MOLECULAR ANALYSIS OF GENETIC VARIATION IN IRISH MOILED CATTLE.

Thesis submitted in accordance with the requirements
of the University of Liverpool for the degree of
Doctor in Philosophy by
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September, 1994.

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ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. J.J.B. Gill and Dr. M.X. Caddick for their help and encouragement throughout the present study. I should also like to thank the Science and Engineering Research Council for providing the funds and also the Rare Breeds Survival Trust for their extra funding.

Many thanks to Adrian Turner for his assistance in collecting blood samples and his input into the creation of the Moilmate programme. Thanks are also due to all the Irish Moiled breeders for their time and assistance in the collection of blood samples. Special thanks to the Clarkes of Lurgan for putting Adrian and myself up and taking us out.

I would also like to thank Dr. A.B. Tomsett for useful discussions throughout the course of the present work and Mr. Dennis Kirk for technical advice and moral support.

For help and advice in the preparation of the final thesis I am grateful to Mrs. Ann Matthewman for the typing and to Phil MacIntyre for the preparation of the photographs.

Finally I would like to say thank you to my parents and Stella for their support and encouragement.

ABSTRACT

DNA was extracted from blood samples collected from every available Irish Moiled animal in the population. These samples were subjected to a series of analyses designed to test genetic relatedness. One procedure was to use a fragment of DNA from the Protein III gene of the bacteriophage M13, excised by restriction digestion, which was found to produce polymorphic banding patterns in Irish Moiled DNA.

In addition an investigation was made into the use of the Arbitrarily Primed PCR technique, using a number of primers, for the study of genetic variation in Irish Moiled cattle. The oligonucleotide probes $(GTG)_5$, $(GGAT)_4$ and $(GT)_8$ were found to produce informative banding patterns in *Hae*III and *Hinf*I digested DNA from Irish Moiled cattle. The use of a standardised electrophoresis and hybridisation system enabled the production of good quality, individual specific, DNA fingerprints. It was found that samples electrophoresed on separate gels could not be compared due to differences in migration distances. The use of the three probes with the two restriction enzymes enabled the analysis of genetic variation at a large number of non-selected loci in every Irish Moiled animal sampled. Some overlap in fragments detected between restriction enzymes was observed.

The use of the oligonucleotide DNA fingerprinting technique in parentage analysis was demonstrated in a case of disputed paternity. The probability of wrongly assigning parentage was shown to be very low.

A computer programme, Moilmate 93, was written, with the assistance of Adrian Turner, to enable the calculation of Inbreeding Coefficients and Coefficients of Co-ancestry in Irish Moiled cattle. The programme was later expanded to include a gene-dropping simulation procedure which has enabled the estimation of the genetic contribution, to the present herd, of

the eight Irish Moiled founder animals and the non-Irish Moiled animals used in upgrading lines. The simulation has also enabled the identification of those founders' genomes at greatest risk of future loss. An estimation of the genetic composition of individual animals identified individuals carrying a high percentage of rare founder alleles.

A regression analysis of band sharing between DNA fingerprints on coefficient of co-ancestry and between the number of bands scored in an individual on Inbreeding coefficient showed no significant relationship between the variables. Possible reasons for this are discussed.

ABBREVIATIONS

A	Adenine
AP-PCR	Arbitarily Primed Polymerase Chain Reaction
bp	base pair
BSA	bovine serum albumin
C	Cytosine
cps	counts per second
d.o.b.	date of birth
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
f	coefficient of co-ancestry
F	inbreeding coefficient
G	Guanine
mki	mean kinship coefficient
OD	optical density
PBS	phosphate buffered saline
PCR	Polymerase Chain Reaction
rpm	revolutions per minute
SD	Standard Deviation.
SDS	Sodium Dodecyl Sulphate
T	Thyamine
T.E.	Tris EDTA buffer
TAE	Tris acetate - EDTA electrophoresis buffer
Tris	tris (hydroxymethyl)-amino methanine
UV	ultra violet
V	volts
w/v	weight per volume

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CHAPTER 1

INTRODUCTION

1.1 IRISH MOILED CATTLE

The Irish Moiled is the only breed to originate in Ulster. It is one of the rarest breeds of cattle in Europe, classed as priority one (less than 150 breeding females) on the Rare Breeds Survival Trust's list of endangered native breeds.

The animals are hornless and it was this trait which originally characterised the breed, indeed the name Moiled is the anglicised form of the old Irish Gaelic word for hornless, 'mael'. The animals are medium sized and coat colour and pattern ranges from red with a continuous white stripe along the back and underparts (line-backed) through a range of intermediates to more or less white animals with red ears and muzzle (Plate 1.1). Another characteristic of the breed is the narrowing of the nasal bones above the muzzle which lends prominence to the lips and nose area, especially in well marked individuals, where the muzzle is surrounded by a striking ring of colour. (Plate 1.2)

The Irish Moiled Cattle Society was first formed in 1926, with the aim of developing and promoting a dual purpose breed of cow for use on the hill farms of Ulster. The breed could produce a reasonable milk yield on relatively poor grazing, and also, when crossed with a beef breed, calves which were suitable for beef production.

Little is known of the history of Irish Moiled cattle prior to the establishment of the society. A number of herds existed in Northern Ireland in the late 19th and early 20th Centuries. Evidence from archaeological excavations and ancient Irish literature suggests that hornless cattle have existed in Ireland for many hundreds of years.

The existence of polled cattle in Finland, phenotypically similar and reputedly closely related to Irish Moiled, has led to speculation that Irish Moiled cattle were plundered by Viking raiders, or that they were introduced to Ireland by Norse or

Danish settlers, although fossil evidence shows that polled cattle existed in Ireland long before this. Quite large numbers of longhorn cattle were imported to Ireland in the early 19th century. The colour pattern of the longhorn is a plum- or red-brindle with a white line down the back and generally a white spot on each thigh. Although it is quite likely that cattle with this colour pattern could be found in Ireland before the longhorn importations, the influx of longhorns undoubtedly increased the frequency of its occurrence and contributed to the origin of Irish Moiled cattle as a recognised breed.

Plate 1.1

Range of coat patterns in Irish Moiled cattle. From red with a continous white line dorsally (cow on right), through a range of intermediates (animals on left and at back) to more, or less, white, with red ears and muzzle (calf at front). Photograph taken at Croxteth Country Park, Liverpool.

Plate 1.2

Glenbrook 1219, showing narrowing of the nasal bases and prominent muzzle, characteristic of Irish Moiled cattle. Photograph taken at Temple Newsam Park, Leeds.

Irish Moiled cattle are mentioned by Professor C. Low of Edinburgh University and Sir WilliamWilde in the mid 19th century but no established breed was recognised at this time. An 18th century poem in Irish, Droimeann Donn Dilis, "beloved brown and white backed cow", may be a reference, but in the context of the poem the beloved brown and white backed cow is also a metaphor for Ireland.

With the formation of the Irish Moiled Cattle Society in 1926 the breed flourished, much support was attracted and classes were held at the Royal Ulster Show. Moiled cows won the cup for best dairy cow in Ulster from 1927 to 1930, and by 1929, 678 animals had been registered as Irish Moiled.

The Irish Moiled Cattle Society was badly affected by the Second World War, and although a number of herds were established, afterwards, from pure animals registered before the war, the Irish Moiled population continued to decline due to competition from new and more specialised breeds. Another factor in this decline was a shortage of bulls, caused by the requirement of the 1949 Agricultural Act (N.I.) that only bulls bred from cows with a recorded milk yield of 6000lbs at first lactation or 8000lbs at subsequent lactations, with a minimum of 3.4% butterfat, could be licensed. As most animals were kept on small farms, in very small herds, most Moiled breeders did not milk record and licensed bulls became scarce.

The population reached a bottleneck in the late sixties and early seventies, only 14 new animals appeared on the pure register between 1965 and 1975. Numbers had dropped to 30 breeding females in the 1970's. These were maintained by two breeders, David Swann of Dunsilly (the Glenbrook herd) and James Nelson of Maymore (the Maymore herd). These had exchanged animals on occasion and the entire present day population is descended from these two herds. Their ancestry can be traced to only eight pure founder animals and a number of upgrading animals.

The lack of breeders and the difficulty in obtaining licensed bulls caused both Swann and Nelson to establish upgrading schemes. Swann upgraded from Shorthorn females and Nelson from Hereford and Red Lincoln males.

In 1982 the Irish Moiled Cattle Society was reformed, with the encouragement of the Rare Breeds Survival Trust. The efforts of the society's members to promote the breed have been very successful and, since the society reformed, the Irish Moiled population has increased to its present level of just under 200 pure registered animals, maintained in herds throughout England as well as Northern Ireland.

1.2 INBREEDING DEPRESSION

Because of their small numbers, rare breeds are prone to inbreeding depression. Inbreeding depression is the decrease in fitness of a population, due to the mating of closely related individuals. Inbreeding results in the reduction of the mean phenotypic value shown by characters connected with reproductive capacity or physiological efficiency. Various characters have been found to be subject to inbreeding depression in a range of species (Falconer, 1960), for example in Friesian cattle milk yield has been found to drop by 3.2% per 10% increase in the inbreeding coefficient (Robertson, 1954).

Inbreeding means the mating together of individuals that are related to each other by ancestry. Over a number of generations, the number of individuals required to provide separate ancestors for all the present individuals becomes higher than any population could contain. Therefore, any pair of individuals will be related to each other through one or more common ancestors in the more or less distant past. The smaller the size of the population in previous generations, the less distant are the common ancestors, or the greater their number (Falconer, 1960).

When two individuals have a common ancestor, they may both carry replicates of one of the alleles present in the ancestor, and if they mate these may be passed on to the offspring. An inbred individual may therefore carry two alleles at a locus that are identical by descent from a common ancestor of its parents.

The coefficient of inbreeding F, first defined by Wright (1922), is the probability that the two alleles at any locus in an individual are identical by descent. In any population, not infinitely large, all alleles present at a locus could be found to be identical by descent if traced far enough back into the distant past. A base population, beyond which ancestry will not be traced, must therefore be specified. The alleles present in this base population are regarded as independent (not identical by descent), and therefore the base population has an inbreeding coefficient of zero. The inbreeding coefficient compares the degree of relationship between the descendants of the base population with that between individuals in the base population. In Irish Moiled cattle,

the eight founder animals, to which the ancestry of the present herd can be traced accurately, is regarded as the base population.

In pedigreed populations, the inbreeding coefficient of individuals can be calculated directly from their pedigrees, by the computation of probabilities at each segregation of alleles as described by Wright (1922).

$$F_I = \Sigma\left[(0.5)^{m+f+1}(1+F_A)\right]$$

F_I = inbreeding coefficient of individual I.

m = number of generations from male parent of I back to a common ancestor.

f = number of generations from female parent of I back to a common ancestor.

F_A = inbreeding coefficient of common ancestor.

The number of alleles shared by pairs of individuals is known as the co-ancestry (*f*) of the two animals (Cruden, 1949). It is equivalent to the inbreeding coefficient of the progeny of these animals, and in pedigreed populations this can be estimated mathematically. Coefficients of co-ancestry can be used to allow the mating of the most distantly related animals. This strategy minimises the accumulation of homozygosity resulting from inbreeding, thus maintaining allelic variation. However, as the effective population size decreases, the variance in the estimates increases, and the technique is less accurate.

An optimal breeding strategy, to maintain genetic variation in a pure breed, will produce homozygosity for those alleles, the expression of which characterise the breed, while maintaining maximal heterozygosity at the loci which are not under direct selection by the breeder. One group of genetic markers which enable the measurement of the genetic variation at non-selected alleles are the blood groups. Because of the number of loci and the large number of alleles existing at some of these loci, blood group analysis can provide a considerable number of genetic markers in large

populations. However, in small populations the blood group data can only provide a limited differentiation between individuals, since as the population size decreases, the variation in the blood groups also decreases. For example, in cattle the B locus is the most variable, exhibiting over 450 alleles. In large populations such as the Friesian, at least 150 alleles are present, however in a sample of 22 pure Irish Moiled cattle only six of the alleles were present (Gill and Kelly, 1990).

The system of DNA fingerprinting developed by Jeffreys *et al.*, (1985a), in humans, may provide a large new set of genetic markers for the examination of genetic variation at non-selected loci. The combination of genetic markers generated from blood group studies and those from DNA fingerprinting may produce sufficient numbers of polymorphic markers to be representative of the entire non-selected genome. The data generated could therefore be used to measure accumulating homozygosity or to measure the 'shared genome' value between potential mates, thus enabling a breeding programme to maintain maximum genetic variation at non-selected loci.

1.3 MULTILOCUS DNA FINGERPRINTS

Multilocus DNA fingerprinting was first descibed in humans by Jeffreys in 1985 (Jeffreys *et al.*, 1985a). Since then the technique has had an enormous impact on many areas of biological science.

Eukaryotic genomes contain many highly polymorphic 'minisatellite' sequences. (Nakamura *et al.*, 1987; Jeffreys *et al.*, 1985a) These regions consist of variable numbers of tandem repeats of relatively short sequences (15-60 bp). Polymorphism results from differences in the number of repeats which is thought to arise by unequal crossing over during meiosis or DNA slippage during replication (Jeffreys *et al.*, 1985a; Jarman and Wells, 1989).

Minisatellite regions are dispersed throughout the genome and share a common short 'core' sequence (10-15 bp) (Jarman and Wells, 1989). Probes which will hybridise to this 'core' are able to detect alleles at many hypervariable loci

simultaneously, revealing DNA band patterns, which resemble bar codes, following autoradiography of the Southern Blot. Due to their individual-specific nature, (only identical twins share the same pattern), these patterns have been termed DNA fingerprints.

The bands which make up a DNA fingerprint are stably inherited and segregate in a Mendelian fashion. Each band in the fingerprint pattern of an offspring can be identified in one or other or both of the parental DNA fingerprint. Each parent contibutes approximately half of the bands in the fingerprint pattern of the offspring.

In providing this set of stably inherited genetic markers, the DNA fingerprinting technique is a powerful tool for determining family relationships. It has been used to resolve paternity disputes in humans, providing positive evidence of relationship, even in cases where samples from important family members were unavailable (Jeffreys *et al.*, 1985b, c).

The individual-specific nature of DNA fingerprints lends their application to forensic medicine. Minisatellite DNA has been shown to be stable in human blood and semen stains, with samples up to four years old able to produce DNA fingerprints of sufficient quality to allow individual identification. The technique has provided forensic scientists with a means of discriminating between individuals which is many orders of magnitude more sensitive than a combination of all the techniques previously available for use in forensic laboratories. The use of eight polymorphic protein systems together, gives a probability of 0.014 for individual specificity, leaving a large degree of uncertainty. The probability of indentical banding patterns occouring by chance, using Jeffreys 33.15 probe is less than 3×10^{-11}, and using both probes 33.15 and 33.6 together, the chance is much less than 5×10^{-19}*. Thus, using this technique, forensic scientists can be virtually certain of any association between samples, whereas previously it was only possible to be certain of non-association (Gill *et al.*, 1985; Gill *et al.*, 1987).

*See Section 5.7 for calculation of probabilities.

In addition to parentage analysis and forensic medicine, the DNA fingerprinting technique has found use in many other applications, including the determination of twin zygosity in multiple births, the identification of post transplant cell populations, linkage analysis for human disease (Schäfer *et al.*, 1988), and evolutionary studies (Reeve *et al.*, 1990; Gill *et al.*, 1990).

The discovery of minisatellites was linked to the development of human molecular genetics. However, the core sequence was found to be similar in length and G content to the Chi sequence, a signal for generalised recombination, in *E. coli*. This suggested that it may be conserved in evolution and therefore probes for human core sequences may cross-hybridise to animal minisatellites (Jeffreys *et al.*, 1985a). Jeffreys sequences have been found to detect hypervariable polymorphic loci in a range of other species, including cats, dogs (Jeffreys and Morton, 1987), birds (Birkhead *et al.*, 1990; Burke and Bruford, 1987; Hanotte *et al.*, 1991; Rabenold *et al.*, 1990), mice (Jeffreys *et al.*, 1987) and whales (Amos and Dover, 1990).

The technique of DNA fingerprinting using Jeffreys probes for minisatellite DNA has been used to determine the level of genetic variability within and between populations of the Californian Channel Island Fox (Gilbert *et al.*, 1990). The level of genetic variability was estimated from the Average Percentage Difference (APD), between DNA fingerprint patterns. The proportion of restriction fragments which differed between pairs of individuals was calculated. A low APD for a population indicates that animals from that poulation share, on average, a greater percentage of their genomes. Foxes of unknown origin were able to be assigned to a particular population, based on APD values or the presence of characteristic restriction fragments.

In an alternative approach, a sequence in the protein III gene of the bacteriophage M13, was found to be able to detect a set of hypervariable minisatellite repeats, distinct from those detected by Jeffreys' probes, in human and animal DNA (Vassart *et al.*, 1987).

Two clusters of 15 bp repeats were found to be responsible for producing DNA banding patterns different to those obtained with Jeffreys probes, under similar conditions. The sequence of these repeats is similar to the core sequences of Jeffreys' probes and, presumably, the repeat sequence in the M13 probe was hybridising to the core sequence of a set of minisatellites not detected by Jeffreys' probes.

As with Jeffreys' probes, the G rich M13 consensus sequence was found to be able to detect hypervariable minisatellites in a range of organisms, including cattle (Vassart *et al.*, 1987; Georges *et al.*, 1988; Georges *et al.*, 1990; Mannen *et al.*, 1993), pigs, horses, dogs (Georges *et al.*, 1988) and chickens (Kuhnlein *et al.*, 1989). Mannen *et al.*, (1993) used the technique of DNA fingerprinting, with the M13 probe, to evaluate the relationship between the coefficient of co-ancestry and the level of band sharing revealed by DNA fingerprinting in Japanese Black cattle. It was suggested that DNA fingerprinting could be applied to estimate the coefficient of co-ancestry in cattle and other species.

A second class of hypervariable loci were found to consist of tandemly repeated short (less than 10 bp) simple sequence motifs. These were termed simple tandem repeats (str) (Schäfer *et al.*, 1988) or 'microsatellites'. Oligonucleotide probes, specific to these simple tandem repeat sequences were used by Ali *et al.*, (1986) to produce individual specific DNA fingerprints with human DNA, thus establishing the oligonucleotide DNA fingerprinting technique. Schäfer *et al.*, (1988) used three different simple repetitive oligonucleotide probes ($(CT)_8$, $(CAC)_5$ and $(TCC)_5$) to produce DNA fingerprints with a panel of human DNAs which had been digested with the restriction enzymes *Alu*I, *Hinf*I and *Mbo*I. The probability of finding the same banding pattern in two individuals was calculated to be 2×10^{-8}*. The DNAs from monozygous twins produced indistinguishable banding patterns and the bands were demonstrated to be inherited according to Mendelian laws. Therefore, the oligonucleotide DNA fingerprinting technique was shown to reveal informative fingerprints that could be used for individual identification, as required in paternity testing or forensic medicine applications.

* See Section 5.7 for calculation of probability.

The simple tandem repeat sequences have been found in all eukaryotic genomes investigated. The occurrance and distribution of the sequences varied considerably betwen species investigated. As with the hypervariable minisatellite loci, polymorphism in simple tandem repeat length and composition is thought to have arisen by unequal crossing over during meiosis or DNA slippage during replication (Epplen, 1988).

The occurrence of simple tandem repeat sequences in all eukaryotic genomes, and the interspersion of the sequences throughout the genome (Tautz and Renz, 1984; Ali *et al.*, 1986; Schäfer *et al.*, 1988; Epplen, 1988; Nanda *et al.*, 1991; Vergnaud *et al.*, 1991) enabled the oligonucleotide DNA fingerprinting technique to be used in a broad range of applications in a variety of species, including mice (Epplen, 1988; Nanda *et al.*, 1991), primates (Nanda *et al.*, 1991), sheep, goats, pigs, horses, dogs, chickens (Buitkamp *et al.*, 1991b) and cattle (Buitkamp *et al.*, 1991a,b).

The species specific occurrence of the simple tandem repeat sequences (Epplen, 1988), requires the investigation of the suitability of a given probe for DNA fingerprinting in a particular species (Buitkamp *et al.*, 1991b; Epplen *et al.*, 1991). The use of a range of oligonucleotide probes was investigated in three German breeds of cattle (Buitkamp *et al.*, 1991a). The probes used are listed in Table 1.1

Table 1.1 (From Buitkamp *et al.*, 1991a).

Average number of polymorphic bands produced by hybridisation of oligonucleotide probes with *HinfI* digested genomic DNA inrelated Holstein Friesian cattle.

Probe	Length (nucleotides)	Average Number of polymorphic bands per individual
$(AT)_8$	16	0
$(CT)_8$	16	0
$(GAA)_6$	18	1
$(GACA)_4$	16	0
$(GATA)_4$	16	0
$(GC)_8$	16	0
$(GGAT)_4$	16	4
$(GT)_8$	16	>10
$(GTG)_5$	15	>10
$(TCC)_5$	15	2

Of the 11 probes tested in cattle, the probes $(GGAT)_4$, $(GT)_8$ and $(GTG)_5$ were found to be informative, revealing at least four polymophic bands with *Hinf*I digested Holstein Friesian DNA (Buitkamp *et al.*, 1991a, b). Using the oligonucleotide probes $(GTG)_5$ and $(GT)_8$ with *Hinf*I, *Hae*III and *Alu*I digested bovine genomic DNA the number of polymorphic bands generated in the three breeds investigated (Holstein Friesian, Red Pied and Simmental cattle), was found to vary from 11 to 23. The probability of finding the same banding pattern in two unrelated individuals ranged from 1.5×10^{-7} to 2.4×10^{-7}*. The oligonucleotide DNA fingerprinting technique therefore allowed the precise identification of individuals and was also shown to be a useful method for paternity analysis in cattle (Buitkamp *et al.*, 1991a).

Oligonucleotide DNA fingerprinting has been shown to have several technical advantages over minisatellite probes (Ali *et al.*, 1986; Schäfer *et al.*, 1988; Buitkamp *et al.*, 1991b). The oligonucleotide probes are synthesised chemically, therefore avoiding problems concerning probe stability, which may exist when tandem repetitive sequences are cloned (Wong *et al.*, 1976). Oligonucleotides can hybridise to digested DNA directly in the dried down agarose gel, avoiding the loss of DNA during Southern transfer (Southern, 1975). Also hybridisation is much faster and exposure time is shorter with oligonucleotide probes. The mutation rates for fragments containing microsatellite loci, were shown to be higher than for conventional loci, but in the same range as those determined by Jeffreys *et al.*, (1985b, 1988) for minisatellite loci (Schäfer *et al.*, 1988; Nürenberg *et al.*, 1989).

The DNA fingerprinting technique has been used to investigate genetic relationships and the degree of inbreeding within populations of various species. Kuhnlein *et al.*, (1990) investigated the correlation between DNA fingerprint pattern and the degree of inbreeding in defined strains of chicken. The technique has been used for estimating genetic variability in wild populations of foxes (Gilbert *et al.*, 1990), naked mole rats (Reeve *et al.*, 1990), sparrows (Wetton *et al.*, 1987) and lions

*See Section 5.7 for calculation of probabilities

(Gilbert *et al.*, 1991; Packer *et al.*, 1991). Piper and Rabenold (1992) have investigated the use of band sharing scores from DNA fingerprinting in the determination of relatedness in the tropical wren. As theoretically expected by Lynch (1990) the DNA fingerprint between two individuals has been shown to be more similar as their relationship increases (Gilbert *et al.*, 1991; Packer *et al.*, 1991; Piper and Rabenold, 1992; Mannen *et al.*, 1993). Despite doubt raised about the reliability of calculating more distant relationships (Lynch, 1990, 1991), Piper and Rabenold have shown that band-sharing analyses provide a useful means of estimating relatedness between pairs of individuals. In Japanese Black cattle, Mannen *et al.*, (1993) have shown that the level of band sharing between individuals correlates strongly with the mathematically derived coefficient of co-ancestry and suggest that DNA fingerprinting could be applied to estimate relatedness between individuals.

The genetic variation present in the hypervariable DNA sequences, detected by DNA fingerprinting, would appear to provide a means to investigate the genetic variation at non-selected loci in Irish Moiled cattle.

1.4 ARBITARILY PRIMED PCR (AP-PCR)

A different DNA polymorphism assay has been described by Williams *et al.*, (1990) and Welsh and McClelland (1990). It was found that simple and reproducible DNA fingerprints of complex genomes could be generated using primers of arbitrary sequence and the polymerase chain reaction. The protocol differed from standard PCR conditions (Erlich, 1989) in that only a single oligonucleotide primer was used. During PCR, segements of DNA which lie between inverted priming sites, are amplified. Small divergences between genomes will often result in the generation of a different DNA fingerprint. Polymorphic banding patterns result from the creation or destruction of priming sites due to changes in the DNA and the insertion or deletion of sequences between priming sites. Each primer tested gave a different pattern of amplification products, each with the potential of detecting polymorphisms between strains or even individuals. The number of polymorphic markers detected can be increased by

increasing the number of primers used. The technique has been shown to be able to reproducibly amplify segments of genomic DNA from a variety of species including humans (Williams *et al.*, 1990), plants, bacteria (Williams *et al.*, 1990; Welsh and McClelland, 1990) and mice (Welsh *et al.*, 1990). Polymorphisms can be detected by electrophoresis of the reaction products and staining with ethidium bromide, thus eliminating the need to use radioactive isotopes.

AP-PCR is quicker to carry out than conventional DNA fingerprinting, it is cheaper, less technically demanding and requires only nanograms of DNA (Hardrys *et al.*, 1992; Arnheim *et al.*,1990; Hedrick, 1992). However, there is some doubt concerning the repeatability of the technique, due to variation in template/primer matching (Hedrick, 1992; Hardrys *et al.*, 1992; Williams *et al.*, 1990).

1.5 AIMS OF THE PRESENT STUDY

This project aimed to utilise molecular biological techniques to study the genetic variation at non-selected loci in the Irish Moiled Cattle population.

A standardised DNA fingerprinting, or similar, system was to be established, and an analysis of the entire extant population, was to be carried out. The size of the population would entail splitting samples between many gels, and as a result, standard conditions and internal markers would be required, to enable between gel comparisons to be made with a minimum of error.

The value of the technique would be investigated for a number of applications including paternity analysis and pedigree verification, the measurement of the actual homozygosity of individual animals, and as an indication of the proportion of the genome shared between animals.

The genetic homozygosity of individual animals and the genetic homology between animals, estimated using molecular biological techniques were to be correlated with mathematical estimates of inbreeding and coancestry, based on Wright's Coefficient.

CHAPTER 2
MATERIALS AND METHODS

2.1 BUFFERS AND CHEMICALS

All chemicals were Analar grade,unless otherwise stated. Distilled water or H_2O refers to sterile distilled water.

TE Buffer

10 mM Tris Base (Analar) pH 8.0

1 mM EDTA

Sterilised by autoclaving.

50 x Tris Acetate - EDTA electrophoresis buffer (TAE) pH 7.7

242 g Tris Base

57.1 ml Glacial acetic acid

18.6 g EDTA

5 x Loading Buffer

50% Sucrose

50 mM EDTA

0.1% Bromphenol blue

0.1% Xylene cyanol FF

Preparation of buffer saturated Phenol

Analar grade phenol was dissolved 1:1 (w/v) in 1M Tris HCl (pH 8.0) and stirred for 30 minutes. After settling, the lower phenol phase was separated from the aqueous phase and mixed with an equal volume of 0.1M Tris HCl (pH 8.0) and stirred for another 30 minutes, as before. The separating and washing process was repeated three more times. 0.1% (w/v) of hydroxyquinoline was then added to the equilibriated

phenol, as an antioxidant, along with 0.5 volumes of 0.1M Tris HCl (pH 8.0). The buffer saturated phenol was aliquotted and stored at 4°C. Long-term storage was at -20°C.

20 x SSC (pH 7.0)

175.3 g NaCl

88.2 g Sodium citrate

per litre of distilled water

Adjusted to pH 7.0 by the addition of drops of a10N NaOH solution

Sterilised by autoclaving.

20 x SSPE (pH 7.4)

174.0 g NaCl

27.6 g NaH_2PO_4 H_2O

7.4 g EDTA

per litre of distilled water

Adjusted to pH 7.4 by the addition of NaOH (~ 6.5 ml of a 10N solution)

Sterilised by autoclaving.

50 x Denharts Solution

5 g Ficoll

5 g Polyvinylpyrrolidine

5g BSA (fraction V)

per 500 mls distilled water

Sterilised by filtration

Aliquotted and stored at -20°C

Phosphate Buffered Saline (PBS) (pH 7.4)

8 g NaCl

0.2 g KCl

1.44 g $Na_2 HPO_4$

0.24 g KH_2PO_4

per litre of distilled water

Adjusted to pH 7.4 by the addition of concentrated HCl

Sterilised by autoclaving.

2.2 COLLECTION OF BLOOD SAMPLES

Blood samples were taken from Irish Moiled cattle throughout England and Northern Ireland between October 1990 and September 1993. It was attempted to obtain samples from every living member of the breed. Samples were collected, with the owner's permission and assistance, at the farms were Irish Moiled cattle were kept. The cattle were immobilised in a 'crush', or were physically restrained using a halter and, after swabbing the relevant area of the animal with absolute ethanol, venous blood for DNA extraction was taken from the tail (coccygeal vein) or neck (jugular vein), using 16 ml vacutainers (Venujet) containing 0.12 mls of 0.34 M EDTA as an anticoagulant.

The use of vacutainers causes blood to be drawn very quickly from the middle of the vein, and the samples taken contain a reduced proportion of nucleated white cells, which tend to adhere to the sides of the vein. However the ease of use of vacutainers and the speed at which samples can be taken, makes them the most convenient method of sampling large numbers of animals.

A number of vacutainers were used to collect approximately 15 ml of blood from each adult. In some cases, such as from young calves, it was only possible to obtain a small amount of blood (less than 5 ml). Immediately upon returning to the laboratory the blood samples were stored at 4°C for up to four days, or frozen, at -20° C indefinitely, depending on the number of samples taken at a particular location (see

Section 2.3.2). The protocol for DNA extraction is different in each case, both are given below.

In addition to the 15 mls of blood taken for DNA extraction, a further sample was collected at the same time, using a 10 ml lithium heparinised vacutainer (Venujet), to be sent to Dr. E. Kelly at University College, Dublin, for blood group analysis.

2.2.1 Friesian Control

A large amount of DNA from one source was needed to use as an internal marker for use on every gel, to enable an accurate comparison of samples which had been electrophoresed on different agarose gels.

Almost 2 litres of blood was obtained from a Friesian cow, by cutting its abdominal or mammary vein shortly after its death at the abattoir of the University of Liverpool Veterinary Field Station, Leahurst. The blood was collected in a large sterile, screw-top container, containing EDTA (to a final concentration of 2.5 mM) as an anticoagulant. The blood sample was mixed, aliquoted into 20 ml sterile plastic universals and stored at -20°C.

The large volume of blood taken enabled the same internal marker to be used on all gels of the present herd. Future studies on the progress of the breed will also be able to use the same internal marker for comparisons with the present herd, as the samples can be stored frozen indefinitely.

2.2.2 Other DNA samples

Blood for DNA extraction was also obtained from a Jersey cow at Croxteth Country Park, Liverpool, and from a Cambridge ram at the University of Liverpool, Veterinary Field Station, Leahurst.

2.3 EXTRACTION AND ASSESSMENT OF DNA

DNA extractions to produce clean high molecular weight genomic DNA, suitable for DNA fingerprinting, were performed by phenol/chloroform treatment using a slightly modified version of the method described by Blin and Stafford (1976).

2.3.1 Extraction from Fresh blood

The following method was used for the extraction of DNA from fresh blood, stored at 4°C for up to four days.

1. The blood samples (~15 ml) were centrifuged at 1300g for 15 minutes (in a Mistral 1000 centrifuge), separating the blood cells from the plasma. The nucleated white cells form a layer between the red cells and the plasma - the 'buffy coat'.

2 The supernatant plasma was discarded and the buffy coat was transferred to a fresh centrifuge tube, using a sterile Pasteur pipette, and re-centrifuged at 1300g for a further 15 minutes, separating the white cells from any plasma and red blood cells which may have been carried over with the isolated buffy coat.

3. The buffy coat was then suspended in 15 ml of DNA extraction buffer, in a 30 ml Sorval centrifuge tube.

DNA extraction Buffer : 10 mM Tris.HCl (pH 8.0)
0.1 M EDTA (pH 8.0)
0.5% SDS

4. 30 µl of freshly prepared 10 mg/ml RNase were added (to a final concentration of 20 µg/ml) and after swirling gently to mix, the solution was incubated at 37°C for one hour.

5. 75 ml (20 mg/ml) Proteinase K were added, the tubes were capped with UV sterilised caps and the viscous solution was mixed by gently inversion. The samples were then incubated in a 50°C water bath for 3 hours.

6. After incubation the samples were allowed to cool to room temperature before the addition of an equal volume of buffer saturated phenol. The 2 phases were

mixed by gentle inversion to form an emulsion and centrifuged at 5000g (6500 r.p.m. in a Sorval centrifuge, Model RC-5B, rotor SS34) for 15 minutes at room temperature.

7. The upper aqueous layer was transferred to a fresh tube using a sterile, wide bore, 1 ml (blue) Gilson tip. One half volume of buffer saturated phenol, and one half volume of chloroform-isomyl alcohol (ratio 24:1) were added and mixed. Tubes were centrifuged as above and the upper aqueous layer was transferred to a fresh tube. This step was repeated until there was no trace of degraded protein at the interphase.

8. The aqueous layer was then mixed with an equal volume of chloroform-isomyl alcohol (ratio 24:1) and centrigued (5000g for 15 minutes at room temperature). The upper aqueous layer was transferred to a fresh tube.

9. Genomic DNA was precipitated by the addition of one-fifth volume of 10 M sodium acetate (pH 5.2), and 2 volumes of absolute ethanol, at room temperature.

10. The high molecular weight DNA, which precipitated out of solution almost immediately was collected by spooling onto a sterile glass rod. The DNA was washed once in 70% ethanol and allowed to air dry before being resuspended in 500 µl distilled water in a 1.5 ml Eppendorf tube.

11. Extracted DNA was stored at -20°C.

2.3.2 Extraction from Frozen Blood

DNA yield dropped considerably if blood samples were stored at 4°C for longer than four days, and the DNA extracted was sometimes degraded as also reported by Breen (1990). Consequently, when a large number of animals were sampled, the extracted blood was frozen, at -20°C, until such time as it was convenient to extract the DNA.

Samples can be stored frozen at -20°C indefinately, and will produce a high yield of undegraded DNA, although repeated thawing and refreezing will reduce this yield. A 10% drop in DNA yield has been reported to occur every time a sample is

frozen and thawed (Ross *et al.*, 1990). Blood samples were stored at -20ºC in the vacutainers in which they were collected. Before freezing, the blood cells and plasma were completely mixed to prevent disruption of the white cells.

Approximately 15 ml of each sample of frozen blood was thawed in a water bath at room temperature. An equal volume of Phosphate Buffered Saline (PBS) was added and mixed. Samples were centrigued at 3500g for 15 minutes in a Mistral 1000 centrifuge.

The nucleated white cells formed a tight pellet at the bottom of the centrifuge tube, and the supernatent plasma and lysed red blood cells was carefully aspirated. The pellet was resuspended in 15 ml of DNA Extraction Buffer, by vigorous vortexing.

The extraction then proceeded from the addition of RNase, as described for the extraction of DNA from fresh blood (step 4).

2.3.3 Measurement of DNA Concentration and Purity

Estimates of DNA concentration by UV. spectrophotometry can be misleading, as genomic DNA does not always dissolve evenly or completely in solution if even small amounts of contaminating protein or phenol are present. The solution can however be homogenised, to some extent, by heating the sample to 55°C and passing it through a 1 ml (blue) micropipette tip several times, to give a more even concentration of DNA (Bruford *et al.*, 1992).

All other manipulations of the DNA solutions are performed using wide-bore micro-pipette tips. These were made by carefully cutting a few millimetres off the end of a normal micropipette tip, to give a wider aperture, before autoclaving. The wide-bore helps to prevent shearing of the DNA as it passes through the aperture. This does not occur at 55°C as the viscosity of the solution is reduced at this temperature (Bruford *et al.*, 1992).

Once the DNA samples have been homogenised, a more accurate estimate of concentration can be obtained by UV spectrophotometry. Samples were diluted 1/100 in distilled water, and their optical density was measured at 260 nm and 280 nm on a

Perkin-Elmer spectrophotometer, using quartz cuvettes. One absorbence unit (a.u.) at 260 nm is equivalent to 5000 µg/ml of double stranded DNA (Sambrook *et al.*, 1990).

The ratio of O.D. 260/O.D. 280 was calculated to give an idea of the purity of the sample. A ratio of approximately 1.8 indicates a pure sample, whereas a ratio of significantly less than this suggests contamination. The presence of associated protein not only reduces the solubility and therefore the estimation of the concentration of the extracted DNA, it also impairs the activity of restriction endonucleases, preventing the digestion of the sample.

2.3.4 Small Scale Purification of DNA

To clean up the sample, the DNA could be re-extracted by a further, small scale phenol/chloroform treatment.

50 µg aliquots of DNA, shown to be contaminated with protein or phenol, were made up to 400 µl with distilled water in 1.5 ml Eppendorf tubes. An equal volume of phenol/chloroform/isoamyl alcohol (ratio 25:24:1) was added and the solutions were mixed by vortexing. The samples were centrifuged at 13000rpm in a bench microcentrifuge for 15 mins, to separate the phases. The upper aqueous phase was removed, using a wide-bore 200 µl (yellow) micropipette tip, taking care not to disturb any degraded, contaminating protein at the interphase and transferred to a fresh 1.5 ml Eppendorf tube. This procedure was repeated until there was no sign of contaminating protein at the interphase. The solution was then treated in the same manner with an equal volume of chloroform/isoamyl alcohol (ratio 24:1), to remove any residual phenol.

The clean DNA was then precipitated by adding 1/10 volume of 10 M sodium acetate and 2 volumes of absolute ethanol (at room temperature) to the aqueous DNA solution, and mixing by gentle inversion. The DNA was pelleted by centrifugation at 13000rpm, in a bench microcentrifuge, for 20 minutes. The DNA pellets were washed with 70% ethanol to remove any remaining salt, and, after air-drying, were resuspended in 60 µl distilled water.

2.3.5 Assessment of the Condition of the Extracted DNA

The integrity of the extracted genomic DNA can be assessed by electrophoresis. 2 µl of each DNA sample (in 7 µl distilled water and 1 µl loading buffer) were electrophoresed on a 1% agarose minigel in 1×TAE running buffer, at 50 V for approximately 30 mins. After electrophoresis, the DNA was visualised by staining the gel in a 0.5 µg/ml solution of ethidium bromide, before examination on a UV transilluminator.

Intact high molecular weight DNA appears as a single band, any DNA seen below this has undergone degradation. Degraded DNA does not sustain the integrity of restriction fragments required to produce clear band patterns and therefore cannot be used for DNA fingerprinting.

2.4 DIGESTION OF DNA SAMPLES

After assessment of the condition and measurement of the concentration of the DNA samples, approximately 10 µg of each sample was digested using a restriction endonuclease with a four base pair recognition sequence. Within regions of DNA with approximately random distribution of nucleotides, these enzymes will cleave the genomic DNA relatively frequently (approximately every 256 bp), producing small fragments of DNA. The larger fragments produced are likely to be repetitive sequences, such as minisatellite and microsatellite DNA, which are devoid of these restriction sites.

Digestions were carried out in 0.5 ml Eppendorf tubes, using 5 units of enzyme per µg DNA, in the presence of 4 mM spermidine trihydrochloride and the manufacturers buffer, at 37°C overnight. The reaction mixture was as follows:

10 µg DNA

4 µl 10× Reaction Buffer

1.6 µl 100 mM Spermidine Trichloride

5 µl (50 units) Restriction Enzyme

made up to 40 µl with Distilled Water

The long incubation at 37°C, the high enzyme concentration and the presence of spermidine trichloride were to ensure the complete digestion of the sample. Partial digestion of the DNA would produce spurious bands in the DNA fingerprint, making interpretation of the results impossible (Sambrook *et al.*, 1990).

2.4.1 Digestion of Friesian Control DNA

The restriction digestion of the Friesian control DNA was carried out in the same manner as for the Irish Moiled DNA samples, five units of restriction enzyme were used per µg of DNA. The reaction volume was much greater, 160 µl, as follows:

41 µl (955 µg/ml) Friesian DNA	40 µg
16 µl 10x Reaction Buffer	1 x
6.4 ml 100 mM Spermidine Trichloride	4 mM
20 µl (200units) Restriction enzyme)	5 units/µg DNA
Made up to 160 µl with Distilled water	

The digestion was carried out in a 0.5 ml Eppendorf tube, at 37°C overnight. One 160 µl reaction produced sufficient digested Friesian DNA for four separate gels, which enabled the Friesian DNA to be used as a control, not only for the relative position of banding patterns after hybridisation, but also for estimation of relative concentrations of digested Irish Moiled DNA samples, in the determination of loading volumes.

2.4.2 Loading Volume

After a minimum of 12 hours incubation at 37°C, a 2 µl aliquot (in 7 µl distilled water and 1 µl loading buffer) of each digested sample was electrophoresed on a 1% agarose 'test' gel, in 1×TAE running buffer, at 60 V for approximately 1 hour, to check the digestion of the sample was complete and to compare concentrations between samples.

After staining the 'test' gel, in a 0.5 µg/ml solution of ethidium bromide for 10 minutes, differences in the concentrations of the digested samples could be visualised

using a UV transilluminator. The concentration of each of the digested Irish Moiled DNA samples, could be compared, by eye, to that of the digested Friesian control DNA and the amount of each sample loaded onto the 'main' gel could be varied accordingly, to approach even loading of the wells.

Approximately 5 µg of digested DNA were loaded into each well, the volume of sample containing this amount of DNA, estimated from the 'test' gel, ranged from 15-25 µl (including 1/10 volume loading buffer). The wells in the 'main' gel could hold a maximum volume of 25 µl. The volume of samples with a very low concentration of digested DNA were reduced by evaporation in an oven at 55°C for several hours. The reduced samples were re-examined on another 1% agarose 'test' gel to estimate, by comparison with digested Friesian control DNA, the approximate volume of concentrated sample which contained 5 µg digested DNA.

DNA samples that had not fully digested could be easily identified from the 'test' gel by the presence of whole genomic DNA as a single band of high molecular weight. These samples were not suitable for DNA fingerprinting. It was important to identify samples which had not fully digested before running the 'main' gel, as the concentration of radiolabelled probe which would bind to the undigested genomic DNA could obscure the results from adjacent lanes. Poor digestion was normally a result of protein or phenol contamination, from the DNA extraction, preventing the action of the restriction enzyme. Undigested samples were re-extracted, as described previously (Section 2.3.4) to remove contaminating protein and phenol before being re-digested.

2.5 ELECTROPHORESIS

In order to be able to accurately compare samples electrophoresed on different agarose gels, running conditions had to be standardised. Every gel was run identically, including the consistant use of the same powerpack (Gibco BRL Model No. 400L) and electrophoresis rig (Gibco BRL Horizon 20.25).

Samples were run on large (25 cm long) 0.8% agarose (Type I, Sigma) gels, using 1 x TAE running buffer. A 20 tooth, 1 mm wide gel comb was used. However, only 16 samples, including control DNAs and the molecular weight marker were electrophoresed on each gel. The two outer lanes at each side of the gel were not used, as the banding pattern produced by samples run in these lanes were consistently found to be distorted, presumably due to effect of proximity to the edge on gel composition or fragment migration.

Greater resolution of the restriction fragments is obtained by electrophoresis at lower voltages for longer periods of time, however this can also result in the diffusion of DNA out of the agarose gel. Electrophoresis at higher voltages, reduces the resolution and (> 2 V/cm between electrodes) can cause overheating, which results in uneven running. After experimenting with a range of voltages and running times, the optimum conditions were found to be 50 V (1 V/cm between electrodes) for 24 hours, this gave good resolution of the fragments, and a minimal loss of DNA by diffusion out of the gel.

The running conditions also ensured that fragments of less than 1 kb in size were electrophoresed off the end of the gel. The very high frequency of small fragments in the population, makes it impossible to identify individual bands in the highly complex low molecular weight area of the banding pattern. The radioactive probe hybridises strongly to low molecular weight fragments, because of their concentration and this can obscure the more informative, higher molecular weight banding pattern.

Ethidium bromide was not used in the agarose gels or running buffers to visualise the DNA under UV light, because of its effect on the mobility of the DNA (Bruford *et al.*, 1992). DNA samples at higher concentrations run faster in the presence of ethidium bromide, so any difference in the concentration of DNA loaded in each well would seriously affect comparisons of band sharing between samples. If insufficient ethidium bromide is added to the loading buffer, the centre of the lane can migrate more quickly than the edges, resulting in smiling bands which are difficult to

score. In addition, ethidium bromide is a powerful mutagen, so from the point of view of user safety it is preferable to keep its use to a minimum.

Initially, when conditions for electrophoresis were being established, visualisation of the molecular weight marker was necessary in order to determine the distance migrated by the DNA samples. This was done by staining the agarose gel in a 0.5 μg/ml solution of ethidium bromide, for 10 minutes, after electrophoresis.

Once standardised running conditions had been established, it was no longer necessary to visualise the DNA after electrophoresis, as the migration distances should have been identical. The presence of Friesian control DNA on every agarose gel and the molecular weight marker, which hybridised to the probes $(GTG)_5$ and $(GGAT)_4$, would enable the determination of relative migration distances after hybridisation and any discrepancies between gels or across the width of a gel could be identified.

The molecular weight marker used was a 1 kb DNA ladder (Gibco BRL). 20μl of a 0.25 μg/μl solution of DNA ladder (in 1 x loading buffer) were loaded into the outer well, of the 16 lanes used, on every gel. The 5 μg of DNA ladder used enabled clear resolution of the marker fragments, after hybridisation with probes $(GTG)_5$ and $(GGAT)_4$. The probes $(GT)_8$ and $(TCC)_5$ did not bind to the molecular weight marker. 5 μg of digested Friesian control DNA (in 1 x loading buffer) were loaded 5 lanes in from the molecular weight marker and another 5 μg of Friesian control DNA were loaded in the outer well at the opposite side of the gel to the DNA ladder. Fourteen 5 μg samples of digested Irish Moiled DNA (in 1 x loading buffer) were loaded into the remaining wells, the outer two lanes at each side of the gel were not used as mentioned previously.

In loading gels in this uniform pattern, the possibility of confusing the identity of the lanes due to examination of the autoradiograph from the wrong side was eliminated, as the correct orientation could easily be established by reference to the distinctive Friesian control DNA and molecular weight marker lanes. In addition, the position of the two Friesian control DNA lanes would enable the detection of any variation in the distance migrated by the samples across the width of a gel.

Family groups were not generally electrophoresed close together on the same gel (samples were loaded randomly, but recorded). This was to enable the investigation of transmission of bands between individuals on separate gels, which would give an idea of how accurate the standardisation between gels had been, and to eliminate any bias that might have occurred during the scoring of the banding patterns.

2.6 GEL DRYING (For Oligonucleotide Probing)

After electrophoresis the agarose gels were dried down using a BioRad Model 583 gel dryer onto Whatman 3MM filter paper for 30 minutes at ambient temperature and 30 minutes at 60°C, as described by Schäfer *et al* (1988). Before drying down, the top right hand corner of the gel was cut off with a scalpel blade, as a further aid to establishing the orientation of the autoradiograph produced.

The Whatman 3MM was wetted with electrophoresis running buffer (1 x TAE) so that the agarose gel retained its own shape as it was carefully manouvered from the gel tray onto the filter paper. The use of dry Whatman 3MM frequently resulted in the distortion of the gel as it was placed on the filter paper. After drying down this distortion became permanent making it difficult to interpret the banding pattern obtained after hybridisation.

Drying down the agarose gel, traps the DNA fragments in the gel matrix, and during hybridisation the small microsatellite oligonucleotide probes can circulate through the gel matrix, to hybridise with the immobilised DNA fragments within the dried gel. This eliminates the need for the transfer of the DNA fragments to the surface of a nitrocellulose or nylon hybridisation membrane by Southern Blotting, which is not always 100% efficient.

