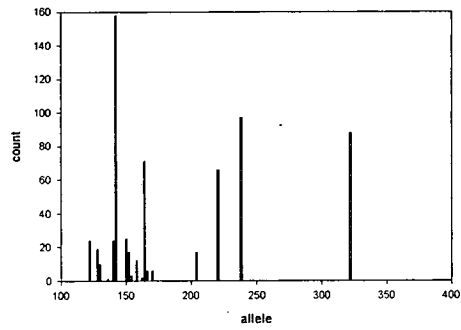
INRA11

*

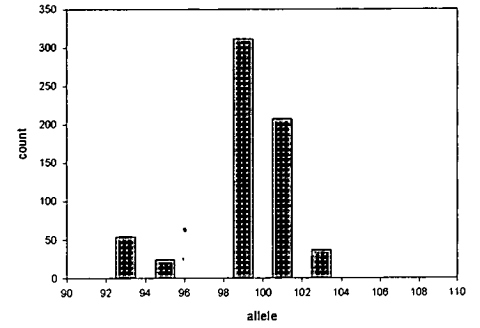


INRA121

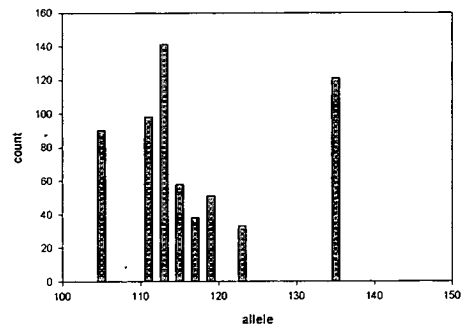
*



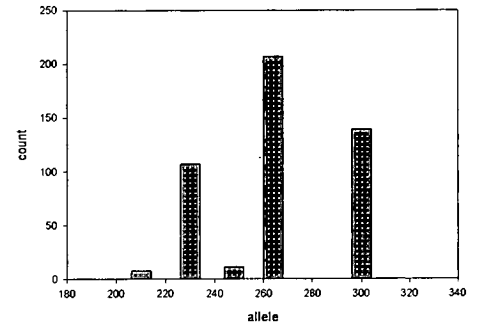
INRA131



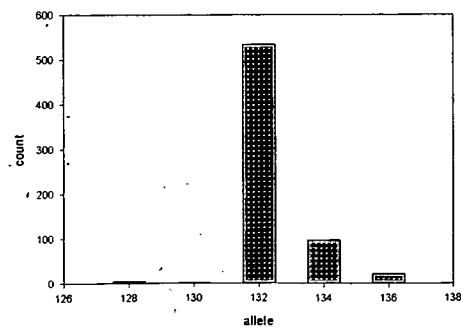
INRA35



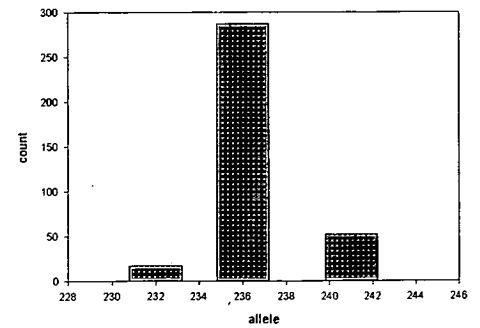
INRA40

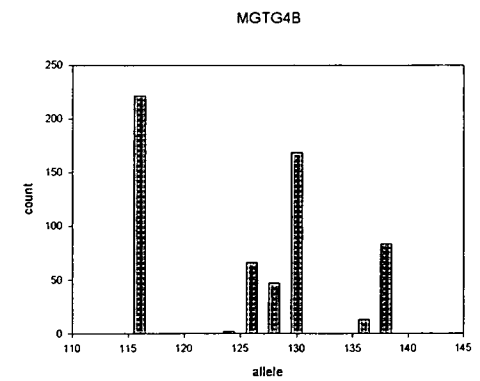
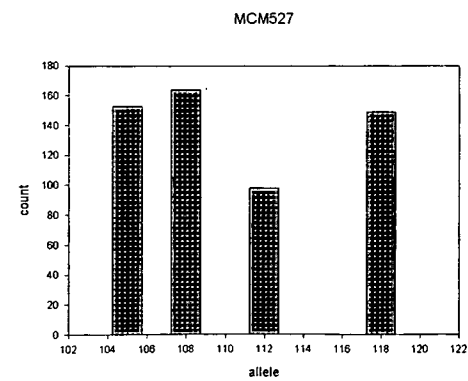
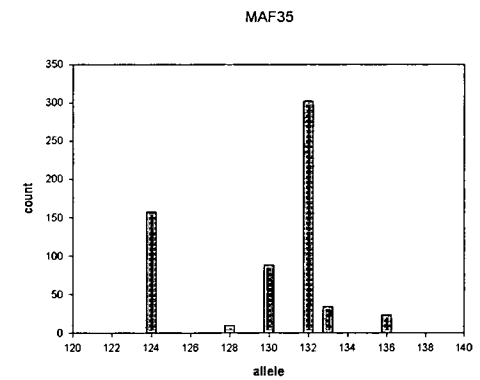
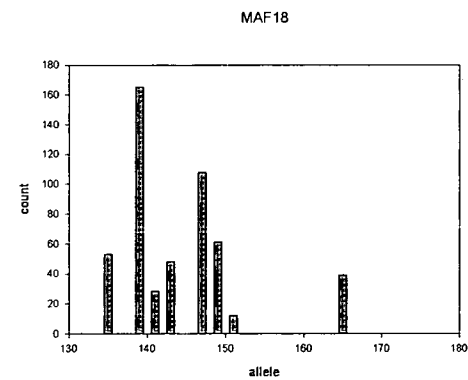
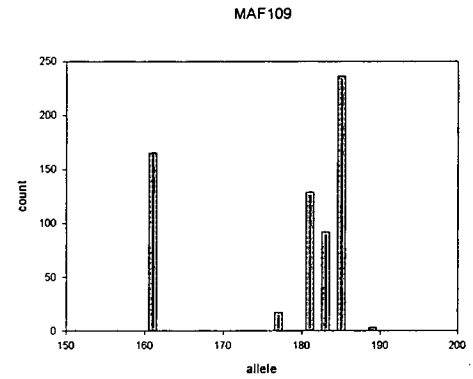
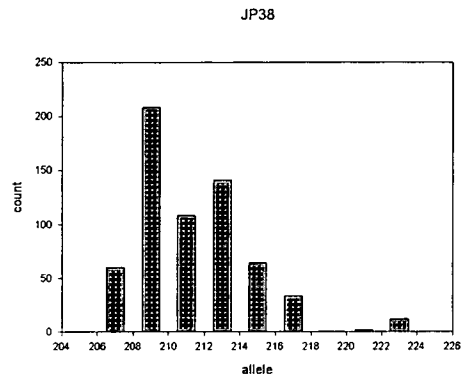
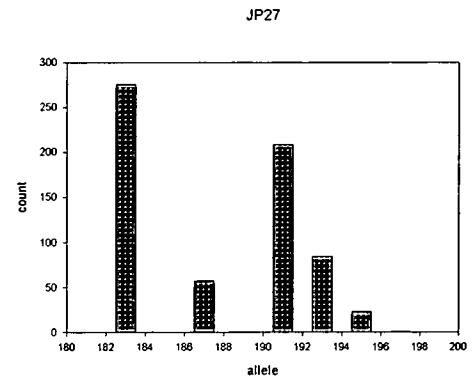
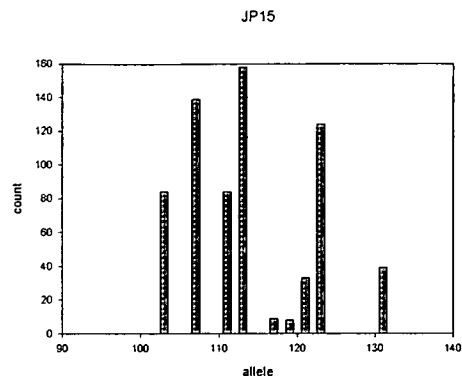


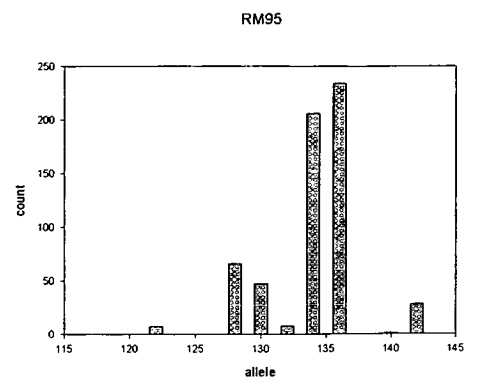
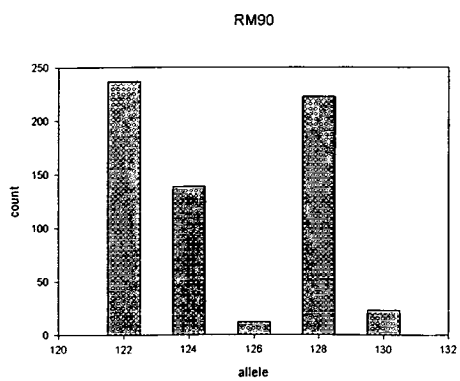
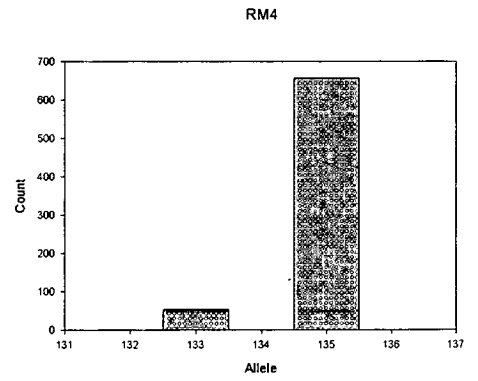
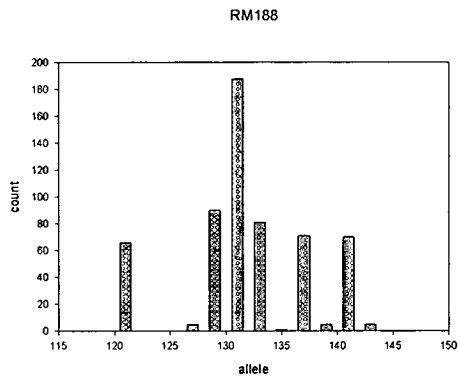
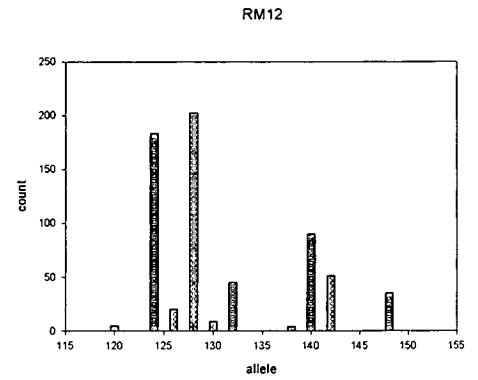
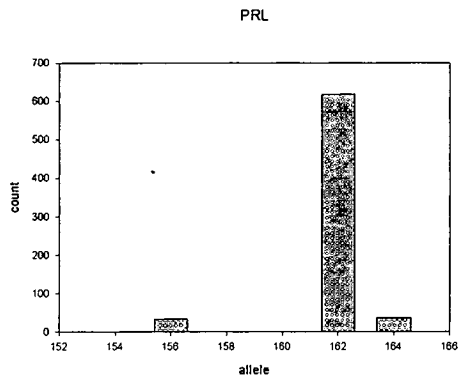
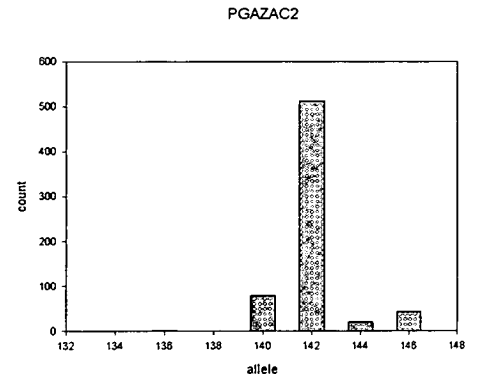
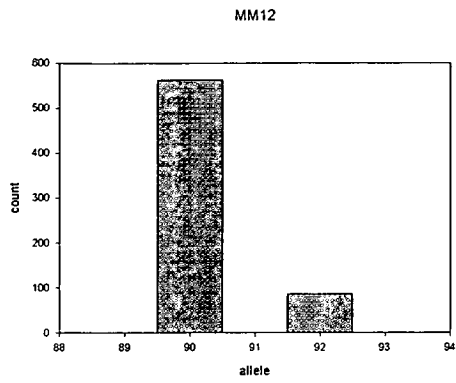
INRA6