Once dried down, the agarose gels were wrapped in Saran Wrap and could be stored flat, away from sunlight until they were needed for hybridisation.

2.7 SOUTHERN BLOTTING

Denaturing Solution :	0.15 M NaCl
	0.5 M NaOH
Neutralising Solution :	1.5 M NaCl
	0.5 M Tris HCl (pH 7.2)
	0.001 M EDTA

Southern transfer of the electrophoresed fragments onto a hybridisation membrane was performed as described by Southern (1975). The hybridisation membrane used was 'Hybond-N' nylon blotting membrane (Amersham). Prior to Southern transfer the DNA fragments were partially hydrolysed by soaking the gel in 0.25 M HCl for 20 minutes, this acid-induced cleavage of the DNA, helps in the transfer of large DNA fragments to the nylon membrane (Wahl *et al.*, 1979).

The gel was then rinsed in distilled water to remove any remaining acid and was placed in Denaturing Solution for 30 minutes at room temperature with constant shaking. The gel was again rinsed in distilled water and then placed in Neutralising Solution for 30 minutes at room temperature with constant shaking, using two changes of buffer.

A capillary blot was set up, using 20 x SSPE as the transfer buffer. After overnight capillary transfer the blotting apparatus was carefully dismantled and the Hybond-nylon hybridisation membrane was briefly washed in 2 x SSC to remove any adhering agarose.

2.7.1 Fixation of DNA to hybridisation membrane

The transferred DNA fragments were fixed to the Hybond-N membrane by UV cross-linking in a Stratagene UV crosslinker. The damp membrane was placed face up in the UV crosslinker, onto a piece of Whatman 3MM filter paper dampened with 10 x SSC. The auto-crosslink function delivers 120 juoules of UV light per cm^2, to the surface of the membrane, over a 30 second period. These conditions had been found

to be optimal for the attachment of DNA to Nylon membranes (Manufacturers instructions). When the membrane was not to be used immediately, in hybridisation experiments, it was stored between two sheets of Whatman 3MM filter paper and wrapped in Saran Wrap.

2.8 OLIGONUCLEOTIDE PROBING

The synthetic oligonucleotide probes $(GT)_8$, $(GTG)_5$, $(GGAT)_4$ and $(TCC)_5$ have been shown to produce individual specific DNA fingerprints in cattle (Buitkamp, 1990a,b). These four probes in conjunction with selected restriction enzymes were to be used to demonstrate hypervariable polymorphic 'minisatellite' loci in the Irish Moiled cattle DNA samples. The large number of animals sampled would entail the use of many agarose gels to accomodate all the samples. In order to enable comparisons to be made between samples electrophoresed on separate gels, the entire system would have to be standardised. Care was taken to ensure that conditions for restriction digestion, electrophoresis and hybridisation were constant in an attempt to minimise variation between gels.

2.8.1 Oligonucleotide Probes

Table 2.1 shows the four oligonucleotide probes used. These were obtained from Pharmacia and had been shown to produce highly polymorphic banding patterns in German Freisian, Red Pied and Simmetal cattle by Buitkamp (1990a).

TABLE 2.1

The four probes used, their length, the concentration and their optimum temperature for hybridisation. Optimum temperature was calculated as 5°C less than the melting temperature (Tm) of the oligonucleotide (Wallace *et al.*, 1979).

OLIGONUCLEOTIDE PROBES USED

Probe	Length	Temperature	Concentration
$(GTG)_5$	15	45°C	350 p mol/µl
$(GT)_8$	16	43°C	120 p mol/µl
$(GGAT)_4$	16	43°C	118 p mol/µl
$(TCC)_5$	15	45°C	106 p mol/µl

2.8.2 End Labelling of Oligonucleotide Probes

[α^{32}P]ATP was used to end label the oligonucleotide probes. The labelling reaction was carried out using T4 polynucleotide kinase as described by Schäfer (1988), but with a lower reaction volume, in an attempt to reduce the level of radioactivity handled. The reaction mixture was as follows:

		Reaction concentration
Distilled water	5.5 µl	
10 x PNK buffer	1.0 µl	1 x
10 p mol/µl probe	0.5 µl	5 pmol
10 µCi/µl γ^{32}P ATP	2.5 µl	25 µCi
(10 units/µl) T4 polynucleotide kinase	0.5µl	5units

The labelling reaction was carried out at 37°C for 45 minutes, followed by 10 minutes at 60°C to inactivate the enzyme and halt the reaction.

2.8.3 Nucleic Acid Hybridisation

2.8.3.1 Pre-treatment of gel

Prior to hybridisation the dried down agarose gels (Section 2.6) were denatured for 30 minutes, and then neutralised for 30 minutes. The Denaturing and Neutralising Solutions used were as described by Schäfer (1988). In each case two changes of solution were used and the gels were constantly agitated on a shaking platform.

Denaturing Solution	:	0.5M NaOH
		0.15M NaCl
Neutralising Solution	:	0.5M Tris Cl (pH 8.0)
		0.15M NaCl

The gels were then washed briefly in 6 x SSC, to remove any traces of neutralising solution, and prehybridised in 6 x SSC for one hour, at the hybridisation

temperature (43°C for probes $(GT)_8$ and $(GGAT)_4$ or 45°C for the probes $(GTG)_5$ and $(TCC)_5$).

2.8.3.2 Hybridisation

All hybridisations were carried out using Techne hybridisation tubes and ovens. Transferring the dried down agarose gel into the hybridisation tubes proved awkward as the damp gel tended to adhere to the sides of the tube. This problem was overcome by folding the gel into thirds, lengthways, before placing it into the hybridisation tube were it could be unfolded. Although dried down agarose gels were tough and flexible enough to be used time and again for repeated hybridisations, care had to be taken in manipulating them, as sharp instruments such as tweezers could easily cause them to tear.

Hybridisations were carried out for four hours in the hybridisation solution described by Schäfer (1988) at the optimum temperature for each probe, as given in Table 2.1.

Hybridisation solution :

		final concentration
5 ml	20 x SSPE	5 x
2 ml	50 x Denharts Solution	5 x
0.2 ml	10% SDS	0.1%
0.2 ml	(1 mg/ml) Sonicated denatured *E. coli* DNA	10 µg/ml

Made up to 20 ml with Distilled Water

2.8.3.3 Washing

After pouring off the hybridisation solution the gels were washed briefly, at room temperature in 6 x SSC (to rinse out the remaining hybridisation solution). This was followed by a 15 minute stringent wash in 6 x SSC at the hybridisation

temperature. This was generally sufficient to produce a low level of background radiation on the final autoradiograph. In cases where a high level of background radiation sufficient to impair scoring of the autoradiograph was observed, a further 25 minute wash in 6 x SSC at the hybridisation temperature was given. This reduced the background to a level where it did not interfere with gel interpretation.

2.8.3.4 Autoradiography

The agarose gel was then blotted dry between two sheets of Whatman 3MM filter paper and sealed in Saran Wrap to prevent it drying out completely. Probe bound to gels which have dried out cannot be removed and the gel cannot be reprobed.

The gels were exposed on GRI blue-sensitive X-ray film at room temperature in X-ray cassettes containing a single intensifying screen. Exposure time varied with the probe used, for gels probed with $(GT)_8$ it was 24 hours, gels probed with $(GTG)_5$ or $(GGAT)_4$ were autoradiographed for three days and exposure for gels probed with $(TCC)_5$ was up to one week. These exposure times were also affected by the age of the isotope used. Due to the short half-life of $\alpha^{32}P$, gels probed using two week old isotope stock required longer exposure times than those probed using fresh isotope.

2.8.3.5 Stripping and Re-probing

Every gel was probed with each of the four oligonucleotide probes in turn, $(TCC)_5$, which gave the weakest signal was used first, then $(GGAT)_4$, then $(GTG)_5$ and finally $(GT)_8$ which gave the strongest signal. This order of probing helped to ensure that isotope from the previous hybridisation was completely removed before re-probing.

To enable reprobing of the gels, bound probe was removed by 30 minutes of denaturation followed by 30 minutes of neutralisation as described under the pre-treatment of gels (Section 2.8.3.1). After this treatment the gels were checked for radioactivity using a Geiger counter. If the counts per second (cps) at the surface of a gel were still above background radiation, a further, more stringent, wash was required

to remove the remaining isotope; the gel would be washed in a 5 mM solution of EDTA at 60°C for 30 minutes (with two changes of wash solution).

Having been stripped for re-use the gels were equilibrated in 6 x SSC for two minutes, before being blotted dry and stored, wrapped in Saran, until required.

2.9 MINISATELLITE PROBING USING M13

Two clusters of 14 bp repeats in the Protein III gene of the bacteriophage M13 have been shown to detect hypervariable minisatellites in human and animal DNA (Vassart *et al.*, 1987; Gatel *et al.*, 1991) and have been used to produce highly polymorphic banding patterns within bovine DNA (Vassart *et al.*, 1987; Georges *et al.*, 1988).

2.9.1 Probe Production

It was attempted to amplify the larger of these clusters, using the polymerase chain reaction, to produce a probe for use with Irish Moiled cattle DNA. The repeated sequence to be amplified spanned from 2283 bp - 2401 bp (co-ordinates as Van Wezembeck *et al.*, (1980))in the M13 nucleotide sequence (see Fig. 2.1).

Figure 2.1

Alignment of tandem repeats present in the Protein III gene of M13. From Vassart *et al.*, (1987).

Using the M13 nucleotide sequence (Van Wezembeck *et al.*, 1980) a pair of 17 nucleotide primers (Biochemistry Department) were designed to bind to sequences flanking the larger of these tandem repeat clusters (see Fig. 2.2). The size of the region which would be amplified was approximately 160 bp (from co-ordinates 2267 bp to 2429 bp).

Figure 2.2

Primers designed to amplify the larger of the two clusters of repeated sequence in the Protein II gene of the bacteriophage M13

PRIMER 1 - $^{5'}$ AA CCT CCT GTC AAT GCT
PRIMER 2 - $^{5'}$ GC GTT TGC CAT CTT TTC

2.9.2 Primer Design

Care was taken in the selection of primers to avoid palindromic sequences, which may have resulted in the primer folding black on itself, complementarity between the two primers at the 3' end which promotes the formation of primer dimers (template independant artifacts - repeats of primers) and also runs of three or more C's or G's at the 3' end which promotes mispairing.

2.9.3 Amplification of Repeat Sequences by PCR

After some experimentation with primer/M13 DNA concentration and PCR conditions (Innis *et al.*, 1989), the following method was found to produce a clear single band of the expected size (~ 160 bp).

The following were mixed in an 0.5 ml Eppendorf tube in the order listed:

		Reaction concentration
74.5 µl	H_2O	
10 µl	10 x Manufacturers PCR buffer	1 x
5 µl	4mM dNTP solution	200 µM
5 µl	Primer 1 (20 mM)	1 µM
5 µl	Primer 2 (20 mM)	1 µM
5 µl	20 µg/ml M13 DNA	1 µg
	0.5 µl Taq polymerase	7.5 units
100µl	reaction volume	

Evaporation was reduced by overlaying the mix with 50 µl of mineral oil, prior to PCR. The PCR was carried out using a Perkin-Elmer Centaur DNA Thermal Cycler programmed for the following cycle:

Denaturation	94°C	40 seconds
Annealing	55°C	1 minute
Extension	72°C	1 minute
x 30 cycles followed by :		
Final Extension	72°C	10 minutes

Following PCR, 50 µl of the reaction mixture (+ 5 µl 10 x loading buffer) were electrophoresed on a 2% agarose gel (in 1 x TAE running buffer). The gel was stained for 10 minutes in a 0.5 µg/ml solution of ethidium bromide and viewed under UV light for the presence of the expected 160 bp amplification product.

The band was then excised from the agarose gel, using a scalpel blade, and transferred to a 1.5 µl Eppendorf tube. This was carried out as quickly as possible, to minimise damage to the fragment from exposure to UV light. The amplified fragment was then isolated from the agarose using the Bio 101 Inc. gene-clean kit according to the manufacturers instructions (Vogelstein and Gillespie, 1979).

The gel slice was found to weigh approximately 0.4 g equivalent to 0.4 mls of agarose. Two point five volumes (1 ml) of NaI stock solution were added to a final concentraion of approximately 4M, preventing denaturing of the DNA. The Eppendorf tube was then placed in a 50°C waterbath, to dissolve the agarose. Once the agarose was completely dissolved 5 µl "Glassmilk" suspension was added and the solution was mixed and placed on ice for 10 minutes. The glassmilk suspension is a silica matrix which binds to the DNA. The silica matrix and bound DNA was pelleted by centrifugation in a bench microcentrifuge at 13000rpm for 10 seconds and the NaI supernatant, containing dissolved agarose was poured off. The pellet was then washed in 'New Wash' solution three times to remove any residual NaI solution. The DNA was then eluted from the silica matrix by resuspension in 10 ml distilled water, and incubated at 50°C for three minutes. The silica matrix was pelleted by centrifugation for 30 seconds and the supernatant, containing the amplified DNA (to be used as a probe) was carefully transferred to a fresh 0.5 ml Eppendorf tube and stored at -20°C.

2.9.4 Radio-labelling Amplified M13 fragments

Two methods of radio-labelling the amplified M13 repeat fragment were used. Random hexanucleotide labelling of the fragment, excised from an agarose gel, after amplification by the PCR, and labelling of the fragment by incorporation of [α^{32}P]dCTP during amplification by the PCR.

2.9.4.1 Random Hexanucleotide Radio-Labelling Of Amplified Fragments

Radio-labelling was carried out using Boehringers' random hexanucleotide labelling kit, according to the schedule provided with the kit (Feinberg and Vogelstein, 1983, 1984).

25 ng of template DNA (amplified repeat sequence) was denatured by heating to 95°C for 10 minutes, then cooled on ice to prevent re-annealing of the DNA strands.

The following were added to the template DNA in a 0.5 ml Eppendorf tube, and mixed well:

9 µl (~25 ng) template DNA
1 µl each of (0.5 m mol/L) dATP, dGTP, dTTP
2 µl hexanucleotide reaction mixutre
5 µl 10 mCi/ml [α^{32}P]dCTP
1 µl Klenow enzyme
(20 µl Reaction Volume)

The reaction mixture was incubated at 37°C for 30 minutes after which the reaction was stopped, by the addition of 1 µl of 0.2 M EDTA (pH 8.0).

2.9.4.2 Labelling Of Amplified Fragment During PCR

Radio-labelled probe could be obtained by including [α^{32}P]dCTP in the PCR mix, which was then incorporated into the amplified M13 repeat sequence during the reaction. The PCR mix was as follows:

		Reaction concentration
10 µl	H_2O	
2 µl	10 x Manufacturers PCR buffer	1 x
1 µl	4 mM dNTP solution (-dCTP)	200 µM
1 µl	10 µM dCTP	0.5 µM
1µl	Primer 1 (20 µM)	1 µM
1 µl	Primer 2 (20 µM)	1µM
1 µl	(20 mg/ml) M13 DNA	1 µg
2 µl	10 µCi/µl [α^{32}P]dCTP	20µCi
1 µl	0.5 units/µl Taq Polymerase (in 1 x PCR buffer)	0.5 units
(20 µl	Reaction volume)	

The mix was overlaid with mineral oil and the PCR was carried out in a Perking-Elmer Centaur thermal temperature cycler as before (Section 2.9.3). After 30 cycles, before the final extension period at 72°C, 1 µl of 1 mM dCTP was added to the mix, for a 'Chase Reaction' to enable complete extension of incomplete products in the event of dCTP becoming limiting in the reaction.

2.9.5 Removal Of Unincorporated Nucleotides

The labelled probe produced by either method, was passed through a Sephadex G-50 column (Pharmacia) to remove unincorporated nucleotides and to assess the level of incorporation of isotope. A freshly prepared Sephadex column was washed through with 300 µl 1 x TE, before the products of the radio-labelling reaction were added at the top. 150 µl aliquots of 1 x TE were then passed through the tube and collected in 0.5 ml Eppendorf tubes at the bottom and labelled with the aliquot number.

The radioactivity of each aliquot was measured using a scintillation counter. The DNA is excluded from the Sephadex gel and larger DNA molecules run through the column more quickly. The first peak in the radioactivity of aliquots should represent the radio-labelled probe and the second peak the unincorporated nucleotides. The relative intensities of these two peaks allows the assessment of the level of incorporation of isotope into the probe. Over a number of experiments the level of incorporation was found to be approximately 30%.

2.9.6 Hybridisation

Prehybridisation/hybridisation solution :

	Reaction concentration
0.4 ml 10% Na-Pyrophosphate	0.2%
0.4 ml 0.5M EDTA (pH 8.0)	10mM
1.8 ml 20 x SSC	1.8%
1 ml 10% SDS	0.5%
0.5 ml 20% Marvel (dried skimmed milk)	0.5%
15.9 ml H_2O	
+ 1.2g PEG 5000	10mM

The size of the M13 probe (~282bp) prevents it from penetrating the dried down agarose gel matrix. Therefore all hybridisations involving the M13 probe were carried out using DNA bound to nylon hybridisation membranes (Section 2.7).

The prehybridisation solution was added to the nylon hybridisation membrane in a (Hybaid) hybridisation tube. Herring sperm competitor DNA has been replaced with dried skimmed milk (Marvel) as the M13 probe hybridises to a hypervariable minisatellite that is also bound by fish DNA. Vassart *et al* (1987) had shown that in the presence of herring sperm DNA the M13 probe was not capable of detecting hypervariable minisatellite repeat sequences. The membranes were prehybridised at 65°C for two hours in a Hybaid hybridisation oven.

The radio-labelled probe was denatured by heating to 95°C for 10 minutes, before being added to the hybridisation tube. The membranes were hybridised overnight at 65°C.

2.9.6.1 Washing and Autoradiography

Following hybridisation a sequence of washes were carried out as follows:

Wash I	briefly, at room temperature with 2 x SSC
Wash II	2 x 30 minutes. at 65°C with 2 x SSC, 1% SDS
Wash III	2 x 30 minutes at room temperature, with 0.1 x SSC.

The membranes were then allowed to air dry for 10 minutes. before being sealed in Saran Wrap. Autoradiography by exposure on GRI blue-sensitive X-ray film, was at -70°C, in X-ray cassettes containing one intensifying screen for up to seven days.

2.9.6.2 Stripping Hybond Membrane

The bound probe was stripped from the Hybond membrane using 0.1 x SSC, 10% SDS solution at 90°C twice for 15 minutes.

2.10 SCORING AND INTERPRETATION OF AUTORADIOGRAPHS

2.10.1 Band sharing

The level of band sharing between pairs of lanes was calculated as described by Piper *et al.*, (1992). Lanes were scored blindly, without knowledge of family relationships or coefficients of co-ancestry in order to prevent any bias in the scoring which might otherwise have occurred.

Autoradiographs were overlaid with overhead projection acetate sheets and the bands were drawn on the acetate so that they could be seen more clearly. Comparison between lanes was made from this acetate, but with reference to the autoradiograph. For each pair of lanes (or 'dyad') scoring began at the origin of electrophoresis and proceeded down the autoradiograph. The presence of a band in the first lane, second lane or common to both lanes was recorded. A band was regarded as unique to one lane if the other lane lacked that band altogether at the same location, or if the band was at least twice as intense in one lane as in the other. In the second case it was assumed that the intense lane was homozygous, containing two copies of the radiolabelled fragment, and that the less intense lane was heterozygous. Alternatively, the occurrence of two bands of such different intensity at a single location may have been the result of the fortuitous co-migration of different fragments (Piper *et al.*, 1992).

Bands were omitted from scoring when it was impossible to judge presence or absence for both lanes of a dyad. This was often the result of irregularity on the autoradiograph or the presence nearby of overlapping bands.

After scoring a pair of lanes, the band sharing between them was calculated using the following formula (Wetton *et al.*, 1987; Westneat *et al.*, 1990; Lynch, 1991a, b; Mannen *et al.*, 1993).

$$S = \frac{2N_{AB}}{(N_A + N_B)}$$

N_{AB} = Number of bands common to both lanes A and B.
N_A = Number of bands in lane A.
N_B = Number of bands in lane B
S = Band sharing score.

The band sharing score represents the portion of bands shared between the lanes. It is weighted according to the number of scorable bands in each lane (Piper, 1992). Theoretically, the DNA fingerprints between two individuals should become more similar and, therefore, the band sharing score should become greater as their relationship increases (Lynch, 1988, 1990).

The difficulty in comparing samples electrophresed on different gels, and in comparing samples run in distant lanes on the same gel has been stressed by many authors (Mannen *et al.*, 1993; Burke *et al.*, 1987; Wetton *et al.*, 1987; Piper *et al.*, 1992). As mentioned previously, standardised electrophoresis conditions and Friesian DNA control lanes were employed on all gels in an attempt to allow the determination of levels of band sharing betwen distant lanes and hopefully even between lanes on different gels.

2.10.2 Segregation Analysis

The occurrence of linkage or allelism in the bands detected in the DNA fingerprint may have been crucial to the idea of an individual-specific DNA fingerprint (Bruford *et al.*, 1992; Burke and Bruford 1987). A segregation analysis on one or more families of two parents and eight or more offspring should be carried out to

establish whether the bands detected are indeed unlinked (Bruford 1992; Burke and Bruford 1987; Jeffreys and Morton 1987). Litter size in Irish Moiled cattle was rarely greater than one and any particular cow was rarely mated with the same bull more than once. The large pedigrees required to perform a segregation analysis were therefore unavailable in Irish Moiled cattle. Each molecular weight band was therefore regarded as a separate microsatellite locus, because of the difficulty in detecting linkage and allelism without many sets of parent-offspring data (Hillel *et al.*, 1989; Mannen *et al.*, 1993).

2.11 ARBITARILY PRIMED POLYMERASE CHAIN REACTION (AP-PCR)

It was attempted to demonstrate DNA polymorphisms in Irish Moiled DNA using the Arbitarily Primed PCR technique described by Welsh *et al.*, (1990, 1991) and Williams *et al.*, (1990). AP-PCR is a simple and fast technique based on the amplification of genomic DNA with single primers of arbitary nucleotide sequences to generate a 'fingerprint' of PCR products (Welsh, 1990; Williams 1990).

2.11.1 Primers Used

Three of the 10 nucleotide primers, described by Williams *et al.*, (1990) were constructed (Biochemistry Department, University of Liverpool).

RAP1 - $^{5'}$TGG TCA GTG A
RAP2 - $^{5'}$CGG CCA CTG T
RAP3 - $^{5'}$GCA AGT AGC T

Any primer with a 40% or greater GC content and a length of at least 9 bases should generate detectable levels of amplification products (Williams *et al.*, 1990). All available primers which fitted the above conditions were also used for AP-PCR of Irish Moiled DNA. These additional primers were as follows:

PRIMER 1 - $^{5'}$AA CCT CCT GTC AAT GCT
PRIMER 2 - $^{5'}$GC GTT TGC CAT CTT TTC

These primers bind either side of the repeat sequence in the protein III gene of the bacteriophage M13 (Section 2.9.1).

C10 $5'$ CCC CCC CCC C
C17 $5'$ CCC CCC CCC CCC CCC CC

In addition, the primer LIV-1, with a GC concent of 18.5% was also used.

LIV - 1 $5'$ CGTCGAC $(T)_{20}$

2.11.2 PCR Conditions

The standard PCR conditions used in an attempt to amplify segments of Irish Moiled genomic DNA are given here. AP-PCR was carried out using 25 µl reaction mixtures in 0.5 ml Eppendorf tubes as follows:

	Reaction concentration
14.5 µl H_2O	
2.5 µl 10 x PCR buffer	1 x
1 µl dNTPs (2.5 mM)	100 µM
5 µl Primer (5 µM)	1 µM
1 µl (25 ng/µl) DNA	25 ng
1 µl (2 units/µl) Taq polymerase	2 units

The reaction was overlaid with mineral oil and cycled through the following temperature profile:

denaturation	94°C	-	40 seconds
annealing	36°C	-	1 minute
extension	72°C	-	1 minute
x 45 cycles, followed by:			
final extension	72°C	-	10 minutes

2.11.3 Electrophoresis of amplification products

Following PCR, the reaction mixtures were taken from under the mineral oil, using a micropipette tip, and transferred to fresh Eppendorf tubes, one-tenth volume of loading buffer was added and 10 µl of product were electrophresed on a 2% agarose gel (with 1 x TAE running buffer) at 50 V for up to four hours.

Amplified DNA fragments were detected by staining the agarose gel, after electrophoresis, in a 0.5 μg/ml solution of ethidium bromide for several minutes before examination on a UV transilluminator.

2.12 COMPUTER ANALYSIS OF PEDIGREE DATA

A computer programme, MOILMATE, was written with the help of Adrian Turner, to assist in the analysis of Irish Moiled pedigree data. So that the programme would be readily available to breeders it was written in Qbasic (version 1.1, Microsoft Corporation) and will run on any IBM compatible computer, although a 386 or faster processor is recommended, to enable calculations to be performed reasonably quickly. A data file (Moiled92.dat) has been written, containing pedigree information for all the Irish Moiled cattle listed in the 1992 Herd Book. The programme assumes pedigrees to be correct and from the information in this data file the Moilmate programme was able to calculate inbreeding coefficients and coefficients of co-ancestry for Irish Moiled cattle. In addition, using a similar procedure ('gene-dropping') (MacCluer, *et al.*, 1986) the relative genetic contribution of the eight founder animals to the extant population were estimated and the risk of future loss of the genes contributed by the various founders could be predicted.

A tutorial designed to allow breeders to use the Moilmate programme is given in Appendix I and a full listing of the programme is given in Appendix II.

2.12.1 Calculation of Inbreeding Coefficients

Inbreeding coefficients (Wright, 1992) were calculated from the pedigree information contained in the Irish Moiled data file. For each individual, the programme finds every possible route by which the animal could receive alleles that are identical by descent. To do this, every possible pathway through which an allele can be transmitted on the individuals father's side is compared with every pathway on its mother's side. Where the pathways first meet a 'hit' is scored. The individual where the pathways meet is a common ancestor, and the chance of the individual under consideration

receiving alleles identical by descent from this common ancestor, by the pathway found, is calculated using Wright's coefficient. A systematic comparison of each possible pathway on the father's side with every possible pathway on the mother's side enables every common ancestor to be found (Fig. 2.3). The sum of the probability of the individual receiving alleles identical by descent from each of these pathways is calculated to give the inbreeding coefficient for the individual.

$$F_I = \Sigma\left[(0.5)^{m+f+1}(1+FA)\right]$$

m = intervening generations on male side.

f = intervening generations on female side.

FA = inbreeding coefficient of common ancestor A.

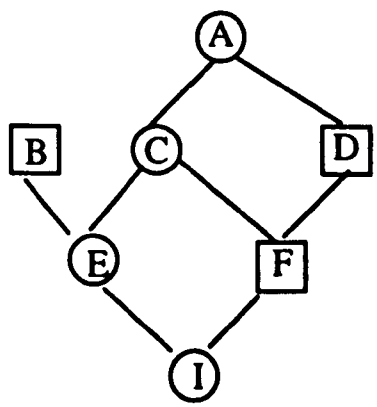
F_I = inbreeding coefficient of Individual I.

Figure 2.3

An illustration of the comparison of pathways to find common ancestors using the Moilmate programme, with a hypothetical pedigree for inbred individual 'I'. The common ancestors 'A' and 'C' were assumed not to be inbred.

□ = male

○ = female



male path	I-F	I-F-D	I-F-D-A	I-F-C
female path	I-E	I-E	I-E	I-E
	I-E-B	I-E-B	I-E-B	I-E-B
	I-E-C	I-E-C	I-E-C	I-E-C
	I-E-C-A	I-E-C-A	I-E-C-A	
	paths do not meet	paths do not meet	paths meet at A	paths meet at C

2.12.2. The Irish Moiled Data file (Moiled92.dat)

The Irish Moiled data file contains 476 entries, each representing a single animal, extant in 1992 or the ancestors of those from the 1992 Herd Book. Each entry contains the following information: Herd Book Number of the individual, Herd Book Number of it's male parent, Herd Book Number of its female parent, Sex, Alive or Dead, Date of Birth and whether or not the individual was one of the founders.

The individuals are not entered into the data file in any particular order, but were sorted into date of birth by the Moilmate programme. The Moilmate programme started calculating inbreeding coefficients for the oldest individuals in the data file and worked steadily through to the youngest, so that the inbreeding coefficients of common ancestors were known before the calculation of inbreeding coefficients for their descendants.

2.12.3 Calculation of Coefficients of Co-ancestry

Coefficients of co-ancestry were calculated in a similar manner to inbreeding coefficients. The coefficient of co-ancestry between two individuals is equivalent to the inbreeding coefficient of their offspring. For each calculation of co-ancestry an imaginary offspring was generated for the two animals and the inbreeding coefficient of this imaginary offspring was calculated as described in the previous section.

In the early stages of the development of the Moilmate programme, a print-out option was available to print out each pathway as it was found. This enabled the calculations and the pathways to be checked. Once the programme was found to be

error free, the print-out option was removed as it was found to slow down the execution of the programme drastically.

2.12.4 Founder Calculations

Macleur *et al.*, (1986) described a computer simulation procedure, called 'gene dropping' which was useful in the genetic management of captive populations. A similar programme, based on Macleur's description of the simulation, was written in Qbasic, as part of the Moilmate programme and applied to the Irish Moiled herd.

The gene dropping procedure has many applications. It can be used to estimate the change in the genetic structure of a population, to estimate the mean genetic contribution of each of the founder individuals to the extant herd, to estimate the percentage of each founder genome lost and predict the percentage of risk of future loss, and also to estimate inbreeding coefficients from identity by descent.

2.12.4.1 Assignment of hypothetical alleles

Each of the eight Irish Moiled founder animals was assigned a pair of unique hypothetical alleles (Table 2.2)

Table 2.2

Unique founder alleles g1-g16 for the eight Irish Moiled founder animals.

Founder No.	Herd Book No.	Name	Alleles
F1	783	Ballydugan Kat	g1, g2
F2	792	Miss Nugent	g3, g4
F3	762	Ballydugan Duke	g5, g6
F4	798	Ballydugan Mimosa	g7, g8
F5	788	Listerdonan	g9, g10
f6	786	Maymore VI	g11, g12
F7	790	Derylecka	g13, g14
F8	723	Derryboy Cyclamen	g15, g16

Using the pedigree information in the Moiled92.dat data file each descendant is given a genotype by Mendelian segregation of its parent's alleles. A random number generator is used to determine which of the two hypothetical alleles is passed on (50/50 chance). Fig. 2.4 demonstrates this Mendelian segregation of founder alelles for a hypothetical pedigree with only four founder animals.

Figure 2.4(a)

Simple pedigree, with four founders (F1-F4) showing the assignment of a pair of unique hypothetical alleles to each founder:

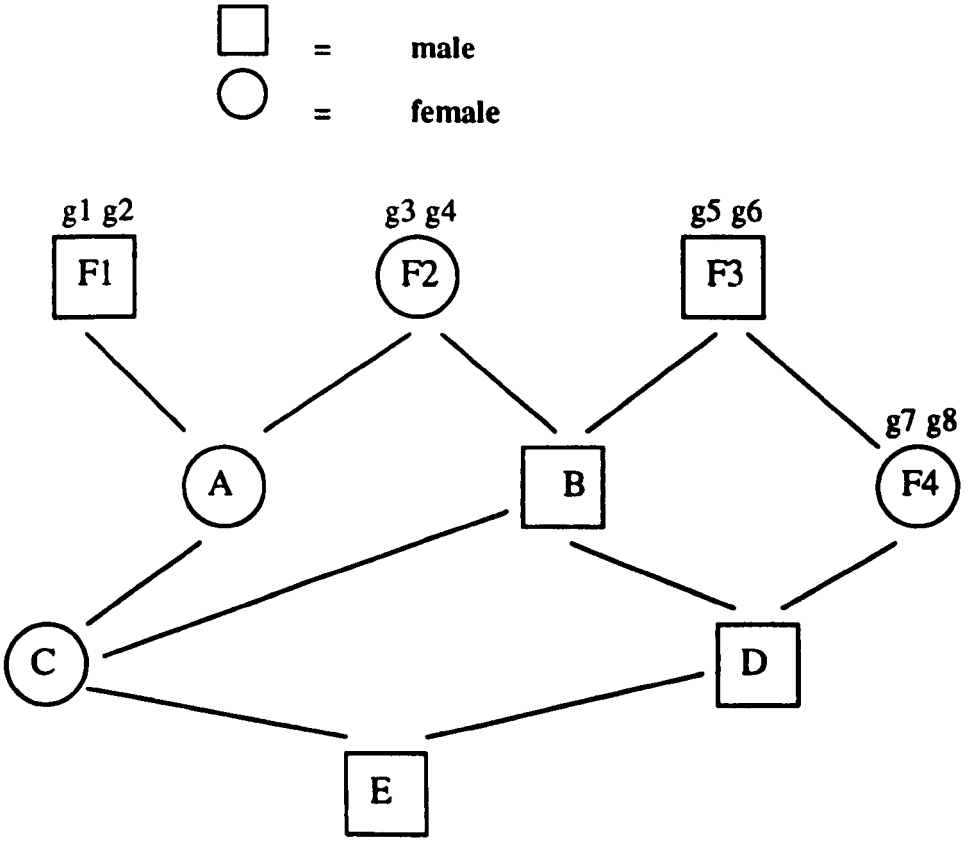


Figure 2.4(b)

Pedigree after gene dropping, with genotypes assigned to descendants by Mendelian segregation of founder alleles.

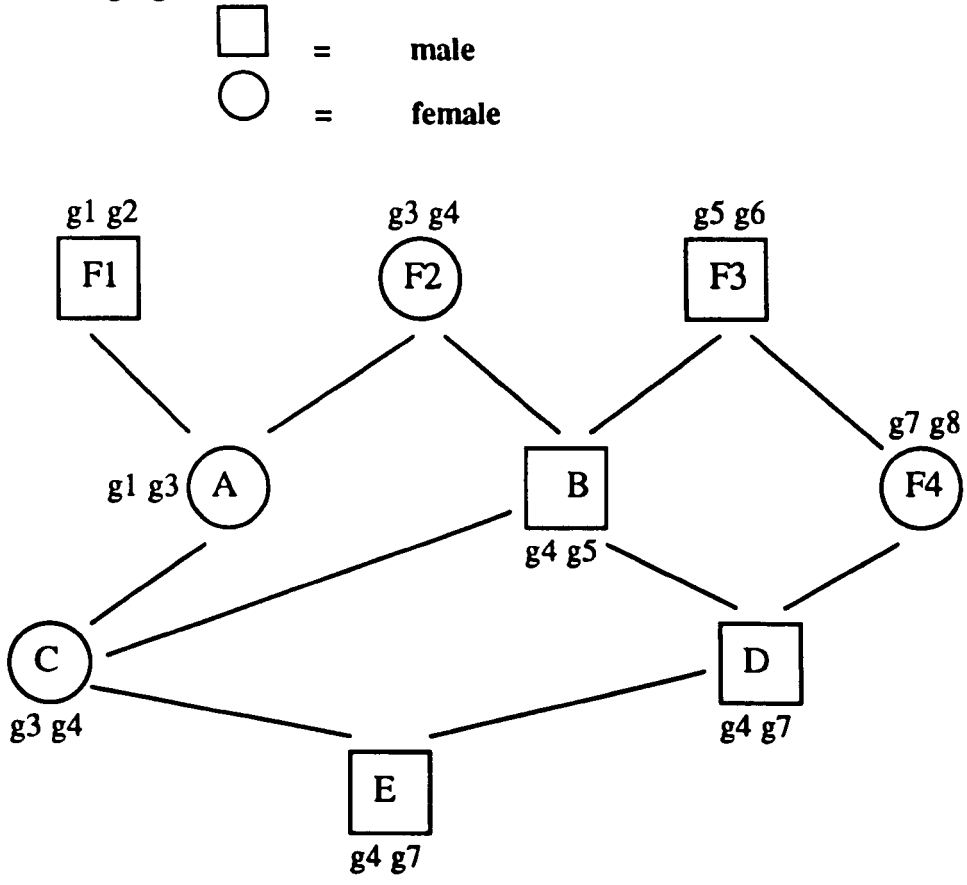


Figure 2.4(b) shows the results of a single cycle of allele assignment. For each founder analysis experiment this process was repeated for a total of one thousand cycles. At the end of each cycle the genotypes assigned to each individual were recorded by the Moilmate programme and used to generate founder analysis results after the completion of 1000 cycles. The founder calculations investigated the transmission of a single pair of alleles from each founder animal. With a sufficient number of cycles the data from the transmission of a pair of alleles can be used as an estimate for the entire genome of a founder.

In addition to the 16 unique founder alleles (g1-g16) used in the analysis of the Irish Moiled population, two other aleleles had to be incorporated into the analysis to accommodate non-Irish Moiled animals used in upgrading schemes and also a number

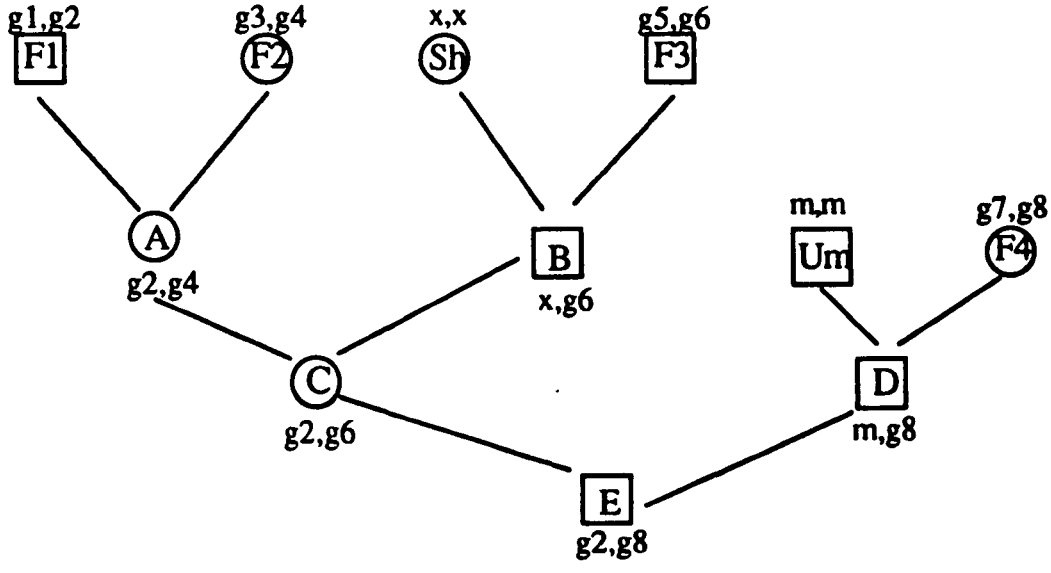
of cases where parentage listed as 'pure registered, but unknown Irish Moiled' in the Irish Moiled Herd Book.

Non-Irish Moiled, or unknown parentage was shown in the Moiled92.dat data file as a blank entry under 'male/female parent' for an individual. All non-Irish Moiled or unknown parents were assigned the alleles"x". Pure registered but unknown Moiled parentage was represented by the letter "M" under 'male/female parent' for an individual, e.g. Appendix III, item 122, female parent of 'C15' was a pure registered but unknown Irish Moiled animal. The blank entry under male parent for C15 indicates that his sire was not an Irish Moiled, in this case the sire was a pedigree polled Lincoln Red bull. Pure registered but unknown Irish Moiled were assigned the genotype "m,m". The alleles for non-Irish Moiled animals and pure registered but unknown Irish Moiled cattle were assigned to descendants in exactly the same way as the unique hypothetical founder alleles (see Fig. 2.5).

Figure 2.5
Simple pedigree, with 4 founders (F1-F4) showing assignment of hypothetical founder alleles (G1-G8), non Irish Moiled alleles (x) and pure registered, but unknown Irish Moiled alleles (m) to descendants by Mendelian segregation.

Sh = Shorthorn,

Um = Unknown but pure registered Irish Moiled.



No distinction has been made between the different breeds of cattle used in various upgrading programmes (Shorthorn, Red Poll, Jersey, Friesian, Highland, Limousin) although it would be a simple procedure to do this. The different breeds could be listed by different letters in the data file, e.g. "S" for Shorthorn, "R" for Red Poll, and each breed could be assigned a different pair of alleles, e.g. Shorthorn "s,s", Red Poll "r,r". In this way the segregation of alleles from each different breed of non-Irish Moiled cattle in the pedigree could be analysed.

2.12.4.2 Percentage Contribution of Founders to Extant Herd

The extant herd consists of all the individuals which are listed as 'alive' in the Moiled92.dat data file. Initially the designation of 'alive' or 'dead' was based on the population census obtained by collection of blood samples. All animals sampled were listed as alive. In addition, bulls for which semen stores were avialable were also listed as alive. The remaining animals in the data file were listed as dead. This population census can, however, only be an estimate of the Irish Moiled population at any given time, as the deaths of individual animals are not reported, making the updating of the data file impossible.

The percentage genetic contribution of each of the founder animals to the extant herd was determined from the frequency at which each of the unique founder alleles were assigned to extant animals each cycle. After a sufficient number of cycles these calculations would give a reasonable estimate of the proportionate contribution of each of the founder animals to the extant herd, allowing the identification of under-represented founders. The frequencies at which non-Irish Moiled alleles were assigned to extant animals were also recorded and the proportionate contribution of non-Irish Moiled and unknown Irish Moiled animals to the present herd could also be shown.

The Moiled92.dat data file had 178 pure-registered and upgrading animals listed as alive. It was possible to alter the data file, changing which individuals were listed as alive or dead, in order to investigate different parameters. For example, all upgrading animals could be removed from the calculation by listing them as dead, in

order to investigate the proportionate contribution of the different founders to the pure-registered Irish Moiled population, or only animals born in a particular year could be listed as alive to investigate the contribution of the founders to calves born that year.

2.12.4.3 Proportion of Founder Genomes lost

The proportion of each founder genome lost was calculated from the proportion of cycles in which a particular founder allele was not assigned to any of the living animals.

2.12.4.4 Proportion of Founder genomes at high risk of loss

The proportion of each founder genome at high risk of loss was calculated from the proportion of cycles in which a particular founder allele was present in the living population at a frequency of less than 10%. This value is an estimate of the percentage of the total founder genome at high risk of loss. An estimate of the percentage of surviving founder genes at high risk of loss was obtained by calculating the percentage of each founder genome lost divided by the percentage of surviving founder genome.

$$\% \text{ Surviving genes at risk} = \frac{\% \text{ at high risk of loss}}{(1 - \% \text{ of founder genome lost})}$$

In an investigation of a colony of 445 grey short-tailed opossum, Macleur (1986) estimated the proportion of founder genomes at risk of loss, from the proportion of cycles in which a particular founder allele was present at less than 1%. Due to the small population size of Irish Moiled cattle this criterion was considered unsuitable and a value of 10% was used, as described by Macleur (1986) for the investigation of the 44 Spekes gazelles in United States zoos.

2.12.4.5 Founder Composition of Animals

The founder composition of individual animals could be estimated from the percentage of different alleles received by an individual during the course of the simulation. This information was then displayed as the proportionate contribution of the eight founders and non-Irish Moiled animals to a particular individual (see Results). This would allow the identification of animals containing a high percentage of rare founder alleles, enabling selective breeding to arrest the loss of under-represented founder genomes.

2.12.4.6 Inbreeding Coefficients

The inbreeding coefficients of individual animals were determined using the 'gene-dropping' computer simulation. The proportion of cycles in which individuals received founder alleles that were identical by descent were tabulated to give an estimation of Inbreeding Coefficient.

The values for Inbreeding Coefficients obtained using the simulation procedure, could be compared with the inbreeding coefficients determined mathematically, by Wright's coefficient, in order to assess how accurate the various estimated results obtained using the simulation procedure were. A low correlation between the values of inbreeding coefficients obtained by the two methods would indicate that the accuracy of the computer simulation results was low. The accuracy of the estimates could then be increased by increasing the number of cycles of 'gene-dropping'.

CHAPTER 3

DNA FINGERPRINTING USING THE M13 PROBE

Two clusters of repeat sequences in the Protein III gene of the bacteriophage M13, have been shown to produce individual specific banding patterns in a range of species including pigs, horses, dogs (Georges *et al.*, 1988), chickens (Kuhnlein *et al.*, 1989), sheep (Gatel *et al.*, 1991) and cattle (Vassart *et al.*, 1987; Georges *et al*, 1988, 1990; Mannen *et al.*, 1993).

3.1 CONFIRMATION OF PROBE INTEGRITY

The use of the amplified M13 fragment produced and radiolabelled as described in Materials and Methods, failed to produce any hybridisation signal with digested Irish Moiled DNA, despite numerous modifications to the hybridisation stringency and conditions (see Section 2.9.6). It was therefore necessary to investigate whether or not the amplified fragment contained the expected sequence from the Protein III gene of the bacteriophage M13.

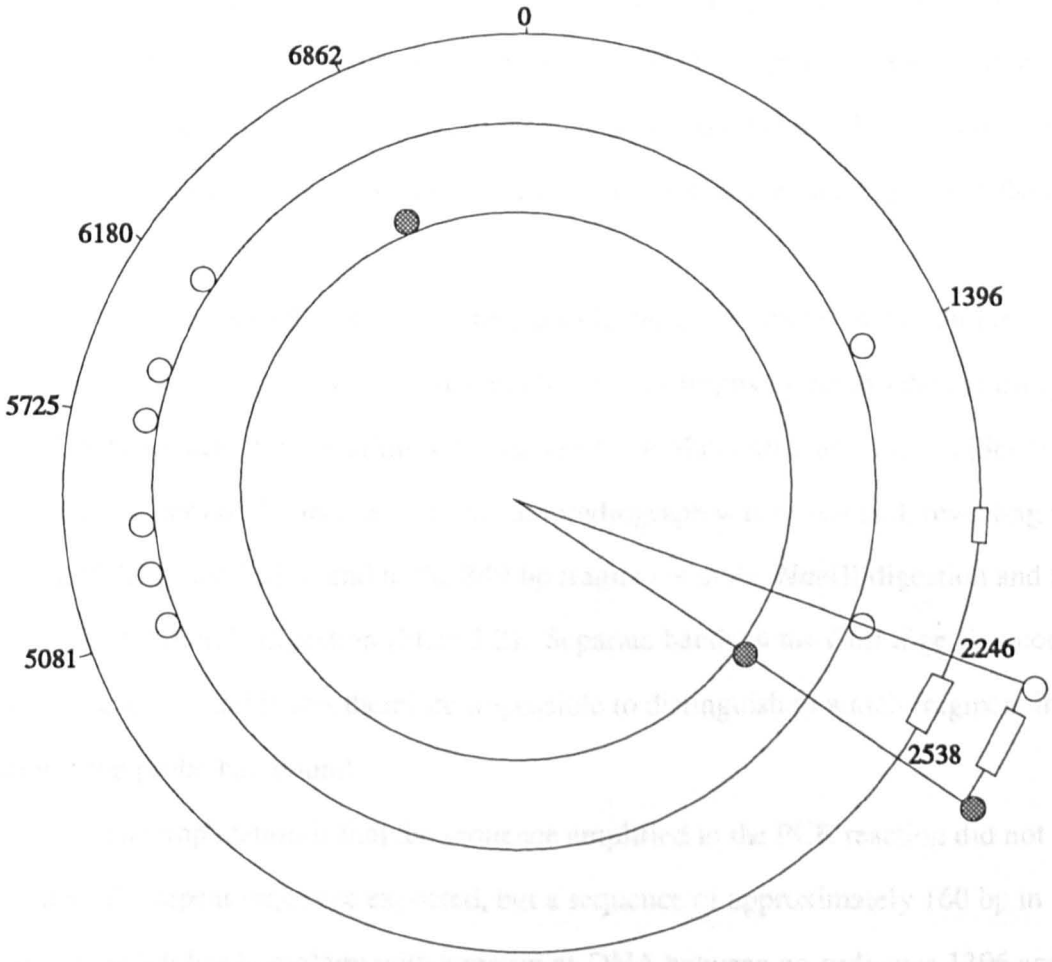
Using the M13 restriction map (van Wezembeck *et al.*, 1980) it was found that the following restriction digestions would produce bands of the size indicated containing the two repeat clusters in the Protein III gene of M13.

Digest	**Band size (bp)**
*Cla*I	2895
*Hae*III	309/849
*Cla*I and *Hae*III	282/849

The locations of these fragments in the M13 genome are shown in Fig. 3.1. The sizes of the other restriction fragments produced by each digestion were also determined, so that the band containing the repeat clusters could be more easily identified after electrophoresis of the restriction products.

Figure 3.1

M13 Restriction Map



***Hae*III (○) and *Cla*I (●) restriction sites are shown. The open boxes give the localization of the tandem repeat sequences. The fragment produced by the *Hae*III/*Cla*I double digestion, containing the larger of these repeat sequences is also shown. The coordinates are as described by van Wezembeck *et al* (1980).**

Following restriction digestion of M13 RF DNA, the restriction products were electrophoresed on a 2% agarose gel (in 1 x TAE running buffer). The gel was stained in a 0.5 μg/ml solution of ethidium bromide for five minutes and viewed over a UV transilluminator. The restriction fragments were well separated and the 282 bp fragment, containing the larger of the two repeat clusters, produced by the *Hae*III and *Cla*I double digest was clearly visible, as was the 309 bp fragment, also containing the larger of the repeat clusters, produced by the *Hae*III digest (Plate 3.1). These bands were identified with reference to the molecular weight marker and the size of the other fragments produced in each digestion.

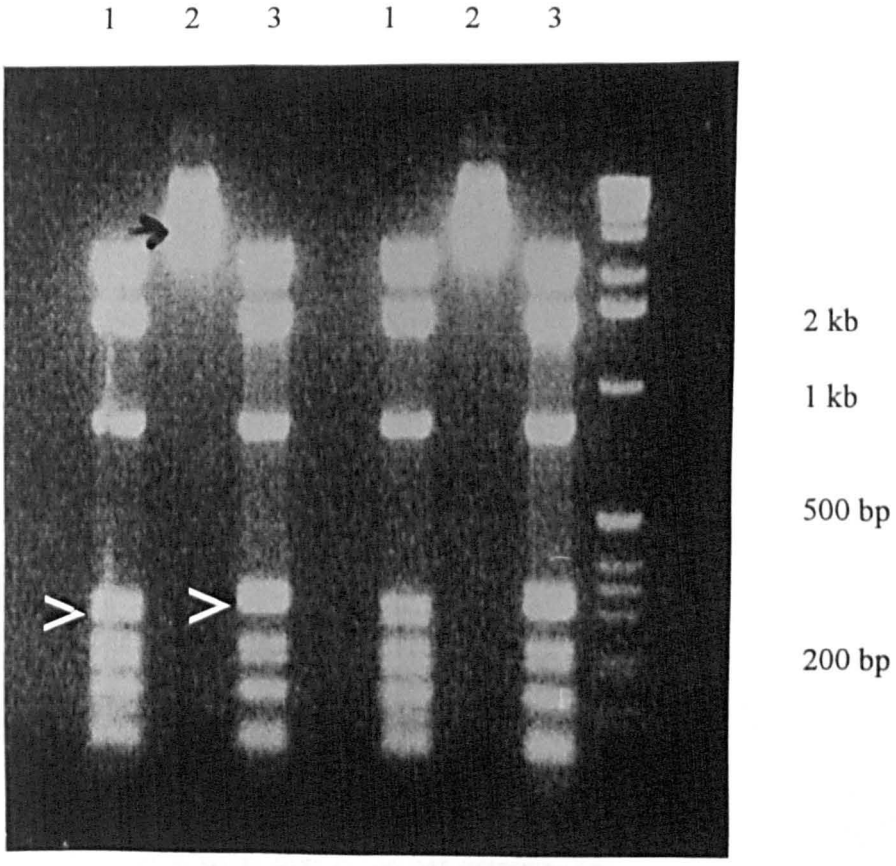
The agarose gel was then blotted onto Hybond hybridisation membrane (Section 2.7) and hybridised with the amplified M13 fragment, radio-labelled using the random hexanucleotide labelling kit as described in Materials and Methods (Section 2.9.4.1). After two hours exposure the autoradiograph was developed, revealing that most of the probe had bound to the 849 bp fragments in the *Hae*III digestion and the *Hae*III/*Cla*I double digestion (Plate 3.2). Separate bands in the *Cla*I digestion could not be resolved and it was therefore impossible to distinguish to which fragment in this digest the probe had bound.

The implication is that the sequence amplified in the PCR reaction did not contain the repeat sequence expected, but a sequence of approximately 160 bp in length, which has homology with a region of DNA between co-ordinates 1396 and 2245 bp in the M13 genome. This region contains three repeats of the same consensus sequence as that of the repeat cluster in the expected amplification product. However, it was unlikely that the probe had hybridised specifically to this sequence, as the signal at the 282 bp band and the 309 bp band, produced by the *Hae*III/*Cla*I double digestion and the *Hae*III digestion respectively, would have been equally intense, if not stronger, than that at the 849 bp fragment.

The DNA Star programme was used to determine any homology between the primers used in the PCR reaction and the rest of the M13 genome. No significant homology was found and the reason for the amplification of the wrong fragment was

Plate 3.1

Restriction digestion of genomic M13 mp9 DNA with the enzymes *Hae*III and *Cla*I

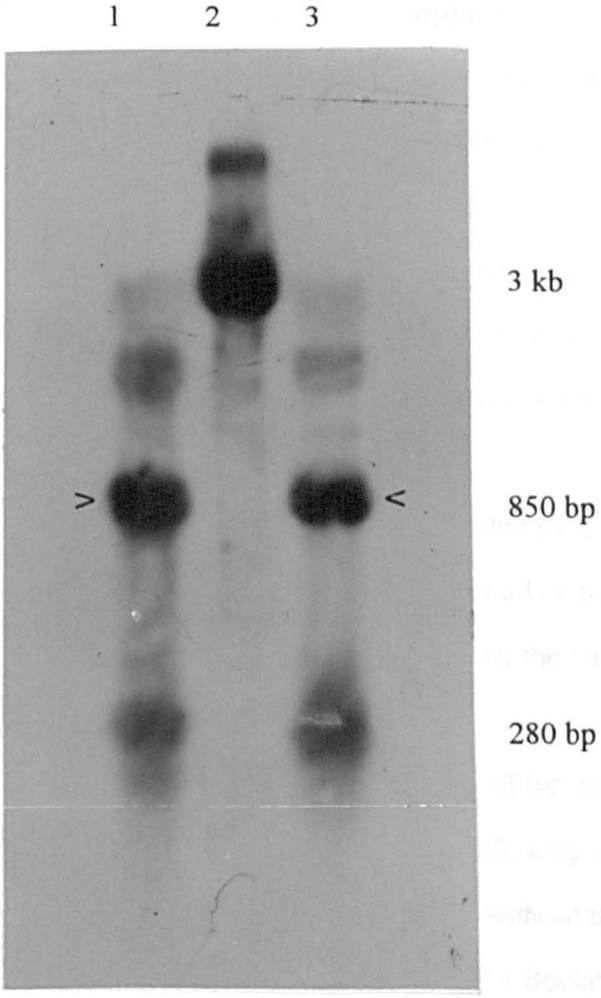


The restriction products from the digestion of genomic M13 DNA are shown in Plate 3.1 The restriction products were analysed on a 2% agarose gel containing ethidium bromide and electrophoresed for two hours at a constant voltage of 60 V. The fragments which contain the larger of the two repeat clusters in the Protein III gene are arrowed.

Lane 1 - M13 DNA digested with *Hae*III and *Cla*I.
Lane 2 - M13 DNA digested with *Cla*I.
Lane 3 - M13 DNA digested with *Hae*III.

Plate 3.2

Hybridisation of M13 probe, produced by PCR amplification of the larger repeat cluster in the Protein III gene, to digested M13 DNA.



The M13 probe, produced by PCR amplification of the larger repeat cluster in the Protein III gene, has hybridised strongly to the 849 bp bands (arrowed) produced by the digestion of genomic M13 DNA with *Hae*III and the double digestion of M13 DNA with *Hae*III and *Cla*I (see Plate 3.1). Individual bands cannot be resolved with the *Cla*I digest.

Lane 1 - M13 DNA digested with *Hae*III and *Cla*I.
Lane 2 - M13 DNA digested with *Cla*I.
Lane 3 - M13 DNA digested with *Hae*III.

unclear. It is possible that the DNA secondary structure or a template independent artefact prevented the amplification of the correct sequence.

3.1.1 New Method of Producing M13 Template

In order to produce a probe containing the larger of the two repeat fragments in the Protein III gene of the bacteriophage M13, the *Hae*III/*Cla*I double digestion was repeated, and the restriction products were electrophoresed on a 2% low melting point agarose gel. The 282 bp fragment, containing the desired sequence, was excised from the gel, using a scalpel, and transferred to a 0.5 ml Eppendorf tube. The gel slice was boiled for seven minutes, to completely melt the agarose and stored at -20°C until used for labelling.

The concentration of the template DNA was estimated by a comparison with a range of M13 DNA samples of known concentrations, over a UV transilluminator, after the addition of ethidium bromide. The concentration of the template DNA was found to be between 50 and 100 ng/µl.

Prior to the labelling reaction the template was boiled for three minutes, and then incubated at 37°C for between 10 and 60 minutes. Following this the DNA in the low melting point agarose could be radio-labelled directly without the need for further purification. The labelling reaction was carried out using the Boehringer random hexanucleotide labelling kit, as described in Section 2.9.4.1.

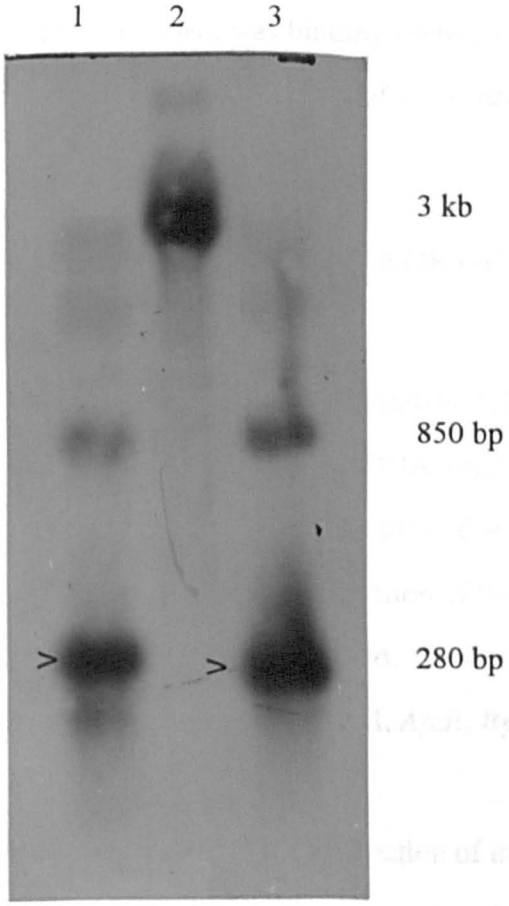
3.1.2 Excised M13 Fragment Hybridised to Correct Band

In order to check that the radio-labelled fragment would hybridise with the repeat sequence in the Protein III gene of M13, the 'digested M13 test membrane' (Section 3.1) was stripped and re-probed with the excised 282 bp M13 fragment, as described in Materials and Methods.

Following autoradiography, the excised M13 fragment was found to bind strongly to the 282 bp band in the *Hae*III/*Cla*I double digestion (Plate 3.3). The fragment was also found to bind readily to the 842 bp fragment produced by the same

Plate 3.3

Hybridisation of the M13 probe, produced by the excision of the 232 bp band (containing the larger of the repeat clusters in the Protein III gene), from the double digestion of M13 DNA with *Hae*III and *Cla*I, with digested M13 DNA.



The M13 probe has hybridised preferentially with the 282 bp band from the *Hae*III/*Cla*I double digestion (arrowed) and the 300 bp band from the *Hae*III digestion (arrowed). Individual bands in the *Cla*I digestion cannot be resolved.

Lane 1 - M13 DNA digested with *Hae*III and *Cla*I.
Lane 2 - M13 DNA digested with *Cla*I.
Lane 3 - M13 DNA digested with *Hae*III.

restriction digestion. This was to be expected as this fragment contains a cluster of three repeats with the same consensus sequence as the repeat sequence in the 282 bp fragment. There was negligible hybridisation to the other restriction fragments, suggesting that the excised M13 fragment was binding preferentially to the repeat cluster in the Protein III gene of M13 and could therefore be used as a probe to detect hypervariable minisatellites in Irish Moiled DNA.

3.2 INITIAL INVESTIGATION USING A RANGE OF RESTRICTION ENZYMES

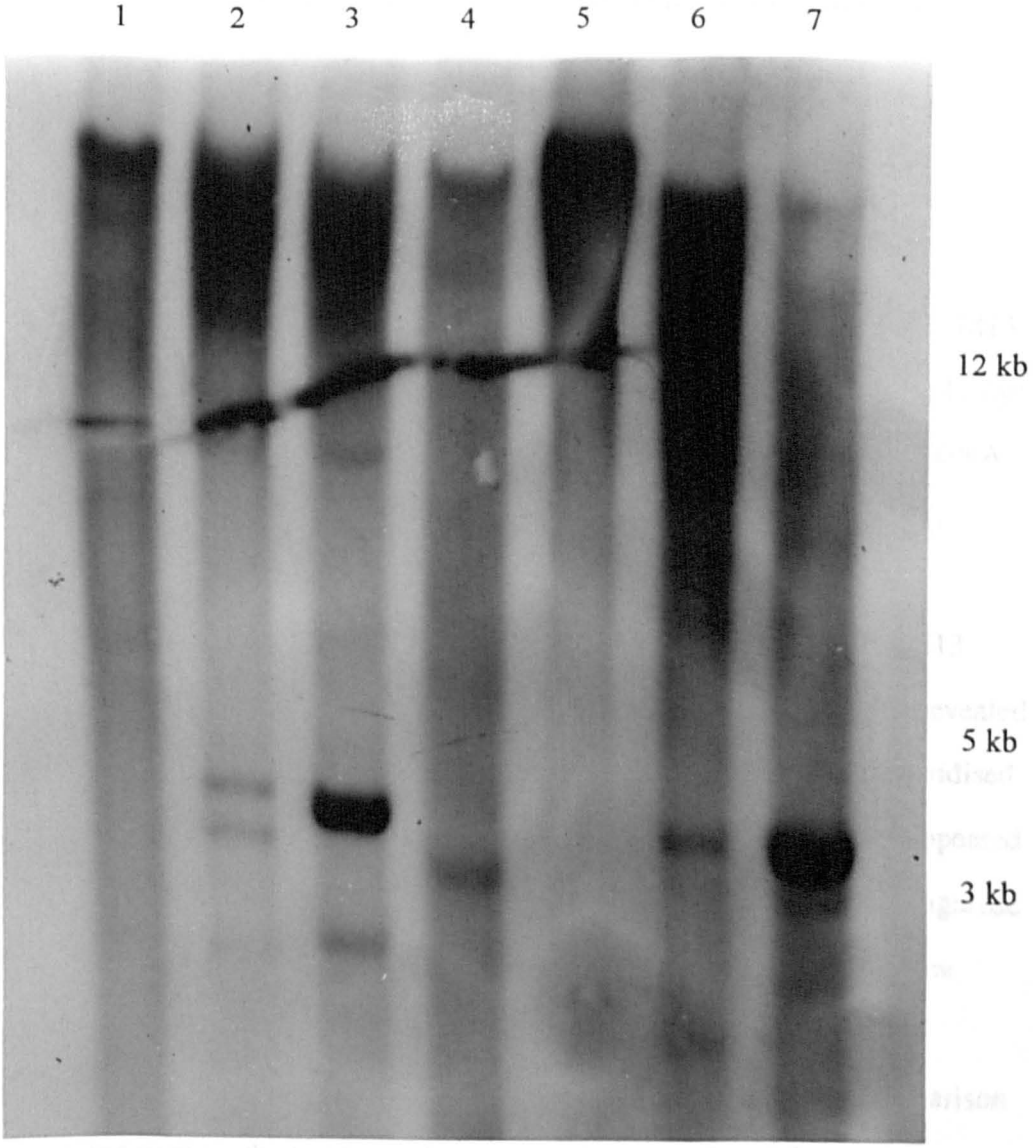
The 282 bp M13 fragment, produced by the *Hae*III/*Cla*I double digest of genomic M13 DNA, was hybridised to Irish Moiled DNA, digested with a range of enzymes. Initially, the restriction enzymes used were those that were available in the laboratory. Plate 3.4 shows the result of the hybridisation of the M13 probe with DNA from the same animal (Dal Conchobar, Herd Book No. 1038), digested with the following range of restriction enzymes: *Bcl*I., *Bam*HI, *Apa*I, *Bgl*II, *Cla*II, *Hind*III, *Kpn*I.

None of the enzymes used gave complete digestion of the Irish Moiled DNA. Large amounts of undigested genomic DNA gave intense hybridisation signals with the M13 probe in each lane on the autoradiograph. In the lanes where Irish Moiled DNA had been digested with the enzymes *Bam*HI, *Apa*I, *Bgl*II, *Kpn*I and *Hind*III one or two bands could be seen. These are shown on Plate 3.4. Irish Moiled DNA digested with the remaining restriction enzymes, *Bcl*I and *Cla*I, did not appear to produce any bands on hybridisation with the M13 probe. Because of the small number of restriction fragments which could be radiolabelled using these probe/enzyme combinations, and the poor digestion of Irish Moiled DNA, these restriction enzymes were not used for further hybridisations.

The inferior quality of the initial autoradiographs may have been due to poor experimental technique in the early stages of this project. The poor digestion of the Irish Moiled DNA samples was likely to be caused by the presence of contaminating

Plate 3.4

Hybridisation of the excised M13 probe with Irish Moiled DNA digested with a range of restriction enzymes.



DNA from a single Irish Moiled animal, Dal Conchobar (Herd Book Number 1038), digested with a range of restriction enzymes, hybridised with the excised M13 probe as described in Materials and Methods. The M13 probe has hybridised strongly to undigested genomic Irish Moiled DNA (>12 kb). Bands visible after autoradiography are arrowed.

Lane 1 - Irish Moiled DNA digested with *Bcl*I.
Lane 2 - Irish Moiled DNA digested with *Bam*HI.
Lane 3 - Irish Moiled DNA digested with *Apa*I.
Lane 4 - Irish Moiled DNA digested with *Bgl*I.
Lane 5 - Irish Moiled DNA digested with *Cla*I.
Lane 6 - Irish Moiled DNA digested with *Kpn*I.
Lane 7 - Irish Moiled DNA digested with *Hind*III.

protein and phenol in the samples, resulting from sub-standard DNA extractions. The presence of additional radiolabelled fragments may have been masked by the intense signal produced by the hybridisation of the M13 probe to the undigested genomic DNA.

3.2.1 Single Intense Band Produced by Hybridisation with *Nco*I Digested Irish Moiled DNA

Plate 3.5 shows the autoradiograph produced by the hybridisation of the M13 probe with eight different Irish Moiled DNA samples, and a sample from a Cambridge ram (Lane 9), digested with the restriction enzyme *Nco*I. The digestion of the DNA samples with *Nco*I was considerably superior to that achieved with the previous enzymes, although the quality of the extracted Irish Moiled DNA was greatly improved. There was no intense signal produced by the hybridisation of the M13 probe with undigested genomic DNA. Although no polymorphic bands were revealed by the hybridisation of the M13 probe with *Nco*I digested DNA, the probe hybridised strongly to a region between two and three kb in size (Plate 3.5). This band appeared in all of the bovine samples, but was absent in the Cambridge ram. The 0.8% agarose gel through which the *Nco*I digested samples had been electrophoresed had been stained in a solution of ethidium bromide and photographed, prior to Southern blotting, in order to determine the distance migrated by the samples. On comparison with the autoradiograph, the band which produced the strong hybridisation signal was found to be visible on this photograph of ethidium bromide stained *Nco*I restriction products (Plate 3.6). This suggested that the band was a region of satellite DNA.

The band was of interest as it had been present in the bovine samples, but not the Cambridge ram, and appeared to be a region of bovine satellite DNA. However initial attempts to clone the restriction fragments in this band into a Bluescript vector (Sambrook *et al.*, 1990) failed. Eventually, further investigation into the use of this band as a bovine minisatellite probe was curtailed, in order to concentrate on the oligonucleotide DNA fingerprinting technique.

Plate 3.5

Hybridisation of *Nco*I digested bovine and ovine DNA, with the excised M13 fragment.

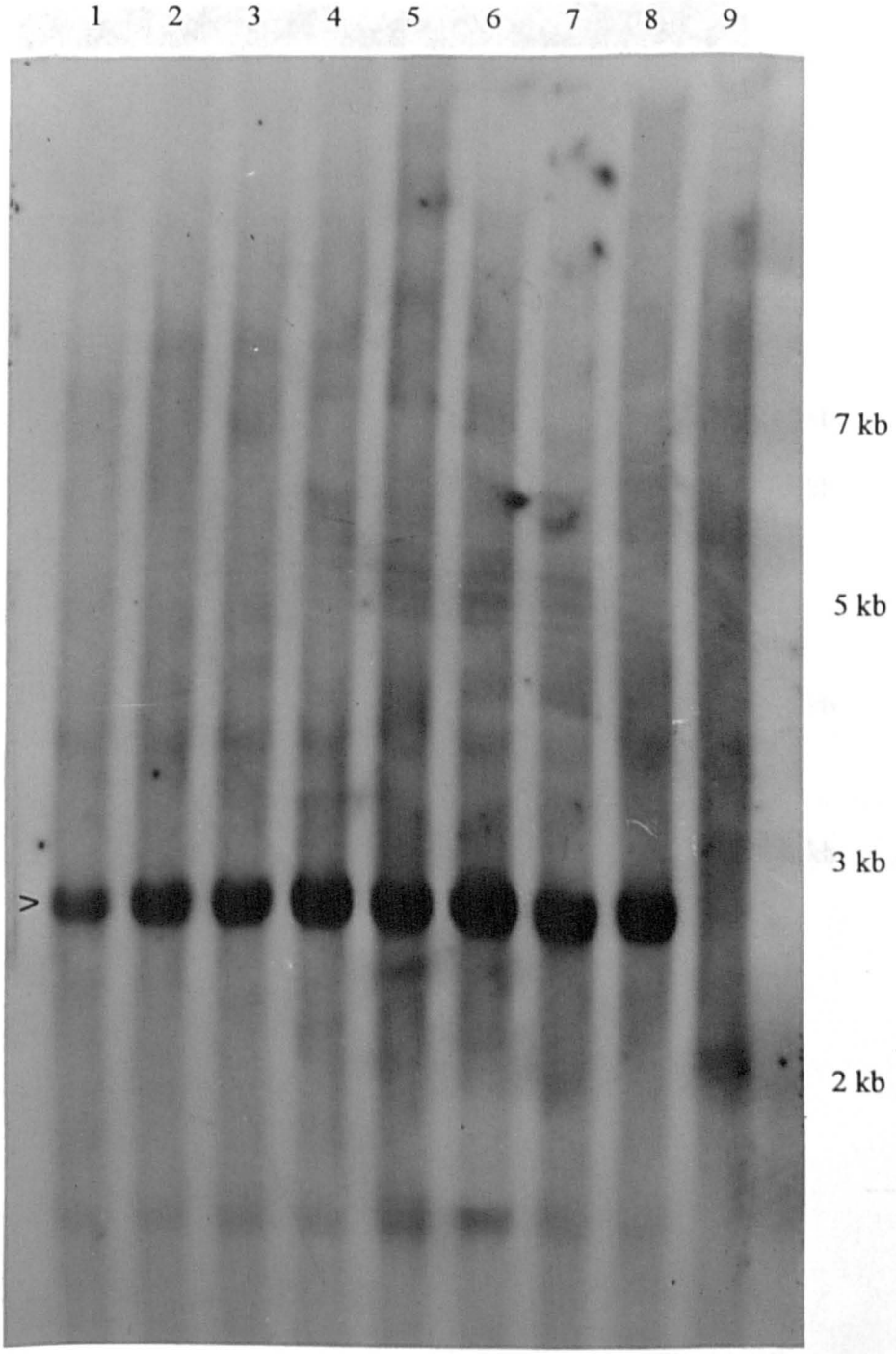


Plate 3.5 shows the hybridisation of DNA from eight different Irish Moiled animals and a Cambridge ram (Lane 9); digested with the restriction enzyme *Nco*I, with the excised M13 fragment, as described in Materials and Methods. The probe has hybridised strongly to a band between two and three kb in size, in the bovine samples (arrowed).

Plate 3.6

Restriction digest of bovine and ovine DNA with the enzyme *Nco*I

Plate 3.6 shows the restriction products from the digestion of eight different Irish Moiled DNA samples, and a sample from a Cambridge ram (Lane9). Digestion and electrophoresis of the samples was as described in Materials and Methods. The gel was photographed over a UV transilluminator after five minutes staining in a 0.5 μg/ml solution of ethidium bromide. The band which produced the strong hybridisation signal with the M13 probe (see Plate 3.5), is arrowed.

3.3 'DNA FINGERPRINTS' OF IRISH MOILED CATTLE USING M13

Reeve *et al* (1990), have produced individual specific DNA fingerprints in naked mole rats, using *Hae*III digested DNA probed with an M13 minisatellite probe. Irish Moiled DNA was digested with the restriction enzyme *Hae*III and hybridised with the M13 probe, produced by the *Hae*III/*Cla*I double digestion of genomic M13 DNA, in an attempt to demonstrate polymorphic banding patterns between closely related Irish Moiled cattle.

Plate 3.7 is the autoradiograph produced from the hybridisation of the M13 probe to DNA samples from eight related Irish Moiled individuals, together with a Jersey and a Friesian cow, digested with *Hae*III. The matrix of coefficients of co-ancestry calculated using the Moilmate computer programme (see Section 2.12) for the eight Irish Moiled cattle is given in Table 3.1. Individual fragments less than approximately 2.5 kb in size could not be resolved on the autoradiograph due to the large number of fragments below this size (Plate 3.7). Georges *et al.*, (1988) had shown that with *Hinf*I digested bovine DNA samples, electrophoresed using the same conditions (1% agarose gel, 3 V/cm between electrodes for 24 hrs - see Materials and Methods), probed with wild type M13mp9 phage, the lower limit for precise analysis of bands was also 2.5 kb.

Plate 3.7

Hybridisation of *Hae*III digested bovine DNA with the excised M13 fragment.

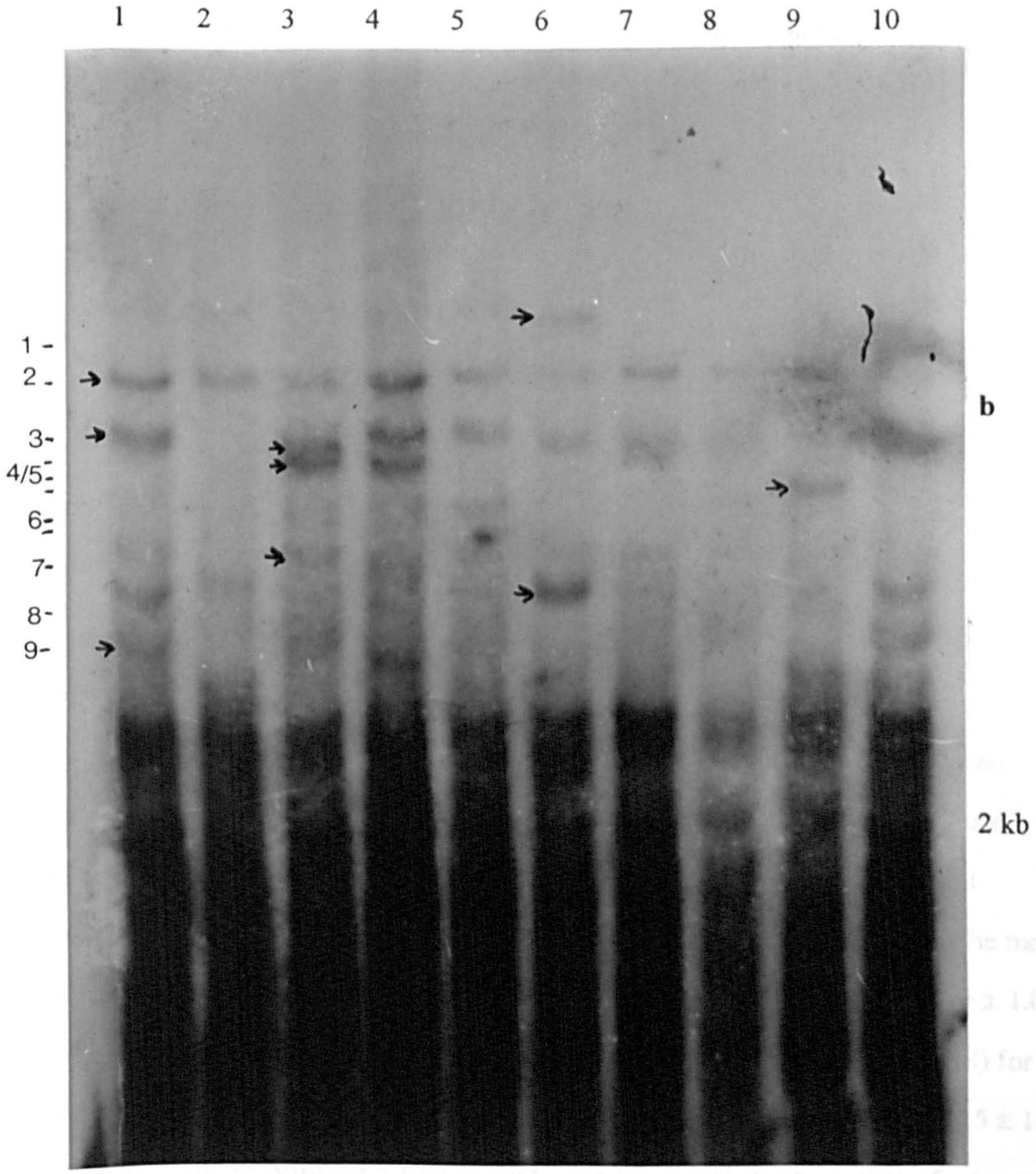


Plate 3.7 shows the banding pattern produced by the hybridisation of the excised M13 fragment with *Hae*III digested DNA from 8 different Irish Moiled animals, a Friesian (Lane 2) and a Jersey cow (Lane 9). The nine different bands which can be resolved in the Irish Moiled samples are arrowed and numbered. Band number '2' is the only band which is present in all samples.

Lane 1	**Irish Moiled**	**(C70)**	**Lane 2**	**Friesian**	
Lane 3	**Irish Moiled**	**(C57)**	**Lane 4**	**Irish Moiled**	**(1064)**
Lane 5	**Irish Moiled**	**(1037)**	**Lane 6**	**Irish Moiled**	**(C63)**
Lane 7	**Irish Moiled**	**(1040)**	**Lane 8**	**Irish Moiled**	**(976)**
Lane 9	**Jersey**		**Lane 10**	**Irish Moiled**	**(1036)**

Table 3.1

Coefficients of Co-ancestry

Matrix of coefficients of co-ancestry for the eight Irish Moiled samples hybridised with the M13 probe as shown in Plate 3.7

Herd Book No.	C70	C57	1064	1037	C63	1040	976	1036
C70	-							
C57	.002	-						
1064	.118	.021	-					
1037	.253	.004	.226	-				
C63	.151	.002	.118	.253	-			
1040	.230	.004	.225	.399	.230	-		
976	.125	.004	.387	.236	.125	.259	-	
1036	.214	.004	.312	.371	.214	.360	.422	-

A total of nine different bands, eight of which were polymorphic, could be resolved in the eight Irish Moiled samples, hybridised with the M13 probe. These bands have been arrowed and numbered 1-9 on Plate 3.7. Only band 2 was not polymorphic and is present in all lanes, including the non-Irish Moiled lanes. The mean number of resolvable fragments per Irish Moiled individual was found to be 3.5 ± 1.07. this value is very low in comparison with that obtained by Georges *et al.*, (1988) for samples from 12 unrelated Belgian Blue cattle, digested with *Hinf*I, in which 7.5 ± 1.8 fragments could be resolved per individual. The mean probability, x, that a fragment present in one Irish Moiled individual would also be present in another was estimated from the presence or absence of each band in the eight Irish Moiled samples (Jeffreys *et al.*, 1985; Georges *et alt.*, 1988). 'x' was estimated to be 0.45 for this group of eight related Irish Moiled cattle. This was considerably higher than the value of 0.35 obtained by Georges *et al.*, (1988) for Belgian Blue cattle and was much higher than that obtained by Jeffreys *et al.*, (1985), who found in humans that with *Hinf*I digested

DNA hybridised with the probe 33.6, the mean probability of finding a fragment of another randomly chosen individual was approximately 0.2 in northern Europeans.

The high probability that a fragment present in one Irish Moiled individual would also be present in another may have been largely due to the sample of related Irish Moiled observed. Very few Irish Moiled animals can be found whose ancestry, even only as far back as their grandparents, is not shared with most other animals. In the early stages of this project, DNA had only been obtained from two Irish Moiled herds; the Croxteth herd in Liverpool and the Temple Newsam herd in Leeds. This made the selection of unrelated individuals for analysis impossible.

The bands arrowed in Plate 3.7 were not particularly clear. The presence of ethidium bromide in the gel and running buffer during electrophoresis has affected the mobility of the DNA and produced 'smiling' bands. Air bubbles under the Hybond membrane during hybridisation prevented the M13 probe from reaching and binding to some areas of the membrane. These areas appeared as clear, bubble-shaped, circles surrounded by a ring of hybridisation signal (Plate 3.7, labelled 'b'). The identification of radiolabelled restriction fragments in and around these areas was impossible. The resolution of the bands produced by the hybridisation of *Hae*III digested Irish Moiled DNA, with the M13 probe, was not thought to be clear enough to allow an accurate comparison between a large number of samples electrophoresed on separate gels.

Various aspects of the hybridisation protocol were altered in an attempt to improve the resolution of the bands. Ethidium bromide was omitted from the gel and running buffer to eliminate its effect on DNA mobility during electrophoresis. Samples were run at lower voltages for longer periods of time in an attempt to increase the separation of the bands. The problem of air bubbles was avoided by rolling a sterile glass spooling rod around the inside of the hybridisation tube to expel any air from under the membrane, prior to hybridisation. The stringency of washes was increased to reduce the level of background radiation.

However, while working on improving the resolution of bands detected with the M13 probe, initial investigations into the possibility of using microsatellite

oligonucleotide probes were being made. It soon became apparent that the oligonucleotide technique would yield considerably more information than the M13 probe. In view of this, and the fact that improvement in the resolution of the M13 bands had been slight, this line of approach was suspended, in favour of microsatellite oligonucleotide DNA fingerprinting.

CHAPTER 4

ARBITRARILY PRIMED-POLYMERASE CHAIN REACTION

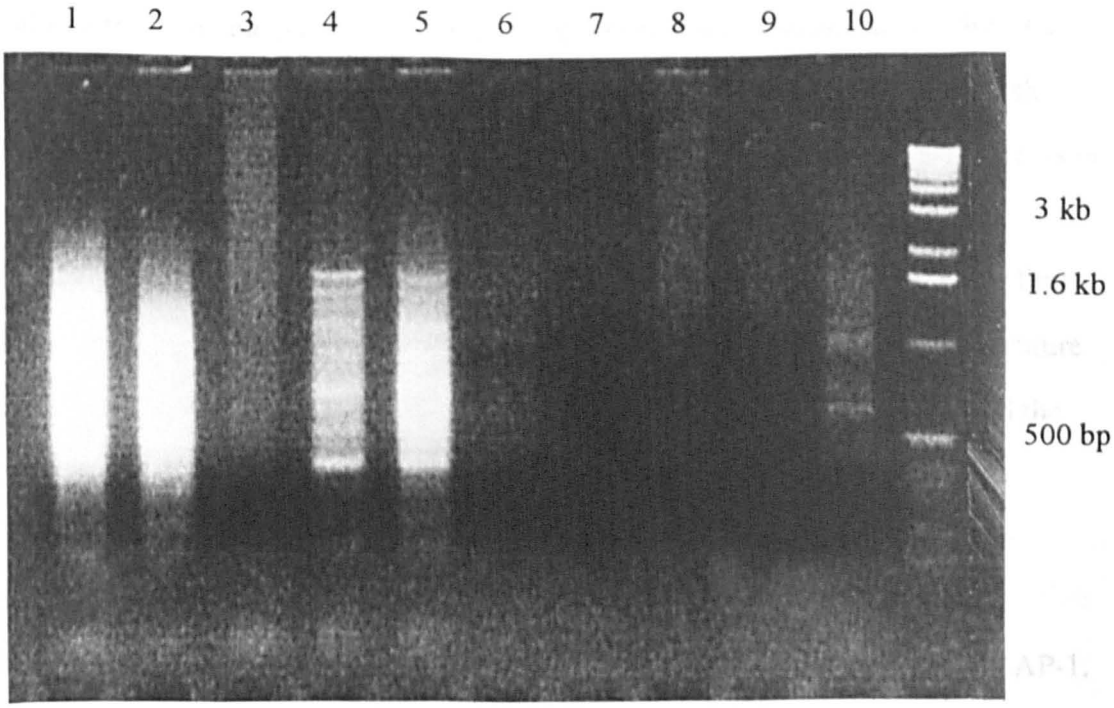
Single primers of arbitrary sequence have been used to produce reproducible fingerprints of complex genomes, using a slightly modified PCR protocol (Williams *et al.*, 1990; Welsh and McClelland 1990, 1991).

Amplification of segments of Irish Moiled genomic DNA by arbitrarily primed PCR proved problematic. Most of the primers used did not produce any visible amplification products. Some combinations of primer and DNA template produced a 'smear' of amplification products when visualised on an agarose gel. Within this smear, it was however impossible to resolve individual bands (Plate 4.1). Williams *et al.*, (1990) were able to convert such smears into discretely sized bands by reducing the concentration of either the *Taq* polymerase or the genomic DNA, however despite numerous modifications to the ratio of the concentration of polymerase to the concentration of genomic DNA individual bands remained 'unresolvable' within the smear of amplification products.

The stringency of the PCR was altered by changing the annealing temperature (see Section 2.11.2). In an attempt to promote the production of amplification products in reactions where the primer-template combination had failed to produce any amplified DNA, the annealing temperature was lowered. This would reduce the specificity of the pairing between primer and template, enabling the primer to anneal and amplification to be initiated at a greater number of sites on the genomic DNA. Conversely, increasing the annealing temperature of the reaction, would increase the stringency. The annealing temperature was increased for reactions which had produced a 'smear' of amplification products in an attempt to reduce the number of amplified segments of genomic DNA produced, so that individual bands might be resolved. Arbitrarily primed PCR was carried out at annealing temperatures ranging from 30°C to 55°C, however temperature differences did not appear to have any affect on the resulting amplification products.

Plate 4.1

Arbitrarily Primed PCR of Irish Moiled DNA using the primer LIV-1



AP-PCR was carried out on 10 different Irish Moiled DNA samples (Lanes 1-10), as described in Section 2.11.2. Lanes 1, 2, 3 and 8 are examples of the smear of amplification products within which individual bands cannot be resolved. AP-PCR of the DNA samples in Lanes 6, 7 and 9 has not produced any amplification products. Bands can be seen in Lanes 4, 5 and 10, although these are not clearly resolved.

Welsh *et al.*,(1990, 1991) has shown that a temperature profile consisting of two low stringency cycles (35°C-50°C) followed by 40 high stringency cycles (60°C) produces reproducible patterns of bands, using DNA from bacteria, rice (Welsh *et al.*, 1990) and mice (Welsh *et al.*, 1991). This temperature profile was applied to Irish Moiled DNA in an attempt to generate individual bands of amplified DNA fragments in reactions where the primer-template combination had previously produced unresolvable smears of amplification products. Despite considerable effort, very little success was achieved. Various aspects of the reaction conditions and the temperature profile for arbitrarily primed PCR were modified. For most of the primers tested the amplification products were either absent, after agarose gel electrophoresis and detection by staining with ethidium bromide, or appeared as smears within which individual bands could not be resolved.

The exceptions to the above were the results obtained with the primers RAP-1, RAP-2, RAP-3 and C10 (see Section 2.11.). Using the arbitrarily primed PCR protocol described in Section 2.11.2 the primers RAP-1 and RAP-3 sometimes produced two to three discrete fragments of amplified DNA with Irish Moiled template, although these were frequently obscured by a smear of amplification product or were absent altogether. Despite numerous modifications to the reaction protocol the pattern of amplified fragments produced was not reproducible, and the individual bands could generally only be resolved with difficulty.

4.1 AMPLIFICATION OF FRAGMENTS OF IRISH MOILED DNA WITH THE PRIMER RAP-2

Of all the primers used in the amplification of DNA by arbitrarily primed PCR, the primer RAP-2 produced the most consistent results and the greatest number of amplification products.

Plate 4.2 shows the amplification products from four different Irish Moiled DNA samples, using the primer RAP-2, as described in Section 2.11.2. Up to 10

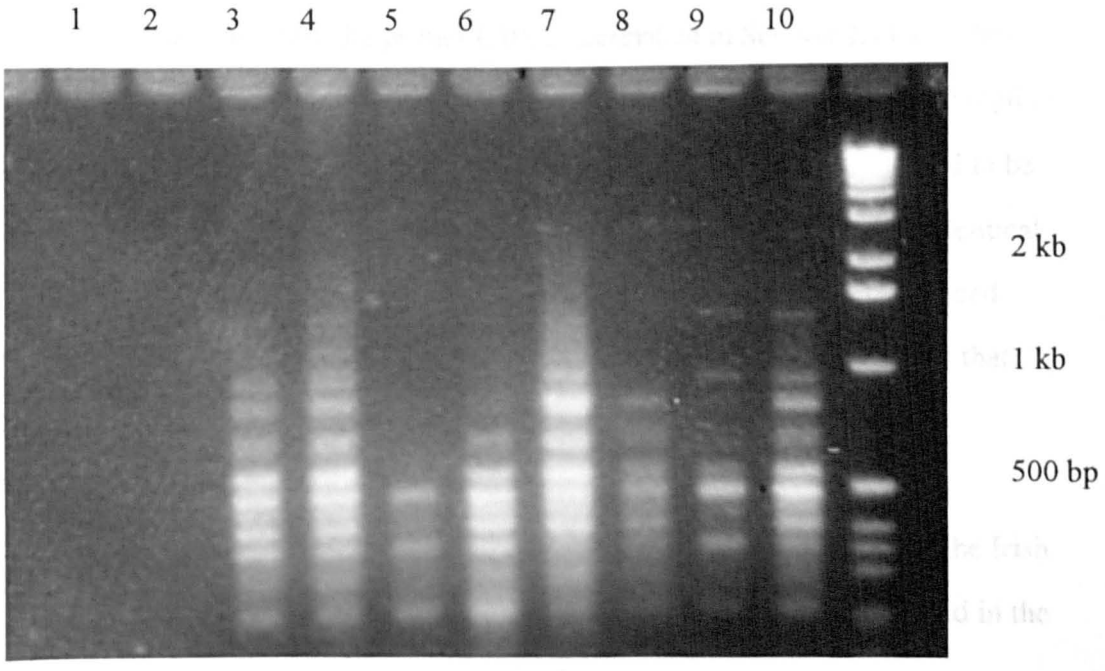
segments of DNA were amplified from each Irish Moiled DNA template tested. The size of the fragments amplified ranged from approximately 250 bp to 1000 bp.

Each reaction was carried out in duplicate, in order to establish whether or not the technique was reproducible. The amplification products from these identical reactions were electrophoresed in adjacent lanes (Plate 4.2). Genomic DNA was omitted in the control reactions (lanes 1 and 2) in order to determine whether any of the bands seen with genomic Irish Moiled DNA are in fact primer artefacts. No bands were visible for any of the control reactions.

The difference between the patterns of amplified fragments produced by identical reactions (for example lanes 5 and 6) illustrates how sensitive the technique is to even small changes in reaction conditions. Many bands present in lane 6 were absent from lane 5 It appears that the production of some bands is variable, depending perhaps on the primer-template specificity in the early rounds of PCR. This inconsistency between apparently identical reactions prevents the identification of polymorphic amplification fragments between individuals. The pattern of bands produced appears to be similar for each individual tested, the only difference between samples being the variable production of some of the bands, as observed between identical reactions. Because the polymerase chain reaction amplifies segments of template DNA exponentially, any slight variation in the initial reaction conditions which may have affected the initial cycles of amplification, would drastically affect the amplification fragments produced. Many factors could have affected the primer/template specificity in the early rounds of PCR, leading to inconsistency between apparently identical reactions. Minute differences in the concentrations of the reaction mixtures, differences in the conduction of heat to the samples, due to the thickness of the Eppendorf tubes, or the position of the samples in the heating block, and the presence of contaminating DNA could all have affected the early stages of amplification (Welsh *et al.*, 1991; Innis *et al.*, 1989).

Plate 4.2

Arbitrarily primed PCR of Irish Moiled DNA using the Primer RAP-2



Reaction conditions were as described in Section 2.11.2. Plate 4.2 shows the amplification products from pairs of duplicate reactions with four different Irish Moiled DNA samples (Lanes 3 and 4, 5 and 6, 7 and 8,and 9 and 10). Lanes 1 and 2 were control samples.

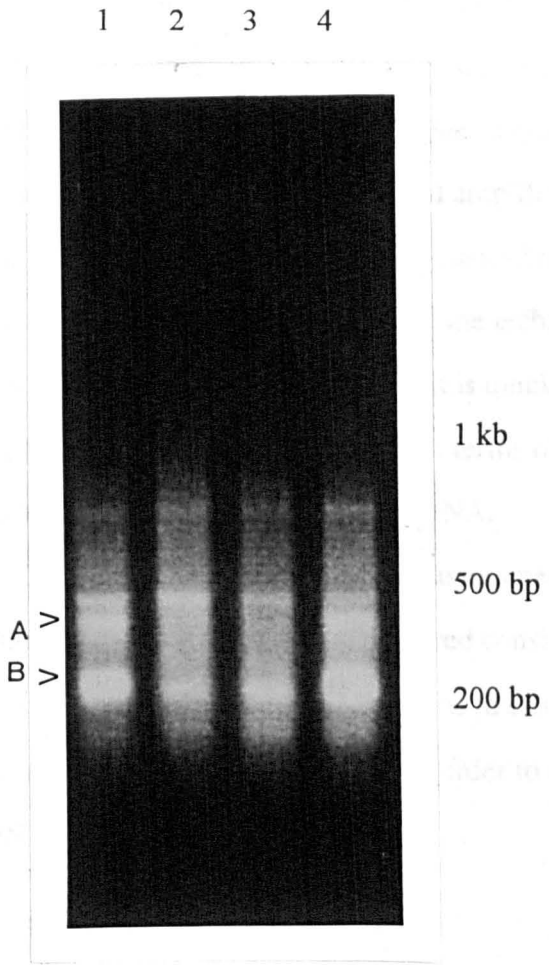
4.2 DIFFERENCES IN BANDING PATTERN PRODUCED WITH IRISH MOILED AND JERSEY DNA SAMPLES

DNA samples from two Irish Moiled cattle and a Jersey cow were amplified by Arbitrarily Primed PCR using the primer C10, as described in Section 2.11.2. After electrophoresis and the detection of the amplification products by staining in a solution of ethidium bromide the banding patterns produced by the two breeds appeared to be different (Plate 4.3). The two different Irish Moiled DNA samples produced identical patterns of amplification fragments (lanes 1 and 4). The banding pattern produced from the duplicate reactions with the Jersey DNA (lanes 2 and 3) differed from that produced with the two Irish Moiled samples. A band of approximately 500 bp (labelled 'A' on Plate 4.3) in the Irish Moiled samples was absent in the Jersey samples. In addition, what appears to be a duplex at approximately 350 bp in the Irish Moiled reaction products (labelled 'B' on Plate 4.3), appears to be a single band in the Jersey lanes.