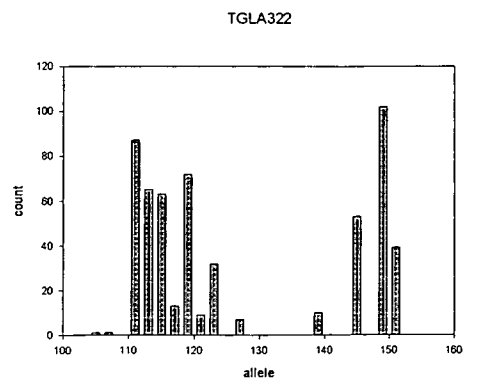
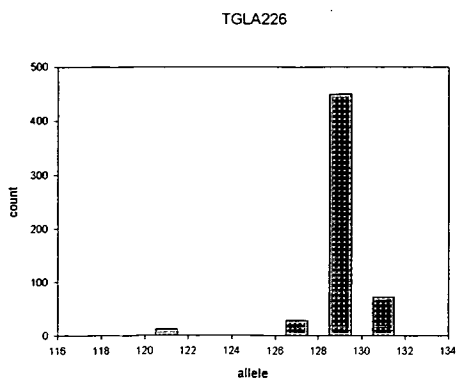
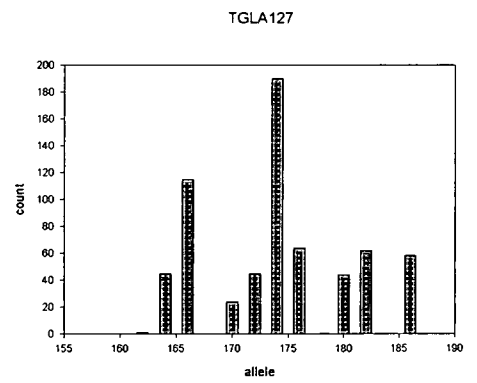
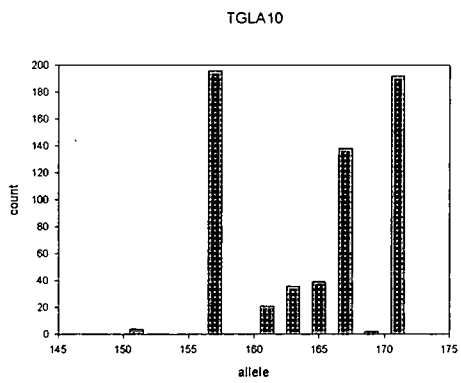
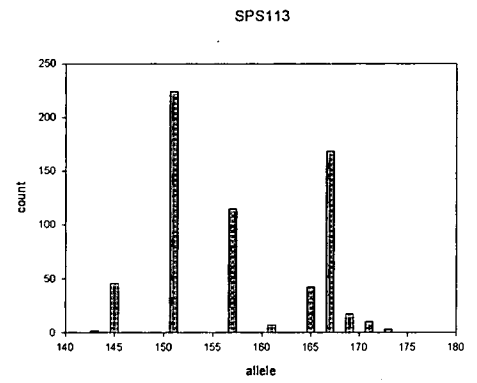
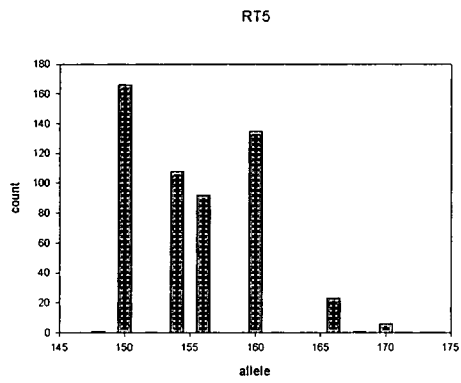
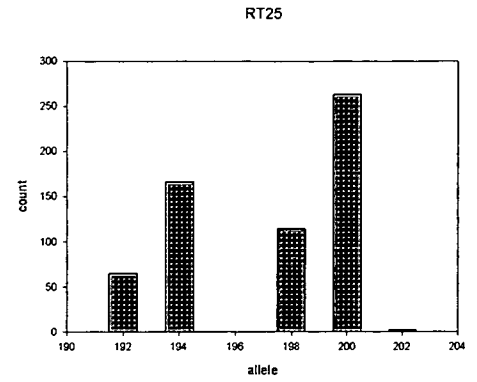
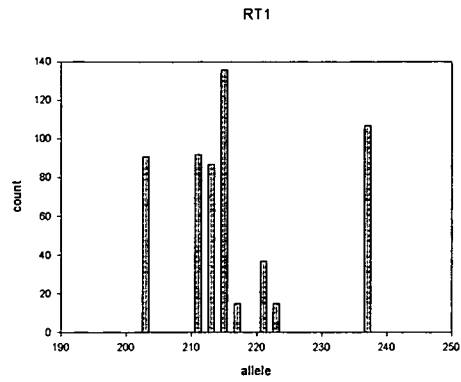


JAB8

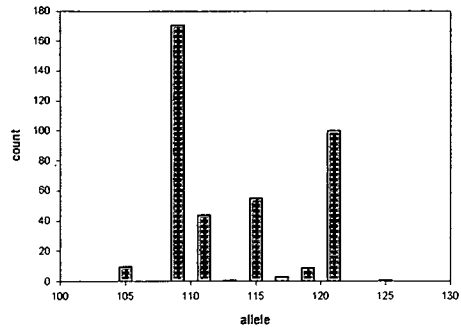




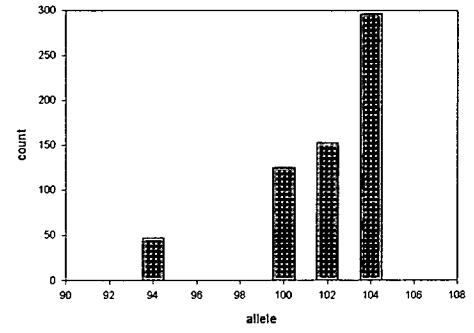




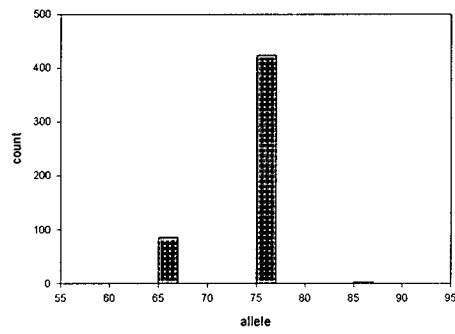
TGLA378



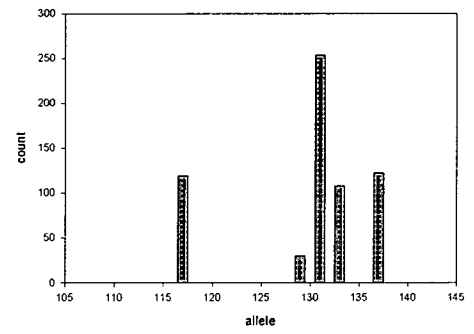
TGLA40



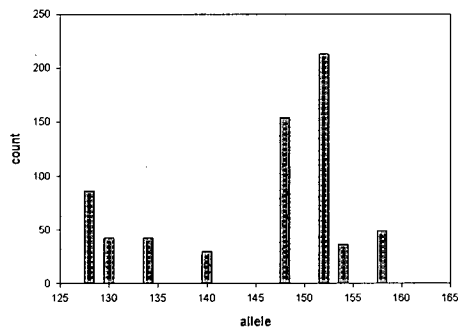
TGLA48



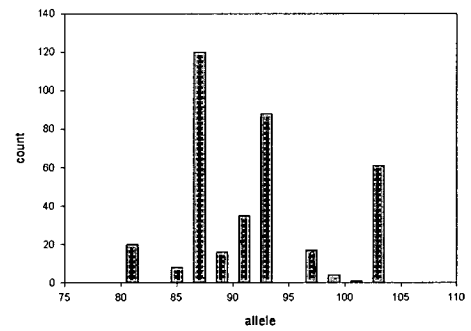
TGLA86



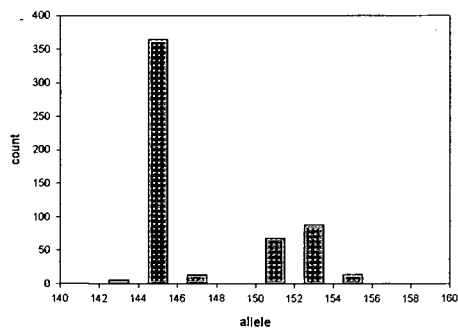
TGLA94



VH54



VH98



Bovine microsatellite loci are highly conserved in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*) and Soay sheep (*Ovis aries*)

J Slate, D W Coltman, S J Goodman, I MacLean, J M Pemberton, J L Williams

Summary

We tested 174 bovine microsatellite primer pairs for use in a primitive breed of sheep and two species of deer. Of 173 markers, 127 (73.4%) gave a product in Soay sheep (*Ovis aries*) of which 54 (42.5%) were polymorphic. One hundred and twenty-nine of 174 (74.1%) markers gave a product in red deer (*Cervus elaphus*) of which 72 (55.8%) were polymorphic. In sika deer (*Cervus nippon*) 126 of 171 (73.7%) microsatellite primers gave a product with 47 (37.3%) polymorphic. The proportion of bovine microsatellite loci conserved across artiodactyl species was significantly greater in this study than previously reported. Reasons for this high degree of microsatellite conservation are discussed. We suggest that a high resolution comparative map of the artiodactyls can be constructed using microsatellites.

Keywords: cattle, comparative mapping, deer, microsatellite, sheep

Introduction

Evolutionary biologists and ecologists have a long-standing interest in the nature of quantitative genetic variation for fitness (Fisher 1958; Gustafsson 1986; Mousseau & Roff 1987). With the advent of genetic maps, highly polymorphic markers and recent advances in the statistical techniques used to detect quantitative trait loci (QTLs) it is now possible to map genes affecting fitness and fitness components in natural populations (Mitchell-Olds 1995). Our interest is in the nature of additive genetic variation for fitness in natural populations of red deer (*Cervus elaphus*) and Soay sheep (*Ovis aries*), and also in natural selection within a hybrid zone between Japanese sika deer (*Cervus nippon*) and red deer in Scotland (Abernethy 1994).

The marker of choice for most population genetics, linkage mapping and parentage studies, particularly in mammal populations, is the microsatellite locus (Bruford & Wayne 1993; Queller *et al.* 1993; Jarne & Lagoda 1996). However, obtaining a suitably large panel of polymorphic markers can be both time-consuming and expensive if there is as yet no genetic map for the study organism. It has been demonstrated that some microsatellite loci are conserved across species (Moore *et al.* 1991; Crawford *et al.* 1994; FitzSimmons *et al.* 1995; de Gortari *et al.* 1997) and even higher taxa (Gemmell *et al.* 1997).

Cross-species utilisation of microsatellite loci not only saves time and effort in the laboratory, but also enables the construction of comparative maps between related species. Comparative maps provide information on conservation of synteny between species and give an estimate of genome coverage of a map (O'Brien *et al.* 1993). They may also be useful in QTL detection (Cockett *et al.* 1994), particularly if a candidate gene is known from another species. O'Brien *et al.* (1993) suggest that evolutionary conserved, gene-encoding loci be termed Type 1 loci, and that less conserved, anonymous DNA markers such as microsatellites termed Type 2 loci. Although Type 1 loci are better conserved across mammalian orders, Type 2 loci are still useful as comparative markers within an order, e.g. the artiodactyla. In addition, Type 2 microsatellite markers are generally easier to screen and are more polymorphic within a species. Earlier studies that tested cattle microsatellite primers across species estimated that around 60% amplify a product in both sheep (Moore *et al.* 1991; Crawford *et al.* 1995; de Gortari *et al.* 1997) and goat (Pepin *et al.* 1995). Indeed the first sheep genetic map contained only 86 microsatellites cloned in sheep compared to 126 taken from the bovine map (Crawford *et al.* 1995).

Here we evaluate the use of primers for 174 bovine microsatellite markers in Soay sheep, red deer and sika deer. We expected to find that about 60% of primers would amplify a microsatellite in Soay sheep, with fewer amplifying a product in the two deer species, reflecting the

Correspondence: J Slate.

Accepted 4 June 1998

J Slate
D W Coltman
S J Goodman
J M Pemberton
I.C.A.P.B., King's Buildings,
University of Edinburgh,
Edinburgh EH9 3JT, UK
I MacLean
J L Williams
Roslin Institute, Roslin,
Midlothian EH25 9PS,
UK

greater evolutionary divergence between cattle and deer, than between cattle and both sheep and goat (Modi *et al.* 1996).

Materials and methods

DNA extraction and purification

DNA was extracted from either ear punches or white blood cells by standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation procedures (Sambrook *et al.* 1989).

Markers tested

We tested primers for 174 bovine microsatellites. These markers are a subset of those published in the most recent bovine genetic map (Barendse *et al.* 1997) and were chosen by two of us (I.M. and J.L.W.) for detection of economically important QTLs in cattle. They were selected for their high degree of polymorphism in cattle, to give even spacing throughout the cattle genome, and because they would reliably amplify loci that were easy to score in cattle.