Williams *et* al., (1990) demonstrated a strain specific banding pattern by applying arbitrarily primed PCR to the identification and verification of mouse strains. The possibility of a breed specific banding pattern in cattle could have been explored further. However, the polymorphic bands that distinguished the two breeds of cattle were difficult to resolve, due to their low molecular weight and the proximity to other amplified fragments in the banding pattern. On repeating the arbitrarily primed PCR using the primer C10, the polymorphisms between the Jersey and Irish Moiled samples were not apparent. This could have been due to poor resolution of the amplified fragments, after electrophoresis and staining with ethidium bromide, concealing the polymorphic bands, or as a results of the variable penetrance of the bands of amplified DNA as mentioned in the previous section.

Plate 4.3

Arbitrarily Primed PCR of bovine DNA with the Primer C10.



Reaction conditions were as described in Section 2.12.2. The amplification products of two identical reactions with Jersey DNA (Lanes 2 and 3) and two reactions with different Irish Moiled samples (Lanes 1 and 4) are shown. Differences in the banding pattern obtained with the two breeds are arrowed 'A' and 'B'.

4.3 AP-PCR TO DETERMINE GENETIC SIMILARITY BETWEEN ANIMALS

The reproducibility of the AP-PCR reactions, in this study, was poor. The sensitivity of the technique to slight changes in the reaction conditions, the production of random non-reproducible amplification patterns due to non-specific primer annealing or heteroduplex formation between related amplification products, has been reported by many authors (Williams *et al.*, 1990: Caetano-Anollés *et al.*, 1991; Hendrick, 1992 and Hadrys *et al.*, 1992). However, the technique does have several advantages over conventional DNA fingerprinting; it is quick to carry out, the complexity of the procedure is lower, it is cheaper in terms of both labour and materials, and it requires only minimal amounts of DNA.

The technique may have been useful for the assessment of genetic similarity between Irish Moiled cattle, but it would have required considerable work in finding informative primers and setting up reaction conditions to ensure reproducible results. This avenue of research was eventually curtailed in order to concentrate on the oligonucleotide DNA fingerprinting technique.

CHAPTER 5

DNA FINGERPRINTING WITH OLIGONUCLEOTIDE PROBES

5.1 EXTRACTION OF DNA FROM BLOOD SAMPLES

5.1.1 Blood samples collected

One hundred and ninety-four animals were sampled in all. Of the 163 samples taken from animals born before 1993, 99 were from pure registered Irish Moiled and 62 were from upgrading animals. This provides a reasonably comprehensive population census for pre-1993 animals, with a maximum of 18 pure registered Irish Moiled unavailable for sampling. The remaining 31 samples were taken from calves born in 1993. This does not encompass every Irish Moiled born in 1993, since many animals were born at various locations after blood samples had been collected at these sites. The animals and locations from which blood samples were collected are given in Appendix III.

Blood samples from the animals in Northern Ireland were collected over a period of one week, in September, 1992. To prevent degradation of the DNA, the samples collected on the first day of this week were posted back to the laboratory. On arrival these 42 samples were mistakenly centrifuged and only the supernatant plasma was retained. The lost samples were collected again in February 1993, however six of the animals previously sampled were unavailable at this time.

5.1.2 Extraction of DNA

As most blood samples were collected in bulk from all the Irish Moiled cattle at a particular location, it proved convenient to store the samples frozen at -20°C to prevent degradation of the DNA before it could be extracted.

Using the protocol for DNA extraction from frozen blood (Materials and Methods), on average 15µg high molecular weight genomic DNA was obtained per ml of blood. Generally 15 ml of blood had been collected from each animal, yielding 225 µg DNA. The concentrations of DNA extracted from the Irish Moiled blood samples are given in Appendix III.

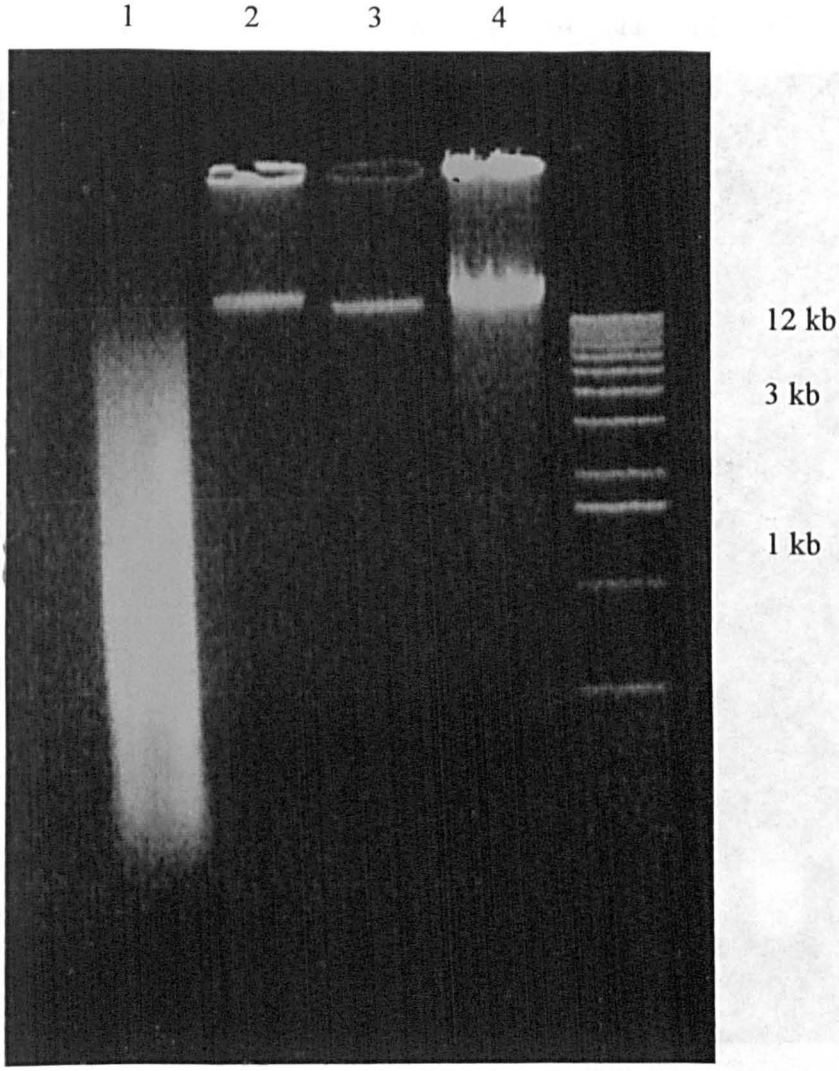
5.1.3 DNA concentration and purity

Homogenisation of the DNA samples as described in Materials and Methods helped to reduce the fluctuation in readings of optical density obtained by UV spectrophotometry, enabling reasonable estimates of concentration and purity to be made.

The extracted DNA was generally undegraded and free from contamination with phenol or protein. DNA samples which were degraded were detected by electrophoresis, as described in Materials and Methods (see Plate 5.1). These samples were not used for DNA fingerprinting. In the very few cases where DNA from a particular animal was found to be degraded, a further blood sample was obtained and the DNA extraction was carried out again.

Plate 5.1

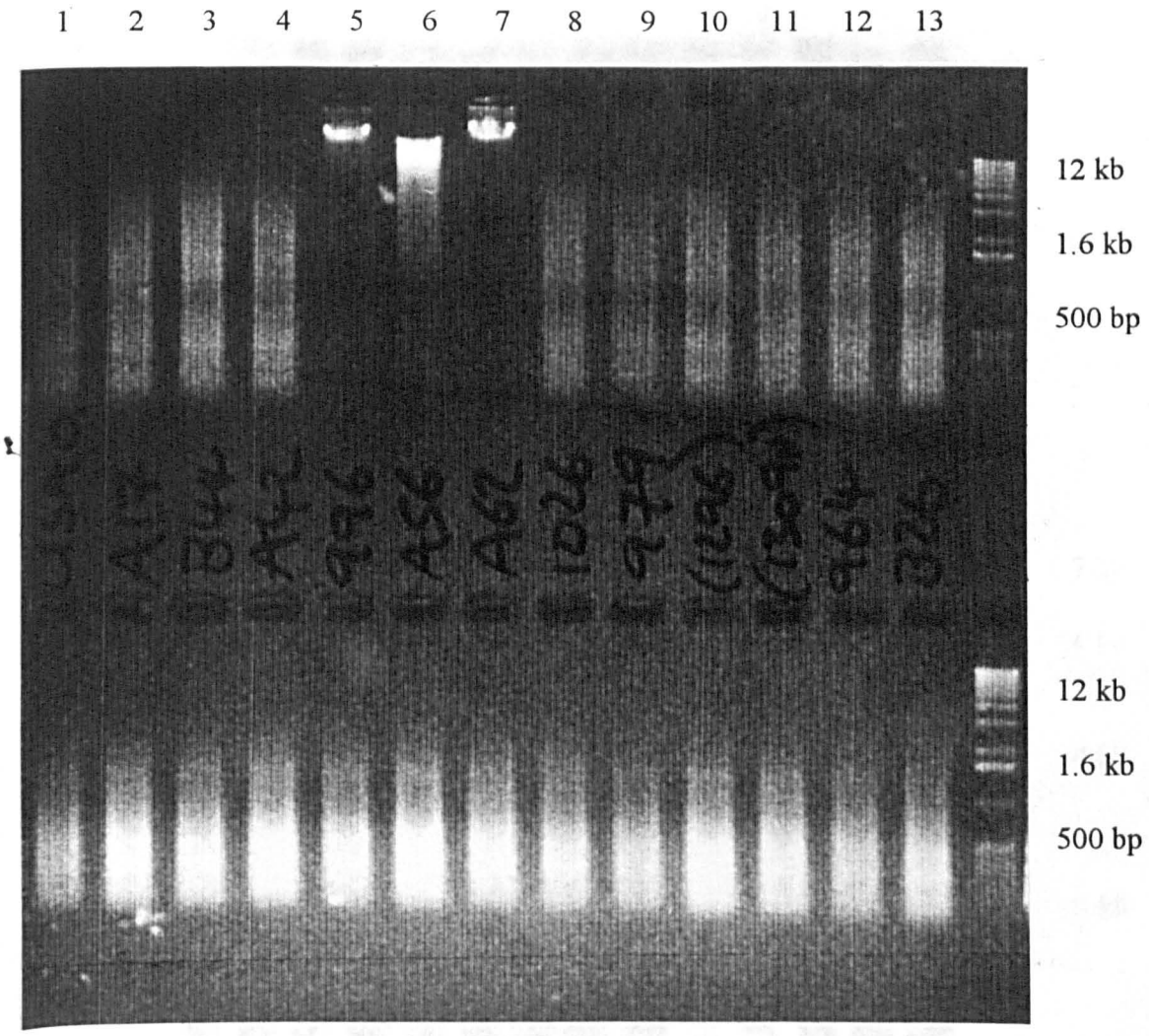
Agarose gel electrophoresis to determine integrity of extracted DNA.



Extracted DNA was electrophoresed and viewed over a UV transilluminator as described in Section 2.3.5. Plate 5.1 shows four Irish Moiled DNA samples. The sample in Lane 1, from the animal B66 was clearly degraded, and could not be used for DNA fingerprinting. The single sharp band of high molecular weight observed in Lanes 2-4 indicates that these samples were intact.

Plate 5.2

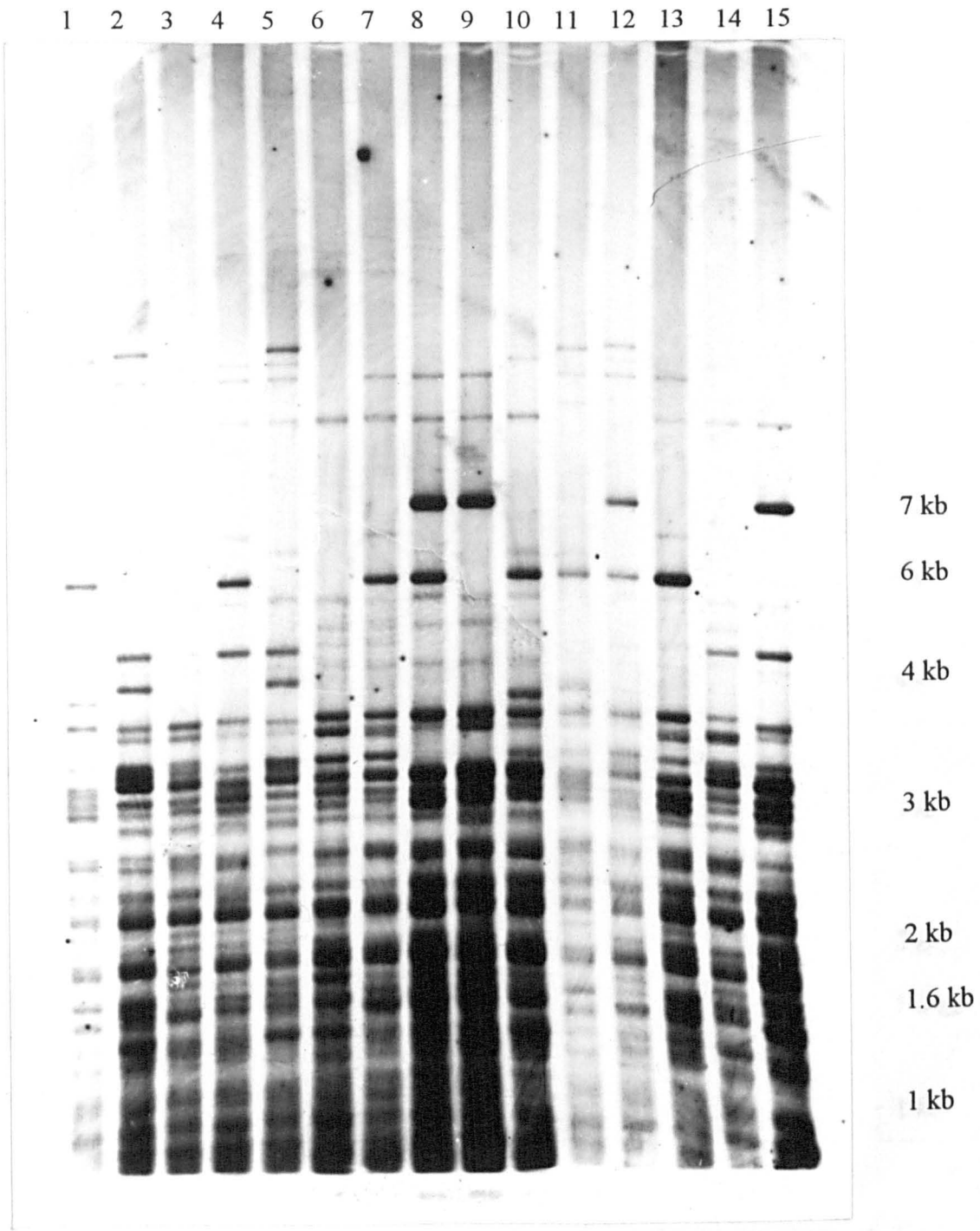
Electrophoresis of Restriction Digested DNA on an agarose 'test gel' to determine relative concentrations.



The top half of plate 5.2 shows 13 Irish Moiled DNA samples digested with the restriction enzyme *Hae*III. The bottom half shows the same samples digested with *Hinf*I. The herd book numbers of the Irish Moiled samples can be seen at the centre of the plate. The similar fluorescence of the samples indicates that they were of approximately equal concentration. However, the presence of a high molecular weight band in Lanes 5, 6 and 7, with *Hae*III digested DNA indicates that these samples (996, A56 and A62) have not been fully digested by the restriction enzyme.

Plate 5.3

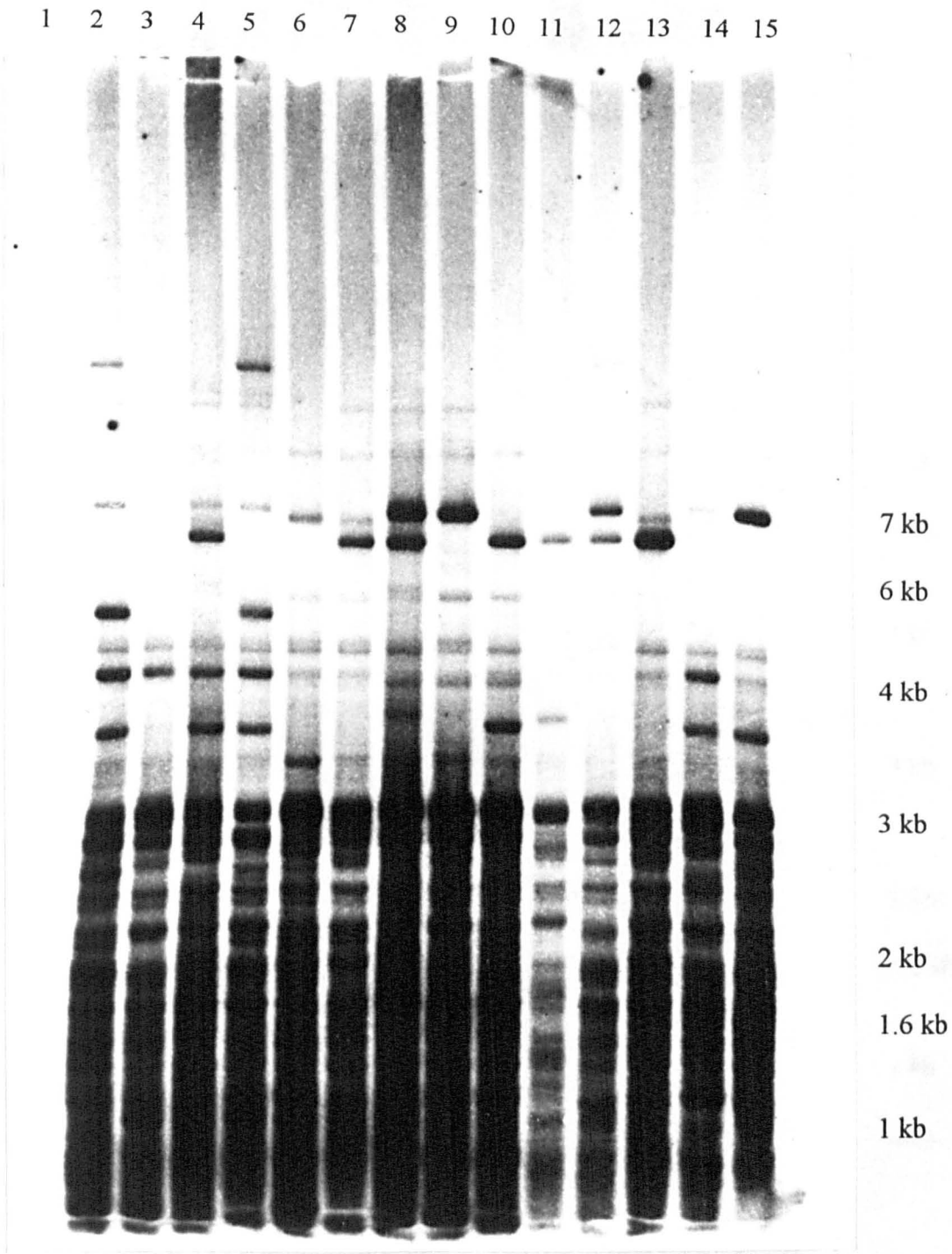
Hybridisation of *Hinf*I digested bovine DNA with the oligonucleotide probe $(GTG)_5$



Example of an autoradiograph produced by the *Hinf*I/$(GTG)_5$ probe/enzyme combination. Lanes 1 and 11 were Friesian control DNA lanes, the remaining 13 lanes were Irish Moiled DNA. The Irish Moiled DNA samples are identical to those seen in Plates 5.4, 5.9 and 5.10.

Plate 5.4

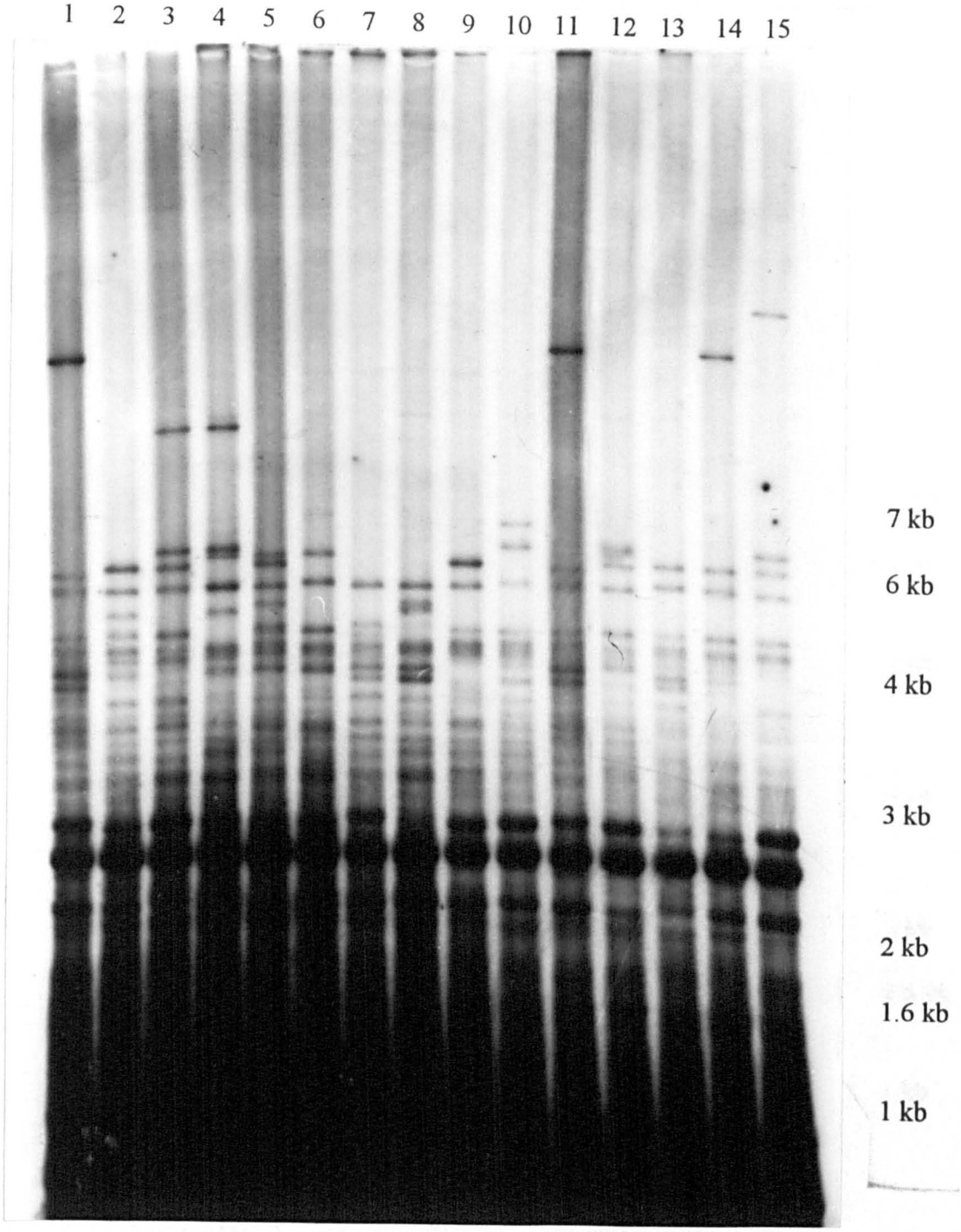
Hybridisation of *Hae*III digested bovine DNA with the oligonucleotide probe $(GTG)_5$



Example of an autoradiograph produced by the *Hae*III/$(GTG)_5$ probe/enzyme combination. Lane number 11 was a Friesian DNA control lane. Fresian DNA had not been loaded in lane 1. he remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.3, 5.9 and 5.10.

Plate 5.5

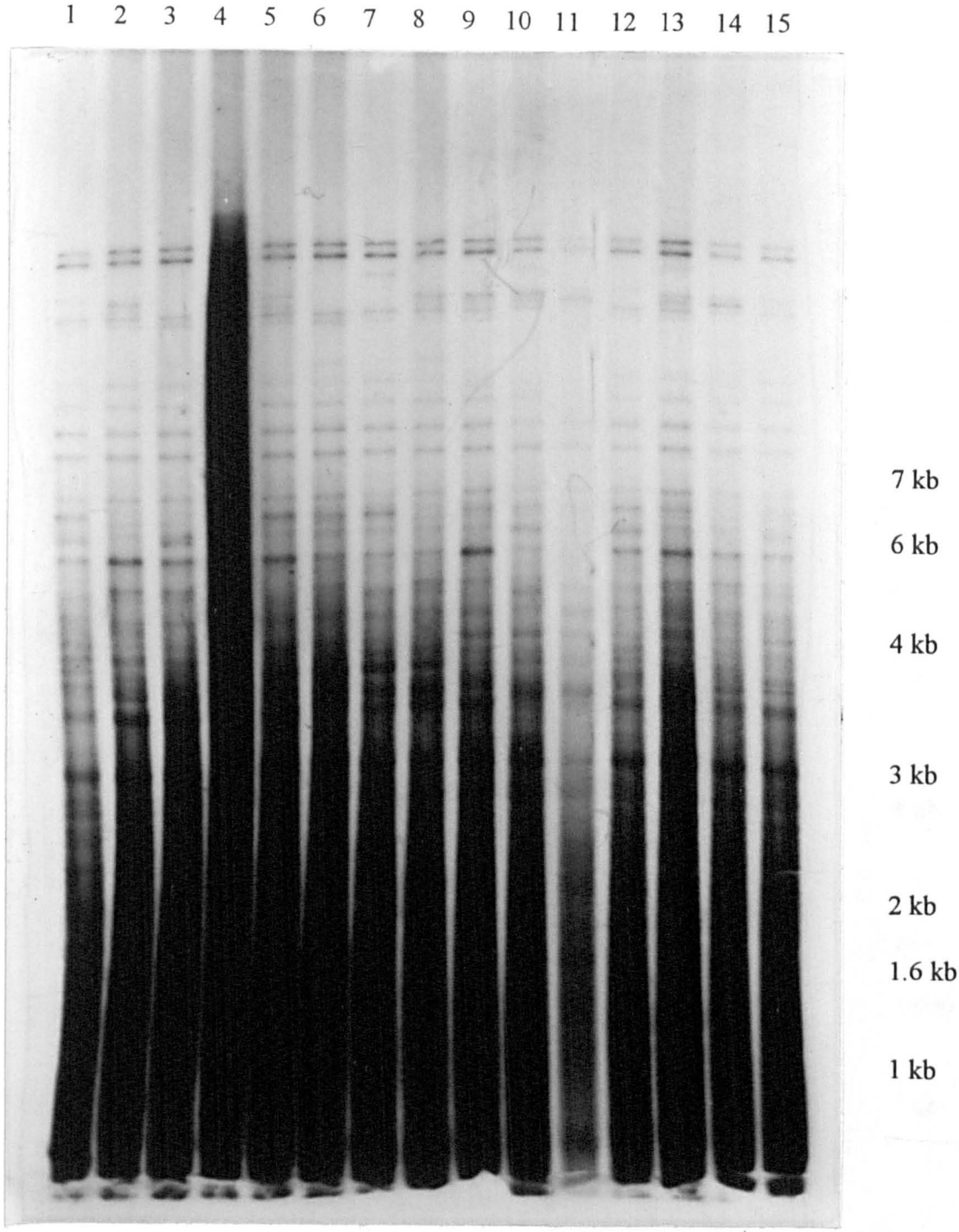
Hybridisation of *Hinf*I digested bovine DNA with the oligonucleotide probe $(GT)_8$



Example of an autoradiograph produced by the *Hinf*I/$(GT)_8$ probe/enzyme combination. Lanes 1 and 11 were Friesian DNA control lanes. The remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.6, 5.7 and 5.8.

Plate 5.6

Hybridisation of *Hae*III digested bovine DNA with the oligonucleotide probe $(GT)_8$

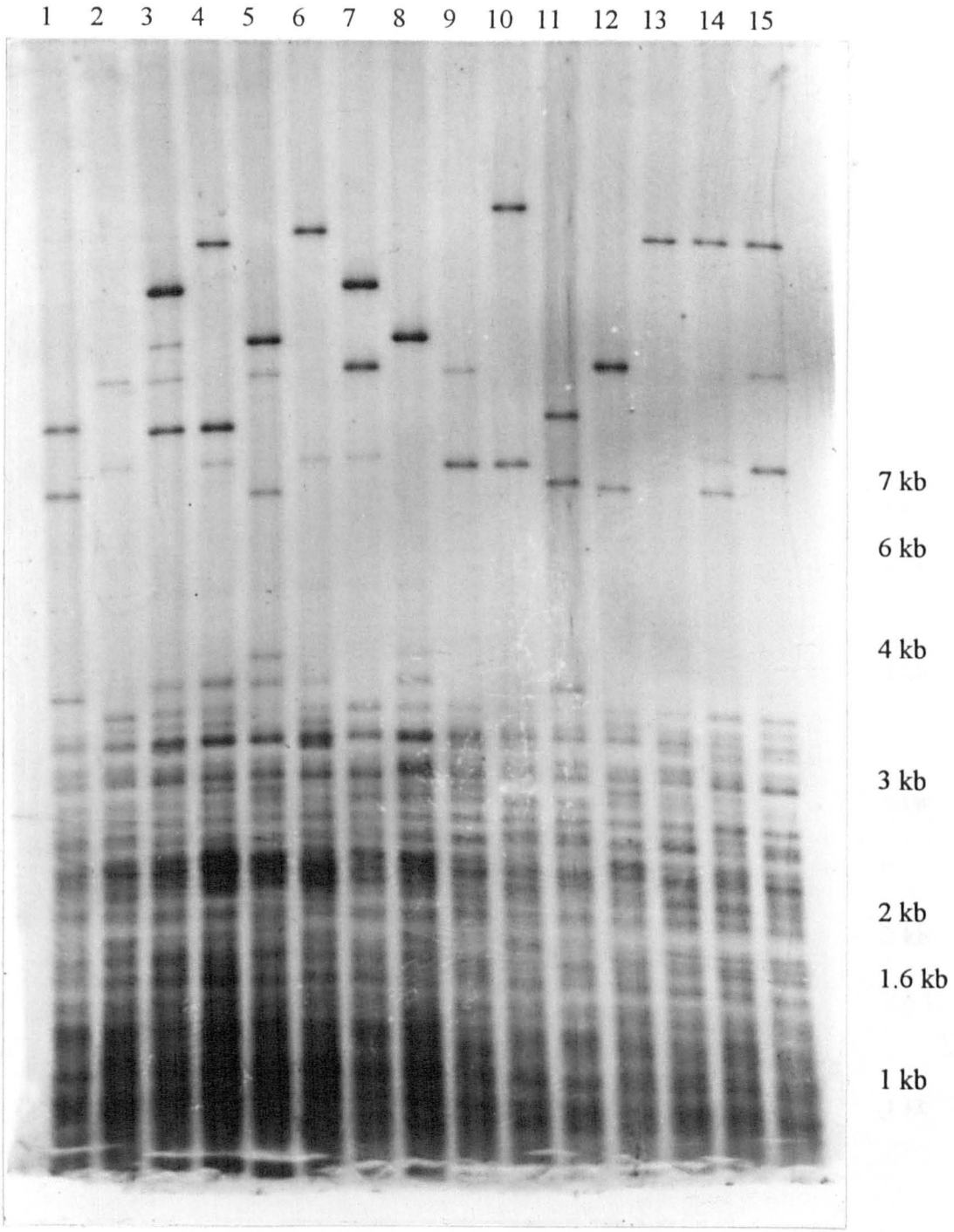


Example of an autoradiograph produced by the *Hae*III/$(GT)_8$ probe/enzyme combination. Lane 11 was a Friesian DNA control lane. Friesian DNA had not been loaded in lane 1. The remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.5, 5.7 and 5.8.

The Irish Moiled DNA sample in Lane 4 has produced a smear of signal, resulting from poor digestion of the sample.

Plate 5.7

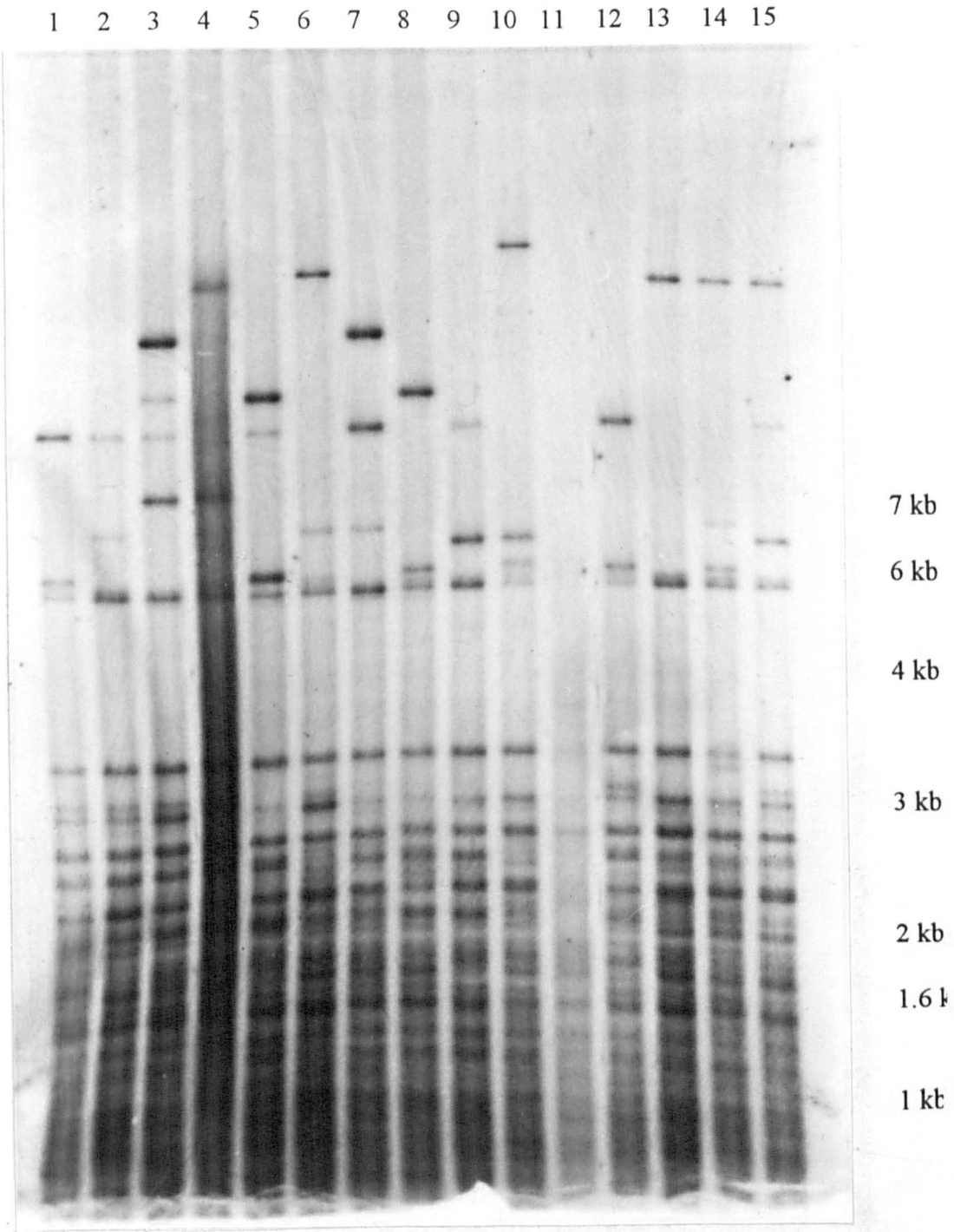
Hybridisation of *Hinf*I digested bovine DNA with the oligonucleotide probe $(GGAT)_4$



Example of an autoradiograph produced by the *Hinf*I/$(GGAT)_4$ probe/enzyme combination. Lanes 1 and 11 were Friesian DNA control lanes. The remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.5, 5.6 and 5.8.

Plate 5.8

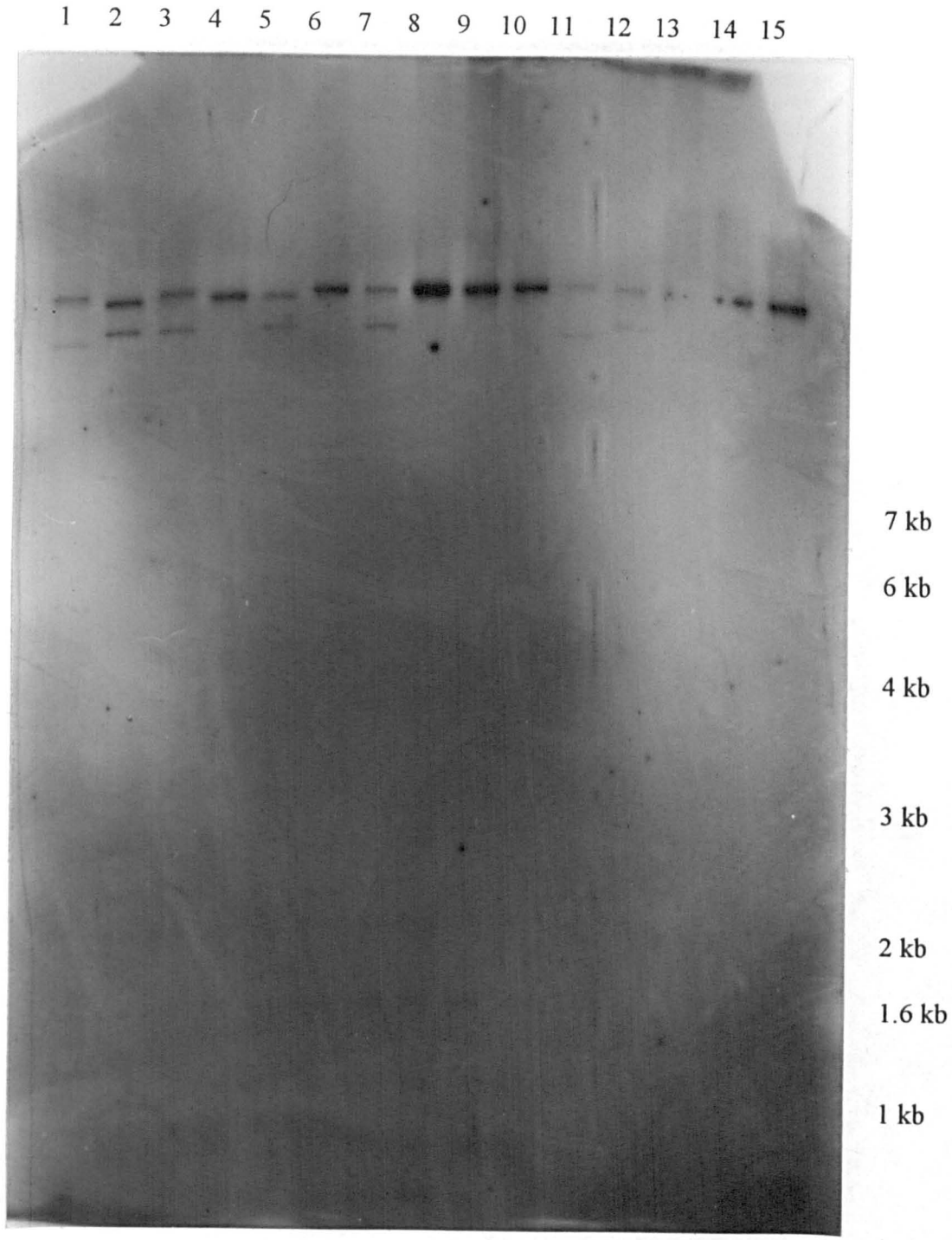
Hybridisation of *Hae*III digested bovine DNA with the oligonucleotide probe $(GGAT)_4$



Example of an autoradiograph produced by the *Hae*III/$(GGAT)_4$ probe/enzyme combination. Lanes 1 and 11 were Friesian DNA control lanes. The remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.5, 5.6 and 5.7.

Plate 5.9

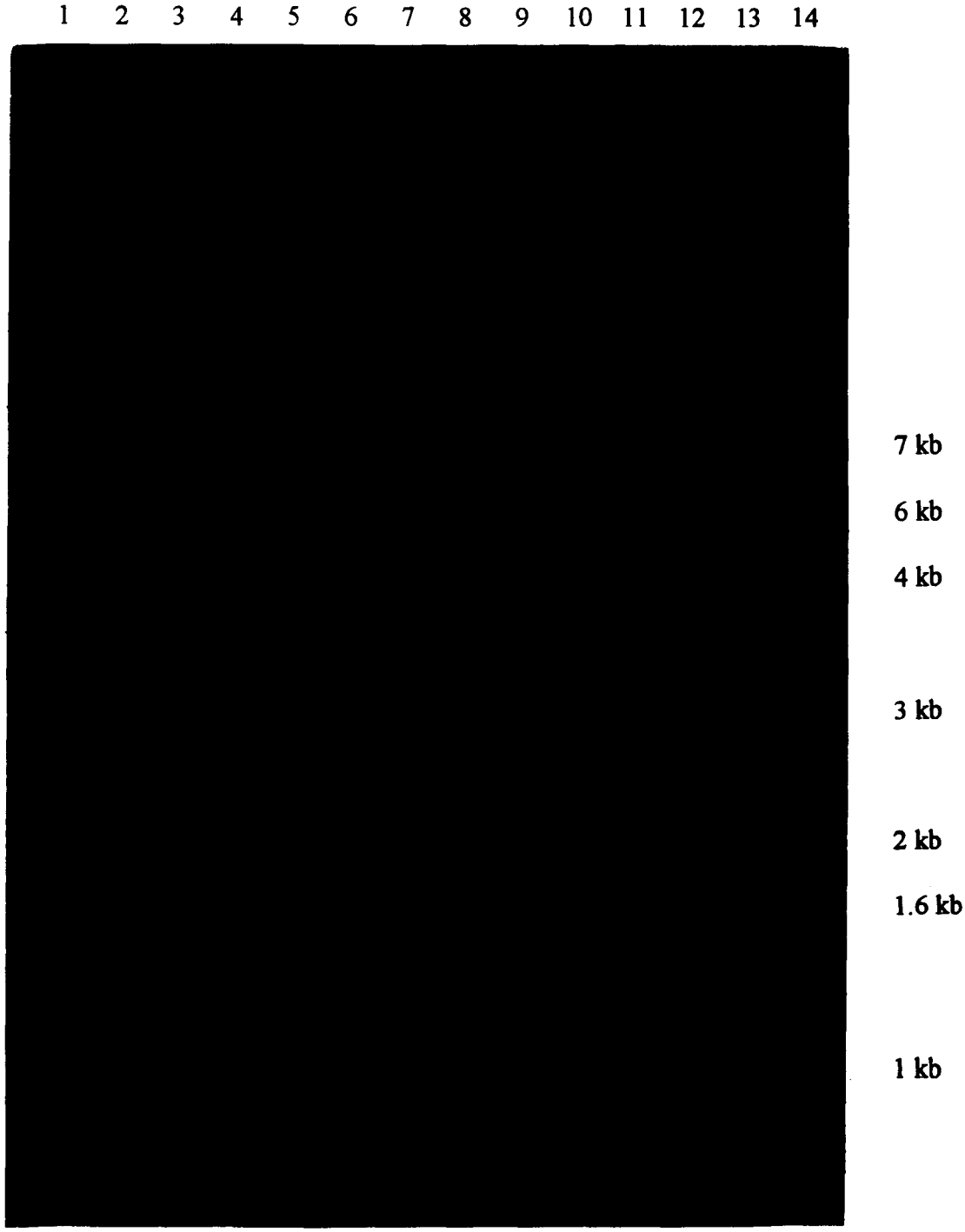
Hybridisation of *Hinf*I digested bovine DNA with the oligonucleotide probe $(TCC)_5$



Example of an autoradiograph produced by the *Hinf*I/$(TCC)_5$ probe/enzyme combination. Lanes 1 and 11 were Friesian DNA control lanes. The remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.6, 5.7 and 5.8.

Plate 5.10

Hybridisation of *Hae*III digested bovine DNA with the oligonucleotide probe $(TCC)_5$



Example of an autoradiograph produced by the *Hae*III/$(TCC)_5$ probe/enzyme combination. Lane 10 was a Friesian DNA control lane. A second Friesian control lane was not loaded on this gel. The remaining 13 lanes were Irish Moiled DNA samples, identical to those seen in Plates 5.3, 5.4 and 5.9.

5.1.4 Restriction Digestion of DNA samples

The standard restriction digestion protocol described in Materials and Methods, consistently resulted in the complete digestion of the DNA samples. Samples which had not properly digested were detected following electrophoresis of the digested samples on a 'test' gel (Materials and Methods). These samples were identified by the presence of a single intense band of high molecular weight (Plate 5.2). Once any contaminating phenol or protein had been removed by the small scale purification of the DNA sample, as described in Materials and Methods, further restriction digestion generally resulted in the complete digestion of the DNA.

To verify that no spurious bands, in the DNA fingerprint, would result from the possible partial digestion of DNA samples using the standard restriction digestion protocol, the banding pattern, produced by hybridisation to the oligonucleotide probe $(GTG)_5$ of a DNA sample digested as described in Materials and Methods was compared with that produced by the same sample digested with a massive concentration (50 units/μg DNA) of restriction enzyme. The banding patterns were found to be identical, confirming that the standard restriction digestion protocol does indeed result in the complete digestion of the DNA.

5.2 DNA FINGERPRINTING WITH OLIGONUCLEOTIDE PROBES

Plates 5.3 to 5.10 show examples of the autoradiographs produced by the eight combinations of oligonucleotide probe and restriction enzyme used. It is obvious from these that the bands produced were generally sharp and clear, and the level of background radiation was low. Distortion of the banding pattern was minimal. It was unfortunate that some lanes produce weaker hybridisation signals than others (e.g.

Plate 5.3 Lanes 11 and 12), despite attempts to ensure even loading of the wells (Materials and Methods). It was often difficult to identify bands in these lanes where a lower concentration of digested DNA had been loaded. This problem was overcome by using a longer period of exposure, two times the normal exposure time (see Materials and Methods) for those gels which contained lanes with weaker hybridisation signals.

The probe $(TCC)_5$ was found not to be sufficiently informative to merit the long exposure times (up to two weeks) required to produce a hybridisation signal. As observed by Buitkamp *et al.*, (1990a) with German Friesian cattle, the hybridisation of the oligonucleotide probe $(TCC)_5$ to *Hinf*I digested Irish Moiled DNA, produced only one or two polymorphic bands (e.g. Plate 5.9). the same was found to be true for the hybridisation of $(TCC)_5$ to *Hae*III digested DNA (e.g. Plate 5.10). In addition, due to the faint hybridisation signal obtained with the $(TCC)_5$ probe, the level of background radiation was relatively high and often obscured the banding pattern. After examining the hybridisation of the probe $(TCC)_5$ with the first six gels, it was decided not to continue to use this probe for the remaining gels.

5.3 STANDARD ELECTROPHORESIS CONDITIONS

Despite rigorous attempts to standardise electrophoresis conditions (Materials and Methods), differences were observed in the distances migrated by the bands in the Friesian DNA control lanes, electrophoresed on separate gels. In some cases the variation in the distance migrated was as much as 20% between two autoradioraphs. Initially this was not considered a problem. A DOS based computer programme 'DNA SIMDEX' (Scott *et al.*, 1993), had been obtained from Dr. Fred Leung at the Pacific Northwest Laboratory, Richland, U.S.A. This computer programme had been

designed to assist in the visual scoring of DNA fingerprint data. One of it's functions was the ability to normalise migration distances of samples electrophoresed on separate gels, by comparison to standard DNA lanes (the Friesian control lanes) on each of the gels. This would enable the comparison of lanes on separate gels, despite inconsistencies in the migration of the samples during electrophoresis (Scott *et al.*, 1993).

However, the usefulness of this programme was found to be limited. After comparison of two lanes with reference to standard Friesian DNA lanes, a final adjustment to the normalised banding pattern was necessary. This adjustment involved assigning which bands in the two lanes matched. Matching bands had to be determined by eye and this proved to be as difficult as a direct visual comparison between lanes on different gels. Unfortunately, it was decided that, as stated by many authors previously (Mannen *et al.*, 1993; Burke *et al.*, 1987; Wetton *et al.*, 1987; Piper *et al.*, 1992), it would be impossible to compare samples electrophoresed on separate gels. However, a great deal of information could be obtained by a comparison of samples electrophoresed on the same gel.

5.4 CALCULATION OF BAND SHARING

Pairwise comparisons were made between all lanes on each gel, comparisons between lanes on separate gels were not made, due to the difficulty in determining whether bands were matching. Matching and non-matching bands were assigned by eye, with reference to the Friesian DNA control lanes for samples which were more distant on the same gel. Only samples which had produced clear DNA fingerprints with all probe and enzyme combinations were scored.

For each probe/enzyme combination individual bands could not generally be resolved below approximately 2-2.5 kb, due to the large number of similar sized fragments below this size. On a minority of autoradiographs, this region was clearer than on others, and individual bands could be resolved, however since these bands could not be scored for every individual, the minimum size limit for scoring bands was set at 2.5 kb.

A total of 15 gels per restriction enzyme, were required to accommodate the 187 Irish Moiled DNA samples. Including those samples which had had to be re-electrophoresed due to distorted banding patterns. A total of 96 hybridisations were carried out and each of these produced several autoradiographs of different intensities. As calculations of band sharing were determined between every sample on an autoradiograph in a pairwise manner, between 55 and 105 values of band sharing between individuals were obtained for each of the autoradiographs. The numbers of matching and non-matching bands scored from each of the six probe/enzyme combinations were totalled to calculate the overall level of band sharing, using the formula given in Materials and Methods (Section 2.10.1). These results could then be correlated with the mathematically derived coefficients of co-ancestry (see Discussion).

5.5. *HINF*I AND *HAE*III DIGESTIONS PRODUCE SIMILAR BANDING PATTERNS WITH THE PROBE $(GGAT)_4$

The autoradiographs produced using the probe $(GGAT)_4$ with *Hinf*I digested DNA were very similar to those produced using the same probe with *Hae*III digested DNA (Plates 5.7 and 5.8). The difference in the sizes of the bands produced was minimal and could be ascribed to the variation in migration between gels. The only difference between the banding patterns produced by the hybridisation of $(GGAT)_4$

with *Hinf*I and *Hae*III digested DNA was the presence of a band at approximately 3 kb in many of the *Hae*III samples which was absent in the *Hinf*I samples.

It was apparent that in each case, the same loci were being detected by the $(GGAT)_4$ probe and as a result of this the DNA fingerprints produced by hybridisation of $(GGAT)_4$ to *Hinf*I digested DNA were excluded from the band sharing analysis.

5.6 NUMBER OF POLYMORPHIC BANDS

The numbers of polymorphic bands observed for each probe/enzyme combination were as shown in Table 5.1.

Table 5.1

Maximum, minimum and total bands observed with each probe/enzyme combination for Irish Moiled cattle

		Probe		
Enzyme	**Bands per Lane**	$(GTG)_5$	$(GT)_8$	$(GGAT)_4$
*Hae*III	maximum	10	15	8
	minimum	5	7	6
	Total	22	19	17
*Hinf*I	maximum	14	8	7
	minimum	9	4	4
	Total	25	16	12

No band was common to every individual in the herd, except for those under 2.5 kb which were not scored. All bands were considered polymorphic and unlinked and therefore used in the calculation of band sharing between individuals. Total bands

in Table 5.1 refers to the total number of different bands, produced by each probe/enzyme combination, in the population.

The number of bands scored for each individual was also recorded to investigate the correlation between number of bands and the mathematically derived inbreeding coefficient of individuals (see Discussion).

5.7 FREQUENCY OF BANDS IN POPULATION

A sample of 10 randomly chosen individuals was studied to calculate the probability of two individuals having identical banding patterns (Buitkamp *et al.*, 1991b; Jeffreys and Morton 1987 Jeffreys *et al.*, 1985b; Hanotte *et al.*, 1991; Burke and Bruford 1987). The results are shown in Table 5.2.

Table 5.2

Variability of oligonucleotide DNA Fingerprints in Irish Moiled Cattle

The mean number of polymorphic bands per individual (n), and the standard deviation (S.D), is shown for each probe/enzyme combination, for a sample of 10 Irish Moiled animals. The mean similarity (x) between banding patterns was calculated from a pairwise comparison of the 10 samples, as described by Jeffreys *et al.*, (1985a). This is the probability that a band present in one individual will be present in a second individual. The probability 'p' that two individuals both either contain, or lack, a given band was calculated from $= 1\text{-}2x+2x^2$ (Jeffreys and Morton, 1987). The probability $\hat{p}$ that two individuals are concordant for all bands was calculated from $\hat{p} = p^{n/x}$ (Jeffreys and Morton, 1987). This is the probability that any two individuals will produce identical banding patterns.

Enzyme	Probe	Mean Number of Bands per Individual (n)	S.D.	mean similarity (x)	p	$\hat{p}$
*Hae*III	$(GTG)_5$	7.0	± 1.3	.64 ± .10	.54	1.2×10^{-3}
	$(GT)_8$	9.2	± 3.2	.77 ± .28	.65	5.8×10^{-3}
	$(GGAT)_4$	7.1	± 0.7	.58 ± .12	.51	2.6×10^{-4}
*Hinf*I	$(GTG)_5$	11.3	± 1.8	.68 ± .08	.56	6.5×10^{-5}
	$(GT)_8$	6.2	± 1.1	.67 ± .08	.56	4.7×10^{-3}

The mean similarity between banding patterns is quite high for each probe/enzyme combination. However, the 10 individuals used to determine these values were all related to some extent, with coefficients of co-ancestry ranging from f = 0.111 to f = 0.381. The probability $\hat{p}$ of two individuals having the same banding pattern for all five probe/enzyme combinations is 5.5×10^{-16}.

5.8 PARENTAGE ANALYSIS USING OLIGONUCLEOTIDE DNA FINGERPRINTING

Buitkamp (1991a) has shown that the oligonucleotide DNA fingerprinting procedure is a useful method for paternity testing in cattle. If the mother was known with certainty the probability of mis-identification of the sire when the putative fathers were unrelated was found to be 5×10^{-2} for Red Pied cattle and 9×10^{-3} for Simmental cattle, using *HinfI* digested DNA hybridised with the oligonucleotide probe $(GTG)_5$ (Buitkamp 1989).

Oligonucleotide DNA fingerprinting was used to establish paternity in Irish Moiled cattle in a case involving two male calves of uncertain parentage. The calves were born of pure-registered Irish Moiled cows (Beltany Tulip, Herd Book Number 982, and Beltany Lilly, Herd Book Number 1034), but it was not known whether they had been sired by a pure-registered Irish Moiled Bull (Beltany Dandy, Herd Book Number 1058) or by an escaped Limousin bull. As the Irish Moiled coat pattern and hornless nature were dominant characteristics, and the Limousin coat colour was red, the physical appearance of the calves gave no indication of their sire. The appearance of the calves was that of very well marked Irish Moiled cattle (Plate 5.11).

DNA samples were obtained from both calves, their mothers, and the putative Irish Moiled sire, Beltany Dandy. The Limousin bull was not available for sample, as it had been slaughtered before blood samples were taken. A DNA sample was also available from a different Irish Moiled calf, known to have been sired by Beltany Dandy.

After restriction digestion, the DNA samples were electrophoresed and hybridised with the oligonucleotide probes as described in Materials and Methods. The autoradiographs used to determine paternity are shown in Plates 5.12, 5.13 and

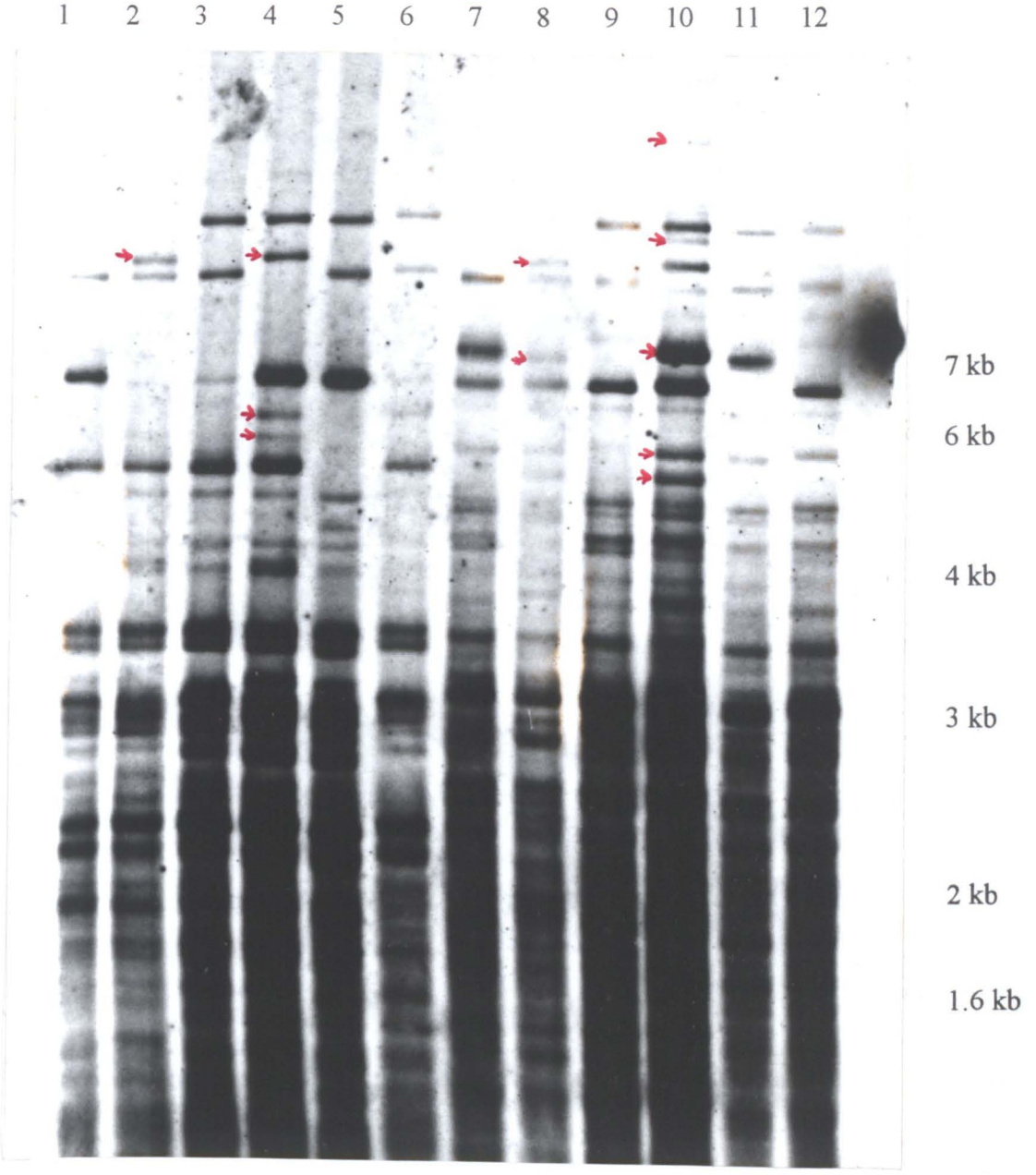
Plate 5.11

Calves putatively sired by the Irish Moiled bull Beltany Dandy.

Both calves were male. The calf on the left was from Beltany Tulip (dam 1), the calf on the right was from Beltany Lilly (dam 2).

Plate 5.12

Paternity test gel, probed with $(GTG)_5$

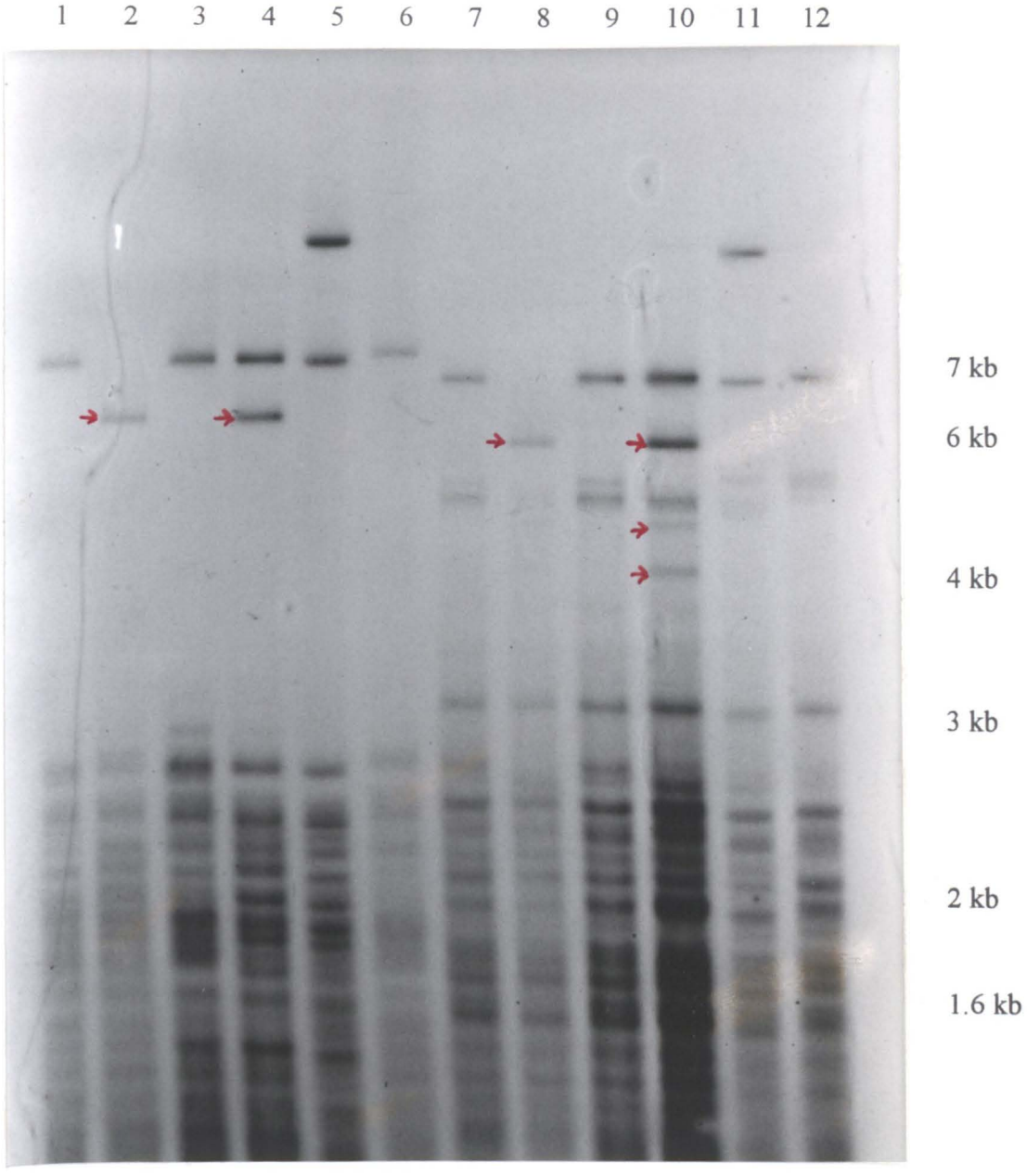


Bands which are present in the calves, but absent in the dam and putative sire are arrowed.

Lanes 1 - 6	***Hinf*I digested DNA.**
Lanes 7 - 12	***Hae*III digested DNA.**
Lanes 1 and 7	**Dam 1**
Lanes 2 and 8	**Calf of Dam 1**
Lanes 3 and 9	**Putative Irish Moiled Sire**
Lanes 4 and 10	**Calf of Dam 2**
Lanes 5 and 11	**Dam 2**
Lanes 6 and 12	**Calf known to have been sired by Beltany Dandy**

Plate 5.13

Paternity Gel, probed with $(GGAT)_4$

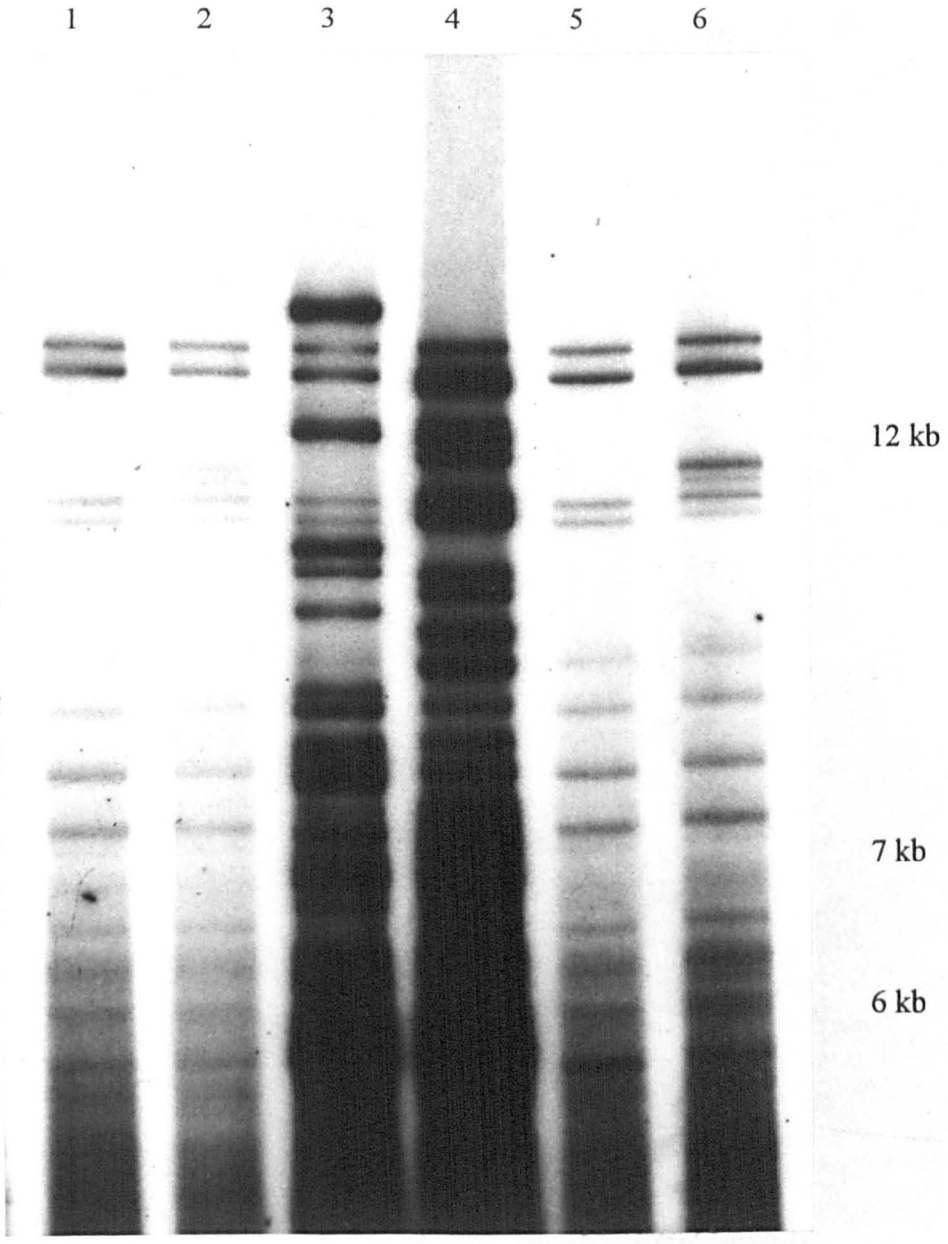


Bands which are present in the calves, but absent in the dam and putative sire are arrowed.

Lanes 1 - 6	***Hinf*I digested DNA.**
Lanes 7 - 12	***Hae*III digested DNA.**
Lanes 1 and 7	**Dam 1**
Lanes 2and 8	**Calf of Dam 1**
Lanes 3 and 9	**Putative Irish Moiled sire**
Lanes 4and l0	**Calf of Dam 2**
Lanes 5 and 11	**Dam 2**
Lanes 6 and 12	**Calf known to have been sired by Beltany Dandy**

Plate 5.14

Paternity gel probed with $(GT)_8$, *Hae*III digested samples



The *Hinf*I digested samples proved uninformative when probed with $(GT)_8$, only the *Hae*III digested samples are shown. Bands which are present in the offspring, but absent in the dam and the putative Irish Moiled sire are arrowed.

Lane 1	**Dam 1**
Lane 2	**Calf of Dam 1**
Lane 3	**Putative Irish Moiled Sire**
Lane 4	**Calf of Dam 2**
Lane 5	**Dam 2**
Lane 6	**Calf known to have been sired by Beltany Dandy.**

5.14. It was obvious from these that the two calves under investigation had not been sired by the Irish Moiled bull, Beltany Dandy. DNA fragments were seen in the calf samples (arrowed) which were not present in either the banding pattern of their mother or that of the putative Irish Moiled sire. Since the maternity of these animals was not in doubt, these bands must have been transmitted from a sire other than Beltany Dandy.

CHAPTER 6
COMPUTER ANALYSIS OF PEDIGREE DATA

6.1 INBREEDING COEFFICIENTS

Inbreeding coefficients have been calculated for every individual in the Moiled92.dat data file. A complete listing of the individuals in the datafile, including their calculated inbreeding coefficients is given in Appendix IV.

From these results the mean inbreeding coefficient of pure-registered Irish Moiled animals born each year was calculated (Fig. 6.1). The graph shows a rapid increase in the mean inbreeding coefficient in the late sixties and early seventies. This corresponds to the predicted narrowing of the genetic base of the population caused by the drop in the number of breeders and difficulty in obtaining bulls at that time. The increase in the Irish Moiled herd size, the management of its breeding to maintain the lines suggested by Gill and Kelly (1991) and their crossing have produced some control over the levels of accumulating homozygosity. Figure 6.1 shows that the mean inbreeding coefficient of pure-registered animals born each year appears to be stabilising at approximately F = 0.2.

Figure 6.2 shows the number of pure Irish Moiled calves registered per year. The numbers have been steadily increasing since the Irish Moiled Cattle Society was reformed as an active body in 1982.

The large error bars associated with the mean inbreeding coefficient of animals registered each year (Fig. 6.1) were due to the small number of animals born each year, especially pre-1980 (see Fig. 6.2). The inbreeding coefficients calculated for each individual, using Wright's coefficient, were themselves only estimates. Due to the small number of animals and the reduced number of polymorphic loci in inbred lines the calculations are subject to large errors (Gill and Harland 1992).

The calculations did not take into account the level of inbreeding in the founder animals which were assumed to be unrelated to each other and to have inbreeding coefficients of F = 0. Individuals which are listed in the Herd Book as pure registered

Figure 6.1

The Mean Inbreeding Coefficient of Pure-Registered Irish Moiled Cattle Born Per Year.

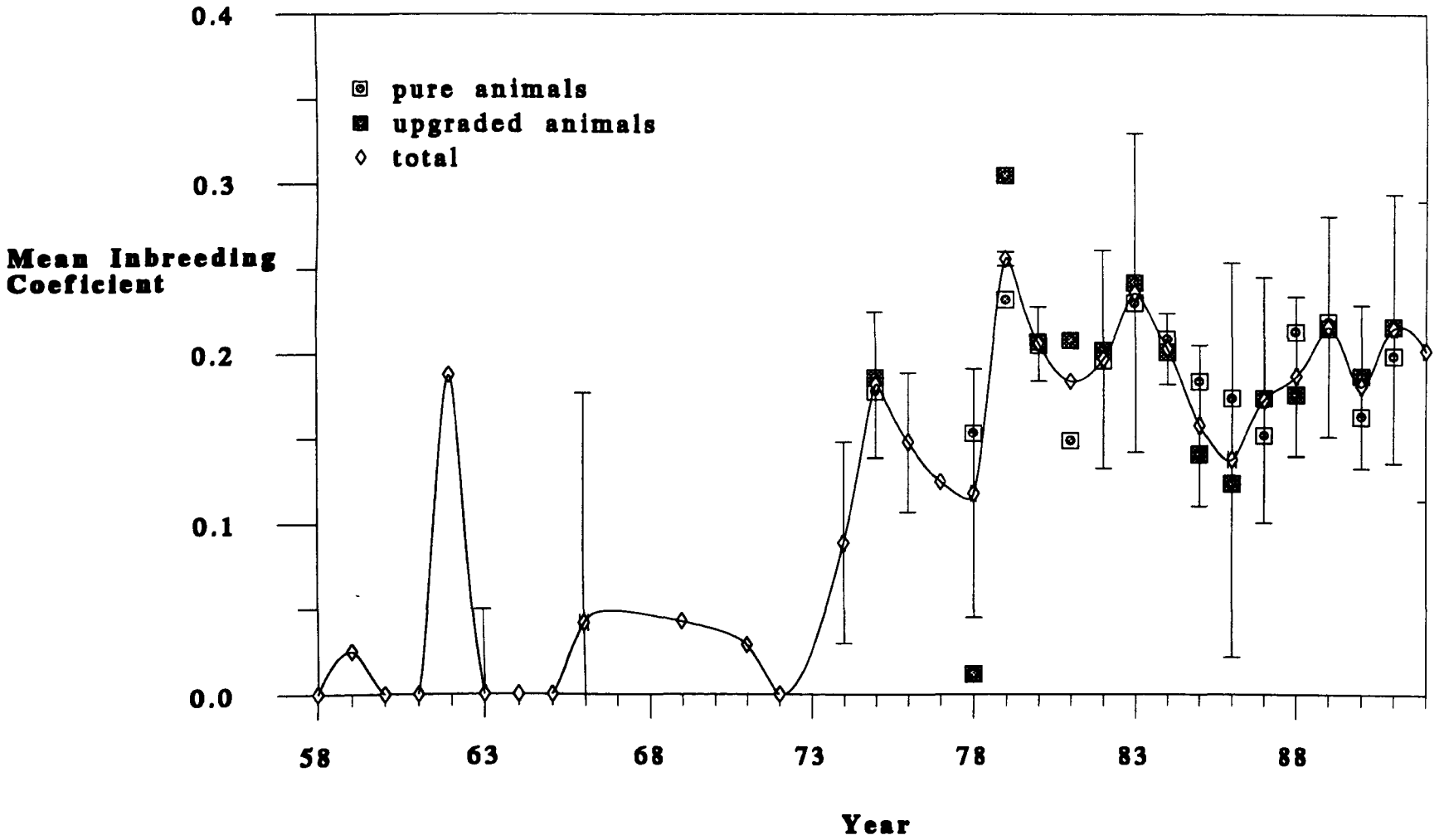
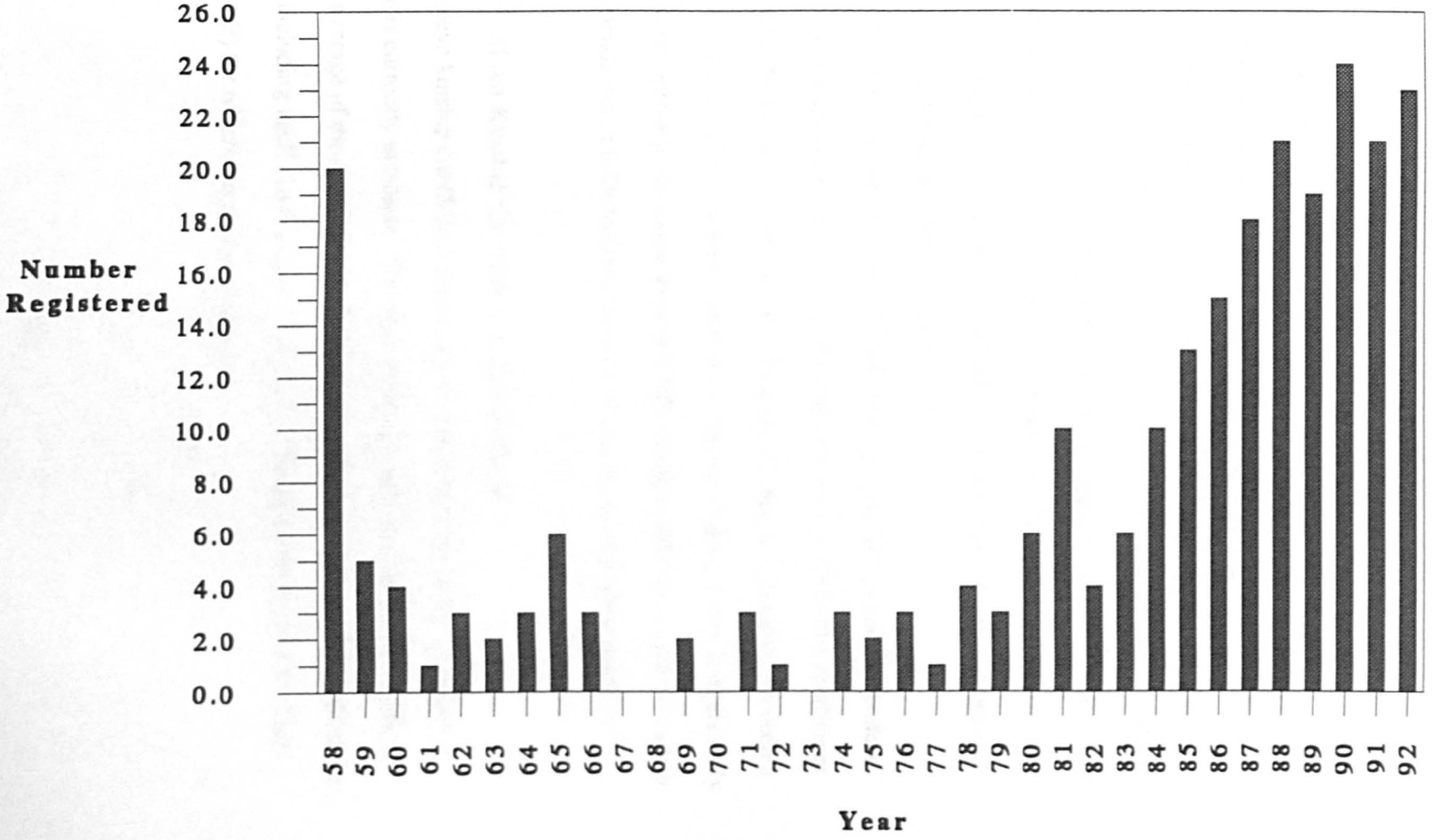


Figure 6.2

Number of Irish Moiled Cattle Registered as Pure per Year



but unknown Irish Moiled, also had to be considered unrelated to any other animal. These two factors may have resulted in misleadingly low inbreeding coefficients, although the contribution of these animals to the inbreeding coefficient of the present herd is negligible. The calculations do however, show the general trend in the levels of accumulating homozygosity in the breed.

6.2 COEFFICIENTS OF CO-ANCESTRY

Using the Moilmate programme, coefficients of co-ancestry were calculated in a pair-wise matrix between every animal from which a DNA sample had been obtained. The results were used for correlation with data generated from a comparison of band sharing between individuals after oligonucleotide DNA fingerprinting. For a pair-wise comparison of the 198 samples taken, over 15000 calculations of coefficients of co-ancestry had to be made. This volume of calculations required over twelve weeks of processing time on a model 486 personal computer.

The Moilmate programme is available for use by Irish Moiled cattle breeders, to determine which bulls would produce calves with the lowest inbreeding coefficient when mated with their cows. Coefficients of co-ancestry can be calculated between a breeders cows and all available semen stocks to enable the mating of the most distantly related individuals either in the entire herd or within constrained lines. Care was taken in the recommendation of bulls to avoid the over-use of any particular animal.

6.2.1 Mean Kinship Coefficient of Semen Bulls

The mean kinship coefficient has been calculated for each of the 10 bulls for which semen is currently available. The mean kinship coefficient (Mace and Ballou, 1990) is the average of the coefficients of co-ancestry between an animal and all other individuals, including itself. In this case between a particular bull and all other bulls (including itself) for which semen was available.

$$mKi = \frac{\Sigma f}{N}$$

mKi = mean kinship coefficient of individual

Σf = sum of coefcicients of co-ancestry between individual and all other individuals (including itself).

N = total number of individuals.

A low mean kinship value indicates that an animal is genetically distinct from the others and might therefore be of greater use in conservation programmes. The uniqueness of an animal is given by (1 - mKi)

The uniqueness of each bull can be used to determine the ratio at which each semen store should be used for artificial insemination with the more genetically distinct animals being used at a greater frequency.

$$frequency = \frac{1}{\sum uniqueness} \times uniqueness$$

In this instance the mean kinship values of the bulls were found to be very similar and the ratio at which they should be used was almost exactly even (see Table 6.1).

The mean kinship coefficient can also be used to assist in the selection of male calves to be used for breeding, as it enables the identification of the most genetically distinct individuals from a selection of phenotypically similar calves

Table 6.1

Mean kinship coefficient for Irish Moiled Bulls.

Semen Bull	Herd Book No.	mKi	uniqueness	frequency to use
Glenbrook Defender	922	.282	.718	.105
Maymore Red Hugh	925*	.189	.811	.119
Glenbrook Toro	939	.277	.723	.106
Maymore Finn-og	948*	.228	.772	.113
Argory Edward	969	.230	.77	.113
Myra Silken Thomas	997	.219	.781	.114
Springfield Unique	1026	.219	.781	.114
Templeson Carnelian	1054	.25	.744	.109
Bellevue Owen	1067	.25	.748	.109
			Σ 6.843	

Table showing the mean kinship coefficient, uniqueness and optimum frequency for use of Irish Moiled bulls for which semen is available

***semen only available directly from Mr. J. Nelson (Maymore, Killyleagh, Downpatrick, N.I.).**

6.3 FOUNDER ANALYSIS

Three different data files were used in the founder analysis of the Irish Moiled population. The Moiled92.dat data file, with every animal sampled, plus all bulls for which semen was available listed as alive (a total of 187 individuals) was used to investigate the entire extant herd. The data file was altered so that only the pure-registered animals (126 in total) were listed as alive in order to study the pure-registered population. To examine the upgrading animals the Moiled92.dat data file was altered so that only these animals (61 in total) were listed as alive.

6.3.1 Inbreeding Coefficients by Gene Dropping

Inbreeding coefficients for extant pure-registered Irish Moiled cattle were determined using the gene dropping simulation. Values were recorded for 250 and 1000 cycles of gene dropping. These values were compared with the calculations of inbreeding using Wright's coefficient in an attempt to determine the accuracy of other estimates made using the gene dropping procedure. The closer the results obtained by gene dropping were to the mathematically derived inbreeding coefficients, the more accurate was the founder analysis. However, the percentage difference between the inbreeding coefficient estimated mathematically and that derived from gene dropping for a particular individual, does not reflect the percentage error in other gene dropping applications for that individual, as the results for each application were measured independently. The correlation between the two sets of results for inbreeding coefficient, together with the standard error, can be used as an indication of the accuracy of the founder analysis (Table 6.2).

Table 6.2

Showing the correlation between values of inbreeding determined using 250 and 1000 cycles of gene dropping with those calculated using Wright's coefficient.

No. Cycles	Correlation	Standard Error
250	.915	4.04
1000	.964	1.89

The correlation between the values of inbreeding calculated using Wright's coefficient and those determined by gene dropping increased, although not proportionally, with the number of cycles of gene dropping. The accuracy of both 250 and 1000 cycles of gene dropping, by a comparison of the estimated values of inbreeding with those calculated mathematically using Wright's coefficient, were found to be highly significant at $p<0.001$. Increasing the number of gene dropping cycles to a

level sufficient to further increase this accuracy would require an inordinate amount of computer processing time. It was decided that all founder analyses were to be made using 1000 cycles of gene dropping. This required three days processing on a model 486 personal computer for each of the three data files described in the previous section.

6.3.2 Contribution of Founder Animals to Extant Herd

Table 6.3 shows the relative genetic contribution of each of the eight founder animals to the extant Irish Moiled herd. It was clear from column 1 that founder 5 (Listerdonnan, Herd Book No. 788) and founder 8 (Derryboy Cyclamen, Herd Book No. 723) were under-represented in the extant Irish Moiled population. Columns 2 and 3 show the estimates obtained for proportionate founder representation in the pure-registered and upgrading populations respectively. The distribution of founder contributions was found to be essentially the same in these two sub-sets, as that found for the entire population. Founders 1 (Ballydugan Kat, Herd Book No. 783) and 4 (Ballydugan Mimosa, Herd Book No. 798) were found to be well represented, while founders 5 and 8 were very poorly represented. One difference between the two sub-sets is the very large (over 30%) contribution of non-Irish Moiled alleles to the upgrading population. this is to be expected, as the animals were upgraded from non-Irish Moiled cattle. On examining the relative genetic contributions to the whole herd (column 1), the proportion of non-Irish Moiled alleles appeared high in comparison to Irish Moiled founder alleles. By examining the pure-registered and upgrading populations separately it could be shown that the pure-registered Irish Moiled cattle population contained only a small proportion of non-Irish Moiled alleles.

Table 6.3

Founder Contributions to Extant Irish Moiled Populations

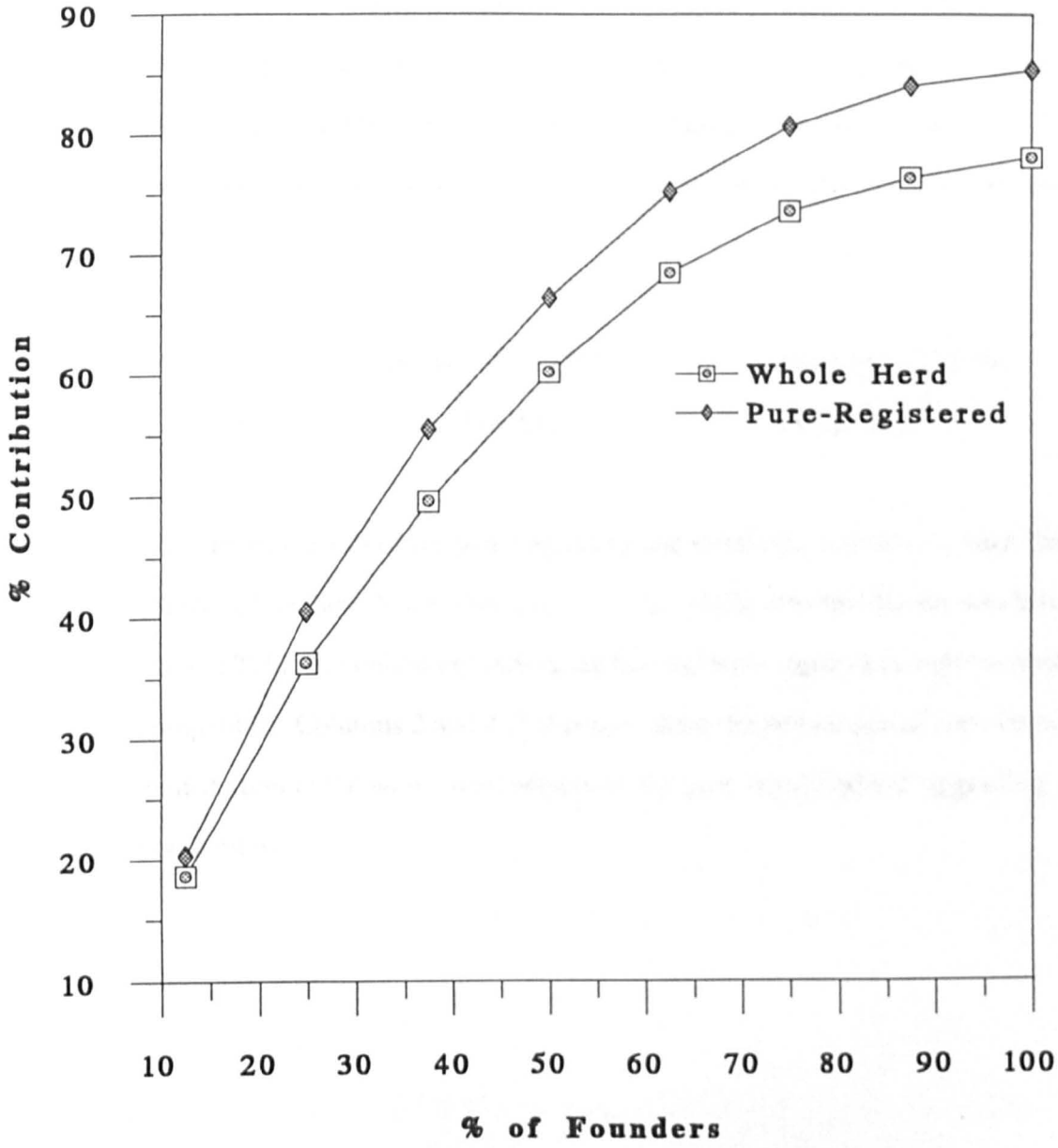
	Whole Herd N=187	Pure-registered N=126	Upgraders N=61
F1(783)	17.56	20.29	14.35
F2 (792)	8.26	8.85	6.69
F3 (762)	5.13	5.43	4.03
F4 (798)	18.60	20.08	14.93
F5 (788)	1.66	1.44	1.33
F6 (786)	13.30	15.05	10.93
F7 (790)	10.65	10.89	8.75
F8 (723)	2.85	3.44	1.79
Other Moiled	9.22	10.96	5.67
Non-Irish Moiled	12.78	3.58	31.54

Showing the relative contribution of each of the founder individuals (F1-F8), pure-registered but unknown Irish Moiled (other Moiled) and non-Irish Moiled individuals to the whole extant Irish Moiled herd (column 1), the pure-registered population (column 2) and the upgrading animals (column 3).

Alderson (1991) has shown an unequal founder effect in White park cattle whereby 30-40% of founders contribute 80% of the ancestry, of 25 bulls studied, and 10-15% of the founders contribute 50% of the ancestry. Figure 6.3 shows the cumulative contribution of the Irish Moiled founders to the whole extant population and to the pure-registered population. Although the trend was similar to that observed by Alderson in White park cattle, 30-40% of Irish Moiled founder animals were found

Figure 6.3

The Cumulative Contribution of the Eight Irish Moiled Founder Animals to the Total Extant Population and to the Pure Registered Population



to contribute only 50% of the ancestry of the pure-registered Irish Moiled population. This suggests that although the number of founders is much smaller [eight in the Irish Moiled pedigree compared with 30 for White Park cattle (Alderson, 1991)], the relative influence of each of the Irish Moiled founders to the present population is not as disproportionate as found in White Park cattle.

The data shown in Table 6.3, for the pure-registered and upgrading Irish Moiled population sub-sets, can be adjusted to show the proportionate contribution of the founder individuals to each sub-set, in relation to the total extant herd of 187 animals (Table 6.4 columns 1 and 3). The values from table 6.3 were adjusted as follows:

Pure animals (N = 126)	:	multiply by 126/187
Upgraders (N = 61)	:	multiply by 61/187

The sum of the values for pure-registered and upgrading animals for each founder (Table 6.4, column 5) are very similar to the results obtained for the whole herd (Table 6.3 and Table 6.4 column 6) indicating that the three separate founder analyses are compatible. Columns 2 and 4 (Table 6.4) show the percentage of each founder contribution to the whole herd present in the pure-registered and upgrading populations.

Table 6.4

Percentage of each founder in the pure and upgrading populations as a percentage of the total herd.

Column	1 Pure N = 126	2 %	3 Upgrading N = 61	4 %	5 Sum N = 187	6 (Whole) N = 187	7 % Diff.
F1 (783)	13.67	74.5	4.68	25.5	18.35	17.56	4.5
F2 (792)	5.96	73.2	2.18	26.8	8.14	8.26	1.5
F3 (762)	3.66	73.6	1.31	26.4	4.97	5.13	3.1
F4 (798)	13.53	73.5	4.87	26.5	18.4	18.6	1.1
F5 (788)	0.97	69.3	0.43	30.7	1.4	1.66	15.7
F6 (786)	10.14	74.0	3.57	26.0	13.71	13.30	3.1
F7 (790)	7.34	72.0	2.85	28.0	10.19	10.65	4.3
F8 (723)	2.32	80.0	0.58	20.0	2.90	2.85	1.8
Moiled	7.38	80.0	1.85	20.0	9.23	9.22	0.1
Others	2.41	19.0	10.29	81.0	12.70	12.78	0.6

Columns 1 and 3 show the founder contributions to the pure and upgrading populations, from Table 6.3, adjusted for a total population of N = 187. Columns 2 and 4 express these values as a percentage of the sum of column 1 plus column 3. The sum of columns 1 and 3 is shown in column 5. The difference between column 5 and the values obtained for the whole herd, from Table 6.3, shown in column 6, are given in column 7.

The data for the total extant herd (column 6) represents the available Irish Moiled genepool. The proportionate contribution of each founder and non-Irish Moiled ancestors is the frequency at which alleles from these individuals would be found in the Irish Moiled population. Approximately 70-80% of the founder alleles were found to be present in the pure-registered Irish Moiled population (column 2) and

approximately 20-30% were found to be present in the upgrading population (column 4).

The problems associated with obtaining a population census prevented an investigation of the change in the genetic composition of the Irish Moiled herd with time to the present date. However, if a population census is taken in the future, the gene-dropping simulation can be used to compare the present herd with the future population. Any change in the proportionate contribution of the founders to the herd may indicate a shift in the popularity of different lines with breeders, for example the preference of breeding beef suckler animals over dairy. The upgrading register is now closed, so future investigations should reveal a drop in the contribution of non-Irish Moiled alleles to the Irish Moiled population.

6.3.3 Percentage Founder Genome Lost

The percentage of each founder genome lost is shown in Table 6.5, for the whole herd and for just the pure registered Irish Moiled animals. The values for percentage of founder genomes lost for the pure registered animals should be higher than for the whole herd, as founder alleles present in the upgrading population are not taken into account. The difference between the two values represents the percentage of each founder genome which is unique to the upgrading animals, not present in the pure-registered population.

The percentages shown in Table 6.5 are in fact the probability of loss of a single pair of founder alleles. However, using 1000 cycles of gene-dropping, these values effectively represent an estimate of the proportion of founder genomes lost. The fact that these values were only estimates was highlighted by the results for founders 6 and 8. A greater proportion of these founders genomes appeared to have been lost in the analysis of the whole Irish Moiled herd than in the analysis of the pure-registered animals. This is, of course, impossible, as all animals present in the pure-registered population were also present in the whole herd. The aberration was due to the variance in the estimation of percentage founder genome lost in the two analyses.

Table 6.5

Percentage of Irish Moiled Founder Genomes Lost.

Founder	whole herd % lost	% remaining	Pure registered % lost	difference
F1	22.4	77.6	24.75	2.35
F2	55.75	44.25	58.9	3.15
F3	64.35	35.65	70.25	5.9
F4	31.3	68.7	33.3	2.0
F5	76.2	23.8	79.3	3.1
F6	37.4	62.6	36.5	-0.9*
F7	57.0	43.0	58.75	1.75
F8	80.05	19.95	79.4	-0.8*

Showing percentage of each founder genome lost in analyses of the whole Irish Moiled herd and the pure-registered population. The percentage of each founder genome remaining in the whole herd is also given.
Difference indicates the difference in the proportion of founder genomes lost between analyses of the whole herd and the pure-registered population, these values represent the proportion of each founder genome unique to the upgrading population.
*** - See text.**

The percentage of each founder remaining (Table 6.5) represents the proportion of each founder genome remaining in the Irish Moiled population. The success of any conservation programme could be monitored by gene-dropping analyses of future Irish Moiled populations. The rate of loss of each founder genome remaining, enables the identification of founder genomes which are being lost most rapidly.

6.3.4 Percentage of Founder Genomes at Risk of Loss

Table 6.6 shows the estimated percentage of founder genomes at high risk of loss. As described in Materials and Methods these values represent the proportion of cycles in which a particular founders' alleles were present in the extant population at a frequency of less than 10%. The results shown in Table 6.6 are from the founder analysis of the whole Irish Moiled herd, after 1000 cycles of gene-dropping. As with the estimates for proportion of founder genomes lost, the values for proportion of founder genomes at risk of future loss represent the probability of loss of a single pair of founder alleles. However, after 1000 cycles of gene-dropping these values effectively represent an estimate of the proportion of each founder genome at risk of future loss.