PCR protocol

Microsatellite loci were amplified as follows: genomic DNA (50 ng) was used as a template in a 10 µl reaction mix containing 0.1 mM dATP, dGTP and dTTP; 0.01 mM dCTP; 2 pmol each primer; 1× 'Parr' buffer containing 1.5 mM MgCl₂ (Cambio, Cambridge, Cambridgeshire, UK); 0.25 U Taq polymerase (Advanced Biotechnologies, Epsom Surrey, UK) and <1 µCi [α -³²P]-dCTP. If these initial low stringency reaction conditions did not yield a satisfactory product, then between 0 and 1.5 mM MgCl₂ (total MgCl₂ 1.5–3.0 mM), 0–10% dimethylsulphoxide (DMSO) and 60 mM tetramethylammonium chloride/2.5% formamide (TMACIDE) were added to the reaction mix (Gemmell 1997). Optimised PCR conditions for loci amplified in the two deer species are contained in Table 1. In general, more optimisation was performed with deer than with Soay sheep. Reactions were overlaid with one drop of mineral oil and amplified in a Hybaid Omnigene Temperature Cycler (Ashford, Middlesex, UK). PCR was performed as follows: 2 min denaturation step at 94 °C; seven cycles of 30 s at 94 °C denaturation, 1 min of 50 °C annealing, 30 s of 72 °C extension; then 30 cycles of 30 s at 94 °C denaturation, 1 min at 52 °C annealing and 30 s of 72 °C extension.

Products were separated on 6% polyacrylamide sequencing gels and visualised on X-ray film as described elsewhere (Bancroft *et al.* 1995).

Animals screened

Primers were initially screened in a panel comprising of DNA from two Soay sheep, two red deer, two sika deer, one Père David's deer (*Elaphurus davidianus*) and one cow. MgCl₂ (1.0 mM) was added to the PCR mix giving an overall MgCl₂ concentration of 2.5 mM. Loci that gave a product in any of the deer or sheep species were then evaluated over a range of PCR conditions for each species using the following larger sets of individuals.

Eighty-nine progeny of two Père David's red deer stags backcrossed to red deer hinds were examined to estimate the number of alleles in red deer. These progeny are part of the deer International Mapping Herd (IMH) produced by AgResearch, Invermay, New Zealand (Tate *et al.* 1995). In these crosses the paternal Père David's allele can usually be distinguished from the maternal red deer allele by running the Père David F0 sire alongside the F1 hybrid sires. In addition seven red deer from Cowal, Scotland were screened for all loci. Eight sika deer were tested, four from Kintyre, Scotland and four from Dawyck, Scotland. Six unrelated Soay sheep from Hirta, St Kilda, Scotland were tested.

Description of PCR products

A single-band PCR product was only considered to be the conserved target microsatellite if the observed band showed a distinctive 'stutter' pattern and was close to the size range of the observed cattle product. If a single-band was not close to the observed size in cattle, or did not show a 'stutter' pattern typical of a microsatellite, then the PCR product was considered non-specific and the locus under test was considered not conserved in the test species. If no PCR product was amplified the same conclusion was reached. Polymorphic loci with 'stutter' bands and of similar size to that observed in cattle were regarded as the target bovine microsatellite locus, conserved across species.

Results

A summary of the results for the 174 loci tested is given in Table 1. Details of the optimised PCR conditions for deer are given as well as size of product in cattle, cattle chromosomal location, and the number of alleles found in each of the

Table 1. Summary of each cattle microsatellite evaluated in Soay sheep, red deer and sika deer

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc. (mM)	Reference ^b
BM3205	1	204–221	3 ^a	1	1			1
TGLA130	1	126–201	–	3	1	10% + TMAC	2.0	2
TGLA415	1	106–126	1 ^c	1	1			2
TGLA213	1	500	–	–	–			2
TGLA49	1	120	– ^c	–	–			2
TGLA135	1	110	1	–	–			2
TGLA57	1	80–104	–	1	1			2
INRA128	1	182	3	2	4			3
CSSM42	2	165–222	–	1	1			4
IDVGA37	2	210	2	7	3	0	2.0	5
BM4440	2	121–147	1	4	2			1
IDVGA2	2	140	1	–	–			5
INRA40 (1)	2		Not tested	3	Not tested	5%	2.0	^d
INRA40 (2)	2	205–230	1	8	5	5%	2.0	3
TGLA431	2	134–163	–	7	3	0	3.0	2
TGLA226	2	145–151	–	6	2	10%	2.0	6
INRA23	3	197–223	3	1	1			7
INRA6	3	105	3 ^a	2	1	0	2.0	8
HUJ1177	3	210	– ^a	7	–	0	2.0	9
IDVGA35	3	230	1	–	–	10% + TMAC	2.0	5
TGLA127	3	170	–	9	2	5%	2.0	2
IDVGA27	3	136–140	1	2	1			5
TGLA263	3	100–136	7	–	–			2
IOBT250	3	105–117	1	1	1			10
RM188	4	124–150	1	6	1	10% + TMAC	2.0	6
INRA37	4	130	1	1	1	5%	2.0	3
HEL25	4	139–149	–	–	–			10
MGTG4B	4	134–164	2	6	1	5%	2.0	10
AGLA293	5	196–260	2 ^c	3	1		2.0	2
BM6026	5	148–168	1	2	1			1
ETH152	5	196–204	1	1	1			11
BM1819	5	114–128	1	1	1			1
BM315	5	107–163	1	–	–			1
BM2830	5	149–203	1 ^a	1	1			1
IDVGA9	5	200	1	1	1			5
ETH10	5	210–226	1	1	1			12
BM2320	6	128–152	–	6	2			1
BM4528	6	238–276	–	1	1			1
ILSTS90	6	150–190	–	–	–			13
BM1329	6	145–161	1 ^c	6	1	10% + TMAC	2.5	1
ILSTS93	6	160 (202?)	3	5	2	10% + TMAC	3.0	13
ILSTS97	6	225	1	1	1			13
RM28	6	96–110	1	1	1			6
AFR227	6	113	4	6	1	10% + TMAC	1.5	14
BM741	7	172–184	1 ^c	1	1			1
ILSTS6	7	283–295	–	9	2	10% + TMAC	3.0	15
BP41	7	312–326	–	–	–			1
INRA53	7	100	1	–	–			3
CSSM29	7	230	1	2	5	10% + TMAC	3.0	4
RM12	7	107–111	1	8	1	10% + TMAC	2.0	6
IDVGA11	8	230	1	1	1			5
HEL9	8	143–165	1	1	1			16
BM4006	8	97–119	1 ^a	3	1	10% + TMAC	2.0	1
CSSM47	8	141–171	2 ^c	1	1			4
TGLA10	8	150	3 ^c	6	3	10% + TMAC	3.0	2
UWCA47	8		3	1	1			10
CSSM37	8	172	2	1	1			4

Table 1. Continued

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc. (mm)	Reference ^b
TGLA13	8	183–209	5 ^c	—	1			2
CSSM56	9	230–240	1 ^a	—	—			4
UWCA9	9	100	1 ^c	—	—			17
BM757	9	182–220	3 ^c	7	2	10% + TMAC	2.0	1
BM2504	9	130–146	3 ^c	1	1			1
TGLA73	9	110–128	3 ^c	1	1			2
SPS113	10	133–165	1	8	2	10% + TMAC	3.0	
CSSM38	10	180	—	—	—			4
BM888	10	173–183	1	11	1	10% + TMAC	3.0	1
CSSM39	10	195–208	1	5	2	10% + TMAC	3.0	4
JAB10	10	276?–288	1	1	2			18
TGLA378	10	100–120	6	6	4	10% + TMAC	3.0	2
INRA107	10	166	2	10	2	10% + TMAC	3.0	7
CSSM46	10	152	—	—	—			4
HEL13	11	177–197	1	—	—			16
IDVGA3	11	150	—	4	1		2.0	5
BM716	11	114–155	2	1	2			1
INRA131	11	125	3	3	1		2.5	3
JAB7	11	360	2	1	1		2.5	18
INRA169	11		1	5	1		2.5	32
BM6108	12	116–144	2 ^c	1	1			1
TGLA36	12	77–107	— ^c	1	1			2
RM178	12		—	3	1	10% + TMAC	3.0	6
BM6404	12	127–149	3 ^a	1	1			1
INRA5	12	119–123	5	1	1	10% + TMAC	3.0	8
INRA209	12	140–150	1	2	1			19
RM162	12	121–139	1	—	—			20
DIK16	12		2	1	1			21
HUJ616	13	140	4 ^c	1	1	10% + TMAC	2.0	22
JAB3	13	226–230	1	—	—			18
BM1222	13	272–303	— ^a	5	1	10% + TMAC	3.0	1
ETH7	13	270	1	—	—			12
TGLA23	13	92–116	2 ^c	—	—			2
L6003	13		1	—	—			
TGLA131	13	150	Not tested	Not tested	Not tested			2
ABS10	13		1	5	1			23
CSSM36	14	163–185	1	1	1			4
BM4513	14	141–161	1 ^c	12	3	10% + TMAC	2.0	1
BM4630	14	151–165	3 ^c	—	—			1
BM6425	14	172–192	1	1	1			1
INRA94	14	157	1	—	—			3
BM2934	14	81–103	1	6	4	10% + TMAC	3.0	1
BR3510	15	90–114	3 ^c	5	1	10% + TMAC	3.0	1
JAB8	15	220	3	5	1	10% + TMAC	3.0	24
JAB1	15	224–234	2	1	1	10% + TMAC	3.0	24
JAB4	15	187–193	1	1	1		2.5	24
IDVGA10	15	190	3	1	1			5
FSHB	15	200	2 ^a	6	2	10% + TMAC	3.0	30
SPS115	15		3	3	2		2.0	
BM6430	16	105–164	—	—	—			1
BM1706	16	233–259	—	5	1		2.5	1
BR6504	16	125–145	— ^c	1	1			1
ETH11	16	214	—	1	1			12
BM719	16	147–155	3 ^c	—	—			1
BM1862	17	191–224	2	—	—			1
OarVH98	17	133–161	3	6	2	10% + TMAC	3.0	25
CSSM33	17	179	1	2	1		2.0	4
INRA25	17	220	—	1	1			3

Table 1. Continued

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc. (mM)	Reference ^b
IDVGA40	17	240	1	1	1			5
INRA110	17	213	1	—	—			3
INRA38	18	115	2	2	1			3
IDVGA31	18	210–220	—	—	—			5
INRA121	18	128–152	1	15	2	10% + TMAC	3.0	3
IDVGA55	18	195	1	7	4		2.5	5
BP20	19	219–233	1	1	1			1
HEL10	19		3 ^c	2	Not tested			16
IDVGA44	19		1	1	1			5
IDVGA46	19	190	3 ^a	4	2	10% + TMAC	3.0	5
AFL361	19		—	—	—			10
BM713	20	100–112	1	—	—			1
BM3517	20	104–124	—	1	1			1
TGLA304	20	86–100	2	3	2		2.5	2
TGLA126	20	109–127	2	1	2			2
BM5004	20	120–154	1 ^a	5	4	10% + TMAC	3.0	1
AFR2215	20	96	1	—	—			14
BM1225	20	227–253	1 ^c	6	2	10% + TMAC	3.0	1
TGLA337	21		1 ^a	6	2		2.5	2
IDVGA39	21	185–196	1	4	1	10% + TMAC	3.0	5
CSSM26	22	240–250	1	3	2		2.5	4
HRH1	22	133–141	1	—	—			1
CSSM41	22	130–140	3 ^a	6	4	10% + TMAC	3.0	4
BM3628	22	243–263?	1 ^c	2	2	10% + TMAC	3.0	1
HUJ175	22	130–160?	3	5	3		2.0	9
INRA130	22	100	3	1	1			3
BM47	23	94–126	1	—	—			1
UWCA1	23	102–130	1	—	—			31
BM1905	23	170–199	2 ^d	1	1			1
DRB	23	131–170	Not tested	Not tested	Not tested			
IOBT528	23	182	—	—	Not tested			10
BM226	24	128–164	— ^c	1	—			1
CSSM23	24	214	1	—	—			4
ILSTS101	24	171	1	1	1			13
INRA90	24	168	1	2	2			3
TGLA86	25	128–140?	2	6	3	10% + TMAC	3.0	2
RM44	25	100	—	—	—			33
JAB5	25		—	—	—			18
HEL11	26	181–193	— ^a	—	—			16
BM4505	26	204–248	— ^c	1	1			1
BM804	26	144–152	—	—	—			1
ABS12	26		—	9	4		2.0	23
HAUT27	26		—	—	—			26
BM203	27	203–244?	6 ^c	8	3		2.0	1
BM3507	27	159–187	—	—	—			1
RM209	27	115–145	1	1	1	10% + TMAC	3.0	34
HUJ113	27		—	1	1			35
CSSM43	27	253–261	3 ^c	11	4	10% + TMAC	3.0	4
BP23	28	272–288	—	3	1	10% + TMAC	3.0	1
IDVGA29	28	144–154	1	2	3		2.0	5
IDVGA8	28	212–226	—	4	2	10% + TMAC	3.0	5
RBP3	28	132–144	3 ^c	7	2		2.5	27
IDVGA43	28	170–184	3	—	—			5
AF4 (L6068)	29		3	1	1			28
BM4005	29	108–122	1 ^c	2	3	10% + TMAC	3.0	1
AF5	29	130–164	—	6	3	10% + TMAC	3.0	28
IDVGA71	29	192–201?	1	1	1			29
TGLA40	29	100–118?	1	4	2	10% + TMAC	3.0	2

Table 1. Continued

Marker	Cow chromosome	Size in cow	Soay alleles	Red alleles	Sika alleles	DMSO	Mg conc. (mM)	Reference ^b
BM6017	X	112–139	–	–	–			1
INRA30	X	156–166	– ^a	1	1			3
HEL26	X	180–193?	–	1	1			10

–, no product amplified in test species.