Table 6.6

Percentage of Irish Moiled founder genomes at risk of loss

Founder	% at risk	% surviving genes at risk of loss
F1	46.85	60.37
F2	30.05	67.91
F3	27.25	59.61
F4	30.9	44.98
F5	22.55	94.75
F6	38.00	60.70
F7	22.65	52.67
F8	15.55	77.94

Showing the proportion of each founder genome at high risk of future loss and the calculated percentage of surviving genes at risk of future loss. At risk of loss is represented by the proportion of cycles in which a particular founders alleles are present in the total extant herd at a frequency of less than 10%.

The results for percentage of each founder genome at risk were not informative. The proportion of cycles in which each founders' allele is present at less than 10% did not include those cycles where the alleles were absent, hence the low values of percentage at risk for founders which were estimated to have lost a large proportion of their genome. The values for percentage at risk represented the percentage of the total founder genome at risk. More informative results were obtained by calculating the percentage of surviving founder genes at high risk of future loss, as described in Materials and Methods. The estimates for the percentage of surviving founder genes at risk enable the identification of founder genomes which are at the greatest risk of future loss and measures can be taken to arrest the loss of alleles from these founders (Table 6.7).

The values for the proportion of Irish Moiled founder genomes at risk of future loss were high. This was because the measure of risk used was 10%. Similar values were obtained for Spekes Gazelles (Macleur, 1986) using the same criteria for measure of risk (9/88, approximately 10%). Although 10% was a stringent measure of risk of future loss, it was useful in determining the relative risk of loss of the eight Irish Moiled founder genomes.

6.3.5 Compilation of Founder Results

Table 6.7 is a compilation of the results for the whole extant Irish Moiled herd from the previous sections. The estimates of percentage founder genomes lost and percentage at risk of future loss could generally have been predicted logically from the estimates of founder contribution to the present herd. Those founders which had contributed the least to the extant herd had lost a larger proportion of their genome and were at the greatest risk of future loss. Those that had contributed the most to the current population had lost a smaller proportion of their genome and were at a lower risk of future loss.

Table 6.7

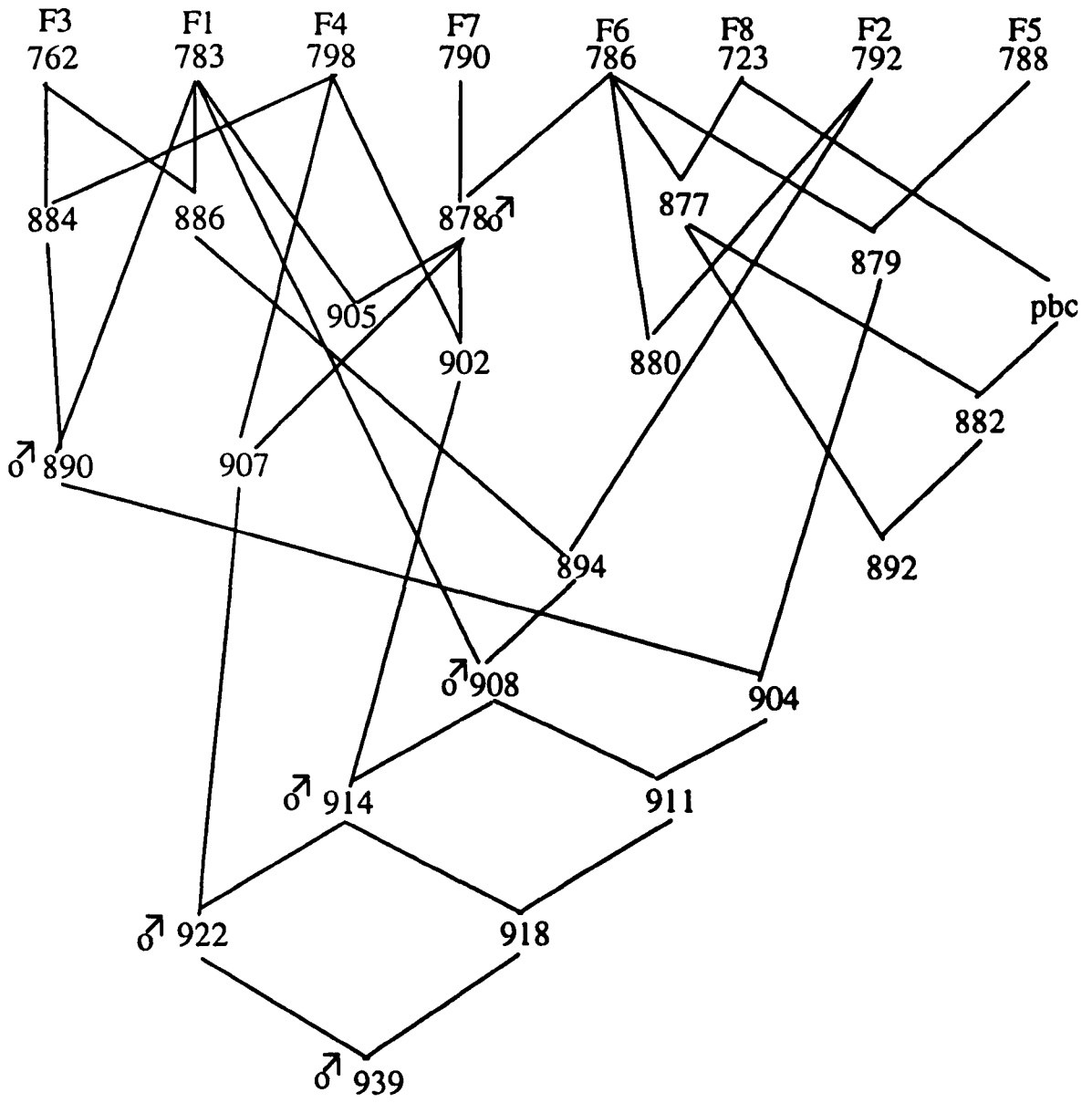
Compilation of founder analysis results for total extant Irish Moiled herd

Founder	% contribution to extant herd	% genome lost	% surviving genes at high risk of loss
F1 (783)	17.56	22.4	60.37
F2 (792)	8.26	55.75	67.91
F3 (762)	5.13	64.35	59.61
F4 (798)	18.6	31.3	44.98
F5 (788)	1.66	76.2	94.75
F6 (786)	13.3	37.4	60.70
F7 (790)	10.05	57.0	52.67
F8 (723)	2.85	80.05	77.94

The contribution of each founder to the present herd appeared to depend on two factors. First, the number of calves produced by each founder that were bred from, and secondly the number of intervening generations between the founder and the prolific bulls 878, 890, 908, 914, 922 and 939 (see Fig. 6.4). For example founder 7 (Herd Book No. 790) had only one calf that was kept and bred from, so an average of 50% of the genome of founder 7 was lost in the first generation. However, this calf was the prolific bull 878, and only a further 7% of the genome of founder 7 was lost in subsequent generations.

Figure 6.4

Immediate Descendants of the Eight Irish Moiled Founders, and the Ancestry of Prolific Bulls (Marked ♂)



The founders which were heavily represented in the present population, founders 1 (Herd Book No. 783) and 4 (Herd Book No. 798) had, respectively, four and three calves which were kept and bred from. The calves produced by founder 1 included the prolific bulls 890 and 908. The calves produced by founder 4 were parents of the bulls 890, 914 and 922. Therefore only a small proportion of the

genome of these founders has been lost. Less has been lost from founder 1, as this animal produced more calves and the number of intervening generations between the founder and its prolific descendants was less. The risk of future loss of genes from these founders is relatively low, especially for founder 4. Presumably the larger number of prolific descendants of founder 4 reduces the proportion of her surviving genome at risk of loss.

Founders 5 (Herd Book No. 788) and 8 (Herd Book No. 723), which were under-represented in the present extant herd, produced only one and two calves respectively, which were kept and bred from. These calves were not ancestors of any of the prolific bulls mentioned previously. This would explain the high proportion of these founder genomes lost and also the very large proportion of surviving genes at high risk of future loss.

6.3.6 Founder Composition of Individual Animals

The founder composition of every living Irish Moiled animal, plus those bulls for which semen straws were available, was estimated using 1000 cycles of the gene-dropping simulation (These results are given in Appendix V).

Only 31 of the extant, pure-registered Irish Moiled population were found to be completely pure (i.e. 0% non-Irish Moiled alleles). These animals are listed in Table 6.8. The remaining pure registered Irish Moiled animals were estimated to have from 1.2% (Rosecorner Patricia, Herd Book Number 1121) to 10.7% (Laurelgrange Wych Elm, Herd Book Number 1050), non-Irish Moiled alleles.

The results of the gene-dropping analysis have enabled the identification of those animals containing a high percentage of rare founder alleles. These animals are shown in Table 6.9. Preferential breeding from these animals may help to arrest the loss of rare founder alleles from the Irish Moiled population.

Table 6.8

Extant, pure-registered Irish Moiled animals, found to be completely pure

Herd Book Number	Name	d.o.b.
916	Glenbrook Tulip 2nd	1974
918	Glenbrook 779	1974
922	Glenbrook Defender	1976
928	Glenbrook Tulip 3rd	1978
929	Glenbrook 964	1979
930	Glenbrook Tulip 4th	1979
935	Glenbrook 1962	1980
939	Glenbrook Toro	1981
950	Glenbrook Catherine	1982
957	Argory Daisy May	1983
955	Laurelgrange Wisp	1983
967	Glenbrook 1198	1984
964	Glenbrook Tulip 5th	1984
983	Maymore Ivor	1985
986	Argory Eva	1986
988	Argory Edith	1986
989	Argory Ethel	1986
1040	Templeson Tansy	1988
1026	Springfield Unique	1988
1027	Springfield Mistletoe	1988
1024	Springfield Feather	1988
1054	Templeson Cornelian	1989
1053	Springfield Orchid	1989
1047	Springfield Frolic	1989
1052	Springfield Echo	1989
1042	Bellevue Iris	1989
1067	Bellevue Owen	1990
1061	Springfield Melody	1990
1066	Bellevue Clover	1990
1083	Springfield Promise	1990
1091	Springfield Enchantment	1991

Table 6.9

Percentage Contribution of Rare Founders F5 (788) and F8 (723) to Irish Moiled Genomes

Herd Book Number	%F5	%F8
918	5.45	-
925	-	11.95
941	7.20	-
950	9.75	-
957	6.55	-
996	1.05	6.10
976	8.0	5.45
977	2.45	5.85
990	-	6.25
982	-	6.25
1011	-	6.35
998	-	6.1
1040	6.15	-
1036	5.45	-
1038	-	6.7
1034	-	6.0
1033	0.7	6.7
1054	6.5	-
1044	5.05	-
1043	1.65	6.4
1063	5.25	2.35
1075	-	6.3
1105	5.55	2.5
1093	-	8.95
1092	1.45	6.25
1107	1.2	6.35
1109	1.15	6.05
1134	-	6.50
B26	6.7	-
A23	-	6.1
B27	7.0	-
B47	1.4	6.75

CHAPTER 7

DISCUSSION

7.1 OLIGONUCLEOTIDE DNA FINGERPRINTING IN IRISH MOILED CATTLE

The large number of polymorphic bands detected in the Irish Moiled population, using five combinations of oligonucleotide probes and restriction enzymes, has provided a substantial number of genetic markers for the analysis of the genetic variation in each animal. Several assumptions had to be made in the analysis of the banding patterns. Firstly, linkage and allelism were ignored. The independence of the fragments detected may be important in regarding the banding patterns produced by the technique as truly individual specific DNA 'fingerprints' (Burke and Bruford 1987; Wetton *et al.*, 1987; Birkhead *et al.*, 1990,; Jeffreys and Morton, 1987; Jeffreys *et al.*, 1986; Bruford *et al.*, 1992). However, due to the difficulties in assigning allelism and linkage in a population where large families are unavailable to perform a segregation analysis, each molecular weight band was regarded as a separate minisatellite locus (Hillel *et al.*, 1989; Bruford *et al.*, 1992; Buitkamp *et al.*, 1991a, b and Mannen *et al.*, 1993). Buitkamp *et al.*, (1991b), did not observe any linkage of bands in the fingerprints of seven half-sibs of one bull, in German Friesian cattle. This was considered to be an indication that the simple tandem repeat sequences detected may assort independently from parent to offspring in cattle, as shown in man by Nürnberg *et al.*, (1989).

A second assumption was that there had been no overlap in the loci detected using the different probe/enzyme combinations. Piper and Rabenold (1992) investigated the possibility of overlap in the fragments detected using Jeffreys probes 33.15 and 33.6 with *Hinf*I and *Hae*III digested DNA in the study of a population of stripe-backed wrens. It was shown that with *Hinf*I digested DNA there was some overlap in the fragments detected with each probe, and that there was a small amount of overlap between the enzymes probed with 33.6. In this study, the overlap in the banding patterns produced by the hybridisation of *Hinf*I and *Hae*III digested DNA

with the probe $(GGAT)_4$ was obvious. The banding patterns produced were almost identical. As it was obvious that the same loci were being detected in each case, only one of these probe/enzyme combinations, $(GGAT)_4$/*Hae*III, was used in the analysis.

The quasi-continuous variation in minisatellite allele lengths makes the unequivocal identification of alleles impossible (Wong *et al.*, 1987; Jeffreys *et al.*, 1985,1991). In this study all shared bands were assumed to be identical alleles from the same locus, rather than co-migrating non-homologous alleles.

7.1.1 Variability of Oligonucleotide DNA Fingerprints in Irish Moiled Cattle

The mean similarity (x) between the banding patterns of 10 randomly chosen individuals was shown to be high, for each of the probe/enzyme combinations. The values of x ranged from 0.58 for *Hae*III digested DNA probed with $(GGAT)_4$ to 0.77 for *Hae*III digested DNA probed with $(GT)_8$. These values were considerably higher than those observed in humans, using oligonucleotide probes (Schäfer *et al.*, 1988). This may reflect the lower nucleotide diversity in cattle, which has been found by Hibert *et al.*, (1989) to be about three times less than in humans. In unrelated samples of German Friesian and Simmental cattle, Buitkamp (1991b) found that the mean similarity between banding patterns produced using the oligonucleotide probe $(GTG)_5$ with *Hinf*I digested DNA was x = 0.4 and x = 0.57 respectively. Although the value of x = 0.68 obtained for the same probe/enzyme combination with Irish Moiled DNA, in this study, is relatively high, the 10 samples used in the analysis of band frequency came from related animals, and the mean similarity is therefore not comparable with values obtained for unrelated individuals.

The values of average number of polymorphic bands (n) and the mean similarity of the banding patterns (x) have been used to determine the probability $\hat{p}$ that any two unrelated individuals will have identical DNA fingerprints. $\hat{p} = \left(1 - 2x + 2x^2\right)^{n/x}$ Jeffreys *et al.*, 1985b, Buitkamp *et al.*, 1991a; Jeffreys and Morton, 1985; Burke and Bruford, 1987). Due to the unavailability of unrelated Irish Moiled animals, the

calculations of band frequencies in this study were carried out using randomly chosen related animals, and so the values obtained relate to animals chosen at random from the population. Since almost all the Irish Moiled animals were related to some extent, the values of $\hat{p}$ for each probe/enzyme combination calculated from 10 related individuals reflects the ability of the technique to produce individual specific DNA fingerprints in the population of Irish Moiled cattle better than a comparison of unrelated animals.

The probabilities of any two animals having identical DNA fingerprints with any of the probe/enzyme combinations was shown to be low (Table 5.2). The probability, using all five probe/enzyme combinations was shown to be 5.5×10^{-16}. Thus, the oligonucleotide DNA fingerprints produced in Irish Moiled cattle are likely to be completely individual specific, even amongst closely related individuals, allowing the precise identification of individuals, and the control and verification of semen used in artificial insemination.

7.1.2 Paternity Analysis by Oligonucleotide DNA fingerprinting

The oligonucleotide DNA fingerprinting technique has been shown to be a valuable tool in the determination of parentage in Irish Moiled cattle, as illustrated by a case in which a pure registered Irish Moiled bull was excluded from the paternity of two calves (see Section 5.8). Without a DNA sample from the Limousin bull, it was impossible to assign paternity to this animal. However, the results clearly ruled out the possibility that the calves had been sired by Beltany Dandy, and as a result of this investigation they were excluded from registration in the Irish Moiled Herd Book. Jeffreys and Morton (1987) calculated that the probability of a wrong paternity diagnosis in a typical dog mating was 10^{-3}. For unrelated Red Pied and Simmental cattle, Buitkamp (1989) calculated the probability of a wrong paternity diagnosis to be 5×10^{-2} and 9×10^{-3} respectively, in cases where the mother was certain and the putative fathers unrelated. In a typical Irish Moiled mating, the probability of a wrong paternity diagnosis with each of the probe/enzyme combinations is shown in Table 7.1.

With the battery of five different probe/enzyme combinations, the probability of missing a wrong paternity was shown to be only 3×10^{-4}.

Table 7.1

Probability of incorrect diagnosis of paternity in Irish Moiled cattle, given that the mother is known unambiguously

		Mean No. Bands (n)	Mean similarity (x)	Probability of incorrect diagnosis
*Hinf*I	$(GTG)_5$	11.3	.68	.113
	$(GT)_8$	6.2	.67	.289
Hae*III*	$(GTG)_8$	7.0	.64	.210
	$(GT)_8$	9.2	.77	.301
	$(GGAT)_4$	7.1	.58	.145

The probability was calculated from x^{Pf} (Jeffreys and Morton 1987) where Pf is the number of paternal specific fragments, estimated as half the mean number of bands (n).

As an independent check on the probe genetic analysis of peidgree data, calves which showed bands incompatible to the purported parentages were compared to the data assembled from more than 100 Moiled cattle independently blood typed over 10 conventional genetic loci by Dr. E.P. Kelly at Serology, University College Dublin. The factors used in common to all Moiled were as follows:

Locus A : A2; Z'

Locus B : B; G1; G3; K; O3; OX; P; Q; T; A'; B'; D'; E'3; F'; G'; I'; J'2; K'; O'; P'2; Q'; A"; B".

Locus C : C2; R1; R2; W; X1; X2; E; C'; L'; C"1; C"2.

Locus F : F; V; N'

Locus L	:	L
Locus Z	:	Z
Locus T'	:	T'
Locus R	:	R'; S'
Locus S	:	S; H'; U; U'; U''; S''

The best apportionment of the reactions observed in these two parentage sets could be phenotyped as shown in Fig. 7.1.

Figure 7.1

Results of Blood Typing for Paternity Analysis

	A	B	C	L
Calf 1	-/-	OX A' O' P'2/ **PI'**	C2 W **XI** E/C2...	-/-
Dam 2	A2	AX A' O' P'2/...	C2 R2 WE/C2...	-/-
Sire	A2	OX A' O' P'2/...	C2 R2 W E/C2...	L/?
Calf 2	A2	BI'/G3 OX E'3 O'	O2 W X' E/C2 R2...	-/-
Dam 2	A2	OX A' O' P'2/G3 T2 Y2 B' Q'	C2 R2 W X2 (L') E/C2...	L/?
Sire	A2	OX A' O' P'2/...	C2 R2 W E/C2...	L/?

Results of blood typing for paternity analysis, for the same samples as described in Section 5.8. Relevant alleles, present at the A, B, C and L Loci are shown. Alleles present in calves which were not present in either their dam or the putative Irish Moiled sire are shown in bold.

Figure 7.1 would suggest that the parentages of these two calves are incompatible. Whereas the phenogroups of the B-locus can be organised into its allelic format, the C-locus whilst showing the same pattern of incompatibility has too many allelic combinations within the factors expressed to formulate an accurate phenotype. For example, whilst both calves one and two show incompatibility, calf 2 shows no B-

system common in either the proposed sire or dam. It therefore requires at least two dam/sire pairs to establish the allelic segregation in this C-system.

These results are in agreement with the DNA band structure which showed exclusions in both these parentage sets.

7.1.3 Standard Electrophoresis Conditions

The standard conditions employed in the electrophoresis of samples were shown to be inadequate to enable the comparison of samples electrophoresed on separate gels. A comparison of the banding patterns produced by the Friesian control DNA on different gels revealed up to a 20% difference in the distance migrated by fragments. The difference in migration distances could have been due to slight differences in the composition of the agarose gels, or differences in the laboratory temperature at the time of electrophoresis. The use of in lane molecular weight markers, to which the hypervariable fragments can be compared, as described by Galbraith *et al.*, (1991), may enable the comparison of samples electrophoresed on separate gels.

Samples electrophoresed on the same gel could be compared by reference to the two Friesian DNA control lanes, even if the samples were widely separated. No investigation into the effect of the distance between samples on the values obtained for band sharing has been made. Piper and Rabenold (1992) have demonstrated the inflation of band sharing scores with increasing distance between lanes and also the decrease in the number of scorable bands with increasing separation. Although the possibility of increased mis-matching of bands due to increasing distance between samples cannot be excluded in this study, reference to the Friesian DNA control lanes and a knowledge of the banding patterns produced certainly helped to limit the effect of lane separation on the number of scorable bands and on the magnitude of band sharing scores. Piper *et al.*, (1992) suggest that samples more than three lanes apart should not be compared and emphasise the importance of organisation of samples on gels. However, in the study of even a small population such as Irish Moiled cattle, the

electrophoresis of each sample within three lanes of every other sample would be very expensive in terms of both time and materials.

7.1.4 Overlap between Enzymes in Fragments Detected

The autoradiographs produced have shown a clear overlap in the patterns produced by *Hae*III and *Hinf*I digested DNA when probed with $(GGAT)_4$. Remarkably, DNA digested with each of the two different restriction enzymes produced almost identical banding patterns when probed with $(GGAT)_4$. Piper and Rabenold (1992) observed a small amount of overlap in the fragments detected between samples digested with *Hinf*I and *Hae*III, probed with Jeffreys probe 33.6, in the tropical wren. The almost identical banding patterns observed in this study could indicate that the recognition sites for the restriction enzymes *Hinf*I and *Hae*III occur very close to each other on either side of the minisatellite loci detected by $(GGAT)_4$. The restriction fragments, containing the repeat sequence to which the probe binds, produced by each of the enzymes, would therefore be similar in size. It is not entirely improbable that this could have occurred for each of the fragments which hybridise with the $(GGAT)_4$ probe, as both enzymes recognise a four base pair sequence and would therefore be expected to cleave DNA of random nucleotide sequence approximately every 256 bp. Another possibility is that the restriction sites for both enzymes are conserved, close to one another at each side of the repeat sequence detected by the probe $(GGAT)_4$. Each possibility would produce the same result, namely identical banding patterns.

Extra bands appear in the *Hae*III digested samples, that do not appear in the *Hinf*I digested samples, when probed with $(GGAT)_4$ (see Plate 5.13). These extra bands were the only difference observed between the almost identical banding patterns produced by the two enzymes. This would suggest that these extra fragments detected in the *Hae*III digested samples may contain internal restriction sites for the enzyme *Hinf*I. The fragments resulting from the internal cleavage of these repeat sequences may therefore be too small to be resolved.

No investigation has been made into the possibility of overlap between enzymes in fragments detected by the probes $(GTG)_5$ or $(GT)_8$. However, the fragments detected by hybridisation of *Hae*III and *Hinf*I digested samples with the probe $(GTG)_5$, appeared to be similar in pattern, although not in size (Plates 5.3 and 5.4). It is therefore possible, that as with $(GGAT)_4$, the probe $(GTG)_5$ was detecting the same loci in both *Hae*III and *Hinf*I digested samples.

The possibility of overlap between probes in fragments detected was discounted. Autoradiographs produced by the hybridisation of the same samples with the probes $(GTG)_5$, $(GGAT)_4$ and $(GT)_8$, were overlaid to check for matching bands. The majority of bands did not match and the few that appeared to match were assumed to be a result of the fortuitous co-migration of separate fragments. Although there had been no overlap between probes in fragments detected, the possibility of overlap between restriction enzymes, in fragments detected with the probe $(GTG)_5$, could have affected the band sharing analysis. This is discussed in Section 7.4.

7.2 COMPUTER ANALYSIS OF PEDIGREE DATA

The pedigree information contained in the 1992 Irish Moiled Herd Book, upon which all computer analyses of the herd were based, was, of necessity, assumed to be entirely correct. Before the reformation of the Irish Moiled Cattle Society in 1982, the registration of stock had been fragmented. The waning interest in the breed and the difficulty in licensing bulls had resulted in neglect of the upkeep of written breeding records, and by 1962 Irish Moiled cattle had ceased to be registered in the Herd Book. Upon the reformation of the Irish Moiled Cattle Society, Mr. A. Cheese was able to trace accurately and collate breeding records for the ancestors of those animals alive in 1982, as far back as 1958. Breeding records from before 1958 could not be traced with the same degree of accuracy, and were therefore not included in the 1992 Herd Book.

The use of the 'format' option in the Moilmate programme, to sort the animals into order of date of birth (see Appendix I) revealed several minor mistakes in the 1992

Herd Book. These mistakes were generally typing errors, animals listed as the wrong sex, or parents given the wrong Herd Book Number, and were easily corrected. A copy of these corrections was passed on to the Registrar of the Irish Moiled Cattle Society.

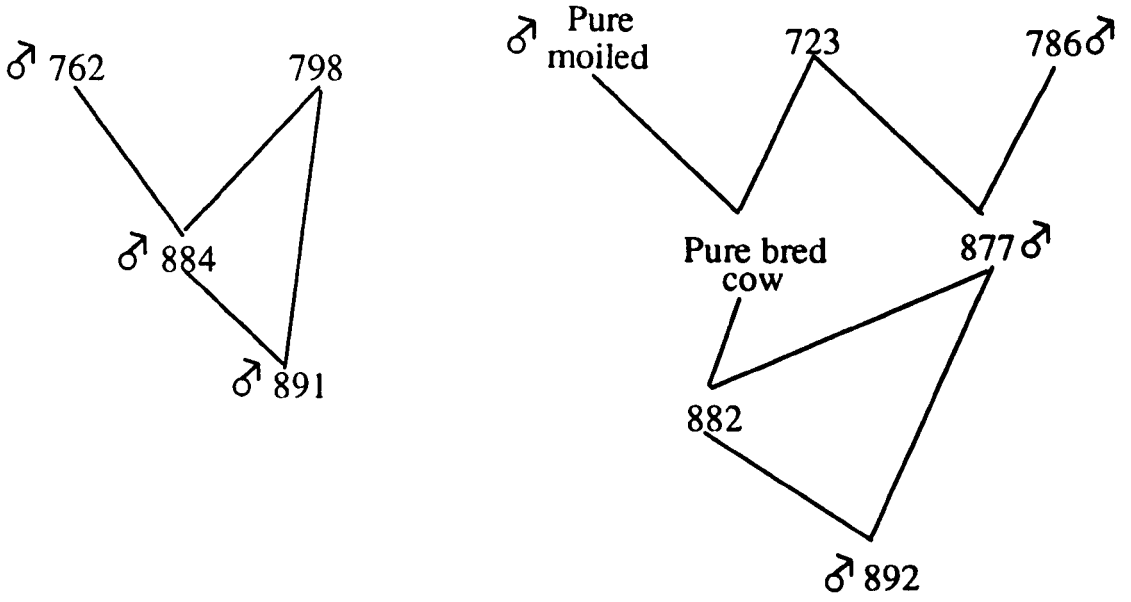
One important assumption was made when transferring the pedigree information in the 1992 Irish Moiled Herd Book into a data file for use with the Moilmate programme. In the Herd Book, the dam of the bull Ballydugan King (Herd Book No. 886) was listed as being either Ballydugan Kat (Herd Book No. 783) or Ballydugan Kate (Herd Book No. 75). It was decided to list the dam as Ballydugan Kat (Founder No. 1) in the data file. The effect of this on the inbreeding coefficients and the co-ancestry of the present herd would be minimal, as the number of intervening generations between animals in the present herd and the founder animals was high. However, the effect of this single uncertain parentage on the results obtained from the founder analysis would be marked. If Ballydugan Kate were the dam of Ballydugan King, rather than Ballydugan Kat, she would effectively have been an extra founder animal (nine in total), contributing a small amount to the present herd. The contribution of founder 1 would drop by the amount contributed by this additional founder.

7.2.1 Coefficients of Inbreeding

A definite trend has been shown in the mean inbreeding coefficients calculated for pure registered animals born each year (see Fig 6.1). This trend was related to the history of the breed. From 1958 up to 1973 the mean inbreeding coefficient remained low, at less than $\bar{x}$ F = 0.05. The only exception to this was the mean value for 1962 which was $\bar{x}$ F =0.188. Of the three animals born in this year, two had exceptionally high inbreeding coefficients. Ballydugan Monarch 3rd (Herd Book No. 891) had an inbreeding coefficient of F=0.25 and Maymore 8th (Herd Book No. 892) had an inbreeding coefficient of F=0.313. The reason for these anomalously high values could be seen from the ancestry of the two animals (Fig. 7.2)

Figure 7.2

Ancestry of Animals 891 and 892



$$F = \Sigma\left[(0.5)^{m+f+1}(I + FA)\right]$$

$$891 = (.5)^2 = 0.25$$

$$892 = (.5)^2 + (.5)^4 = .3125$$

The rapid increase in the mean inbreeding coefficient in the mid to late seventies was related to the population bottleneck and the limited number of bulls working from the 1960s onwards. The lack of different breeders at this time had severely limited the number of bulls available and from 1969 to 1974 only a single bull, Glenbrook Victor (Herd Book No. 908) was used. From 1974 to 1979 the only bull to be used was Glenbrook Young Cooper (Herd Book No. 914), the son of Glenbrook Victor.

Two animals were born in 1975, Glenbrook 795 (Herd Book No. 919) and Glenbrook 815 (Herd Book No. 920). The second of these was the first pure-registered Irish Moiled to come from the Glenbrook x Shorthorn upgrading line. The calculated inbreeding coefficients for these two animals were very similar, F = 0.178 for the pure animal 919, and F = 0.186 for the upgraded animal 920. Although the upgrading line was established in response to fears about rising levels of inbreeding,

due to the narrowing of the genetic base of the population, the inbreeding coefficient of the upgraded animal 920 was not considerably different from that of the pure animal 919, and was in fact slightly higher. The use of the same low number of sires in both the upgrading and pure lines, has resulted in similar levels of inbreeding in the animals produced from each line.

The calculated inbreeding coefficients for absolutely pure and upgraded animals born each year show that the upgrading lines have not reduced the overall level of inbreeding in the population. The mean inbreeding coefficient for upgraded cattle was very similar to that for the pure cattle each year. The only exceptions to this occurred when only a single upgraded animal was produced, with an inbreeding coefficient significantly different to the mean inbreeding coefficient of the pure animals born in the same year (see Fig. 6.1). For example, in 1979 the upgraded animal Glenbrook Pinky (Herd Book No. 931) was born. The inbreeding coefficient of this animal, F = 0.305, was much higher than the mean of the two pure animals born this year, F = 0.232. However, the standard deviation for the mean of the two pure animals was ± 0.107 and therefore the inbreeding coefficient of Glenbrook Pinky was not significantly greater than the mean of the two pure animals born in the same year.

The inbreeding coefficient of the upgraded bull Maymore Red Hugh (Herd Book No. 925) in 1978 was found to be significantly lower than the mean inbreeding coefficient of the three pure animals (Herd Book Nos. 926, 927 and 928) born in the same year.

$$925 \quad F = 0.012$$

$$926, 927, 928 \; \tilde{x} \; F = 0.153 \pm 0.022$$

This low inbreeding coefficient had arisen by the use of a Glenbrook bull, the upgrading animal Glenbrook Nelson (Herd Book No. A6) on a Maymore cow, Maymore 82 (Herd Book No. 907). The only common ancestor from which Red Hugh could receive alleles identical by descent is the founder Maymore VI (Herd Book

No. 786) which is common to both the Glenbrook and Maymore foundation stocks. Red Hugh became a very important bull, despite carrying 0.0625 alleles from his shorthorn ancestry, as he represented a unique sample of alleles from the Maymore foundation stock. The ancestry of many animals in the present herd could be traced to Red Hugh and therefore these animals contained a proportion of his shorthorn alleles.

The number of pure animals registered each year from 1959 to 1980, was low due to the lack of breeders. The standard deviation for the calculation of mean inbreeding coefficient per year was therefore high. The increase in the number of Irish Moiled cattle registered per year from 1981 onwards indicates the resurgence of interest in the breed at that time. The calculated inbreeding coefficients for pure animals registered after the reformation of the Irish Moiled Cattle Society as an active group in 1982, showed that the mean inbreeding coefficient, per year, had stabilised at approximately $\bar{x}$ F =0.2 by 1992. Year by year variations were dependent on the bulls used for breeding in a particular year. This stabilisation in the rate of increase in the mean inbreeding coefficient was brought about by the expansion of the herd. More breeders had become interested in the breed and the population increased. More bulls became available and the effective, as well as the actual, population size increased.

The accuracy of the values calculated for inbreeding coefficients and coefficients of co-ancestry, using Wrights coefficient, was dependent on the accuracy of the pedigree information from which the values were calculated. Founder individuals were assumed to have an inbreeding coefficient of zero. If the founders were themselves inbred or related, the inbreeding coefficients would have been greater. However, the effect of this on the coefficient calculated for the present herd would be minimal.

7.2.2 Founder Analysis

The founder analysis, using the gene dropping simulation, suggested that founders 5 and 8 (Listerdonan (788), and Derryboy Cyclamen (723) respectively) were very poorly represented in the present population, each contributing less than 3%.

Founders 1 and 4 (Ballydugan Kat (783), and Ballydugan Mimosa(798)) were shown to be well represented, each contributing approximately 18% to the extant herd. The remaining founders each contributed between 5% and 13% (see Table 6.3). Examination of the Irish Moiled pedigree information revealed that those founders which had contributed the most to the present population were the founders that had produced the most offspring which were kept and bred from. Founder 1 had produced four calves which were kept and bred from and founder 4 had produced threee, whereas founder 5, the least represented founder in the present population, had only produced one calf. The exception to this was founder 7, Derylecka (Herd Book No. 790) which, although it had only produced one calf, was better represented in the present population (10.6%), than founders 2 (8.3%), 3 (5.1%) and 8 (2.9%) which had produced two calves each. Examination of the pedigree information revealed that the success of Derylecka was due to the fact that its single calf was the very prolific bull, Derylecka Maymore (878), which was common to the ancestry of many of the present Irish Moiled herd.

A comparison of founder contributions estimated using the three different population parameters (whole extant herd, pure registered population and upgrading population), revealed that the upgrading population contained a very small proportion of unique founder alleles (see Table 6.5). This result was not surprising as the same bulls which had been used to produce the pure line had also been used as sires for the upgrading population. Hence the alleles transmitted to the upgrading animals would have been the same as those present in the pure line. The bulls 908 (Glenbrook Victor), 914 (Glenbrook Young Cooper), 922 (Glenbrook Defender) and 926 (Glenbrook Young Cooper 2nd) were used in both the pure line and the upgrading line from 1962 until 1983, with a single bull working at a time in a series of father to son generations.

Although the alleles present in the upgrading population were shown to be very similar to those present in pure Irish Moiled animals, the results indicate that the upgrading population contains a significant proportion of Irish Moiled founder alleles

(see Table 6.5). For example alleles from the under-represented founder F1 were estimated to be present in the extant Irish Moiled population at a frequency of 0.209. Just over 20% of these alleles were shown to be present in the upgrading population. The upgrading animals are therefore an important reservoir of Irish Moiled alleles.

7.2.2.1 Percentage Founder Genomes lost and at Risk of Future Loss

The estimation of the percentage of each founder genome lost (see Table 6.6) gave a starting point for the conservation of founder alleles. The proportion of surviving alleles at high risk of loss gave an indication of those founder genomes which were at the greatest risk of future loss. These values did not, however, take into account the age of extant animals and their future reproductive value. Very old animals containing rare founder alleles may not have had many breeding years left. The alleles present in these animals would therefore have been at a higher risk of loss than those present in younger animals capable of producing many more calves. For example, the cow Glenbrook Tulip 2nd (Herd Book No. 916) was born in 1974 and was still alive at the time the computer analysis was performed. This animal was therefore listed as alive in the Irish Moiled data file, and the founder alleles that she received contributed to the population average. 916 was however, approaching the end of her reproductive life-span, and was unlikely to produce further calves. In the estimation of the proportion of each founder genome lost, the alleles received by 916 were considered safe, as they were present in the living population. However, if 916 produced no more offspring, then these alleles, although present in a living animal, would be effectively lost from the Irish Moiled population. Glenbrook Tulip 2nd could effectively have been listed as dead in the Irish Moiled data file. In the case of 916, which had produced seven pure offspring, this would have made very little difference to the estimates of the proportion of each founder genome lost, as the proportion of her genome which would not be represented in her seven offspring would have been only 0.5^7 (0.0078125). If 916 had been listed as dead, then the risk of loss of her alleles would only have been fractionally higher.

The gene-dropping simulation assumed that every animal had the same reproductive life-span ahead of them. Alleles present in older animals, which had only produced a small number of offspring would have been at a higher risk of future loss than suggested by the results. Conversely alleles that were present in bulls, especially those used for artificial insemination, would have been at a much reduced risk of loss, because of the potentially large number of calves which were likely to be sired by these animals. It is clear that one method of increasing the frequency or arresting the loss of an under-represented founders alleles in the population would be to establish a store of semen from bulls with a high proportion of that founders alleles, for use in an artificial insemination programme.

7.2.2.2 Genetic Composition of Individual Animals

The genetic composition of individual animals enabled the identification of those animals with a high proportion of alleles from under-represented founders or founders that were at high risk of future loss. The percentage of non-Irish Moiled alleles present in each individual was also shown. This enabled the identification of pure-registered Irish Moiled that were completely pure, and those which were descended from upgraded stock. Many of the present population had an ancestry which could be traced to the upgraded bull Maymore Red Hugh. These individuals therefore contained a proportion of non-Irish Moiled (shorthorn) alleles. The estimated proportion of non-Irish Moiled alleles in these animals was shown in the gene-dropping results.

The estimates of the genetic composition of animals can be used in an attempt to arrest the loss of a particular founders alleles or to increase the frequency in the population of an under-represented founder. Animals with a high proportion of a rare founders alleles can be bred from preferentially, or semen could be collected from bulls for artificial insemination, as described in the previous section. However, several factors must be considered. Individuals with high proportion of the same rare founders alleles were likely to be related, as the alleles were probably transmitted via the same

route, therefore mating these animals will increase the level of inbreeding in the herd. Increasing the frequency of one founders alleles in the population would result in the decrease in the frequency of other founders alleles. This would not be a problem if the frequencies of the very well represented founders, F1 (783) and F4 (798), were reduced. Care would have to be taken to ensure that the frequency of other rare founders alleles were not reduced. If the Irish Moiled population continued expanding, it may be possible to increase the level of representation in the herd of a rare founders alleles, without increasing the rate of loss of other founder alleles.

In theory, the optimum frequency for each of the eight founders in the population would be 12.5%, each of the founders would be represented equally in the herd. However, due to the irreversible loss of large portions of many founder genomes, this would be impossible to achieve. The optimum frequency of each founders alleles could be recalculated, to accommodate this loss. The target founder representations could be calculated from the proportion of a particular founders genome remaining as a percentage of the sum of the proportion of the eight founder genomes remaining (Table 7.2)

Table 7.2

Target Founder Representations for Irish Moiled Cattle

	% loss	% remaining	target	actual
F1	.224	.776	.207	.176
F2	.558	.442	.118	.0830
F3	.644	.356	.095	.0510
F4	.313	.687	.183	.186
F5	.702	.238	.063	.017
F6	.374	.626	.167	.133
F7	.570	.430	.115	.106
F8	.801	.199	.053	.029
		Σ 3.754		

7.2.2.3 Population Census

One of the problems associated with founder analysis of gene-dropping was that of obtaining an accurate population census. Without this the results generated could only have been at best a rough idea of the genetic composition of the population. A reasonably accurate population census was obtained by taking blood samples from every living animal. Each animal sampled was listed as alive. No account could be taken of animals which had died or were born after sampling.

A future population census would enable a number of other factors to be investigated, by comparison of gene-dropping results from the future herd with those in this present study.

The change in the estimates of percentage contribution to the extant herd would enable the change in the frequency of each founder animal in the population to be observed. The percentage contribution of non-Moiled alleles should drop as the C grade register (which is the first out-cross in the upgrading system) has now been closed.

The increase in the percentage of each founder genome lost indicates the rate of loss of particular founders alleles. This, together with any change in the percentage of each founder gene at high risk of future loss, would enable the effectiveness of any breeding policy designed to arrest the loss of particular founders alleles to be gauged.

7.3 AVAILABILITY OF THE MOILMATE PROGRAMME FOR USE BY BREEDERS

The Moilmate programme has now been made available to Irish Moiled cattle breeders, together with instructions on how to use it (Appendix I). Perhaps the most obvious use of the programme is in the calculation of most distantly related animals for the purposes of reducing the inbreeding coefficient of calves. It must be stressed however, that the co-ancestry of two animals should not be the only factor considered in choosing which animals to mate. Care must be taken to avoid the over-use of particular bulls and the animals used should be functionally correct and of good type.

It is possible to update the moiled92.dat data file as new herd books become available, enabling the same calculations to be performed on future generations of Irish Moiled cattle. One major drawback to the Moilmate programme was the programming language, Qbasic, which is very slow. At present the calculation of the inbreeding coefficient for a single animal born in 1992 takes approximately 45 minutes on a model 486 computer. As future generations are added to the data file, and the number of intervening generations to the founders increases, the time taken to calculate inbreeding coefficients will also increase. To overcome this problem the Moilmate programme is in the process of being converted to a faster language.

The Moilmate programme can be applied for use with other rare breeds, as described in the tutorial (Appendix I). However, since the programme was written specifically for Irish Moiled cattle, the gene-dropping simulation is only capable of dealing with eight founder animals. Only a small change in the programme is necessary to change the number of founder animals analysed to that required for a different breed or species.

7.4 GENERAL DISCUSSION

7.4.1 Relation between Band Sharing and Co-ancestry

The relation between mathematically derived coefficients of co-ancestry and the genetic similarity determined by the level of band sharing in the oligonucleotide DNA fingerprint pattern was investigated.

Theoretically the similarity between the DNA fingerprint patterns of two individuals should increase as their relationship increases (Lynch 1990, 1991). If the number of loci detected is large enough to be considered representative of the entire genome, the similarity between the loci detected in a pair of individuals should reflect their genetic relatedness. In this study, linear regression of the level of band sharing (y) on coefficient of co-ancestry (x), using 175 pairwise comparisons with coefficients of co-ancestry ranging from 0.05 to 0.4, yielded the relationship $y = 0.63 + 0.15x$ (see

Figure 7.3

Regression Analysis for Pair-wise Comparisons of Mathematically Derived Coefficients of Coancestry and the Level of Band Sharing Calculated by DNA Fingerprinting

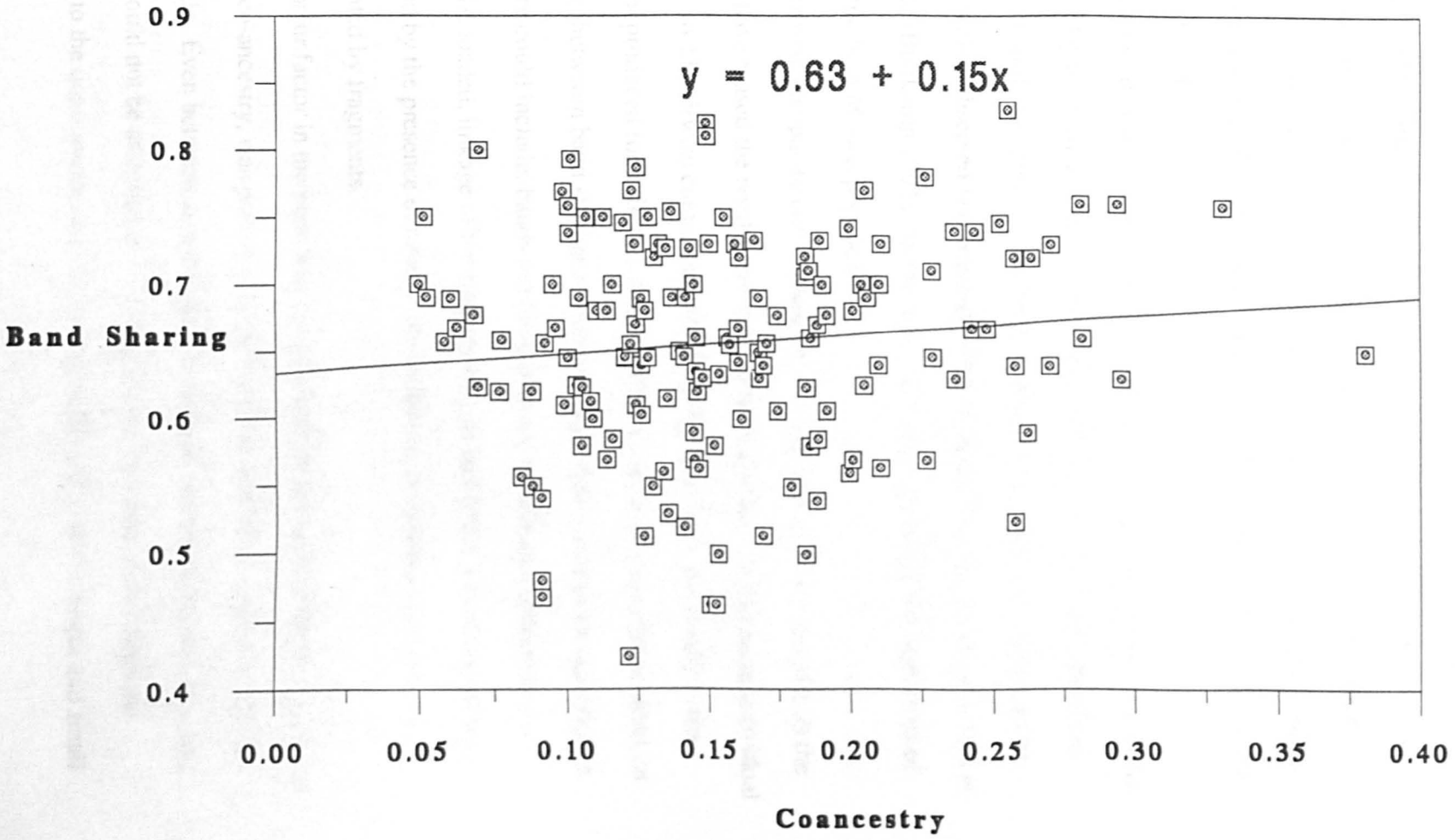


Fig. 7.3). However, the regression analysis was found not to be significant (significant at $P = 0.117$).

In a similar investigation in Japanese Black cattle, Mannen *et al.*, (1993) had shown that the similarity of DNA fingerprints using an M13 probe, increases proportionally to inbreeding and relationship. The linear regression of band sharing level (y) on relationship coefficient (x) was significant at $P > 0.001$. Many other authors have also shown a relationship between similarity in DNA fingerprint pattern and relatedness in a range of species (Kuhnlein *et al.*, 1990; Gilbert *et al.*, 1990, 1991; Wetton *et al.*, 1987; Packer *et al.*, 1991).

Linkage and allelism in the bands produced in this study were ignored, as in the investigation of Japanese Black cattle by Mannen *et al.*, (1993). Any linkage of the bands detected would have elevated the band sharing scores obtained. Although the level of band sharing observed was relatively high compared to that observed in similar studies in cattle (Buitkamp 1991a, b), this may have been a result of the high level of inbreeding in the Irish Moiled population.

The pairwise comparison of all samples on a gel with all other samples on the same gel may have biased the results obtained for band sharing. As the same individual was used in up to 13 pairwise comparisons of banding sharing, any anomaly in the banding pattern produced for a single individual could have had a significant effect on the relationship betwwen band sharing and co-ancestry. Anomalies in an individual's banding pattern could include, bands too faint to be scored due to variation in the amount of DNA loaded, linkage of the fragments in an individual's banding pattern, bands obscured by the presence of nearby intense bands, or localised variation in the distance migrated by fragments.