TMAC = 60 mM tetramethylammonium chloride and 25% formamide.

Standard PCR conditions were 2.5 mM MgCl₂, 0% DMSO and 0% TMACIDE unless otherwise listed.

PCR conditions for Soay sheep are not listed. Most loci were evaluated using 2.5 mM MgCl₂ in the absence of DMSO and TMACIDE.

^aMapped previously in domestic sheep. de Gortari *et al.* (1998).

^b1, Bishop *et al.* (1994); 2, Georges & Massey (1992); 3, Vaiman *et al.* (1994c); 4, Moore *et al.* (1994); 5, Mezzalana *et al.* (1995); 6, Barendse *et al.* (1994); 7, Vaiman *et al.* (1994a); 8, Vaiman *et al.* (1992); 9, Shalom *et al.* (1994); 10, Barendse *et al.* (1997); 11, Steffen *et al.* (1993); 12, Solinas-Toldo *et al.* (1993); 13, Kemp *et al.* (1995); 14, Jorgenson *et al.* (1995); 15, Brezinsky *et al.* (1993); 16, Kaukinen & Varvio (1993); 17, Sun *et al.* (1994); 18, Williams *et al.* (1996); 19, Bahri-Darwich *et al.* (1994); 20, Kossarek *et al.* (1996); 21, Inoue *et al.* (1995); 22, Shalom *et al.* (1993); 23, Pfister-Genkow *et al.* (1995); 24, Williams *et al.* (1995); 25, Hanrahan *et al.* (1993); 26, Thieven *et al.* (1997); 27, Fries *et al.* (1993); 28, Konfortov *et al.* (1996); 29, Zhang *et al.* (1995); 30, Moore *et al.* (1992); 31, Sun *et al.* (1993); 32, Vaiman *et al.* (1994b); 33, Kossarek *et al.* (1995); 34, Kossarek *et al.* (1994); 35, Shalom *et al.* (1995).

^cMapped previously in domestic sheep. Crawford *et al.* (1995).

^dAdditional locus amplified in red deer only.

test species. Markers that have previously been shown to amplify a product in sheep are also indicated. Table 2 summarises the number and proportion of bovine loci conserved in each species tested.

Twelve of the markers tested gave a heterozygous product in the Père David deer. They were TGLA226, INRA37, TGLA431, BM757, ILSTS93, RM12, SPS113, ILSTS6, INRA107, INRA5, BM1225, and IDVGA46.

Discussion

In this paper we describe a panel of bovine microsatellite loci which are conserved in three species of artiodactyl, namely Soay sheep (54 polymorphic loci), red deer (72) and sika deer (47). These loci can be used to measure genetic variation in natural populations of these species and in the case of red deer can be mapped in what is an economically important species (Tate *et al.* 1995).

The proportion of primers amplifying a locus in each of the three test species was higher than

found in other similar studies. de Gortari *et al.* (1997) tested 1036 bovine primers in sheep and found that 605 (58%) amplified a locus. Our proportion of bovine markers giving a product was significantly higher in all three species tested (Soay sheep (this study) vs. domestic sheep (de Gortari): $\chi^2 = 13.99$, d.f. = 1, $P < 0.001$; red deer (this study) vs. sheep (de Gortari study): $\chi^2 = 15.47$, d.f. = 1, $P < 0.001$; sika deer (this study) vs. sheep (de Gortari): $\chi^2 = 14.36$, d.f. = 1, $P < 0.001$). Kuhn *et al.* (1996) found that 14/27 cattle microsatellites primers (52%) amplified a product (all 14 loci polymorphic) in red deer. Engel *et al.* (1996) tested 12 bovine primer sets across a range of six cervid species. Only 40% of tests resulted in a product being obtained in deer, although a higher figure was obtained when ovine or caprine derived primers were used.

The higher level of cross-species amplification obtained in the present study may be because PCR conditions were varied and optimised; we tried a variety of conditions including adjusting the magnesium chloride

Table 2. Summary of the number and proportion of loci amplifying a microsatellite, the number and proportion of microsatellites polymorphic, and mean number of alleles in Soay sheep, red deer and sika deer

Species	Loci giving microsatellite product	Proportion giving microsatellite (%)	Loci polymorphic	Proportion polymorphic (%)	Mean no. of alleles (SD)
Soay sheep	127/173	73.4	54/127	42.5	1.8 ± 1.2
Red deer	129/174	74.1	72/129	55.8	3.4 ± 2.9
Sika deer	126/171	73.7	47/126	37.3	1.6 ± 1.0

concentration and testing the effect of DMSO and TMACIDE, especially in deer. Of the other studies mentioned above, only Kuhn *et al.* (1996) optimised PCR conditions for all primers tested.

Another explanation for the higher rate of cross-species amplification found in the present study may be that there was a bias in the loci used (de Gortari *et al.* 1997), since they were initially selected for ease of use and high polymorphism content in cattle. Therefore our results were compared with those of de Gortari *et al.* (1997) in greater detail. Seventy-nine of the markers used in the present study were also used by de Gortari *et al.* (1997). They found that 49 (62.0%) of these markers gave a product in domestic sheep. This is not significantly different from the remainder of the markers giving a product in domestic sheep in that study (49/79 vs. 556/907: $\chi^2 = 0.463$, d.f. = 1, $P = 0.496$, NS). Furthermore, in the de Gortari study, these 49 markers did not have a greater mean number of alleles in domestic sheep (2.9 ± 1.8 SD), than did the remainder of the 605 loci (3.2 ± 2.1 SD). On this basis, there is no reason to suspect that the loci used in our study are biased in their degree of conservation across species or have an upwards bias in the number of alleles in non-source species.

A third source of variation between studies could be different criteria for designating amplification of monomorphic loci. It could be argued that where a monomorphic PCR product is observed in the test species, DNA sequencing is the only way of determining whether the product is the actual target microsatellite. All of our test species were run on gels alongside a cattle control. A monomorphic product was only regarded as a microsatellite if it was within 10 bp of the cattle size range and showed the same banding pattern, often a 'stutter' band typical of microsatellites.

Ideally, to demonstrate that we were amplifying homologous loci we would require sequence comparisons of PCR products and map positions in both source and test species. Therefore, we only claim provisionally that we are amplifying homologous loci. However, 46 of the markers tested here have been independently mapped in domestic sheep and shown to be homologues of cattle markers (see Table 1).

We expected to find a greater proportion of primers amplifying a product in Soay sheep than in either deer species. Deer are thought to have shared a common ancestor with cattle 20–25 Ma, while sheep are thought to have diverged from cattle more recently at 15 Ma (Modi *et al.* 1996). However, we found a similar

proportion of markers yielding a product in all three species. It is likely that this observation results from our using a wider range of PCR conditions in the deer than sheep. In particular, if a PCR product was found in one deer species, a concerted effort would be made to successfully amplify a product in the other. Only two loci (HUI177 and TGLA13) amplified a product in just one deer species. We suspect that an even greater proportion of cattle microsatellite markers might be useful in sheep if a further effort is made in adjusting PCR conditions (e.g. by adjusting annealing temperature during the PCR). Redesigning the primer pairs for loci that do not amplify a product across species may result in a higher still conservation rate. This approach was successfully used by Cockett *et al.* (1994) to map the callipyge gene in sheep.

Ninety-nine (56.8%) of the 174 markers tested here amplified a microsatellite in all three species. However, only 18 of these were informative in all three species. Additionally 10 of these 18 loci have previously been mapped in goat (Vaiman *et al.* 1996). These loci (IDVGA37, TGLA10, BM757, TGLA378, FSHB, VH98, IDVGA46, TGLA304, CSSM41 and CSSM43) are on 10 different bovine chromosomes and offer the greatest potential as markers useful in comparative mapping projects. Given the relatively low level of genetic variation in Soay sheep and sika deer (see below) it is not particularly surprising that a higher number of loci were not polymorphic across all species.

We found that red deer had a greater mean number of alleles than either Soay sheep or sika deer. This may in part be due to the fact that we tested more red deer than either of the other species. However, it should be noted that in contrast to red deer both of the other species have a history of small population sizes. The sika deer sampled were the result of introductions of small numbers to Scotland (Abernethy 1994) and Soay sheep are an ancient breed isolated on islands in the St Kilda archipelago, Scotland for many generations (Bancroft *et al.* 1995) and so probably have depressed levels of genetic variation. The number of alleles observed in Soay sheep should not be taken as typical for domestic sheep.

In conclusion, we suggest that the primer sites flanking artiodactyl derived microsatellites (and presumably the repeat itself) may be more highly conserved across closely related species than previously reported. Fries (1993) suggested that the bovine map can be used as a master map to establish genetic maps of other artiodactyls. This study provides further support that it should be possible to construct a

relatively dense comparative map of this agriculturally important taxon using Type 2, as well as Type 1 loci.

Acknowledgements

We thank Mike Tate (AgResearch, New Zealand) for providing DNA from the deer International Mapping Herd, and Stephen Kappes for providing data on the bovine microsatellite primers that did not amplify a product in sheep in the de Gortari *et al.* paper. The authors are funded by BBSRC, NERC, The Wellcome Trust and MAFF (grant LK0602).

References

- Abernethy K. (1994) The establishment of a hybrid zone between red and sika deer (genus *Cervus*). *Molecular Ecology* **3**, 551–62.
- Bahri-Darwich I., Vaiman D., Olsaker I., Oustry A. & Cribiu E.P. (1994) Assignment of bovine synteny group -U27 and group -U8 to R-banded chromosome -12 and chromosome -27, respectively. *Hereditas* **120** (3), 261–5.
- Bancroft D.R., Pemberton J.M. & King P. (1995) Extensive protein and microsatellite variability in an isolated, cyclic ungulate population. *Heredity* **74**, 326–36.
- Barendse W., Armitage S.M., Kossarek L.M. *et al.* (1994) A genetic linkage map of the bovine genome. *Nature Genetics* **6**, 227–35.
- Barendse W., Vaiman D., Kemp S.J. *et al.* (1997) A medium-density genetic linkage map of the bovine genome. *Mammalian Genome* **8**, 21–8.
- Bishop M.D., Kappes S.M., Keele J.W. *et al.* (1994) A genetic linkage map of cattle. *Genetics* **136**, 619–39.
- Brezinsky L., Kemp S.J. & Teale A.J. (1993) ILSTS6 a polymorphic bovine microsatellite. *Animal Genetics* **24** (1), 73.
- Bruford M.W. & Wayne R.K. (1993) Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development* **3**, 939–43.
- Cockett N.E., Jackson S.P., Shay T.L. *et al.* (1994) Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proceedings of the National Academy of Sciences of the United States of America* **91** (8), 3019–23.
- Crawford A.M., Montgomery G.W., Pierson C.A. *et al.* (1994) Sheep linkage mapping: nineteen linkage groups derived from the analysis of paternal half-sib families. *Genetics* **137**, 573–9.
- Crawford A.M., Dodds K.G., Ede A.J. *et al.* (1995) An autosomal genetic linkage map of the sheep genome. *Genetics* **140**, 703–24.
- Engel S.R., Linn R.A., Taylor J.F. & Davis S.K. (1996) Conservation of microsatellite loci across species of artiodactyls: implications for population studies. *Journal of Mammalogy* **77** (2), 504–18.
- Fisher R.A. (1958) *The Genetical Theory of Natural Selection*. Dover Publications, New York.
- FitzSimmons N.N., Moritz C. & Moore S.S. (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution* **12** (3), 432–40.
- Fries R. (1993) Mapping the bovine genome: methodological aspects and strategy. *Animal Genetics* **24**, 111–6.
- Fries R., Eggen A. & Womack J.E. (1993) The bovine genome map. *Mammalian Genome* **4**, 405–28.
- Gemmell N.J. (1997) Enhancement of microsatellite amplification using tetramethylammonium chloride and formamide (TMACIDE). *Technical Tips Online* (<http://www.elsevier.nl/locate/tto>) T40029.
- Gemmell N.J., Allen P.J., Goodman S.J. & Reed J.Z. (1997) Interspecific microsatellite markers for the study of pinniped populations. *Molecular Ecology* **6**, 661–6.
- Georges M. & Massey J.M. (1992) Polymorphic DNA markers in *Bovidae*. Patent W092/13102.
- de Gortari M.J., Freking B.A., Kappes S.M. *et al.* (1997) Extensive genomic conservation of cattle microsatellite heterozygosity in sheep. *Animal Genetics* **28**, 274–90.
- de Gortari M.J., Freking B.A., Cuthbertson R.P. *et al.* (1998) A second generation linkage map of the sheep genome. *Mammalian Genome* **9**, 204–9.
- Gustafsson L. (1986) Lifetime reproductive success and heritability: empirical support for Fisher's fundamental theorem. *The American Naturalist* **128** (5), 761–4.
- Hanrahan V., Ede A.J., Pierson C.A. & Hill D.F. (1993) Ovine microsatellites at the *OarVH98*, *OarVH110*, *OarVH116*, *OarVH117* and *OarVH130* loci. *Animal Genetics* **24**, 223.
- Inoue M., Hirano T., Nakane S. *et al.* (1995) Five bovine polymorphic dinucleotide loci (DIK008, DIK010, DIK015, DIK016, DIK020). *Animal Genetics* **26** (6), 447.
- Jarne P. & Lagoda P.J.L. (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11** (10), 424–9.
- Jorgenson C., Konfortov B.A., Barendse W. & Miller J.R. (1995) Two polymorphic bovine microsatellite loci (AFR227 and AFR2215). *Animal Genetics* **26** (2), 132.
- Kaukinen J. & Varvio S.L. (1993) Eight polymorphic bovine microsatellites. *Animal Genetics* **24**, 148.
- Kemp S.J., Hishida O., Wamburger J. *et al.* (1995) A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Animal Genetics* **26**, 2993–06.
- Konfortov B.A., Jorgensen C.B., Barendse W. & Miller J.R. (1996) Five bovine polymorphic markers (AF1AF5). *Animal Genetics* **27** (3), 220.
- Kossarek L.M., Grosse W.M., Findlay O., Barendse W., Hetzel D.J.S. & McGraw R.A. (1994) Bovine dinucleotide repeat polymorphism RM209. *Journal of Animal Science* **72**, 528.
- Kossarek L.M., Finlay O., Grosse W.M. *et al.* (1995) Five bovine dinucleotide repeat polymorphisms: RM040, RM044, RM096, RM153 and RM179. *Animal Genetics* **26**, 57–8.
- Kossarek L.M., Finlay O., Grosse W.M., Su X. &