One major factor in the poor relationship between the level of band sharing and coefficient of co-ancestry, was probably the difficulty in assigning matching and non-matching bands. Even between samples which had been electrophoresed in adjacent lanes, bands could not be assigned as matching or non-matching with complete certainty, due to the quasicontinuous variation in minisatellite allele length and small

differences in the distance migrated by samples across the width of the gel. In this study, band sharing had been calculated between samples up to 13 lanes apart on the same gel. Piper and Rabenold (1993) reported the strong tendency for band sharing scores to increase with lane distance, and the decrease in consistency between separate scorings of the same autoradiograph. They suggest that for the determination of relatedness between individuals, samples more than three lanes apart cannot be compared with any degree of accuracy.

Any overlap between enzymes or between probes in the fragments detected, as described in the previous section, would serve to increase the inaccuracy in the measurement of the level of band sharing. The effects of linkage and difficulty in assigning matching bands would affect the scoring of each of the overlapping bands.

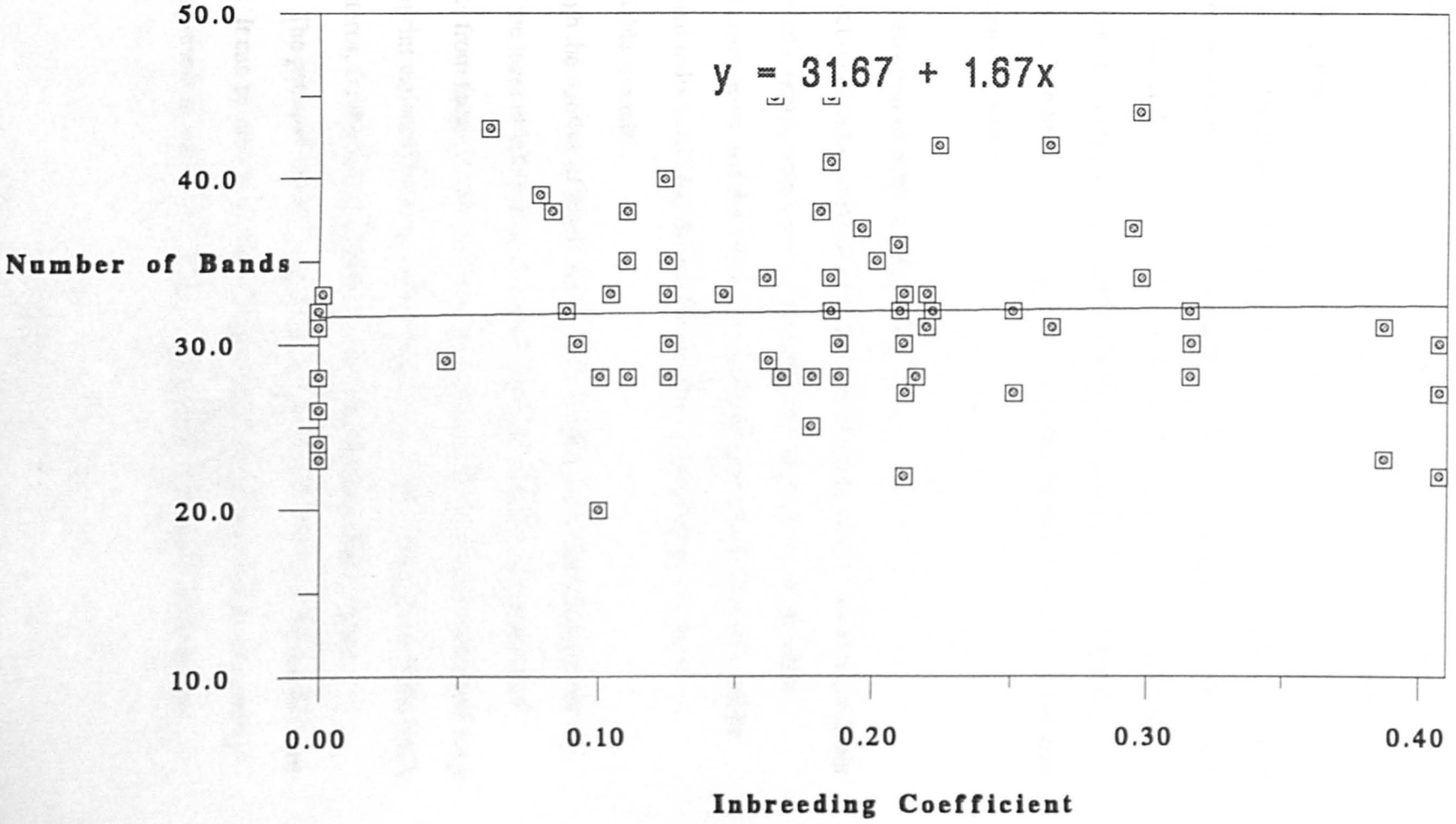
The relationship between band sharing and coefficient of co-ancestry could have been affected by selection or mutation at the loci detected with the oligonucleotide probes. The rate of mutation at the loci detected by these probes is high in comparison to that at other loci. However, the rate of mutation is not likely to be high enough significantly to affect estimations of genetic variability, based on band sharing analysis, between Irish Moiled individuals. The repeat sequences detected produce no discernible phenotypic effect and are therefore not directly subject to selection. However, linkage of these alleles with loci which have been selected for in Irish Moiled cattle may possibly have resulted in their indirect selection; a process referred to as the hitch-hiking effect.

7.4.2 Relation Between Band Number and Inbreeding

The relation between the level of inbreeding and the number of bands scored was also investigated. In theory the more homozygous an individual becomes, the more homozygous its DNA fingerprints, therefore fewer bands might be visible. Although the assignment of allelism was impossible, without the family pedigrees required to perform a segregation analysis, the possession of many bands by one individual and few by another may suggest that the first carries more genetic variation

Figure 7.4

Regression analysis for Relationship Between Inbreeding Coefficient and the Number of Bands present in a DNA Fingerprint



than the second. The lower number of bands in the second individual could be the result of the co-migration of identical sized restriction fragments from homologous chromosomes producing a single band in the DNA fingerprint. If there is no homozygosity two bands would result (Kuhlein 1990; Gill and Kelly 1990).

Linear regression of number of bands scored (y) on inbreeding coefficient (x), yielded the relationship $y = 31.67 + 1.67x$ (see Fig. 7.4), however, the regression analysis was not significant (significant at less than $P = 0.805$).

Again, as with the relationship between band sharing and co-ancestry, the poor relationship between number of bands and the level of inbreeding may be a reflection of the inability to consistently score lanes electrophoresed on separate gels. Differing hybridisation intensities may have revealed bands in one individual, which although present in another, were too faint to be scored. Linkage between bands may have also resulted in inaccurate scoring.

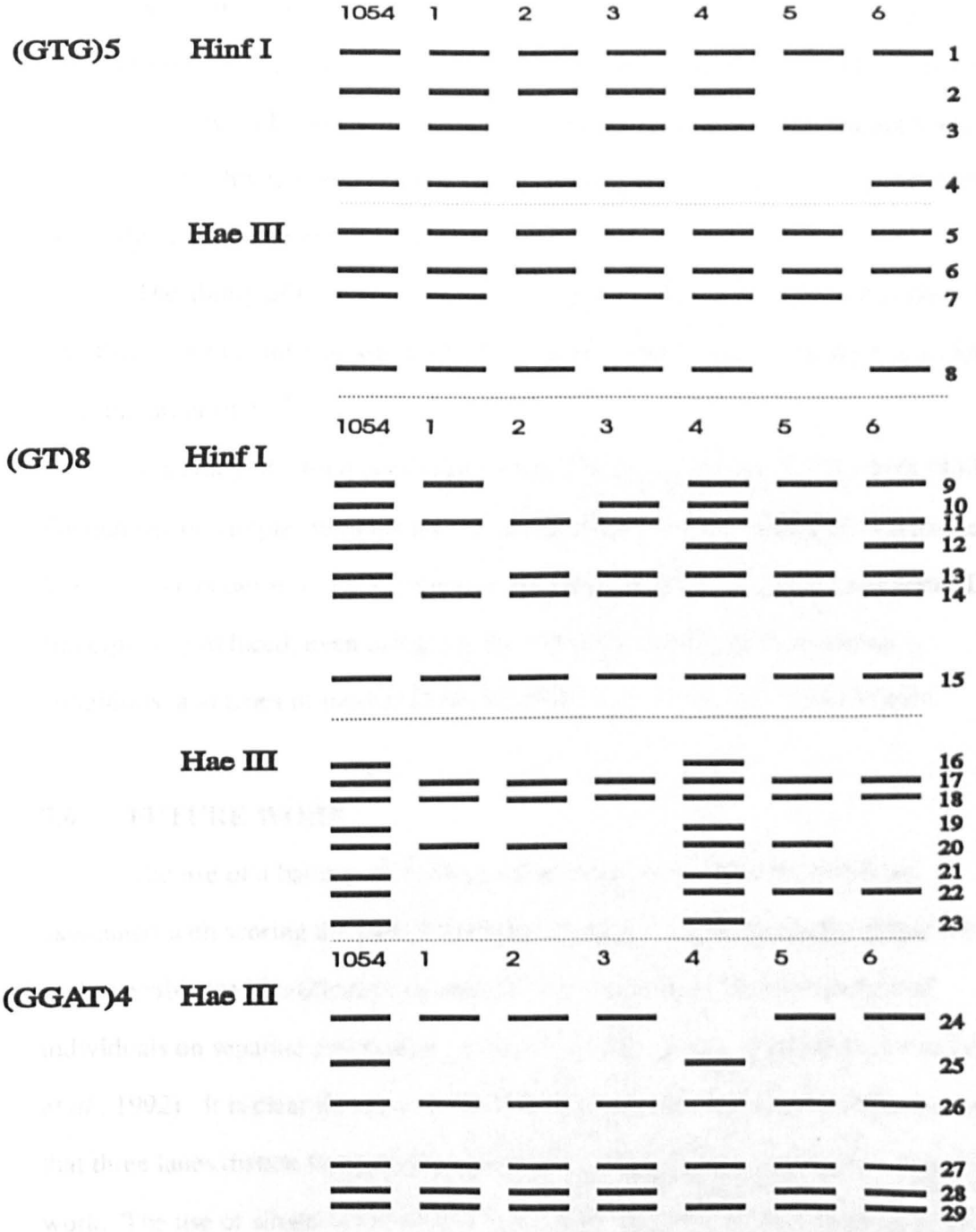
7.4.3 Selection of Bulls for Breeding

The selection of bulls for breeding from a number of closely related individuals has generally been random, with a few animals rejected on phenotypic grounds. Coefficients of co-ancestry and the mean kinship coefficient (see Chapter 6) will be similar for related individuals and therefore cannot be used to select the most genetically variable animals.

Although the number of bands present in an individual's DNA fingerprint did not depend on the level of inbreeding, Figure 7.5 shows how the transmission of genetic variants from father to son can be used to select breeding bulls with a full range of DNA fingerprint variants from the father. Figure 7.5 is the compilation of the DNA fingerprint patterns, for the bull 1054 with respect to the five probe/enzyme combinations. The paternal specific fragments in the six half-sibs sired by this bull are also compiled. It can be seen from Fig. 7.5 that if offspring 1 and 4 were maintained all the variants present in bull 1054 would be preserved. The bands are however,

Figure 7.5

Composite, interpreted DNA fingerprint for the bull 1054 and six of his calves



The DNA fragments resolved in each of the probe/enzyme combinations for the bull 1054 are numbered (1-29). The paternal Bands which appear in the DNA fingerprint patterns of six of his offspring are shown.

equivalent to any other genetic marker and can only represent a small portion of the genome.

7.5 CONCLUSIONS

No relationship could be observed between the level of band sharing determined from the DNA fingerprints and the coefficient of co-ancestry determined mathematically, or between the number of bands scored and the inbreeding coefficient. This was probably the result of inaccurate scoring between samples electrophoresed distantly on the same gel or on separate gels.

The ability of the technique to assign paternity is clear. The probability of mis-identification of a putative sire with the oligonucleotide DNA fingerprinting technique is of the order of 10^{-4}.

In a study of a total population, even if of small size such as the Irish Moiled, the number of samples requires that some samples be electrophoresed on separate gels. The distance between samples prevents accurate interpretation of the multilocus DNA fingerprints produced, even using strictly controlled standard electrophoresis conditions, and lanes of marker DNA for comparison across the width of gels.

7.6 FUTURE WORK

The use of a battery of single locus probes would avoid the problems associated with scoring the multilocus DNA fingerprint. Hypervariable single locus probes enable the identification of specific loci and alleles. The comparison of individuals on separate gels is also possible with reference to internal markers (Bruford *et al.*, 1992). It is clear that to analyse an entire population with all samples no more that three lanes distant from any other sample would require an inordinate amount of work. The use of single locus probes, such as those described by Crawford *et al.*, (1990) and Swarbrick *et al.*, (1992) for sheep may help to overcome this problem.

Blood and DNA stores are available for all the Irish Moiled animals sampled in this study. This will provide a reference point for any future investigation into the progress of the conservation programme in this breed.

The Moilmate computer programme is available for use by the Irish Moiled breeders, to assist in future calculations of co-ancestry and to enable the mating of the most distantly related individuals within or between lines. A future population census could be taken to investigate any drift in the representation of founder allele frequencies, and assess the conservation of rare founder alleles.

REFERENCES

Alderson G.L.H. (1992). A system to maximise the maintenance of genetic variability in small populations. In : GENETIC CONSERVATION OF DOMESTIC LIVESTOCK, VOLUME 2. L. Alderson (ed.). C.A.B. International, Wallingford. pp. 18-29.

Ali S., Muller C.R. and Epplen J.T. (1986). DNA fingerprinting by oligonucleotide probes specific for simple repeats. Hum. Genet. **74**: 239-243.

Amos W. and Dover G.A. (1990). DNA fingerprinting and the uniqueness of whales. Mammal Review **20**: 23-30.

Arnheim N., White T. and Rainey W.E. (1990). Application of PCR : Organismal and population biology. BioScience **40(3)**: 174-182.

Birkhead T.R., Burke T., Zann R., Hunter F.M. and Krupa A.P. (1990). Extra-pair paternity and intraspecific brood paratism in wild zebra finches, *Taeniopygia guttat*, revealed by DNA fingerprinting. Behav. Ecol. Sociobiol. **27**: 315-324.

Blin N. and Stafford D.W. (1976). A general method for the isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Research **3**: 2303.

Breen M. (1990). CHROMOSOME STUDIES IN THE EQUIDAE. Ph.D. Thesis, University of Liverpool.

Bruford M.W., Hanotte O., Brookfield J.F.Y. and Burke T. (1992). In: MOLECULAR GENETICS ANALYSIS OF POPULATIONS : A PRACTICAL APPROACH. (ed. Hoetzel, A.R.) pp. 225-269. IRL Press,Oxford.

Buitkamp J., Zischler M., Epplen J.I. and Geldermann H. (1991a). DNA fingerprinting in cattle using oligonucleotide probes. Animal Genetics **22**: 137-146.

Buitkamp J. (1989). DNA fingerprinting in cattle. Thesis, Tierärztliche Hochshule. Hannover, Germany.

Buitkamp J., Ammer H. and Geldermann H. (1991b). DNA fingerprinting in domestic animals. Electrophoresis **12**: 169-174.

Burke T. and Bruford M.W. (1987). DNA fingerprinting in birds. Nature **327**: 149-152.

Caetano-Anollés G., Bassan B.J. and Gresshoff P.M. (1991). DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology **9**: 553-557.

Crawford A.M., Buchanan F.C. and Swarbrick P.A. (1990). Ovine dinucleotide repeat polymorphism at the MAF18 locus. Animal Genetics **21**: 433-434.

Cruden D. (1949). The computation of inbreeding coefficients in closed populations. J. Hered. **40**: 248-251.

Epplen J.T. (1988). On simple repeated GATA/GACA sequences in animal genomes : a critical reappraisal. Journal of Heredity **79**: 409-416.

Epplen J.T., Ammer H., Epplen C., Kammerbauer C., Mitreiter R., Roewer L., Schwaiger W., Steimle V., Zischler H., Albert E., Andreas A., Beyermann B., Meyer W., Buitkamp J., Nanda I., Schmid M., Nürnberg P., Pena S.D.J., Pöche H., Sprecher W., Schartl M., Weising K. and Yassouridis A. (1991). Oligonucleotide fingerprinting using simple repeat motifs : a convenient, ubiquitously applicable method to detect hypervariability for multiple purposes. In : DNA FINGERPRINTING : APPROACHES AND APPLICATIONS. Burk, T. (ed.). Burkhäuser Verlag, Basel. pp. 50-69.

Erlich H.A. (ed.) (1989). PCR TECHNOLOGY : PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION. Stockton Press, New York.

Falconer D.S. (1960). In : INTRODUCTION TO QUANTITATIVE GENETICS. Olive and Boyd, London.

Feinberg A.P. and Vogelstein B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 6-13.

Feinberg A.P. and Vogelstein B. (1984). Addendum. Anal. Biochem. **137**: 266-267.

Galbraith D.A., Boag P.T., Gibbs H.L. and White B.N. (1991). Sizing bands an autoradiograms : a study of precision for scoring DNA fingerprints. Electrophoresis **12**: 210-220.

Gatel M.H., Chen P.M., Daniel R.C.W. and Lavin M.F. (1991). DNA fingerprints of sheep using an M13 probe. Animal Genetics **22**: 285-289.

Georges M., Lathrop M., Hilbert P., Marcotte A., Schwers A., Willens S.S., Vassart G. and Hanset R. (1990). On the use of DNA fingerprints for linkage studies in cattle. Genomics **6**: 461-474.

Georges M., Lequarre A.S., Castelli M., Hanset R. and Vassart G. (1988). DNA fingerprinting in domestic animals using four different minisatellite probes. Cytogenetics and Cell Genetics **47**: 127-131.

Gilbert D.A., Lehman N., O'Brien S.J. and Wayne R.K. (1990). Genetic fingerprinting reflects population differentiation in the California Channel Island fox. Nature **244**: 764-766.

Gilbert D.A., Packer A., Pusey A.E., Stephens J.C. and O'Brien S.J. (1991). Analytical DNA fingerprinting in lions : parentage, genetic diversity and kinship. J. Heredity **82**: 378-386.

Gill J.J.B. and Kelly E.P. (1990). Genetic variation in rare breeds and the measurement of homozygosity. In: GENETIC CONSERVATION OF DOMESTIC LIVESTOCK. Alderson, L. (ed.). C.A.B. International, Wallingford. pp. 175-185.

Gill J.J.B. and Harland M. (1992). Maximal maintenance of genetic variation in small populations. In: GENETIC CONSERVATION OF DOMESTIC LIVESTOCK 2. Alderson, L. (ed.). C.A.B. International, Wallingford. pp. 3-17.

Gill P., Jeffreys A.J. and Werrett D.J. (1985). Forensic applications of DNA fingerprints. Nature **318**: 577-579.

Gill P., Lygo J.E., Fowler S.J. and Werrett D.J. (1987). An evaluation of DNA fingerprinting for forensic purposes. Electrophoresis **8**: 38-44.

Hadrys H., Balick M. and Schierwater B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology **I**: 55-63.

Hanotte O., Burke T., Armour J.A.L. and Jeffreys A.J. (1991). Hypervariable minisatellite DNA sequences in the India peafowl, *Pavo cristatus*. Genomics **9**: 587-597.

Hedrick P. (1992). Shooting the RAPDs. Nature **355**: 679-680.

Hilbert P., Marcotte A., Schwers A., Hansett R., Vassart G. and Georges M. (1989). Analysis of genetic variation in the Belgian Blue cattle breed using DNA sequence polymorphism at the growth hormone, low density lipoprotein receptor, α-subunit of glycoprotein hormones and thyroglobulin loci. Animal Genetics **20**: 383-94.

Hillel J., Plotsky Y., Haberfield A., Lavi U., Cahaner A. and Jeffreys A.J. (1989). DNA fingerprints of poultry. Animal Genetics. **20**: 25-35.

Innis M.A. and Felford D.H. (1980). Optimization of PCRs. In: PCR TECHNOLOGY : PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION. Erlich H.A. (ed.) Stockton Press, New York. pp. 3-12.

Jarman A.P. and Wells R.A. (1989). Hypervariable minisatellites: recombinators or innocent bystanders? Trends in Genetics **5(II)**: 367-371.

Jeffreys A.J., Brookfield J.F.Y. and Semeonoff R. (1985c). Positive identification of an immigration test-case using human DNA fingerprints. Nature **317**: 818-819.

Jeffreys A.J., Macleod A., Tamaki K., Neil D.L. and Monckton D.G. (1991). Minisatellite repeat coding as a digital approach to DNA fingerprinting. Nature **354**: 204-209.

Jeffreys A.J. and Morton D.B.(1987). DNA fingerprints of dogs and cats. Animal Genetics **18**: 1-15.

Jeffreys A.J., Royle N., Wilson V. and Wang Z. (1988). Spontaneous mutation rates to new length alleles at tandem repetitive hypervariable loci in human DNA. Nature **332**: 278-281.

Jeffreys A.J., Wilson V., Kelly R., Taylor B.A. and Bulfield G. (1987). Mouse DNA 'fingerprints' : analysis of chromosome localisation and germ-line stability of hypervariable loci in recombinant inbred strains. Nucleic Acids Research **15**: 2823-2836.

Jeffreys A.J., Wilson V. and Thein S.L. (1985a). Hypervariable 'minisatellite' regions in human DNA. Nature **314**: 67-73.

Jeffreys A.J., Wilson V. and Thein S.L. (1985b). Individual specific 'fingerprints' of human DNA. Nature **316**: 76-79.

Kuhnlein U., Dawe Y., Zandwoirny D. and Gavora J.S. (1989). DNA fingerprinting: A tool for determining genetic distances between strains of poultry. Theor. Appl. Genet. **77**: 669-672.

Kuhnlein U., Zadworny D., Daw Y., Fairfull R.W. and Gavora J.W. (1990). Assessment of inbreeding by DNA fingerprinting : Development of a calibration curve using defined strains of chickens. Genetics **125**: 161-165.

Lynch M. (1990). The similarity index and DNA fingerprinting. Mol. Biol. Evol. **7**: 478-484.

Lynch M. (1991). Analysis of population genetic structure by DNA fingerprinting : In: DNA FINGERPRINTING : APPROACHES AND APPLICATIONS. Burke T. (ed.). Birhaüse Verlag, Basel. pp. 113-126.

Mace G.M. and Ballou J.D. (1990). Population management for conservation. Trends in Ecology and Evolution Vol. **5** No. 4: 102-104.

Mannen H., Tsuji S., Mukai F., Goto N. and Ohtagaki S. (1993). Genetic similarity using DNA fingerprinting in cattle to determine relationship coefficient. Journal of Heredity **84**: 166-169.

McCleur J.W., Vandeberg J.L., Read B. and Ryder O.A. (1986). Pedigree analysis by computer simulation. Zoo Biology **5**: 147-160.

Nakamura Y., Leppert M. O'Connell P., Wolff R., Holm T., Culver M., Martin C., Fujimoto E., Hoff M., Kumlin E. and White R. (1987). Variable number of tandem repeat (VNTR) markers for human gene mapping. Science **235**: 1616-1627.

Nanda I., Zischler H., Epplen C., Guttnebach M. and Schmid M. (1991). Chromosomal organisation of simple repeated DNA sequences used for DNA fingerprinting. Electrophoresis **12**: 193-203.

Nürnberg P., Rower L., Neitzel H., Sperling K., Pöppel A., Hundrieser J., Pöche H., Epplen C., Zischler H. and Epplen J.T. (1989). DNA fingerprinting with the oligonucleotide probe $(CAC)_5/(GTG)_5$: somatic stability and germline mutations. Human Genetics **84**: 75-78.

Packer C., Gilbert D.A., Pusey A.E. and O'Brien S.J. (1991). A molecular genetic analysis of kinship and co-operation in African lion. Nature **351**: 562-565.

Piper W.H. and Rabenold P.P. (1992). Use of fragment sharing estimates from DNA fingerprinting to determine relatedness in a tropical wren. Molecular Ecology **1**: 69-78.

Reeve H.K., Westneat D.F., Noon W.A., Sherman P.W. and Aquardro C.F. (1990). DNA 'fingerprinting' reveals high levels of inbreeding in colonies of the eusocial naked mole-rate. Proc. Natl. Acad. Sci. USA **87**: 2496-2500.

Rabenold P.O., Rabenold K.N., Piper W.H., Haydock J. and Zock S.W. (1990). Shared paternity revealed by genetic analysis in co-operatively breeding tropical wrens. Nature **348**: 538-540.

Robertson, A. (1954). Inbreeding and performance in British Friesian cattle. Proc. Brit. Soc. Anim. Prod. 1954. pp. 87-92.

Ross K.S., Haites N.E. and Kelly K.F. (1990). Repeated freezing and thawing of peripheral blood and DNA in suspension : effects on DNA yield and integrity. J. Med. Gen. **27**: 569-570.

Sambrook J., Firtsch E.F. and Maniatis T. (1990). MOLECULAR CLONING : A LABORATORY MANUAL (2nd edn.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schäfer R., Zischler H., Birsner U., Becker A. and Epplen J.T. (1988). Optimised oligonucleotide probes for DNA fingerprinting. Electrophoresis **9**: 369-374.

Scott D.A., Welt M. and Leung F.C. (1993). A computer program to aid in calculating similarity indexes from DNA fingerprinting. Biotechniques **14(6)**: 980-983.

Southern E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503.

Swarbrick P.A., Schmack A.E. and Crawford A.M. (1992). MAF4S, a highly polymorphic marker for the pseudoautosomal region of the sheep genome, is not linked to the FecX (Inverdale) gene. Genomics **13**: 849-851.

Tautz D. (1989). Hypervariability of simple sequences as general source of polymorphic DNA markers. Nucleic Acid Res. **17**: 6463-6471.

Tautz D. and Renz,M. (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research **12**: 4127-4138.

Van Wezembeck P.M., Hulsebos T.J.M. and Schoemakers J.G. (1980). Nucleotide sequence of the filamentous bacteriophage M13 DNA genome : Comparison with phage *f*d. Gene **11**: 129.

Vassart G., Georges M., Monsieur R., Brocas H., Lequarre,A.S. and Christophe D. (1987). A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science **235**: 683-684.

Vergnaud, G., Mariat, D.,Zoroastro,M. and Lauthier, V. (1991). Detection of single and multiple polymorphic loci by synthetic tandem repeats of short oligonucleotides. Electrophoresis **12**: 134-140.

Vogelstein B. and Gillespie D. (1979). Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76**: 615-619.

Wahl G.M., Stern M. and Stark G.R. (1979). Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridisation using dextran-sulphate. Proc. Natl. Acad. Sci. **76**: 3683.

Wallace R.B., Shaffer J., Murphy R.F., Bonner J., Hirose T. and Itakwa K. (1979). Hybridisation of oligodeoxyribonucleotides to $\Phi\chi$174 DNA: the effect of single base pair mismatch. Nucleic Acids Research **6**: 3543.

Welsh J., Peterson C. and McClelland M. (1990). Polymorphisms generated by arbitrarily primed PCR in the mouse : application to strain identification and genetic mapping. Nucleic Acids Research **19(2)**: 303-306.

Welsh and McClelland (1990). Fingerprinting genomes with arbitrary primers. Nucleic Acids Research **18(24)**: 7213-7218.

Westneat D.F., Noon W.A., Reeve H.K. and Aqadro C.F. (1988). Improved hybridisation conditions for DNA fingerprints probed with M13. Nucleic Acids Research **16**: 4161.

Wetton J.H., Carter R.E., Parkin D.T. and Walters D. (1987). Demographic study of a wild house sparrow population by DNA fingerprinting. Nature **327**: 147-149.

Williams J.G.K., Kabelik A.R., Lwak K.J., Rafalski J.A. and Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research **18(22)**: 6531-6535.

Wong Z., Wilson, V. Jeffreys A.J. and Thein S.L. (1986). Cloning a selected fragment from a human DNA 'fingerprint': isolation of highly polymorphic minisatellite. Nucleic Acid Research **14**: 4605-4616.

Wright, S. (1992). Coefficients of inbreeding and relationship. Amer. Nature **56**: 330-338.

APPENDIX I

MOILMATE '93 TUTORIAL

The following tutorial was written to accompany the Moilmate programme, which is now available to the Irish Moiled cattle breeders. The tutorial was designed to demonstrate the basic functions of the Moilmate programme, with reference to a data file (tutor.dat) containing pedigree information for a small hypothetical population. The application of the programme to the Irish Moiled population is also explained.

MOILMATE '93 TUTORIAL

CONTENTS :

- Return to File Manager
- Quit Moilmate '93

Other Options (from the Current Menu)

- Hardcopy option
- Add data

Other Options (from the File Manager Menu)

- Rename a data file
- Create a new data file
- Delete a data file
- Specify new drive path

The Moiled92.dat Data File

About MOILMATE '93

MOILMATE'93 is a computer programme, written in Qbasic for the calculation of Inbreeding Coefficients and coefficients of co-ancestry from pedigree information. The programme will run on any IBM compatible computer, although a model 386, or faster processor is recommended. MOILMATE'93 was written specifically for use with Irish Moiled cattle, and a data file, "moiled92.dat" is included with the programme. This data file contains peidgree data for all Irish Moiled cattle, and their ancestors, listed in the 1992 Herd Book. The programme can also be used with data files containing pedigree information for other breeds/species.

Care has been taken to try to ensure that the programme is bug-free. However, if any bugs do crop up, please contact Mark Harland, c/o Department of Genetics and Microbiology, The University of Liverpool.

Installation

To copy the Moilmate directory from the floppy disk onto your hard-drive, insert the 'Moilmate' disk into your floppy drive (drive A or B) and at the 'C>' prompt type:

"xcopy a: c:/s <return>"

(if you are using drive B, type "xcopy b: c:/s <return>")

Loading

To enter the Moilmate directory, type "cd moilmate <return>" at the "C>" prompt them type "moilmate <return>" to run the program.

Getting started

As the tile screen appears, you will be asked to "specify a drive path for your data files". since the data files provided with this program (in the Moilmate directory) have been copied onto the hard drive, type "C<return>" to specify the moilmate directory on the hard drive. The 'file manager menu' should now be displayed on your monitor (Fig. 1).

Figure 1

FILE MANAGER MENU

1. List data files on drive c:\moilmate\
2. Rename a data file
3. Create a new data file
4. Delete a data file
5. Load data file
6. Specify new drive path.
7 Quit MOILMATE

List data files on Drive c:

Press "1" to display the data files currently in the moilmate directory on the hard drive. There should be 2 files included with the program (Fig. 2)

Figure 2

```
C:\MOILMATE
TUTOR      .DAT          MOILED92.DAT
134443008 Bytes free

C:\MOILMATE
C:\moilmate\*.res
No files present

hit space bar to continue...
```

Return to the file manager menu by pressing any key.

About the data files

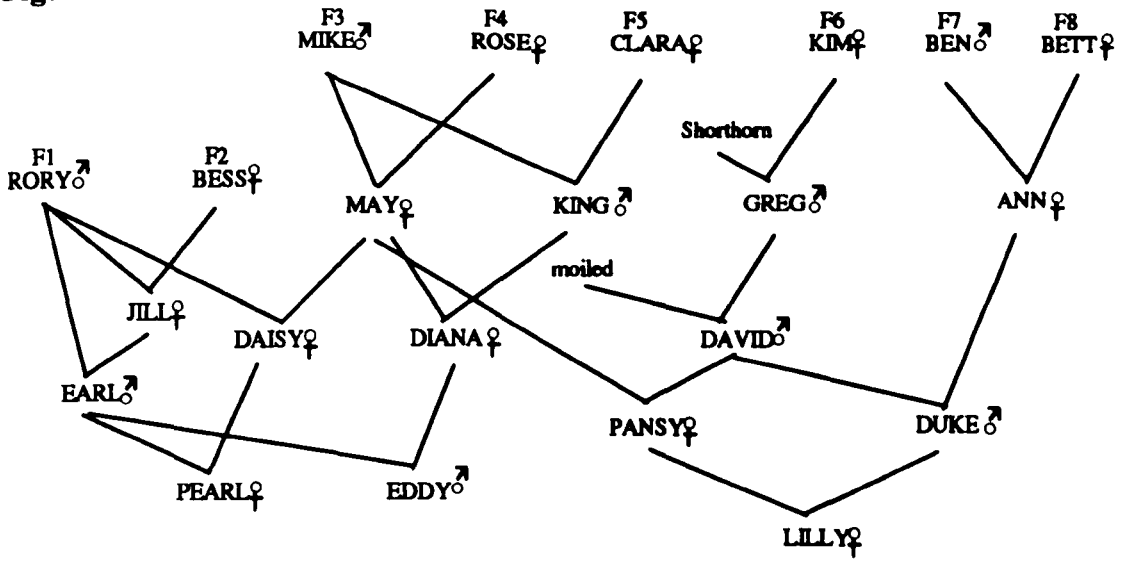
Moiled 92.dat was created from the Irish Moiled Cattle, 1992 Herd Book, it contains every animal listed in the herd book (476 in all) traced back to 8 pure founder animals.

Tutor.dat is a simple file containing data from an imaginary herd of 22 Irish Moiled cattle (including 8 founders). It is intended for use with this tutorial, to demonstrate the basic functions of the Moilmate program. The pedigree information contained in this data file is shown in the pedigree diagram (Fig. 3).

Loading a data file

Before a file can be examined, it must be loaded into the 'Moilmate' program. Press '5' to select the 'Load a data file' option from the File manager menu. The 2 data files present in the Moilmate directory should again be displayed. At the prompt 'File to be loaded?' type "tutor.dat<return>" (always us the **full** name of the data file). The 'current menu' should now be displayed on your monitor with the name of the loaded data file "tutor.dat" at the top right of the screen (Figure 4). "Current Menu" is short for currently loaded data file menu.

Figure 3



Pedigree diagram for a Hypothetical herd of 22 Irish Moiled cattle, with 8 founders (F1-F8). 'Moiled' = pure registered but unknown Irish Moiled animal.

Figure 4

CURRENT MENU

1. Display or edit the file.
2. Add data to the file.
3. Format the file.
4. Output file data to a line printer.
5. Calculate inbreeding coefficients.
6. Calculate coefficients of coancestry (bestmate)
7. Founder calculations.
8. Return to file manager.

Displaying the data file

Press "1" to display the information in the tutor.dat data file, the following should appear on the screen (Fig. 5a).

Figure 5a

MOILMATE 93 **23 individuals in set** **c:\moilmate\tutor.dat**

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born			
1	pearl	earl	daisy	y	f	?	1989			n
2	eddy	earl	diana	y	m	?	1990			n
3	lilly	duke	pansy	y	f	?	1992			n
4	earl	rory	jill	y	m	?	1985			n
5	daisy	rory	may	y	f	?	1985			n
6	diana	king	may	y	f	?	1984			n
7	pansy	david	may	y	f	?	1986			n
8	duke	david	ann	y	m	?	1987			n
9	jill	rory	bess	y	m	?	1980			n
10	may	mike	rose	y	f	?	1981			n
11	daisy	rory	may	y	f	?	1985			n
12	ann	ben	betty	y	f	?	1980			n
13	greg		kim	n	m	?	1975			n
14	rory			n	m	?	1970	g1	g2	f
15	bess			n	f	?	1971	g3	g4	f
16	mike			n	m	?	1971	g5	g6	f
17	rose			n	f	?	1972	g7	g8	f

The name of the data file being displayed, "tutor.dat" is shown at the top right of the screen. The second line indicates that there are 23 individuals in the data set.

The information is shown a screen at a time, press any key (except 'f' - see "Large data sets") to advance the display by one page and show the remaining 6 data entries (Fig. 5b).

Figure 5b

MOILMATE 93 **23 individuals in set** **c:\moilmate\tutor.dat**

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born			
18	clara			n	f	?	1972	g9	g10	f
19	kim			n	f	?	1972	g11	g12	f
20	ben			n	m	?	1974	g13	g14	f
21	betty			n	f	?	1975	g15	g16	f
22	david	greg	M	n	m	?	1981			n
23	king	mike	clara	n	m	?	1978			n

edit (y/n)?

Each individual in the pedigree is represented by a line of data containing the following information:

Item : This is simply the order in which the data entries are stored in the data file.

Name : Name of the individual, can use herd book Nos., E.T. Nos. or actual name.

♂/♀ Parent : Name or Herd book No. of the individuals parents. A blank entry (except in the case of the founder animals) indicates that the parent is not Irish Moiled (see item 13, ♂ parent of greg was a short-horn). The entry "M" indicates that the parent was an unknown, but pure-bred Irish Moiled (see item 22, 'david').

Alive : whether animal is extant yes or no.

Sex : Male (m) or female (f).

Inbreeding Coefficient : all entries contain a question mark to indicate that the inbreeding coefficient has not yet been calculated.

Year Born : Date of birth of individual.

Founder alleles : The pair of unique founder alleles assigned to each founder (this is covered later in the section on 'Founder Calculations').

Founder : Whether or not the individual is a founder (f/n).

Deliberate mistakes

Careful examination of the data entries will reveal discrepancies between the pedigree diagram and the information contained in the data file. Item 9 "jill" has been listed as male, and 23 animals are listed, rather than the 22 shown in the pedigree. These deliberate mistakes have been included to demonstrate how the format option works (see 'formatting the file').

Large data sets

With large data files (such as moiled92.dat) it is often useful to scroll through the data entries more quickly than a page a time. Pressing the 'f' key will set the display mode to 'fast' scrolling. The return to the page by page display press the 's' key at any point during the rapid scrolling.

Editing the data file

At the moment the tutor.dat data file does not need to be edited, so at the edit (y/n) prompt type 'n<return>' and then press any key, as instructed on screen, to return to the current menu.

Formatting the File

The individuals in the tutor.dat data file were not entered in any particular order, but for the program to be able to use the data file they must be arranged in order of date of birth. To do this the format option must be used. Type "3" to select the format option.

After a few seconds the data file will be displayed with the individuals listed in order of date of birth. The format option then checks the file for any errors. The following error messages should appear (Fig. 6).

Figure 6

MOILMATE 93 **23 individuals in set** **c:\moilmate\tutor.dat**

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	
18	daisy	rory	may	y	f	?	1985	n
19	pansy	david	may	y	f	?	1986	n
20	duke	david	ann	y	m	?	1987	n
21	pearl	earl	daisy	y	f	?	1989	n
22	eddy	earl	diana	y	m	?	1990	n
23	lilly	duke	pansy	y	f	?	1992	n

sex mismatch - female parent at item 16 (earl)
is male individual at item 11 (jill)
duplicate entries - items 17 (daisy) and 18 (daisy)
no date mismatches found
individual data entries found for all quoted parents

Press 'h' for help on format errors or any other key to continue...

Press 'h' to see an explanation of these error messages. Once you have read the first screen of explanations, press any key to proceed to the second, the press any key to return to the "Current menu".

Editing a datafile

Press "1" to display the tutor.dat data file. The first screen of the data file will be shown (Fig. 7). Note that the animals have been arranged in order of data of birth, with the youngest at the top, oldest at the bottom.

Figure 7

MOILMATE 93 **22 individuals in set** **c:\moilmate\tutor.dat**

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles		
1	rory			n	m		1970	g1	g2	f
2	bess			n	f		1971	g3	g4	f
3	mike			n	m		1971	g5	g6	f
4	rose			n	f		1972	g7	g8	f
5	clara			n	f		1972	g9	g10	f
6	kim			n	f		1972	g11	g12	f
7	ben			n	m		1974	g13	g14	f
8	betty			n	f		1975	g15	g16	f
9	greg		kim	n	m		1976			n
10	king	mike	clara	n	m	.0000	1978			n
11	jill	rory	bess	y	f	.0000	1980			n
12	ann	ben	betty	y	f	.0000	1980			n
13	may	mike	rose	y	f	.0000	1981			n
14	david	greg	M	n	m		1981			n
15	diana	king	may	y	f	.1250	1984			n
16	daisy	rory	may	y	f	.0000	1985			n
17	daisy	rory	may	y	f	.0000	1985			n

any key to continue....

Press any key (except 'f') to display the remaining data entries (Fig. 8)

Figure 8

MOILMATE 93 **22 individuals in set** **c:\moilmate\tutor.dat**

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
18	pansy	david	may	y	f	.0000	1986	n
19	duke	david	ann	y	m	.0000	1987	n
20	pearl	earl	daisy	y	f	.187	1989	n
21	eddy	earl	diana	y	m	.0000	1990	n
22	lilly	duke	pansy	y	f	.1250	1992	n

edit (y/n)?

The format error messages (Fig. 6) show that item 11 (jill), the female parent of earl, has been listed as male in the tutor.dat data file. to correct this type "y<return>" at the prompt 'edit(y/n)?'. You are then asked to enter the item number to be edited. type "11<return>" to edit the line of data for Jill.

The line of data being edited is shown at the top of the screen (Fig. 9).

Figure 9

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	
11	jill	rory	bess	y	m	?	1980	n

individual ?

Only the sex of the animal is incorrect in this particular example, with the exception of sex, which should be entered as female "f", enter the information at the various prompts exactly as displayed at the top of the screen (Figure 10).

Figure 10

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	
9	jill	rory	bess	y	m	?	1980	n

individual ? jill<return>
male parent ? rory<return>
female parent ? bess<return>
alive ? y<return>
male / female ? f<return>
inbreeding coefficient, if known?<return>
year of birth ? 1980<return>
founder (y/n) ? n<return>

NOTE : Moilmate data files are case sensitive, so always use lower case letters.

If, as in the case of 'jill', the inbreeding coefficient is unknown, simply press <return> at the prompt 'Inbreeding coefficient, if known?'

Once the information for Jill has been entered as above, it will be displayed at the bottom of the screen. Check this line of information with that at the top of the screen to ensure that no mistakes have occurred in typing in the data. If any mistakes

have occurred, type "y<return>" at the 'edit(y/n)?' prompt and re-edit item 11 (jill) to correct the mistakes.

Deleting a line of data

Press 'y<return>' at the 'edit (y/n) prompt and then enter the item number to be deleted. In the case of tutor.dat items 17 and 18 (daisy) are identical, so one must be deleted. At the prompt "Item No. to be edited?" type "18<return>" and one of the lines of data for Daisy will be shown at the top of the screen (Fig. 11).

Figure 11

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	
18	daisy	rory	may	y	f	?	1985	n

individual ?

Press <return> at the prompt 'individual?' and the entire line of data will be deleted. The data entries will again be displayed. Note that there are now only 22 individuals in the data file.

NOTE : If the wrong line of data is accidently called up to be edited or deleted, re-enter the data exactly as shown at the top of the screen.

Re-formatting the data file

Now that the errors in tutor.dat have been corrected the file should again be formatted. Calculations of coefficient of co-ancestry and Inbreeding coefficient cannot be carried out until the 'format option' has confirmed that there are no remaining errors in the datafile.

Press 'n<return>' at the "edit (y/n)" prompt, then press any key to return to the current menu. Select the format option, by pressing '3'. After a few seconds the message that there are no detectable errors and that tutor.dat is now formatted, should appear on the screen (Fig. 12).

Figure 12

MOILMATE 93 **22 individuals in set** **c:\moilmate\tutor.dat**

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	
18	pansy	david	may	y	f	?	1986	n
19	duke	david	ann	y	m	?	1987	n
20	pearl	earl	daisy	y	f	?	1989	n
21	eddy	earl	diana	y	m	?	1990	n
22	lilly	duke	pansy	y	f	?	1992	n

no duplicate entries found
no sex mismatches found
no date mismatches found
individual data entries found for all quoted parents
c:\moilmate\tutor.dat is now formatted
any key to continue...

Founder calculations, calculations of Inbreeding Coefficients and coefficients of coancestry can now be carried out on the tutor.dat data file. Press any key to return to the current menu.

Calculations of Inbreeding Coefficients

Press '5' to select the 'calculate inbreeding coefficients' option from the current menu. Then press <space> to start the calculations.

The inbreeding coefficients (based on Wrights coefficient) will then be calculated for every individual in the datafile, starting with the oldest animal and working steadily through to the youngest. The information which scrolls rapidly across the screen relates to how the program calculates the inbreeding coefficient for each individual (Fig. 13).

Figure 13

Hit No. : The number of pathways to common ancestors found.

Common ancestor : Common ancestor found

(F=) : Inbreeding coefficient of common ancestor.

Number of individuals in pathway : equivalent to number of interveneing generations on the male side + number on the female side + 1, as a Wright coefficient *(m+f+1)*.

Left path : Names of ancestors on female side.

Right path : Names of ancestors on male side.

Coeff. : Inbreeding coefficient for current pathway.

NOTE : With small data files such as tutor.dat this information will scroll too rapidly to be read. With large data files such as moiled.92 the scrolling will be much slower and the information for each pathway to a common ancestor can be read.

After a few seconds a message should appear informing you that the inbreeding coefficient has now been calculated for every individual in the data file. Press <space> to view the file. Individuals where one or both parents are unknown have an inbreeding coefficient of zero, the program does not need to calculate these, which is why these individuals have no entry under inbreeding coefficient.

When looking at a data file, a question mark ('?') in the column for inbreeding coefficient means an as yet uncalculated inbreeding coefficient, and a blank indicates an inbreeding coefficient of zero.

Large data sets

The calculation of coefficients of co-ancestry may take a considerable amount of time with large data files such as moiled92.dat. to halt the calculations at any point, press 'x'. The programme will finish calculating the present coefficient and then return to the 'Current Menu'. To indicate that the calculations are about to end "(Stopping...)" appears at the bottom of the information for each pathway found. The calculations will re-start with the next unknown inbreeding coefficient the next time option '5', Calculate inbreeding coefficient, is chosen.

Calculation of Coefficient of Coancestry (best-mate)

This option enables the calculation of the coefficient of coancestry (or genetic similarity) between 2 animals. Since the coefficient of coancestry between 2 individuals is the same as the inbreeding coefficient of an offspring of these individuals, this option can be used to determine which matings will produce offspring with the lowest inbreeding coefficient.

Press '6' to selection 'bestmate' option. In the herd represented by tutor1.dat the bulls Duke and Eddy are to be mated with either Daisy, Lilly or Pearl. At the prompt 'individual to be mated?' type "duke<return>". You are now asked for a 'mate?', type "daisy<return>", then type "lilly<return>" at the next 'mate?' prompt and type "pearl<return>" at the next. Now that the 3 cows have been entered at the next 'mate?' prompt type "end<return>".

The prompt 'individual to be mated?' will reappear, type "eddy<return>" and at the 'mate?' prompts enter the cows (and 'end'') as before. When the prompt 'individual to be mated?' appears again type "end<return>" and then press any key to start the calculations (Fig. 14).

Figure 14

MOILMATE 93 **c:\moilmate\tutor.dat**

BEST MATE ANALYSIS

Individual to be mated? duke
Enter your selection of mates, for individual duke
When done, type 'end'
Mate? daisy
Mate? lilly
Mate? pearl
Mate? end
Individual to be mated? earl
Enter your selection of mates, for individual earl
When done, type 'end'
Mate? daisy
Mate? lilly
Mate? pearl
Mate? end
Individual to be mated? end

The program will calculate the coefficient of coancestry between Duke and the 3 cows and between Earl and the 3 cows. In a few seconds the results will be displayed on your monitor (Fig. 15).

Figure 15

BEST MATE ANALYSIS

Calf	Parent one	Parent two	Coefficient of Coancestry
x 1	duke	daisy	.0000
x 2	duke	lilly	.3125
x 3	duke	pearl	.0000
x 4	eddy	daisy	.1719
x 5	eddy	lilly	.0391
x 6	eddy	pearl	.2422

Enter 's' to save these analyses to disc, or 'm' to return to menu?

The coefficients of co-ancestors show that Duke could be mated with either Daisy or Pearl to produce a calf with an inbreeding coefficient of F=0. Mating Eddy with Lilly would produce calves with a lower inbreeding coefficient than mating him with either Daisy or Pearl.

To save the results, type "s<return>". When asked to enter a file name, the results can be added to an existing results file or saved as a separate file. Since there are no existing results files, save these calculations to a new data file by typing "mating.res<return>". The results will be saved under this name and the current menu will reappear. The file mating.res can now be loaded from the file manager menu (option '5') whenever these results need to be viewed.