- McGraw R.A. (1996) Seven bovine dinucleotide repeat polymorphisms: RM042, RM128, RM134, RM154, RM162, RM181 and RM509. *Animal Genetics* **27**, 61.
- Kuhn R., Anastassiadis C. & Pirchner F. (1996) Transfer of bovine microsatellites to the cervine (*Cervus elaphus*). *Animal Genetics* **27**, 199–201.
- Mezzelani A., Zhang Y., Redaelli L. *et al.* (1995) Chromosomal localization and molecular characterization of 53 cosmid-derived bovine microsatellites. *Mammalian Genome* **6** (9), 629–35.
- Mitchell-Olds T. (1995) The molecular basis of quantitative genetic variation in natural populations. *Trends in Ecology and Evolution* **10** (8), 324–8.
- Modi W.S., Gallagher D.S. & Womack J.E. (1996) Evolutionary histories of highly repeated DNA families among the artiodactyla (mammalia). *Journal of Molecular Evolution* **42**, 337–49.
- Moore S.S., Sargeant L.L., King T.J., Mattick J.S., Georges M. & Hetzel D.J.S. (1991) The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* **10**, 654–60.
- Moore S.S., Barendse W., Berger K.T., Armitage S.M. & Hetzel D.J.S. (1992) Bovine and ovine DNA microsatellites from the EMBL and GENBANK databases. *Animal Genetics* **23**, 463–7.
- Moore S.S., Byrne K., Berger K.T. *et al.* (1994) Characterization of 65 bovine microsatellites. *Mammalian Genome* **5** (2), 84–90.
- Mousseau T.A. & Roff D.A. (1987) Natural selection and the heritability of fitness components. *Heredity* **59**, 181–97.
- O'Brien S.J., Womack J.E., Lyons L.A., Moore K.J., Jenkins N.A. & Copeland N.G. (1993) Anchored reference loci for comparative genome mapping in mammals. *Nature Genetics* **3**, 103–12.
- Pepin L., Amigues Y., Lepingle A., Berthier J.-L., Bensaid A. & Vaiman D. (1995) Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis. *Heredity* **74**, 53–61.
- Pfister-Genskow M.A., Hawkins G.A., Doud L.I. *et al.* (1995) Bovine dinucleotide repeat polymorphisms ABS010, ABS011, ABS012, ABS013 and ABS014. *Journal of Animal Science* **73** (10), 3189.
- Queller D.C., Strassman J.E. & Hughes C.R. (1993) Microsatellites and kinship. *Trends in Ecology and Evolution* **8** (8), 285–8.
- Sambrook J., Fritsch E.F. & Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory Press, New York.
- Shalom A., Soller M. & Friedmann A. (1993) Dinucleotide repeat polymorphism at the bovine HUI616 locus. *Animal Genetics* **24** (4), 327.
- Shalom A., Mosig M.O., Barendse W.A., Soller M. & Freidmann A. (1994) Dinucleotide repeat polymorphism at the bovine HUI246, HUII77, HUI223, HUIV174 and HUII75 loci. *Animal Genetics* **25** (1), 56.
- Shalom A., Mosig M.O., Barendse W., Soller M. & Freidmann A. (1995) Dinucleotide repeat polymorphisms at the bovine HUI673, HUI121, HUI174, HUI225, HUII13 and HUII29 loci. *Animal Genetics* **26** (3), 202–3.
- Solinas-Toldo S., Fries R., Steffen P. *et al.* (1993) Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. *Mammalian Genome* **4** (12), 720–7.
- Steffen P., Eggen A., Dietz A.B., Womack J.E., Sranzinger G. & Fries R. (1993) Isolation and mapping of polymorphic microsatellites in cattle. *Animal Genetics* **24** (2), 121–4.
- Sun H.S., Whallon S., de Ponce Leon A. & Kirkpatrick B.W. (1993) Development of polymorphic bovine microsatellites from a cosmid library. *Journal of Animal Science* **71** (Suppl.), 100.
- Sun H.S., Huff B.M., Dentine M.R. & Kirkpatrick B.W. (1994) Polymorphic bovine microsatellites UWCA5, UWCA7 and UWCA9. *Animal Genetics* **25** (1), 59.
- Tate M.L., Mathias H.C., Fennessy P.F., Dodds K.G., Penty J.M. & Hill D.F. (1995) A new gene mapping resource: interspecies hybrids between Père David's deer (*Elaphurus davidianus*) and red deer (*Cervus elaphus*). *Genetics* **139**, 1383–91.
- Thieven U., Solinas-Toldo S., Friedl R. *et al.* (1997) Polymorphic CA microsatellites for the integration of the bovine genetic and physical map. *Mammalian Genome* **8** (1), 52–5.
- Vaiman D., Osta R., Mercier D., Grohs C. & Leveziel H. (1992) Characterization of 5 new bovine dinucleotide repeats. *Animal Genetics* **23** (6), 537–41.
- Vaiman D., Imam-Gahli M., Moazami-Goudarzi K. *et al.* (1994a) Conservation of a syntenic group of microsatellite loci between cattle and sheep. *Mammalian Genome* **5** (5), 310–4.
- Vaiman D., Mercier D., Eggen A. *et al.* (1994b) A genetic and physical map of bovine chromosome-11. *Mammalian Genome* **5** (9), 553–6.
- Vaiman D., Mercier D., Moazami-Goudarzi K. *et al.* (1994c) A set of 99 cattle microsatellites-characterization, syntenic mapping and polymorphism. *Mammalian Genome* **5** (5), 288–97.
- Vaiman D., Schibler L., Bourgeois F., Oustry A., Amigues Y. & Cribiu E.P. (1996) A genetic linkage map of the goat genome. *Genetics* **144**, 279–305.
- Williams J.L., Urquhart B.G. & Barendse B. (1995) 3 bovine chromosome-15 microsatellite markers. *Animal Genetics* **26** (2), 124.
- Williams J.L., Morgan A.L.G., Guerin G. & Urquhart B.G.D. (1996) Ten new cosmid-derived bovine microsatellite markers. *Animal Genetics* **27** (5), 380.
- Zhang Y., Redaelli L., Castiglioni B., Mezzelani A. & Ferretti L. (1995) Five polymorphic bovine microsatellite loci (IDVGA62-A, IDVGA71, IDVGA82, IDVGA88, IDVGA90). *Animal Genetics* **26** (5), 65–6.

Power to detect QTL in a free-living polygynous population

J. SLATE*†, J. M. PEMBERTON† & P. M. VISSCHER‡

†Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT and ‡Institute of Ecology and Resource Management, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG, U.K.

Evolutionary biologists attempting to detect quantitative trait loci (QTL) in natural populations have thus far had to carry out some kind of cross with their study organism. Such techniques often increase the probability of detecting linkage, but are impossible or undesirable in many populations. A potential way of overcoming this problem is to carry out linkage studies in large complex pedigrees. In this paper we contrast the power to detect linkage in a complex pedigree of red deer (*Cervus elaphus*) with that of the widely used half-sib design. When a QTL of magnitude 1 phenotypic standard deviation is segregating and completely linked to a marker, the extended pedigree has power of 0.67, whereas the half-sib design has power of just 0.21. We conclude that detecting QTL may be possible in intensively studied natural populations, provided detailed life history data and good pedigree information are available.

Keywords: *Cervus elaphus*, genetic mapping, major gene, natural population, pedigree, red deer.

Introduction

Evolutionary biologists have long been interested in genetic variation for fitness in natural populations. One unresolved issue is whether genetic variation for fitness and fitness components is caused by many polygenes of small effect, or whether genes of major effect can segregate despite natural selection (Barton & Turelli, 1989; Orr & Coyne, 1992). Recent attention has focused on the possibility of mapping genes for fitness in natural populations, given the advent of suitable markers, maps and statistical methodology (Mitchell-Olds, 1995). However, very few studies have, as yet, attempted to do this, and none has managed it in an unmanipulated population actually *in the wild*. The vast majority of linkage mapping projects have taken place in humans, laboratory model organisms, e.g. *Drosophila* (Shrimpton & Robertson, 1988), mouse (Keightley & Bulfield, 1993) or domestic species, e.g. cattle (Georges *et al.*, 1995) and tomato (Paterson *et al.*, 1988).

A few mapping programmes have been carried out in 'natural' populations of plants, most notably within the genus *Mimulus* (e.g. Bradshaw *et al.*, 1995; Lin & Ritland, 1997). However, these studies have taken place within artificial crosses, a technique which elevates the amount of phenotypic and additive genetic variation

segregating within the cross, relative to the situation in the wild. Furthermore, selective genotyping (Darvasi & Soller, 1992) may have been employed. Both of these techniques improve the chances of detecting QTL but may be impossible in a large number of natural populations. Selective genotyping is applicable only when a single trait (or several phenotypically highly correlated traits) is of interest, whereas in many populations it is neither desirable nor possible to perform crosses. In addition QTL segregating within a cross do not necessarily segregate in a single wild population.

Where it is not possible to create specific crosses, researchers might turn to an alternative experimental design: the use of complex, extended pedigrees. To carry out a QTL mapping programme without manipulating a pedigree is analogous to complex disease mapping in human populations, although considerably more difficult. To obtain a pedigree of a natural population is nontrivial. Additionally, genetic maps of wild species will not be as dense (with the possible exception of wild mouse populations) as the human map. The biggest problem for QTL mappers of natural populations is a lack of power in the pedigrees available (if any) to actually detect linkage.

Considerable attention has been given in the animal breeding literature to the relative power of half-sib designs and more complicated family structures, in detecting QTL (Weller *et al.*, 1990; van der Beek *et al.*,

*Correspondence. E-mail: j.slate@ed.ac.uk

1995). Weller *et al.* (1990) examined the power to detect QTL for the half-sib (or daughter) design and compared this to a three-generation granddaughter design. In the daughter design each sire has a number of daughters by different dams. All animals are genotyped and progeny have recorded trait values. The alternative granddaughter design was developed with dairy cattle in mind: it requires sires to have a medium to large number of sons, who in turn sire a large number of daughters. The sons of the sires are genotyped and trait values of their daughters are recorded. Breeding values of sons are accurately estimated by taking daughter phenotypic means. Weller *et al.* (1990) conclude that although not a particularly complex pedigree, the granddaughter design was a more powerful method than the half-sib design in detecting QTL. Additionally, less genotyping is required in obtaining this enhanced power. Georges *et al.* (1995) successfully used this design to detect milk production QTL in dairy cattle.

Human geneticists have also focused on the relative merits of extended pedigrees vs. nuclear families for detecting linkage. The recent Genetics Analysis Workshop 10 (GAW10) set a challenge to a number of groups to detect QTL for an oligogenic trait segregating in two simulated data sets, one containing nuclear families and the other an extended pedigree (Wijsman & Amos, 1997). The number of phenotypes and genotypes was equal for both data sets. Those analyses that used the extended data set were generally more successful in detecting the simulated disease loci, estimated QTL location more accurately and detected fewer false positives. Two properties of extended pedigrees lead to this increased power. First, there are more meioses in the extended pedigree than the nuclear family design for the same number of genotypes. Secondly, marker phase is estimated more accurately over several generations than over one. Recent statistical advances have enabled geneticists to use information from extended pedigrees rather than their constituent nuclear families or sibships (Almasy & Blangero, 1998). Approaches such as these may enable evolutionary biologists to search for QTL without performing crosses within their study population. However, utilizing whole pedigrees does have disadvantages. Analyses can be computationally demanding, and statistical techniques familiar to those using simple crosses, such as least squares, cannot be performed.

In this paper we investigate, by simulation, the power of QTL analyses in a free-living red deer population, using a large complex pedigree or the series of half-sibships that comprise it. The red deer on Rum, Inner Hebrides, Scotland have been intensively studied for over 25 years, with life history data collected for over 2000 deer. The deer genome is now reasonably well

mapped and includes over 600 markers of which 130 are microsatellites (Tate, 1997). In principle it is possible to screen the Rum red deer for a large number of mapped, polymorphic markers and carry out a search for fitness-related QTL. The main purpose of this paper is to investigate three questions about the power to detect QTL in this population. First, do small half-sibships contribute towards power? Secondly, do least squares and likelihood approaches give similar estimates of power for the half-sib design? Thirdly, the main aim of the paper, do the complex pedigree and the half-sib design give similar estimates of power when using the likelihood approach? If the complex pedigree gives greater power than the half-sib design then this approach may be useful in attempting to map QTL in the wild.

Methods

The study population

The deer in the north block of Rum have been intensively studied since 1971 and are one of the best characterized free-living mammal populations in the world. For a review of the first decade of the study and a description of the study site see Clutton-Brock *et al.* (1982). Since 1982 over 80% of new-born calves have been sampled for genetic purposes. Mother-offspring pairs in the population are determined by observation in the field. Typing of over 1100 individuals for nine mapped microsatellite loci and three allozymes has revealed no mis-assignments of maternity (Marshall *et al.*, 1998). Paternity was assigned using CERVUS (Marshall *et al.*, 1998), a program for inferring paternity in unmanaged populations at any required statistical confidence. CERVUS uses population characteristics including allele frequencies to calculate, by simulation, likelihood thresholds for determining paternity at the desired confidence. 875 calves born between 1982 and 1996 were analysed of which 475 calves were assigned sires with 80% confidence and 203 with 95% confidence (Marshall *et al.*, 1998). The polygynous mating system of red deer results in large numbers of half-sibs, but relatively few full-sibs.

Pedigree used in simulation studies

The Rum pedigree was visualized using PEDVIEW (Kinghorn, 1994). Features such as large half-sibships and successful individuals were identified. One particular stag, MAXI, was investigated more fully for a number of reasons. First, MAXI was extremely successful, siring 21 calves at 95% confidence and a further 12 at 80% confidence, for most of whom we have life history data. Secondly, a number of MAXI's offspring were extremely

successful themselves (see Fig. 1) and to date MAXI has at least 416 known descendants since his birth in 1971. Thirdly, MAXI was an F_1 between a Rum hind and a mainland stag (Lincoln *et al.*, 1973) and so may have introduced novel additive genetic variation into the population. For the same reason marker heterozygosity may be higher within this specific pedigree. For the purposes of our simulations we have analysed the MAXI pedigree. DNA samples and life history data are available for all individuals used in our simulations. The 416 individuals are spread over six generations. In total there are 73 sibships ranging in size from two to 27.

The alternative half-sib design with which we compared the MAXI pedigree was simply the composite half- and full-sibships within the larger pedigree. Breaking down the complex pedigree in this way violates the assumption that the nuclear families are independent, but analysis is simplified such that least squares methods can be employed.

Simple least-squares-based analysis of power

A quick method to estimate the power to detect QTL in the constituent half-sibships of the larger pedigree was

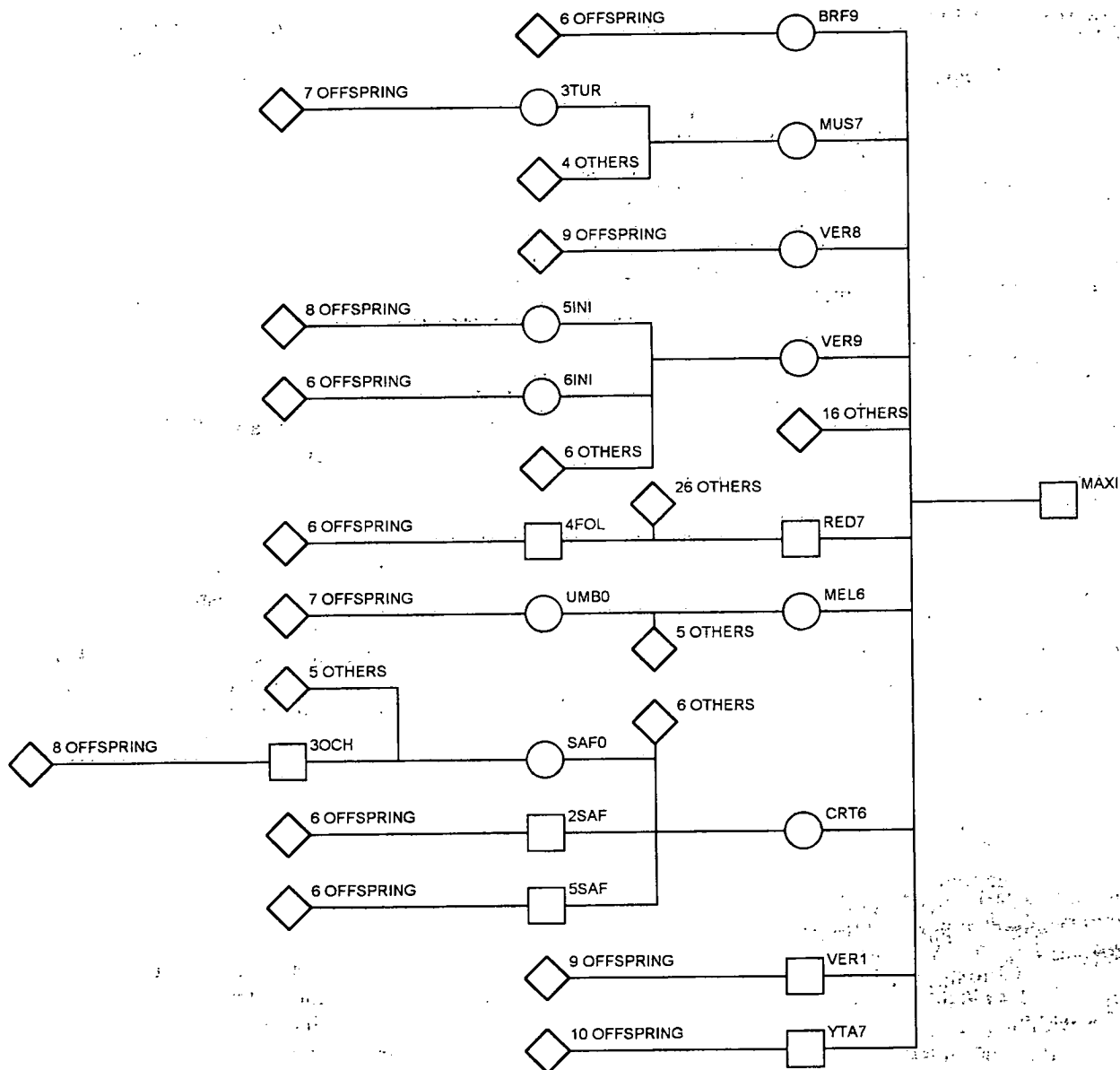


Fig. 1 Pedigree showing MAXI, and his most reproductively successful descendants. Squares represent males, circles females and diamonds five or more individuals.

developed by one of us (P.V.) using computer simulation. We used this 'Weller approach' to investigate whether small sibships contribute to power, and also to compare the power of this approach with a likelihood approach for a half-sib design. This analysis is an extension of that used by Weller *et al.* (1990) when evaluating the daughter design, but allows for sibships of unequal size. In the balanced half-sib design used by Weller *et al.* (1990), the distribution of the test statistic under both the null hypothesis (no QTL segregating) and the alternative hypothesis can be approximated by a central and a noncentral χ^2 , respectively. With unequal family sizes, the test statistic under the alternative hypothesis is not obvious, and we therefore use the following approach.

For each replicated population the sum of squares attributable to the marker contrasts within sires was sampled by:

- 1 determining whether each sire is homozygous or heterozygous at the QTL, by sampling from a binomial distribution;
- 2 binning each progeny into one or other marker allele class (all sires are heterozygous for the marker), again by sampling from a binomial distribution;
- 3 sampling the means of each marker class from a normal distribution with the mean difference between the marker classes equal to the QTL effect. The squared difference between the means, standardized by the variance of the difference between the two means, is a draw from a noncentral χ^2 distribution. It was thus assumed that the within-sire variance was known (or estimated without error).

Summing this marker contrast sum of squares across all sires gives the test statistic. Power is calculated by determining the probability of this test statistic exceeding a central χ^2 of Type I error α , with degrees of freedom equal to the number of sires which have progeny in both marker classes.

Unlike Weller *et al.* (1990) we have investigated half-sibships of differing size, as will be the case for most natural populations. In addition, we have taken into account the finite sampling of progeny group sizes for each marker class. We have assumed that all sires are heterozygous for the marker allele and that there is no recombination between marker and trait loci. We used the following parameter values for simulations. The QTL was assumed to be biallelic, with each allele at frequency 0.5. QTL magnitude was varied in increments of 0.1 of a within-QTL genotype phenotypic standard deviation, so that the difference between the heterozygote and either homozygote was 0.5–1.0 SD. Polygenic heritability was 0.5. We examined the effect of using all sibships (two sibs or more) or those containing greater

than three, four, or five sibs, on the power of detecting linkage. Ten thousand replications were performed throughout.

SIMLINK program

Although the Weller approach gives a rapid and accurate estimate of power within the half-sib design, it cannot be used to analyse more complex pedigrees. To perform power comparisons between the extended pedigree and the half-sib designs (the main purpose of this paper) we used the likelihood-based program SIMLINK (Boehnke, 1986; Ploughman & Boehnke, 1989). SIMLINK also enables investigation of the effects of marker heterozygosity and recombination on power. SIMLINK tests whether a segregating major gene can be detected when linked to a marker, compared to the null hypothesis of a major gene segregating but being unlinked. Note that SIMLINK and the Weller approach are performing subtly different statistical tests. The Weller approach tests for a QTL segregating against the null hypothesis of no QTL, whereas SIMLINK tests for a QTL linked to a marker, compared with a QTL segregating but unlinked to the marker. The implications of this difference are discussed. We compared the power to detect linkage in the extended pedigree and its constituent sibships, whilst also investigating the effect of QTL magnitude, marker heterozygosity and recombination between marker and QTL. One hundred replications were performed throughout.

SIMLINK parameters

- 1 QTL magnitude: this was as for the Weller approach.
- 2 Recombination fraction: the distance between marker and trait locus was set to 0, 10 or 20 cM.
- 3 Marker heterozygosity: the number of marker alleles was varied from two to nine with all alleles at equal frequency. This meant that marker heterozygosity varied from 0.5 to 0.89. Unlike the Weller approach, a marker heterozygosity of unity is impossible with SIMLINK. However, the parameter values used in SIMLINK are more realistic. In practice no single marker has a heterozygosity of unity.

Significance thresholds

The relationship between the χ^2 -test of the Weller approach and the likelihood test of SIMLINK is complicated, but the same significance level for both is essential to make a comparison between the two approaches for a half-sib design. Baret *et al.* (1998) examined the relationship between the test statistics for the *F*-statistic (or its approximation by χ^2) and a likelihood ratio test, for

the half-sib design. They showed for the null hypothesis of no QTL segregating, that a likelihood ratio test (or LOD score) follows an approximate distribution of half times 0 and half times a χ^2 with d.f.=1. This can be approximated by using a χ^2 distribution with 1 d.f. and doubling the probability value. So, if SIMLINK is used to test the power of obtaining a LOD of 3.0 (corresponding to a probability of 0.0001) then to perform the equivalent test with the Weller approach a *P*-value of 0.00005 (half of 0.0001) should be used.

Results

Contribution of smaller sibships to power

We used the Weller approach to examine the effect of the smaller half-sibships on the power to detect linkage to a QTL (see Table 1). Of the 73 sibships within the MAXI pedigree, 31 of them only had two sibs and 17 just three sibs. Power was greatest when all half-sibships were used, but the contribution from the half-sibships with fewer than five progeny was small. In small sibships all offspring often inherit the same sire marker allele. In consequence the average number of informative sires will vary across replications of the simulation, and the Type 1 threshold will not be constant. As the

smaller families contributed little power, all subsequent Weller simulations were performed with the 18 half-sibships containing five or more offspring. This meant that a single Type 1 threshold could be used (assuming that all 18 sires were informative; see Table 1), and comparisons between Weller *et al.*'s (1990) least squares approach and the likelihood method of SIMLINK could be made.

Comparison of the Weller and SIMLINK approaches for the half-sib design

Both methods were used to investigate the power of detecting linkage to a QTL for the constituent half-sib design. For a QTL of effect 0.5–1.0 phenotypic standard deviation, with no recombination between marker and trait loci, both methods yielded similar results (Table 2), although the Weller approach tended to give slightly higher power. This is perhaps unsurprising given that the Weller approach makes the assumption that all sires are heterozygous at the marker allele, whereas for our SIMLINK simulations we used a marker heterozygosity of 0.89 (see Methods). The maximum discrepancy in power between the two programs was 0.08.

Table 1 Effect of small half-sibships on power to detect QTL using a 'Weller approach' half-sib design

Minimum sibship size	Number of sibships	Mean number of informative sires	Average Type 1 threshold (χ^2 units)	Average test statistic (χ^2 units)	Power
2	73	52.0	69.7	84.5	0.81
3	42	36.5	51.5	64.5	0.80
4	25	23.1	36.0	46.7	0.78
5	18	17.5	28.3	37.7	0.76

Heritability was set to 0.5, QTL effect was 1.0 phenotypic standard deviation, and a significance threshold of 0.05 was used. Ten thousand replications were performed. The number of informative sires is less than the actual number of sires because all offspring in small sibships can inherit the same sire marker allele.

Table 2 Comparison of the Weller approach and a LOD score approach (SIMLINK) for power of the half-sib design

QTL effect (SD)	0.5 (W)	0.5 (S)	1.0 (W)	1.0 (S)	2.0 (W)	2.0 (S)	3.0 (W)	3.0 (S)
0.5	0.22	0.22	0.10	0.02	0.01	0.00	0.00	0.00
0.6	0.33	0.30	0.15	0.14	0.03	0.01	0.00	0.00
0.7	0.44	0.38	0.25	0.23	0.06	0.02	0.01	0.01
0.8	0.56	0.49	0.35	0.31	0.10	0.10	0.03	0.01
0.9	0.68	0.62	0.49	0.42	0.18	0.18	0.07	0.03
1.0	0.78	0.72	0.60	0.52	0.30	0.27	0.14	0.10

Columns refer to power of obtaining LOD scores in the range 0.5–3.0 for the Weller (W) and SIMLINK (S) approaches. QTL effect ranged from 0.5 to 1.0 of a phenotypic standard deviation. Marker heterozygosity was 1.00 for the Weller approach and 0.89 for SIMLINK. Heritability was set to 0.5 in the Weller approach. Ten thousand replicates were performed for the Weller method and 100 for SIMLINK. The Weller approach used only sibships with five or more offspring.

Table 3 Comparison of power for an extended pedigree and a half-sib design using SIMLINK. Mean LOD score and power of obtaining LOD score greater than 0.5–3.0 for the two designs

Recombination fraction	QTL effect (SD)	Extended pedigree					Half-sib design				
		Mean LOD ($\pm$ SE)	Power > 0.5	Power > 1.0	Power > 2.0	Power > 3.0	Mean LOD ($\pm$ SE)	Power > 0.5	Power > 1.0	Power > 2.0	Power > 3.0
0	0.5	0.676 (0.069)	0.49	0.24	0.06	0.02	0.246 (0.033)	0.22	0.02	0.00	0.00
	0.6	1.069 (0.093)	0.67	0.41	0.18	0.04	0.377 (0.047)	0.30	0.14	0.01	0.00
	0.7	1.557 (0.122)	0.78	0.56	0.32	0.17	0.551 (0.063)	0.38	0.23	0.02	0.01
	0.8	2.126 (0.150)	0.85	0.75	0.46	0.26	0.769 (0.080)	0.49	0.31	0.10	0.01
	0.9	2.769 (0.177)	0.92	0.82	0.60	0.40	1.033 (0.097)	0.62	0.42	0.18	0.03
	1.0	3.475 (0.203)	0.97	0.90	0.74	0.51	1.346 (0.115)	0.72	0.52	0.27	0.10
10	0.5	0.403 (0.049)	0.34	0.10	0.01	0.00	0.183 (0.028)	0.11	0.03	0.00	0.00
	0.6	0.608 (0.067)	0.43	0.22	0.06	0.01	0.266 (0.039)	0.22	0.05	0.00	0.00
	0.7	0.860 (0.085)	0.55	0.38	0.09	0.03	0.366 (0.050)	0.25	0.13	0.02	0.00
	0.8	1.152 (0.105)	0.63	0.44	0.18	0.07	0.485 (0.062)	0.31	0.17	0.03	0.00
	0.9	1.485 (0.124)	0.77	0.56	0.26	0.11	0.623 (0.073)	0.42	0.19	0.05	0.03
	1.0	1.850 (0.143)	0.85	0.71	0.39	0.19	0.779 (0.084)	0.53	0.27	0.10	0.04
20	0.5	0.239 (0.039)	0.19	0.08	0.00	0.00	0.141 (0.023)	0.05	0.02	0.00	0.00
	0.6	0.334 (0.050)	0.25	0.11	0.01	0.00	0.191 (0.030)	0.12	0.03	0.00	0.00
	0.7	0.448 (0.061)	0.26	0.19	0.05	0.00	0.244 (0.037)	0.20	0.04	0.00	0.00
	0.8	0.577 (0.072)	0.37	0.19	0.08	0.00	0.299 (0.044)	0.26	0.09	0.01	0.00
	0.9	0.729 (0.082)	0.43	0.25	0.08	0.03	0.355 (0.050)	0.27	0.10	0.02	0.00
	1.0	0.895 (0.092)	0.53	0.35	0.14	0.03	0.415 (0.056)	0.29	0.16	0.03	0.00

Marker heterozygosity was set to 0.89, recombination fraction between marker and QTL was set to 0, 10 or 20 cM, and QTL effect was 0.5–1.0 phenotypic standard deviations. One hundred replications performed throughout.

Comparison of mean LOD score and power between an extended pedigree and its constituent sibships

Table 3 compares the results obtained from SIMLINK using the extended pedigree and using the constituent half-sibships analysed separately. Mean LOD score and power are compared when recombination between marker and trait locus is set to 0, 10 or 20 cM. Figure 2 illustrates the effect of QTL magnitude on mean LOD for all six scenarios considered (extended and half-sib pedigrees at three different recombination fractions). The mean LOD score was always greater for the extended pedigree than for the equivalent half-sib design. The relative difference between the two designs was greater when recombination fraction was lower. At 0 cM the extended pedigree mean LOD was 2.5–3.0 times greater than for the half-sib design, whereas at 20 cM the ratio was only 1.5–2.0.

The relationship between pedigree design and power was similar to that of pedigree design and mean LOD score. Figure 3 illustrates the power of obtaining LOD scores in the range 0.5–3.0 for some of the models tested. When there was no recombination between the marker and the QTL, the extended pedigree had more than double the power of the half-sib design to obtain a LOD

score of 2.0 and above, regardless of QTL effect. Power rapidly decreased with increasing recombination fraction between marker and QTL for both designs.

Effect of marker heterozygosity on mean LOD score and power

We also examined the effect of marker heterozygosity on mean LOD score and power, for both extended and half-sib designs (Table 4). When marker heterozygosity was reduced from 0.89 to 0.50, mean LOD score for both designs was more than halved. However, the extended pedigree still had a mean LOD score more than 2.5 times greater than that of the half-sib design. At the lower marker heterozygosity (0.50), the power to obtain a LOD score of 3.0 had decreased from the original value of 0.51 to just 0.10 for the extended pedigree.

Discussion

We have demonstrated that the probability of detecting QTL in an unmanaged free-living population of red deer is greatly increased if an extended pedigree rather than a simple half-sib design is used. This is the first time that complex pedigrees have been considered for mapping QTL in wild populations and the results are encouraging.

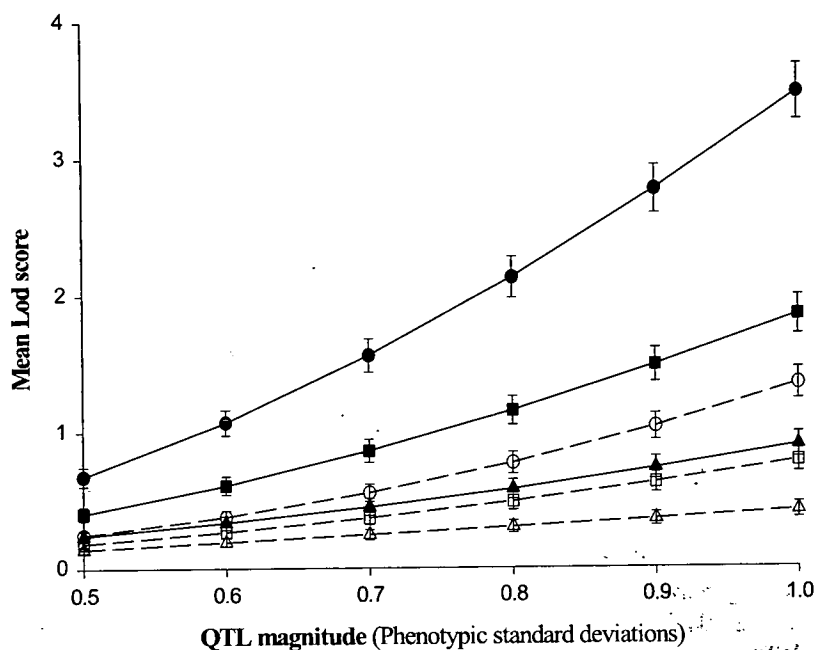


Fig. 2 Plot showing effect of QTL magnitude on mean LOD score for extended pedigree and half-sib design. Marker heterozygosity = 0.89. One hundred replicates performed.

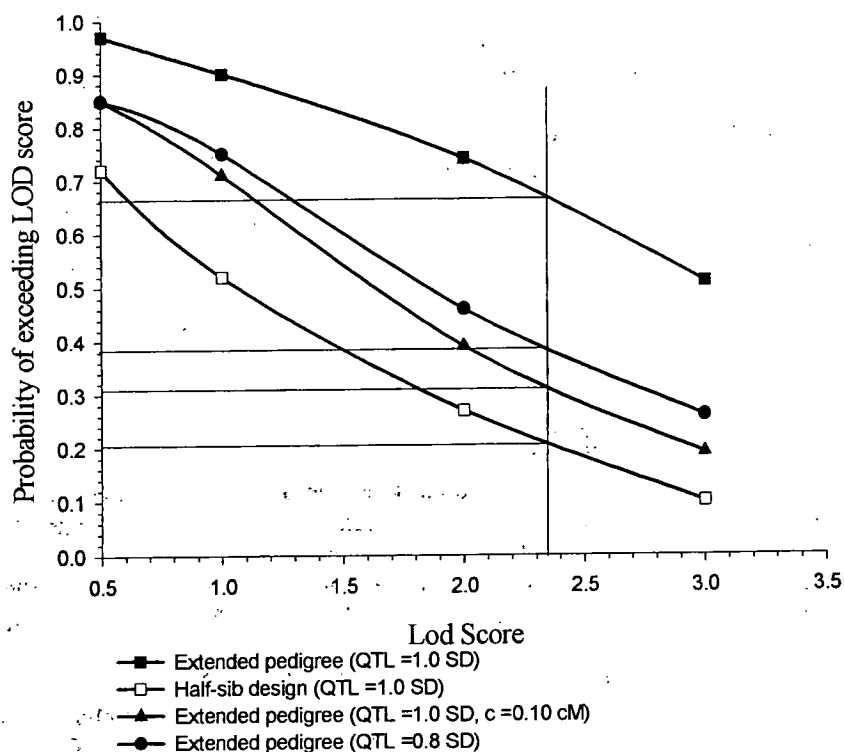


Fig. 3 Power curves for extended pedigree and half-sib designs. Curves show the power of obtaining LOD scores in the range 0.5–3.0. The vertical line represents a LOD score of 2.34, equivalent to a genome-wide significance level of 0.05 when 100 markers are screened (see Discussion). When a QTL of 1.0 SD is segregating and there is no recombination between marker and QTL, the extended pedigree has a power of 0.67 whereas the half-sib design has a power of 0.21. The extended pedigree has a power of 0.31 when recombination is 10 cM. For a QTL of effect 0.8 SD, and no recombination, the extended pedigree has power 0.38.

Table 4 Effect of marker heterozygosity on mean LOD score and power

Marker alleles	Marker heterozygosity	Extended pedigree					Half-sib design				
		Mean LOD ($\pm$ SE)	Power > 0.5	Power > 1.0	Power > 2.0	Power > 3.0	Mean LOD ($\pm$ SE)	Power > 0.5	Power > 1.0	Power > 2.0	Power > 3.0
2	0.500	1.355 (0.110)	0.76	0.51	0.23	0.10	0.518 (0.065)	0.33	0.15	0.05	0.01
3	0.667	2.062 (0.150)	0.85	0.73	0.44	0.25	0.838 (0.081)	0.58	0.31	0.14	0.01
4	0.750	2.565 (0.172)	0.93	0.79	0.57	0.32	0.977 (0.088)	0.62	0.41	0.14	0.03
5	0.800	2.971 (0.181)	0.91	0.87	0.69	0.43	1.158 (0.106)	0.68	0.45	0.16	0.08
6	0.833	3.183 (0.186)	0.97	0.88	0.67	0.50	1.246 (0.107)	0.71	0.49	0.23	0.09
7	0.857	3.230 (0.186)	0.95	0.90	0.73	0.47	1.302 (0.113)	0.72	0.49	0.23	0.09
8	0.875	3.336 (0.195)	0.95	0.90	0.73	0.51	1.322 (0.113)	0.73	0.49	0.24	0.10
9	0.889	3.475 (0.203)	0.97	0.90	0.74	0.51	1.346 (0.115)	0.72	0.52	0.27	0.10

Mean LOD score and power of achieving LOD score greater than 0.5–3.0 for an extended pedigree and a half-sib design. QTL effect was 1.0 SD, recombination between marker and QTL was 0 cM and 100 replicates were performed.

A number of field studies could now produce pedigrees several generations deep, and similar to the one we use in our analysis.

Why does the extended pedigree yield greater power than the half-sib design? The extended pedigree uses genotype and phenotype information on all individuals, and by tracing marker alleles down the pedigree, calculates the likelihood of a major gene segregating. In other words, both between- and within-family information is considered. In contrast, the half-sib design uses only within-family information.

With a real QTL mapping data set, one would probably perform a test that compares a model of QTL and background polygenic effect against a model which had only a polygenic effect. Our comparison of a half-sib design and an extended pedigree used a model of QTL linked vs. QTL present but unlinked. Our own data, and also those of Le Roy & Elsen (1995) suggest that the former test may give a slightly higher test statistic than the SIMLINK comparison. In our half-sib simulations the Weller approach gave marginally higher power than SIMLINK, although this may be caused by the slightly higher marker heterozygosity for the Weller approach. Le Roy & Elsen (1995) also demonstrate that a LOD score approach (testing QTL linked vs. QTL unlinked) gives lower power relative to a Weller approach, particularly for a three-generation design compared to a half-sib design. This would suggest that our test statistic for the extended pedigree may be downwardly biased, making our comparison between the extended pedigree and half-sib design conservative. It should also be pointed out that our approach does not account for any polygenic variation that may be segregating. However, there is no evidence that ignoring a polygenic effect inflates our test statistic. Knott & Haley (1992) simulated a polygenic component in a full-sib design and showed that a QTL linked vs. QTL

unlinked test gave a *lower* test statistic than one where polygenic effect was fitted (QTL linked + polygenic component vs. QTL unlinked + polygenic component). Again this suggests that our test may be conservative.

A contentious area of QTL mapping is that of significance thresholds. Lander & Kruglyak (1995) discussed the criteria that should be used for assigning linkage. A common problem is that when carrying out a genome-wide scan for QTL, multiple testing leads to false assignment of linkage because of Type I error. However, imposing too strict a value for significance may wrongly reject any suggestions of linkage that are found (Type II error). Traditionally a LOD score of 3 has been used to assign linkage. However, Lander & Kruglyak (1995) suggest a different approach: the use of a genome-wide significance level to find what they term 'significant linkage'. This is defined as statistical evidence expected 0.05 times in a genome-wide scan. If one were to carry out a genome-wide scan using 100 markers (a realistic number of markers for a natural population screening), an experiment-wide error rate of 0.05 could be approximated using a Bonferroni correction so that

$$\alpha' = 1 - (1 - 0.05)^{1/100} = 5.13 \times 10^{-4}.$$

Using the approach of Baret *et al.* (1998) this significance level corresponds to a LOD score of 2.34. In other words, rather than use a LOD score of 3.0 to assign linkage, a LOD score of 2.34 could be used for an experiment-wide significance level of 0.05. For the MAXI pedigree this equates to a power of about 0.67 (rather than 0.51) when a QTL of effect 1.0 SD is segregating, and there is no linkage between marker and QTL (see Fig. 3).

Although the power to detect QTL is high only in a somewhat idealized situation (highly polymorphic markers, QTL of very large effect, and low recombination

fraction between marker and QTL), further considerations may increase this power. In a real mapping study researchers may have prior information on environmental factors influencing the trait of interest. Controlling for these variables will serve to increase the proportion of the residual variation explained by the QTL. In other words, the heritability of the trait determined by the QTL will increase, as will the power to detect the QTL. Mapping projects in wild populations may also be able to draw on previous work carried out in closely related laboratory or farmed species where QTL for a trait of interest have been identified. If one knew the likely location of a QTL, then extra markers could be screened in that chromosomal region, hence reducing the recombination fraction between the QTL and marker(s). Alternatively only markers linked tightly to the candidate region could be typed and less stringent significance thresholds used. Again, this would increase the power to detect QTL. There is increasing evidence from the plant breeding literature that QTL affecting particular traits are found in syntenic genomic regions across species (Kearsey & Farquhar, 1998).

In our simulations we have performed linkage analysis only with single-point markers. Interval mapping and multipoint analysis would reduce much of the loss of power observed when recombination occurs between marker and QTL (Lander & Botstein, 1989; Knott *et al.*, 1996). For example, when a QTL of effect 1.0 SD is segregating and there is no recombination, the power for a mean LOD > 3.0 is 0.51 in the extended pedigree. At 10 cM the power is only 0.19, and at 20 cM just 0.03. Interval mapping would partially alleviate this problem, the effect of which is most noticeable when one has markers spaced at wide intervals (Fulker & Cardon, 1994), as will be the case in most studies of natural populations. Interval mapping has the additional advantage over single-point mapping that other QTL parameters such as magnitude and location can be estimated (Lander & Botstein, 1989). Most interval mapping procedures developed thus far are applicable to sib-pair (Lander & Botstein, 1989; Fulker & Cardon, 1994) or half-sib (Haley & Knott, 1992; Knott *et al.*, 1996) families. However, our simulations suggest that QTL detection in unmanipulated natural populations may be possible only if whole, complex pedigrees can be analysed. A recent study (Almasy & Blangero, 1998) described a method in which large complex pedigrees can be analysed by multipoint linkage. The number of alleles shared identically by descent (IBD) at marker loci is used to estimate genes IBD at various points along a chromosome for all relative pairs within the pedigree. Variance-component maximum likelihood linkage analysis is then performed on the overall pedigree to indicate the presence of a QTL. As new statistical approaches

like these are developed, QTL mapping in natural populations will become a possibility.

As expected, marker heterozygosity had a significant impact on ability to detect QTL. For both the extended and the flat pedigrees, a heterozygosity of 0.8 gave about twice the LOD score as one of 0.5. Microsatellite loci typically have heterozygosity values in the range 0.5–0.8, although this varies among species and populations. In species with a very dense map, such as the mouse, it may be possible to use highly variable markers exclusively.

In summary, we conclude that QTL detection in natural populations is possible, but unlikely unless loci of very large effect are segregating. However, if this is the case, those studies with reasonable sample sizes will benefit greatly from being pedigreed for several generations. Mapping of QTL may be possible with pedigrees of well under 1000 individuals. We look forward to the development and application of new, sophisticated statistical software, and remain cautiously optimistic that mapping programmes such as these will be realized in the next few years.

Acknowledgements

We thank all members of the Rum red deer project, in particular T. H. Clutton-Brock, S. D. Albon, F. E. Guinness and the various field assistants who have collected individual specific life-history data. We also thank the numerous volunteers who have helped them over the duration of the project. We thank SNH and its predecessor NCC for permission to work on Rum. The long-term study has been funded by NERC and BBSRC. Chris Haley, Bill Hill, Marg Mackinnon, Dave Coltman and an anonymous referee made helpful comments on earlier versions of the manuscript. J.S. is funded by a BBSRC postgraduate studentship.

References

- ALMASY, L. AND BLANGERO, J. 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am. J. Hum. Genet.*, **62**, 1198–1211.
- BARET, P. V., KNOTT, S. A. AND VISSCHER, P. M. 1998. On the use of linear regression and maximum likelihood for QTL mapping in half-sib designs. *Genet. Res.*, **72**, 149–158.
- BARTON, N. H. AND TURELLI, M. 1989. Evolutionary quantitative genetics: how little do we know? *Ann. Rev. Genet.*, **23**, 337–370.
- BOEHNKE, M. 1986. Estimating the power of a proposed linkage study: a practical computer simulation approach. *Am. J. Hum. Genet.*, **39**, 513–527.
- BRADSHAW, H. D., JR, WILBERT, S. M., OTTO, K. G. AND SCHEMSKE, D. W. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature*, **376**, 762–765.

- CLUTTON-BROCK, T. H., GUINNESS, F. E. AND ALBON, S. D. 1982. *Red Deer: Behaviour and Ecology of Two Sexes*. Edinburgh University Press, Edinburgh.
- DARVASI, A. AND SOLLER, M. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theor. Appl. Genet.*, **85**, 353–359.
- FULKER, D. W. AND CARDON, L. R. 1994. A sib-pair approach to interval mapping of quantitative trait loci. *Am. J. Hum. Genet.*, **54**, 1092–1103.
- GEORGES, M., NIELSEN, D., MACKINNON, M., MISHRA, A., OKIMOTO, R., PASQUINO, A. T. ET AL. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics*, **139**, 907–920.
- HALEY, C. S. AND KNOTT, S. A. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, **69**, 315–324.
- KEARSEY, M. J. AND FARQUHAR, A. G. L. 1998. QTL analysis in plants; where are we now? *Heredity*, **80**, 137–142.
- KEIGHTLEY, P. D. AND BULFIELD, G. 1993. Detection of quantitative trait loci from frequency changes of marker alleles under selection. *Genet. Res.*, **62**, 195–203.
- KINGHORN, B. P. 1994. Pedigree Viewer – a graphical utility for browsing pedigreed data sets. In: Smith, C., Gavora, J. S., Benkel, B., Chesuais, J., Fairfull, W. and Gibson, J. P. (eds) *5th World Congress on Genetics Applied to Livestock Production*, pp. 85–86. University of Guelph, Canada.
- KNOTT, S. A. AND HALEY, C. S. 1992. Maximum likelihood mapping of quantitative trait loci using full-sib families. *Genetics*, **132**, 1211–1222.
- KNOTT, S. A., ELSEN, J. M. AND HALEY, C. S. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theor. Appl. Genet.*, **93**, 71–80.
- LANDER, E. S. AND BOTSTEIN, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, **121**, 185–199.
- LANDER, E. AND KRUGLYAK, L. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genet.*, **11**, 241–247.
- LEROY, P. AND ELSEN, J. M. 1995. Numerical comparison between powers of maximum likelihood and analysis of variance methods for QTL detection in progeny test designs: the case of monogenic inheritance. *Theor. Appl. Genet.*, **90**, 65–72.
- LIN, J.-Z. AND RITLAND, K. 1997. Quantitative trait loci differentiating the outbreeding *Mimulus guttatus* from the inbreeding *M. platycalyx*. *Genetics*, **146**, 1115–1121.
- LINCOLN, G. A., GUINNESS, F. E. AND FLETCHER, T. J. 1973. History of a hummel – Part 3. Sons with antlers. *Deer*, **3**, 26–31.
- MARSHALL, T. C., SLATE, J., KRUIK, L. E. B. AND PEMBERTON, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.*, **7**, 639–655.
- MITCHELL-OLDS, T. 1995. The molecular basis of quantitative genetic variation in natural populations. *Trends Ecol. Evol.*, **10**, 324–328.
- ORR, H. A. AND COYNE, J. A. 1992. The genetics of adaptation: a reassessment. *Am. Nat.*, **140**, 725–742.
- PATERSON, A. H., LANDER, E. S., HEWITT, J. D., PETERSON, S., LINCOLN, S. E. AND TANKSLEY, S. D. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature*, **335**, 721–726.
- PLOUGHMAN, L. M. AND BOEHNKE, M. 1989. Estimating the power of a proposed linkage study for a complex genetic trait. *Am. J. Hum. Genet.*, **44**, 543–551.
- SHRIMPTON, A. E. AND ROBERTSON, A. 1988. The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. II. Distribution of third chromosome bristle effects within chromosome sections. *Genetics*, **118**, 445–459.
- TATE, M. L. 1997. *Evolution of Ruminant Chromosomes*. Ph.D. Thesis, University of Otago, New Zealand.
- VAN DER BEEK, S., VAN ARENDONK, J. A. M. AND GROEN, A. F. 1995. Power of two- and three-generation QTL mapping experiments in an outbred population containing full-sib or half-sib families. *Theor. Appl. Genet.*, **91**, 1115–1124.
- WELLER, J. I., KASHI, Y. AND SOLLER, M. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.*, **73**, 2525–2537.
- WIJSMAN, E. M. AND AMOS, C. I. 1997. Genetic analysis of simulated oligogenic traits in nuclear and extended pedigrees: summary of GAW10 contributions. *Genet. Epidemiol.*, **14**, 719–735.