Never return to the main menu (by typing 'm') without saving or printing important results, as the information will be lost.

The coefficient results screen can be printed by using the print screen key on the keyboard. This avoids the need to create a results data file, but when printing always make sure the printer is on-line and is loaded with paper, if the printer is off-line or runs out of paper while printing, the program will crash and important results may be lost. It is always advisable to make a results data file before attempting to print the results screen.

Founder Calculations

The founder calculations option calculates the % contribution of the 8 founder animals to the present herd. It identifies which founder genomes are at risk of being lost and also gives the founder composition of individual animals, enabling selection to preserve those genomes at risk. Choose option '7' from the current menu - to start founder calculations.

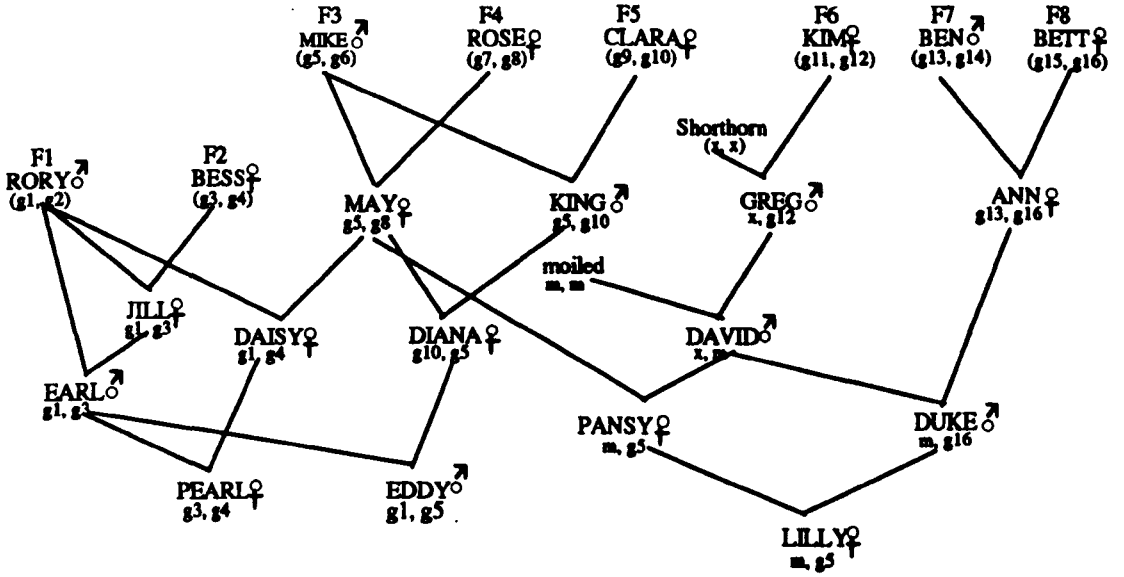
In the tutor.dat file (and moiled92.dat) the 8 found animals have each been assigned a unique pair of alleles g1-g16 (Fig. 16)

Figure 16

Founder	Name	Alleles
F1	Rory	g1, g2
F2	Bess	g3, g4
F3	Mike	g5, g6
F4	Rose	g7, g8
F5	Clare	g9, g10
F6	Kim	g11, g12
F7	Ben	g13, g14
F8	Betty	g15, g16

The founder calculations option gives each of the descendants a genotype at random by Mendelian segregation of these founder alleles. At the end of one cycle every individual in the pedigree will have a genotype (e.g. Fig. 17).

Figure 17



Pedigree diagram for hypothetical Irish Moiled herd. The unique alleles g1-g16 assigned to each founder are shown bracketed. The genotypes of the offspring, after one cycle of segregation of the founder alleles, are given with the allele inherited from the male parent shown first.

'm' = Pure registered but unknown Irish Moiled allele.

'x' = non-Irish Moiled allele.

The process is repeated many times and the results from each cycle are compiled to give estimates of the contribution of each founder to the extant herd and the composition of individual animals. An estimation of % of a founder genome lost is given by the % of cycles in which a founders alleles fail to appear in the extant herd. Similarly the % of a founders genome at risk is given by the % of cycles in which a particular founders alleles are present at less than 10% in the extant herd. The more cycles carried out the greater the accuracy of these estimates. Generally 1000 cycles will give results accurate to within 1.0%.

The founder calculations screen offers the choice of New (n), Continue (c) or View results (r). Type "n<return>" to carry out founder calculations on the tutor.dat data file.

1000 or more cycles are needed for accurate results, but for now type "100<return>" at the 'No. cycles?' prompt to perform 100 cycles.

Once you start the founder calculations the screen shows which individuals are receiving which alleles. Non Irish Moiled alleles are represented by 'x' and pure but unknown Irish Moiled alleles are represented by 'm'. A line in the middle of the screen keeps track of how near the calculation is to completion.

It should take a few minutes for the program to complete 100 cycles with this small pedigree. Once the calculations are complete press any key to get to the 'Founder Results Menu' (Fig. 18).

Figure 18

FOUNDER RESULTS MENU

1. Percentage Contribution of Founders to Extant Population
2. Percentage of Each Founder Genome Lost/At Risk of Loss
3. Founder Composition of Animals (and inbreeding)
4. Information to Restart Analysis
5. Return to main menu.

Choose any of the above options by pressing the appropriate key (1-5). Once you have seen the information displayed for the option, press any key to return to the 'Founder Results Menu'.

Option 1 shows the % contribution of each of the founders to the extant herd. Note that founders 5 and 6 (Clara and Kim) are under-represented in the present herd.

Option 2 shows the % of each founder genome lost and at risk of further loss. The numbers in brackets indicate the % of surviving genes at risk of future loss.

Option 3 gives the composition of each of the extant individuals in the pedigree. type "a<enter>" and press any key to scroll through all 11 animals one at a time. Lilly has the highest % of the under-represented founder 6 (Kim) and could be preferentially bred from to arrest the loss of this founder from the herd.

Option 4 provides information necessary to re-start the founder calculations, e.g. Fig. 19 - make a note of this information before returning to the current menu if you intend to restart calculations in the future.

Option 5 returns you to the Current Menu.

Figure 19

FOUNDER CALCULATIONS **c:\moilmate\tutor.dat**

No. of Cycles Completed = 101

Value of P =	O	D..	36.5	29	21	16	25.5	14	42	36
Value of Y =	2200	L..	10.5	50	40	50	74.5	80	50	50

No. Extant = 11

Keep a record of the above values, you'll need them to restart founder calculations

Continue Founder Calculations

At any time in the future it is possible to re-start founder calculations, to increase the accuracy of the results by performing more cycles. Choose the 'continue' option ('c') when you start the founder calculations and you will be asked to enter the list of data which was given on the 'information to re-start' screen the last time founder calculations were performed.

When asked to enter 'No. of cycles' enter the new **total** number of cycles you wish to be completed. Do not enter a number less than the number of cycles already completed.

View results from previous Founder Calculations

The founder calculations can be viewed at any time by choosing the 'view' option ('r'). You will be required to enter a list of data as above and will then be taken straight to the results screen.

Large data sets

With a large data file the founder calculations can take a considerable length of time. For example, it takes over 24 hours to complete 1000 cycles with the moiled92.dat data file. If you need to halt the program press 'x' and you will be taken to the founder results menu. Make a note of the information to re-start calculations and you can resume the analysis at a later date using the continue option.

Return to File Manager

To return to the File Manager Menu, choose option '8'. This will close the tuto.dat data file, saving the changes that have been made to it. From the File Manager Menu you can load a different data file, such as moiled92.dat or exit from the programme.

Quit MOILMATE'93

Choose option '7' ('quit MOILMATE') from the File Manager Menu to terminate this tutorial session. Type "y<return>" at the prompt 'Are you sure you want to quit (y/n)?' to return to the DOS system prompt: 'c:\moilmate>'.

Other Options (from the Current Menu)

Hard-copy Option

Information from pedigree data files (such as tutor.dat and moiled.dat) and results data files (such as mating.res) can be printed using option '3' 'the hardcopy option' from the current menu. As with all printing please ensure that the printer is on-line and loaded with paper, to prevent the program crashing.

Add Data

As new generations of animals appear in the Herd Book it is necessary to update a data file, choose the 'Add data' option from the current menu by pressing '2' Data is entered as described under "Create data file".

To end data entry, and return to the filemanger menu hit <return> at the prompt 'individual?'. Remember to re-format the data file after entering the data for the new generation of animals.

Other Options (from the File Manager Menu)

Rename a Data file

Enables the names of data files to be changed. Choose option '2' from the File Manager Menu and follow the instructions on screen. The same name cannot be used for more than one file. File names can be maximum of eight letters and must be followed with '.dat' for a data file or '.res' for a results file.

Creat a new Data file

this option allows you to create a data file for breeds, or species, other than Irish Moiled cattle. Choose option '3' from the File Manger Menu and you will be asked for a name for the data file to be created (8 letters maximum, followed by ".dat"). Press <return> after typing the file name and then enter information for each animal in the Herd Book, when prompted on screen, as follows:

Individual? : Maximum of 5 letters, use Name, ear tag number or Herd Book number (but be consistent). Press <return> after typing name.

Male Parent? : Enter male parent of the individual as above. Press <return> without typing a name if the parent is unknown or not of the same breed.

Female parent? : Enter female parent of the individual as above.

NOTE : The above entries are case sentitive so if using letters, use the lower case.

Alive? : Type "y<return>" if alive or "n<return>" if the individual is dead. List animals from which semen is available as alive.

male/female? : type "m<return>" for male or "f<return>" for female.

Inbreeding coefficient, if known? : Press <return> as the inbreeding coefficient is not known.

Year of Birth? : Enter the individual's date of birth (between 1800 and 2050). Press <return>. If the data of birth is unknown, enter a date later than the date of birth of the individuals parents and earlier than the date of birth of its offspring.

founder (y/n)? : type "n<return>" if the individual is not a founder of the breed. type "y<return>" if it is.

founder gene 1?/founder gene 2? : These prompts will only appear if the animal is a founder. Type "g1<return>" and then "g2<return>" for founder number 1, "g3<return>" and then "g4<return>" for founder 2 etc.

NOTE : MOILMATE'93 has been written specifically for use with Irish Moiled cattle and was designed to handle 8 founder animals. If you wish to use the Founder Calculations option to analyse a breed with more or less than 8 founders, please contact Mark Harland, c/o Department of Genetics and Microbiology, The University of Liverpool, for an updated version of the programme. If you do not wish to use the Founder Calculations option, simply list all animals as 'not founders'.

Specify New Drive Path

MOILMATE'93 can run using files stored on floppy disks. Use option '6' to change the drive used, from c:\moilmate to A: or B: (and back). Files which are stored in a sub-directory on a floppy disk cannot be read (transfer them to the root directory). It is not recommended to use a floppy drive to run large data files such as moiled92.dat, as the time taken for the computer to read from the floppy drive will slow down the calculations substantially.

The Moiled92.dat data file

The moiled92.dat data file contains all the information for the Irish Moiled herd. It can be used exactly as described for the tutor.dat data file, to calculate Inbreeding, coefficients of co-ancestry and for Founder analyses. The 8 Irish Moiled founder animals are as follows (Fig. 20):

Figure 20

Founder	Name	Herd Book No.	Founder alleles
F1	Balldugan Kat	783	g1, g2
F2	Miss Nugent	792	g3, g4
F3	Ballydugan Duke	762	g5, g6
F4	Ballydugan Mimosa	798	g7, g8
F5	Listerdonan	788	g9, g10
F6	Maymore VI	786	g11, g12
F7	Derrylecka	790	g13, g14
F8	Derryboy Cyclamen	723	g15, g16

As the moiled92.dat data file is very large, calculations will take considerably longer than with the tutor.dat data file. Options to stop the calculations part-way through (see sections on 'Large data sets') have been included, in case the computer is required for other uses. The time required for the various calculations

depends on the speed of the processor and the size of the data file. A model 486 processor can complete 1000 cycles of founder calculations with the moiled92.dat data file, in approximately 3 days.

The moiled92.dat data file has already been formatted and all inbreeding coefficients have been calculated. There is no need to format the data file before use.

APPENDIX II

MOILMATE '93 PROGRAMME LISTING

```
80 REM full version of program
     REM 16 founders
     REM upto 300 bestmate analyses
     REM strip coefficients/reprogram data files
     RANDOMIZE TIMER
     DIM lmaster$(500)
     DIM c$(500)
     DIM i$(500): DIM f$(500): DIM m$(500): DIM e$(500)
     GOTO 7000

200 CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
     LOCATE 2, 22: PRINT "INBREEDING CALCULATIONS"
     GOSUB 640
270 code% = 1: GET #1, code%
280 pointer = CVI(fi$): form$ = fm$
300 FOR f = 2 TO pointer
305 code% = f: GET #1, code%
315 IF fe$ <> "?     " THEN GOTO 340
310 IF fm$ = "     " OR fm$ = "M    " OR ff$ = "M    " OR ff$ = "     " THEN GOTO 325
320 b$ = fi$
322 GOTO 330
325 LSET fe$ = "      ": PUT #1, code%: GOTO 340
330 GOSUB 560
335 IF stop$ = "yes" THEN stop$ = "": GOTO 370
340 NEXT f

345 CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
     LOCATE 2, 22: PRINT "INBREEDING CALCULATIONS": PRINT
     PRINT "All coefficients for individuals listed in this file have now been calculated"
370 PRINT "Hit 'Space' to display the file..."
     WHILE INKEY$ <> " "
     WEND
400 caller = 1: GOSUB 4995
402 PRINT : PRINT "Any key to return to menu..."
     WHILE INKEY$ = ""
     WEND
     GOTO 7420
430 found = 0
434 code% = 1: GET #1, code%
436 pointer = CVI(fi$): form$ = fm$: f = 2
440 code% = f: GET #1, code%
445 IF INKEY$ = "x" THEN stop$ = "yes"
450 IF individ$(x) = fi$ THEN item = f: i$(x) = fi$: m$(x) = fm$: f$(x) = ff$: l$(x) = fl$: s$(x) = fs$:
e$(x) = fe$: d$(x) = fd$: a1$(x) = fa1$: a2$(x) = fa2$: g1$(x) = fg1$: g2$(x) = fg2$: g3$(x) = fg3$:
g4$(x) = fg4$: g5$(x) = fg5$: g6$(x) = fg6$: g7$(x) = fg7$: g8$(x) = fg8$: g9$(x) = fg9$: g10$(x) =
fg10$: g11$(x) = fg11$: g12$(x) = fg12$: g13$(x) = fg13$: g14$(x) = fg14$: g15$(x) = fg15$: fg16$(x)
= fg16$: fg17$(x) = fg17$: fre$(x) = ffre$: moil$ = fmoil$: found = 1: RETURN
460 IF f < pointer THEN f = f + 1: GOTO 440
```

```
470 IF found = 0 AND bestmate <> 1 AND lprin <> 1 THEN GOTO 1770
480 RETURN
490 found = 0
560 REM individual being processed
610 individ$(1) = b$: master$ = b$
620 GOTO 730
640 PRINT
650 REM PRINT "If you require a hard copy of each pathway calculation, hit 'p'"
    REM PRINT "NOTE - tractor feed printer is required as a 'paper out' error will halt the"
    REM "halt the program. Consult manual for further information."
    REM "Hit 'SPACE' if printout is not required"
660 PRINT "any key to start calculations"
690 p$ = INKEY$
700 REM IF p$ = "p" OR p$ = "P" THEN prin = 1: RETURN
710 IF p$ <> "" THEN RETURN
720 GOTO 690
730  CLS
740 IF prin = 1 THEN LPRINT "Inbreeding Coefficient For "; master$
750 PRINT "Calculating Coefficient for "; master$; " Please Wait"
760 REM tracing right pathway
770 rpath$ = individ$(1)
780 x = 1: GOSUB 430
790 IF m$(1) = "    " OR m$(1) = "M   " OR f$(1) = "M   " OR f$(1) = "   " THEN GOTO 1550
800 IF e$(1) <> "?    " THEN PRINT " coeff on file": END
820 REM up right
830 IF f$(1) = "    " OR f$(1) = "M   " THEN GOTO 880
840 individ$(1) = f$(1)
850 rpath$ = rpath$ + individ$(1)
860 GOSUB 1020
865 x = 1: GOSUB 430
870 GOTO 820
880 REM up left
890 IF m$(1) = "    " OR m$(1) = "M   " THEN GOTO 940
900 individ$(1) = m$(1)
910 rpath$ = rpath$ + individ$(1)
930 GOTO 860
940 REM back a step
950 ROLDIND$ = RIGHT$(rpath$, 5)
960 rpath$ = LEFT$(rpath$, LEN(rpath$) - 5)
970 individ$(1) = RIGHT$(rpath$, 5)
980 IF individ$(1) = master$ THEN GOTO 1530
990 x = 1: GOSUB 430
1000 IF m$(1) = ROLDIND$ THEN GOTO 940
1010 GOTO 880
1020 REM left search routine
1030 lpath$ = master$
1040 individ$(2) = master$
1050 x = 2: GOSUB 430
1060 REM up left
1070 IF m$(2) = "    " OR m$(2) = "M   " THEN GOTO 1120
1080 individ$(2) = m$(2)
1090 lpath$ = lpath$ + individ$(2)
1100 GOSUB 1260
1110 GOTO 1050
```

```
1120 REM up right
1130 IF f$(2) = "    " OR f$(2) = "M    " THEN GOTO 1190
1140 individ$(2) = f$(2)
1150 lpath$ = lpath$ + individ$(2)
1160 GOSUB 1260
1170 GOTO 1050
1180 REM back a step
1190 LOLDIND$ = RIGHT$(lpath$, 5)
1200 lpath$ = LEFT$(lpath$, LEN(lpath$) - 5)
1210 individ$(2) = RIGHT$(lpath$, 5)
1220 IF individ$(2) = master$ THEN RETURN
1230 x = 2: GOSUB 430
1240 IF f$(2) = LOLDIND$ THEN GOTO 1180
1250 GOTO 1120
1260 REM match check
1265 IF INKEY$ = "q" THEN END
1270 IF RIGHT$(lpath$, 5) <> RIGHT$(rpath$, 5) THEN RETURN
1280 IF (LEN(rpath$) + LEN(lpath$)) < 21 THEN RETURN
1290 FOR r = 1 TO LEN(rpath$) / 5 - 2
1300 FOR l = 1 TO LEN(lpath$) / 5 - 2
1310 IF MID$(rpath$, r * 5 + 1, 5) = MID$(lpath$, l * 5 + 1, 5) THEN miss = 1
1320 NEXT l
1330 NEXT r
1340 IF miss = 1 THEN miss = 0: RETURN
1350 hitno = hitno + 1
1360 anc$ = RIGHT$(lpath$, 5): individ$(3) = anc$
1370 x = 3: GOSUB 430
1380 mult = VAL(e$(3))
1400 NUMBER = (LEN(lpath$) / 5) + (LEN(rpath$) / 5) - 3
1405 PRINT
     PRINT "Calculating Coefficient for "; master$
     PRINT "Hitno. "; hitno
     PRINT "Common Ancestor = "; anc$; " F ="; mult
     PRINT "No. of Individuals in Pathway ="; NUMBER
     PRINT "Left Path : "; lpath$
     PRINT "Right Path: "; rpath$
     COEFF = .5 ^ NUMBER * (1 + mult)
     PRINT "Coeff. ="; .5 ^ NUMBER; " * ( 1 +"; mult; ") ="; COEFF
     PRINT "More...."
     IF stop$ = "yes" THEN PRINT ; "(Stopping...)"
     sum = sum + COEFF
1510 IF prin = 1 THEN GOTO 1665
1520 RETURN
1530 REM end of search
1540 IF hitno <> 0 THEN GOTO 1615
1550 PRINT
     PRINT "No common ancestor, inbreeding coefficient = 0"
1562 individ$ = master$: x = 1: GOSUB 430
1564 LSET fe$ = " .0000": code% = item: PUT #1, code%
1570 IF prin = 1 THEN GOTO 1585
1580 RETURN
1585 LPRINT
     LPRINT "No common ancestor, inbreeding coefficient = 0"
     LPRINT "----------------------------------------"
```

```
    GOTO 1580
1615 PRINT
    REM LPRINT master$, sum
    PRINT "sum of coefficients ="; sum; " for individual "; master$;
    PRINT USING "_(##.##_%_)"; sum * 100
    PRINT "-------------------------------------------------"
    individ$ = master$: x = 1: GOSUB 430
    sum = sum * 100000: bosh = INT(sum): bish = bosh / 100000: bish$ = STR$(bish)
    LSET fe$ = bish$: code% = item: PUT #1, code%
    IF prin = 1 THEN GOTO 1724
1655 sum = 0: hitno = 0: pox$ = ""
    RETURN

1665 LPRINT
    LPRINT "Hit No."; hitno
    LPRINT "Common ancestor = "; anc$; "(F="; mult; ")"
    LPRINT "No. of individuals in pathway ="; NUMBER
    LPRINT "left path : "; lpath$
    LPRINT "right path: "; rpath$
    LPRINT "Coeff.="; .5 ^ NUMBER; "* ( 1 +"; mult; ")="; COEFF
    RETURN

1724 LPRINT
    LPRINT "sum of coefficients ="; sum; "for individual "; master$;
    LPRINT USING "_(##.##_%_)"; sum * 100
    LPRINT "-------------------------------------------------"
    GOTO 1655

1770 PRINT "ancestry data missing : "; individ$(x); " Calculation aborted"
    PRINT "Hit any key to return to menu"
    WHILE INKEY$ = ""
    WEND
    GOTO 7420

1810 REM Best Mate
1820 xno = 0: CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
1830 LOCATE 2, 26: PRINT "BEST MATE ANALYSIS": PRINT
1832 code% = 1: GET #1, code%: pointer = CVI(fi$): form$ = fm$: mpoint = pointer
1840 INPUT "Individual to be mated"; b2$: IF b2$ = "" THEN GOTO 7420
1850 IF b2$ = "end" OR b2$ = "END" THEN mateno = mateno - 1: GOTO 2213
1860 b2$ = b2$ + "     ": b2$ = LEFT$(b2$, 5)
1880 master$ = b2$: bestmate = 1
1890 individ$(1) = master$: x = 1: GOSUB 430
1900 IF found = 0 THEN PRINT "no record of animal "; b2$; " - please try another": GOTO 1840
2020 PRINT "Enter your selection of mates, for individual "; b2$
2030 cowno = 1: PRINT "When done, type 'end'"
2040 INPUT "Mate"; c$(cowno)
2050 IF c$(cowno) = "end" THEN c$(cowno) = "": cowno = cowno - 1: GOTO 2180
2060 c$(cowno) = c$(cowno) + "     ": c$(cowno) = LEFT$(c$(cowno), 5)
2100 individ$(1) = c$(cowno): x = 1: GOSUB 430
2110 IF found = 0 THEN PRINT "no record of animal "; c$(cowno); " - please try another": GOTO 2040
2150 lmaster$(cowno) = c$(cowno)
2160 cowno = cowno + 1
2170 GOTO 2040
```

```
2180 REM creating calf filespace
2190 code% = 1: GET #1, code%: pointer = CVI(fi$): form$ = fm$: newpoint = pointer
2200 FOR f9 = 1 TO cowno
2202 xno = xno + 1
2205 i$ = "X" + STR$(xno) + "   ": i$ = LEFT$(i$, 5): l$ = " ": s$ = " ": e$ = "?      ": d$ = "2100"
2210 LSET fi$ = i$: LSET fm$ = b2$: LSET ff$ = c$(f9): LSET fl$ = l$: LSET fs$ = s$: LSET fe$ = e$:
LSET fd$ = d$
2220 bestmate = 0: code% = newpoint + f9: PUT #1, code%
2225 LSET fi$ = MKI$(newpoint + f9): LSET fm$ = form$: code% = 1: PUT #1, code%
2211 NEXT f9
2212 GOTO 1840
2213 GOSUB 640
2214 FOR calf = 1 TO ((newpoint + f9) - mpoint) - 1
2215 code% = mpoint + calf: GET #1, code%
2217 b$ = fi$: GOSUB 560
2218 IF stop$ = "yes" THEN stop$ = "": calf = calf + 1: GOTO 2238
2236 NEXT calf
2238 LSET fi$ = MKI$(mpoint): LSET fm$ = form$: code% = 1: PUT #1, code%
2239 CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 65: PRINT filename$
2240 CLS : LOCATE 2, 30: PRINT "BEST MATE ANALYSIS": PRINT
2250 PRINT "       Parent  Parent  Coefficient"
2260 PRINT "Calf     one     two  of Coancestry": PRINT
2265 FOR f = 1 TO calf - 1
2270 REM FOR f = 1 TO ((newpoint + f9) - mpoint) - 1
2280 code% = mpoint + f: GET #1, code%
2290 PRINT fi$; "   "; fm$; "   "; ff$; "     "; USING ".####"; VAL(fe$)
2295 i$(f) = fi$: m$(f) = fm$: f$(f) = ff$: e$(f) = fe$
2296 LSET fi$ = "     ": LSET fm$ = "     ": LSET ff$ = "     ": LSET fl$ = " ": LSET fs$ = " ": LSET fe$
= "?     ": LSET fd$ = "    ": LSET fend$ = CHR$(13)
2297 PUT #1, code%
2300 NEXT f
2310 PRINT : INPUT "Enter 's' to save these analyses to disc, or 'm' to return to menu"; z$
2320 z$ = LEFT$(z$, 1): IF z$ <> "s" AND z$ <> "S" AND z$ <> "m" AND z$ <> "M" THEN GOTO
2310
2330 IF z$ = "m" OR z$ = "M" THEN calf = 0: GOTO 7420
2335 CLOSE
2340 INPUT "filename"; filename2$: filename2$ = drive$ + filename2$
2350 OPEN "R", #1, filename2$, 108: FIELD #1, 5 AS fi$, 5 AS fm$, 5 AS ff$, 1 AS fl$, 1 AS fs$, 7 AS
fe$, 4 AS fd$, 3 AS fa1$, 3 AS fa2$, 64 AS fill$, 1 AS fg17$, 8 AS ffill$, 1 AS fend$
2360 code% = 1: GET #1, code%: form$ = fm$: pointer = CVI(fi$): IF pointer > 1 THEN GOTO 2367
2365 pointer = 1
2367 FOR f = 1 TO calf - 1
2370 REM FOR f = 1 TO ((newpoint + f9) - mpoint) - 1
2380 pointer = pointer + 1: code% = pointer: l$ = " ": d$ = "2100"
2390 LSET fi$ = i$(f): LSET fm$ = m$(f): LSET ff$ = f$(f): LSET fe$ = e$(f): LSET fl$ = l$: LSET fs$
= l$: LSET fd$ = d$
2400 PUT #1, code%
2410 NEXT f
2415 calf = 0
2420 code% = 1: i$ = MKI$(pointer): LSET fi$ = i$: LSET fm$ = "    ": PUT #1, code%
2430 caller = 1: GOSUB 440
2440 CLOSE
2450 OPEN "R", #1, filename$, 108: FIELD #1, 5 AS fi$, 5 AS fm$, 5 AS ff$, 1 AS fl$, 1 AS fs$, 7 AS
fe$, 4 AS fd$, 3 AS fa1$, 3 AS fa2$, 64 AS fill$, 1 AS fg17$, 8 AS ffill$, 1 AS fend$
```

```
    GET #1, 1: PRINT fm$
2470 caller = 0: GOTO 7420

4000 PRINT : INPUT "Enter old File Name: ", r$: IF r$ = "" THEN ren = 0: GOTO 7000
    r$ = drive$ + r$
    OPEN "R", #1, r$, 108: FIELD #1, 5 AS fi$, 5 AS fm$, 5 AS ff$, 1 AS fl$, 1 AS fs$, 7 AS fe$, 4 AS
fd$, 3 AS fa1$, 3 AS fa2$, 64 AS fill$, 1 AS fg17$, 8 AS ffill$, 1 AS fend$
    code% = 1: GET #1, code%: form$ = fm$
    pointer = CVI(fi$): IF pointer >= 1 THEN CLOSE : GOTO 4090
    PRINT : PRINT "*file not found*": CLOSE
    KILL r$: PRINT : PRINT "any key to continue..."
    WHILE INKEY$ = ""
    WEND: GOTO 7200
4090 INPUT ; "Enter New File Name: ", s$: IF s$ = "" THEN ren = 0: GOTO 7000
    IF LEN(s$) > 12 THEN PRINT "    (8 Charachters max.)": GOTO 4090
    s$ = drive$ + s$
    OPEN "R", #1, s$, 108: FIELD #1, 5 AS fi$, 5 AS fm$, 5 AS ff$, 1 AS fl$, 1 AS fs$, 7 AS fe$, 4 AS
fd$, 3 AS fa1$, 3 AS fa2$, 64 AS fill$, 1 AS fg17$, 8 AS ffill$, 1 AS fend$
    code% = 1: GET #1, code%: form$ = fm$
    pointer = CVI(fi$): IF pointer < 1 THEN GOTO 4180
    PRINT : PRINT "*file already exists*": CLOSE : GOTO 4090
4180 CLOSE : KILL s$
4190 NAME r$ AS s$: ren = 0: GOTO 7200

4500 REM data input
    CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
4520 PRINT "DATA INPUT"
4560 PRINT : IF new = 1 THEN PRINT "Creating new file.." ELSE PRINT "Updating file"
    IF new = 1 THEN INPUT ; "Filename"; filename$: IF filename$ = "" THEN new = 0: GOTO 7000
    IF new = 1 AND LEN(filename$) > 12 THEN PRINT "    (8 Charachters max.)": GOTO 4560
    IF new = 1 THEN filename$ = drive$ + filename$
    IF new = 1 THEN OPEN "R", #1, filename$, 108: FIELD #1, 5 AS fi$, 5 AS fm$, 5 AS ff$, 1 AS
fl$, 1 AS fs$, 7 AS fe$, 4 AS fd$, 3 AS fa1$, 3 AS fa2$, 64 AS fill$, 1 AS fg17$, 8 AS ffill$, 1 AS fend$
    code% = 1: GET #1, code%
    IF new = 1 THEN GOTO 4615
    pointer = CVI(fi$): IF pointer < 1 THEN PRINT "file not found": CLOSE : GOTO 4520
    GOTO 4640
4615 pointer = CVI(fi$): IF pointer < 1 THEN GOTO 4620
    PRINT : PRINT : PRINT "*File already exists*": PRINT : PRINT "Any key to return to menu..."
    WHILE INKEY$ = ""
    WEND: new = 0: GOTO 7000
4620 i$ = MKI$(1): LSET fi$ = i$: code% = 1: PUT #1, code%: GOTO 4640
4630 i$ = MKI$(pointer): LSET fi$ = i$: code% = 1: PUT #1, code%
4640 caller = 1: GOSUB 4980
4645 PRINT : PRINT "hit return to end data entry"
4650 PRINT : INPUT "individual      "; i$: IF i$ = "" THEN GOTO 4830
4660 i$ = i$ + "    ": i$ = LEFT$(i$, 5)
4670 INPUT "male parent     "; m$: m$ = m$ + "    ": m$ = LEFT$(m$, 5)
4680 INPUT "female parent   "; f$: f$ = f$ + "    ": f$ = LEFT$(f$, 5)
4690 INPUT "alive           "; l$: l$ = LEFT$(l$, 1)
4700 IF l$ <> "y" AND l$ <> "Y" AND l$ <> "n" AND l$ <> "N" THEN GOTO 4690
4710 INPUT "male / female   "; s$: s$ = LEFT$(s$, 1)
4720 IF s$ <> "m" AND s$ <> "M" AND s$ <> "f" AND s$ <> "F" THEN GOTO 4710
4730 INPUT "inbreeding coefficient, if known"; e$
```

```
4735 IF e$ = "" THEN e$ = "? ": GOTO 4745
4740 e$ = e$ + "      ": e$ = LEFT$(e$, 7): IF VAL(e$) < 0 OR VAL(e$) > 1 THEN GOTO 4730
4745 INPUT "year of birth              "; d$
4750 IF LEN(d$) <> 4 OR VAL(d$) < 1800 OR VAL(d$) > 2050 THEN GOTO 4740
4760 LSET fi$ = i$: LSET fm$ = m$: LSET ff$ = f$: LSET fl$ = l$: LSET fs$ = s$: LSET fe$ = e$:
LSET fd$ = d$: LSET fend$ = CHR$(13)
4761 INPUT "founder (y/n)"; p$
4762 IF p$ = "y" OR p$ = "Y" THEN p$ = "f": GOTO 4767
4763 IF p$ = "n" OR p$ = "n" THEN p$ = "n": a$ = "": b$ = "": GOTO 4768
4764 GOTO 4761
4767 INPUT "founder gene 1 ", a$: INPUT "founder gene 2 ", b$
4768 LSET fa1$ = a$: LSET fa2$ = b$
4769 LSET fg17$ = p$
4770 code% = pointer + 1
4780 PUT #1, code%
4790 PRINT : IF edit = 1 THEN RETURN
4810 pointer = pointer + 1
4820 GOTO 4630
4830 IF edit = 1 THEN edit = 2: RETURN
4840 IF pointer < 2 THEN GOTO 4890
4850 code% = pointer: GET #1, code%
4860 IF VAL(fd$) <> 0 THEN GOTO 4890
4870 pointer = pointer - 1
4880 GOTO 4850
4890 i$ = MKI$(pointer): code% = 1
4900 LSET fi$ = i$
4910 PUT #1, code%
4915 PRINT : PRINT "any key to continue...";  : WHILE INKEY$ = ""
4917 WEND
4920 IF new = 1 THEN new = 0: CLOSE : GOTO 7000
4925 GOTO 7420

4930 REM data check/edit
4980 code% = 1: GET #1, code%
4990 pointer = CVI(fi$): form$ = fm$
4995 CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
5000 LOCATE 1, 24: PRINT pointer - 1; " individuals in set"
5010 PRINT : PRINT "                Male   Female                Inbr.  Year   Founder"
5020 PRINT "Item   Name   Parent Parent Alive? Sex   Coeff. Born   Alleles": PRINT
5025 IF fullscrn = 1 THEN fullscrn = 0: GOTO 5040
5027 IF caller = 2 THEN GOTO 5040
5030 FOR f = 1 TO pointer - 1
5040 code% = f + 1: GET #1, code%
5050 f$ = STR$(f): f$ = f$ + "    ": f$ = LEFT$(f$, 5)
5055 PRINT f$; "   "; fi$; "   "; fm$; "   "; ff$; "   "; fl$; "      "; fs$; "      ";
5056 IF fe$ = "      " OR fe$ = "?     " THEN PRINT LEFT$(fe$, 5);  ELSE PRINT USING ".####";
VAL(fe$);

5057 a$ = INKEY$
     IF a$ = "s" THEN toggle = 0

5062 IF RIGHT$(filename$, 3) = "res" THEN PRINT : GOTO 5063
     PRINT "   "; fd$; "   "; fa1$; "   "; fa2$; "   "; fg17$
5063 IF caller = 2 THEN caller = 0: RETURN
```

```
5064 IF f / 17 <> INT(f / 17) THEN GOTO 5078
5065 IF f = pointer - 1 THEN GOTO 5080

5066 PRINT "any key to continue..."

5067 REM option to scroll
5068 IF toggle = 1 THEN GOTO 5075
5069 a$ = INKEY$
     IF a$ = "f" THEN toggle = 1: GOTO 5075
     IF a$ = "" THEN GOTO 5069

5075 fullscrn = 1: f = f + 1: GOTO 4995
5078 NEXT f
5080 IF caller = 1 THEN caller = 0: RETURN
5090 PRINT : INPUT ; "edit (y/n)"; z$
5100 IF z$ <> "y" AND z$ <> "Y" AND z$ <> "n" AND z$ <> "N" THEN GOTO 5090
5110 pointer2 = pointer
5120 IF z$ = "n" OR z$ = "N" THEN edit = 0: GOTO 5245
5124 PRINT : INPUT "Item number to be edited"; pointer: edit = 1

5125 REM deletes from item no. to end
5126 REM GET #1, 1: LSET fi$ = MKI$(pointer): PUT #1, 1
5127 REM STOP: GOTO 7420

5131 IF pointer = 0 THEN edit = 0: GOTO 5245
     code% = 1: GET #1, code%: form$ = " ": LSET fm$ = form$: PUT #1, code%
5132 f = pointer: caller = 2: CLS : GOSUB 5010
5140 GOSUB 4650
5150 IF edit = 2 THEN GOTO 5170
5155 f = pointer: caller = 2: GOSUB 5010
5160 pointer = pointer2: GOTO 5090
5170 REM delete an item (edit flag = 2)
5180 pointer3 = pointer:
5190 FOR f = pointer + 2 TO pointer2
5200 code% = f: GET #1, code%
5210 code% = f - 1
5220 PUT #1, code%
5230 NEXT f
5240 PRINT "item "; pointer; " deleted": pointer2 = pointer2 - 1
5245 PRINT : REM caller = 1: GOSUB 4980
5250 code% = pointer2: GET #1, code%
5260 IF VAL(fd$) <> 0 OR pointer2 < 2 THEN GOTO 5280
5270 pointer2 = pointer2 - 1: GOTO 5250
5280 i$ = MKI$(pointer2)
5290 code% = 1: LSET fi$ = i$: LSET fm$ = form$: PUT #1, code%
5300 IF edit = 2 THEN edit = 0: GOTO 4980

5305 PRINT "any key to continue..."
     WHILE INKEY$ = ""
     WEND
     GOTO 7420

5600 REM hardcopy
     CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
```

```
      PRINT : LOCATE 3, 26: PRINT "HARDCOPY OPTION": PRINT : cowno = 1
      PRINT "Enter names of individuals to be printed, or 'a' for all on file"
      PRINT "To output by item number, enter 'i' followed by the item, e.g. i200"
      PRINT "Type 'end' when done"
 5645 INPUT z$
      IF z$ = "" THEN GOTO 7420
      IF z$ = "a" OR z$ = "A" THEN GOTO 5720
      IF LEFT$(z$, 1) = "i" THEN GOTO 5672
      IF z$ = "end" OR z$ = "END" THEN cowno = cowno - 1: GOTO 5690
      z$ = z$ + "     ": individ$(1) = LEFT$(z$, 5): x = 1: lprin = 1: GOSUB 430
      IF found = 0 THEN PRINT "no record of animal "; z$: GOTO 5645
      lprin = 0
      c(cowno) = item: GOTO 5675
 5672 z$ = RIGHT$(z$, LEN(z$) - 1): c(cowno) = VAL(z$) + 1
 5675 cowno = cowno + 1: GOTO 5645
 5690 header = 1: GOSUB 5720
 5695 FOR f2 = 1 TO cowno
      f = c(f2)
      GOSUB 5790
      NEXT f2
      header = 0: GOTO 7420
 5720 LOCATE 21, 1: PRINT "Check printer is loaded & on line... any key to start printing"
      printer$ = ""
      WHILE INKEY$ = ""
      WEND
      ON ERROR GOTO 5835
      LPRINT TAB(8); "MOILMATE 93                    "; filename$
      IF printer$ = "offline" THEN GOTO 5305
      code% = 1: GET #1, code%
      pointer = CVI(fi$): form$ = fm$
      IF header = 1 THEN GOTO 5772
      LPRINT : LPRINT TAB(8); "individuals in set:"; pointer - 1: LPRINT
 5772 LPRINT TAB(8); "          Male   Female          Inbr.  Year  Founder"
      LPRINT TAB(8); "Item  Name   Parent Parent  Alive? Sex  Coeff.  Born  Alleles": LPRINT
      IF header = 1 THEN RETURN
      IF header = 2 THEN RETURN
      FOR f = 2 TO pointer
      IF f = 54 THEN header = 2: GOSUB 5772
      newpage = (f - 54) / 57
      IF newpage = INT(newpage) AND newpage > 0 THEN header = 2: GOSUB 5772
      header = 0
 5790 code% = f: GET #1, code%
      f$ = STR$(f - 1): f$ = f$ + "    ": f$ = LEFT$(f$, 5)
      LPRINT TAB(8); f$; " "; fi$; "   "; fm$; "   "; ff$; "   "; fl$; "      "; fs$; "    ";
      IF fe$ = "      " OR fe$ = "?     " THEN LPRINT LEFT$(fe$, 5);  ELSE LPRINT USING ".####";
 VAL(fe$);
      IF RIGHT$(filename$, 3) = "res" THEN LPRINT : GOTO 5815
      LPRINT "   "; fd$; "   "; fa1$; "   "; fa2$
 5815 IF header = 1 THEN RETURN
      NEXT f
      GOTO 7420

 5835 REM print error capture
      PRINT "Device Fault - Printer Not Ready": printer$ = "offline": RESUME NEXT
```

```
5900 REM format - date sorter
     CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
     PRINT : LOCATE 3, 26: PRINT "FORMAT OPTION": PRINT
     code% = 1: GET #1, code%
     pointer = CVI(fi$): form$ = fm$
     FOR f = 3 TO pointer
     code% = f: GET #1, code%
5970 i$ = fi$: m$ = fm$: f$ = ff$: l$ = fl$: s$ = fs$: e$ = fe$: d$ = fd$: a1$ = fa1$: a2$ = fa2$: g17$ =
fg17$
     code% = f - 1: GET #1, code%
     i2$ = fi$: m2$ = fm$: f2$ = ff$: l2$ = fl$: s2$ = fs$: e2$ = fe$: d2$ = fd$: a12$ = fa1$: a22$ = fa2$:
g172$ = fg17$
     IF VAL(d$) >= VAL(d2$) THEN GOTO 6070
     LSET fi$ = i2$: LSET fm$ = m2$: LSET ff$ = f2$: LSET fl$ = l2$: LSET fs$ = s2$: LSET fe$ =
e2$: LSET fd$ = d2$: LSET fa1$ = a12$: LSET fa2$ = a22$: LSET fg17$ = g172$: code% = f: PUT #1,
code%
     LSET fi$ = i$: LSET fm$ = m$: LSET ff$ = f$: LSET fl$ = l$: LSET fs$ = s$: LSET fe$ = e$:
LSET fd$ = d$: LSET fa1$ = a1$: LSET fa2$ = a2$: LSET fg17$ = g17$: code% = f - 1: PUT #1, code%
     IF f > 3 THEN f = f - 1: GOTO 5970
6070 NEXT f
     caller = 1: toggle = 1
     GOSUB 4980
     toggle = 0: GOTO 6150

6085 PRINT : PRINT "Press 'h' for help on format errors or any other key to continue..."
6090 a$ = INKEY$
     IF a$ = "h" OR a$ = "H" THEN GOTO 6410
     IF a$ = "" THEN GOTO 6090
     GOTO 7420

6150 REM duplicate checker
     mm = 0: dup = 0: dmistot = 0: miss = 0
     code% = 1: GET #1, code%
     pointer = CVI(fi$): form$ = fm$
     FOR d = 2 TO pointer
     code% = d: GET #1, code%
     i1$ = fi$: m1$ = fm$: f1$ = ff$: d1$ = fd$: a11$ = fa1$: a21$ = fa2$: g171$ = fg17$
     FOR d2 = 2 TO pointer
     code% = d2: GET #1, code%
     i2$ = fi$: s2$ = fs$: d2$ = fd$: a12$ = fa1$: a22$ = fa2$: g172$ = fg17$
     IF i1$ = i2$ AND d < d2 THEN PRINT "duplicate entries - items "; d - 1; "("; i1$; ")"; " and "; d2 -
1; "("; i2$; ")": dup = 1
     IF m1$ = i2$ AND VAL(d1$) <= VAL(d2$) THEN dmis = 1
     IF f1$ = i2$ AND VAL(d1$) <= VAL(d2$) THEN dmis = 2
     IF m1$ = i2$ OR m1$ = "    " OR m1$ = "M    " THEN mmatch = 1
     IF m1$ <> i2$ THEN GOTO 6280
     IF s2$ = "m" OR s2$ = "M" THEN GOTO 6280
     PRINT "sex mismatch - male parent at item "; d - 1; "("; i1$; ")": PRINT "   is female individual at
item "; d2 - 1; "("; i2$; ")": mm = 1
6280 IF f1$ = i2$ OR f1$ = "    " OR f1$ = "M    " THEN fmatch = 1
     IF f1$ <> i2$ THEN GOTO 6302
     IF s2$ = "f" OR s2$ = "F" THEN GOTO 6302
```

```
      PRINT "sex mismatch - female parent at item "; d - 1; "("; i1$; ")": PRINT "  is male individual at
item "; d2 - 1; "("; i2$; ")": mm = 1
6302 IF dmis > 0 THEN PRINT "date mismatch - individual at item "; d - 1; "("; i1$; ")"; "  is older than
it's parent at item "; d2 - 1; "("; i2$; ")": dmistot = 1
      dmis = 0
      NEXT d2
      IF mmatch = 0 THEN PRINT "data missing for male parent entered at item "; d - 1; "("; i1$; ")":
miss = 1
      IF fmatch = 0 THEN PRINT "data missing for female parent entered at item "; d - 1; "("; i1$; ")":
miss = 1
      mmatch = 0: fmatch = 0
      NEXT d
      IF dup < 1 THEN PRINT "no duplicate entries found"
      IF mm < 1 THEN PRINT "no sex mismatches found"
      IF dmistot < 1 THEN PRINT "no date mismatches found"
      IF miss < 1 THEN PRINT "individual data entries found for all quoted parents"
      code% = 1: GET #1, code%
      IF dup < 1 AND mm < 1 AND miss < 1 AND dmistot < 1 THEN GOTO 6397 ELSE GOTO 6400
6397 m$ = "f": LSET fm$ = m$: PUT #1, code%
      PRINT : PRINT filename$; " is now formatted": GOTO 5305
6400 m$ = " ": LSET fm$ = m$: PUT #1, code%: GOTO 6085

6410 REM format error messages - help
      CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 65: PRINT filename$
      PRINT : LOCATE 3, 33: PRINT "FORMAT ERRORS": PRINT
      LOCATE 5, 30: PRINT "duplicate entries"
      PRINT "Duplicate entries may cause the program to return an incorrect coefficient."
      PRINT "Return to the Display / Edit option from the 'current' menu and delete one "
      PRINT "of the duplicate entries by entering it's item number and striking return on"
      PRINT "the prompt 'individual?'"
      LOCATE 11, 30: PRINT "sex mismatch"
      PRINT "This occurs when an animal entered as a male individual is also given as"
      PRINT "the dam of another, or vice versa. Return to edit as above and correct"
      PRINT "whichever item number is in error by retyping."
      LOCATE 16, 30: PRINT "data missing for parent"
      PRINT "All animals entered as parents must also be specified individually. Choose"
      PRINT "the update option from the 'current' menu and type in the animal's details."
      PRINT "If no details are available, hit return at both 'parent' prompts, 'y' at"
      PRINT "'alive', 'f' at 'male/female', return at 'coefficient', and enter it's year"
      PRINT "of birth as that of the oldest known animal in the file"
      LOCATE 24, 30: PRINT "any key to continue...";
      WHILE INKEY$ = ""
      WEND
      CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 65: PRINT filename$
      PRINT : LOCATE 3, 33: PRINT "FORMAT ERRORS": PRINT
      LOCATE 5, 35: PRINT "date mismatch"
      PRINT "This occurs when an individual's parent, as detailed in a separate item, is"
      PRINT "the same age or younger than the individual. Using 'edit', correct whichever"
      PRINT "item is in error. If the parent's year of birth is not known, set it to one"
      PRINT "year before that of the calf."
      LOCATE 21, 1: PRINT "NOTE - After each edit return to the formatter. Files not correctly
formatted"
      PRINT "cannot be used for the calculation of coefficients"
      LOCATE 24, 30: PRINT "any key to continue";
```

```
6720 WHILE INKEY$ = ""
     WEND
     GOTO 7420

7000 REM filemanager
     CLOSE
     CLS : LOCATE 2, 26: PRINT "MOILMATE 93"
     LOCATE 3, 13: PRINT "Copyright A. Turner, M. Harland, 1993"
     IF dimi = 1 THEN GOTO 7050
     DIM inbr(600): dimi = 1
7050 IF drive = 1 THEN GOTO 7090
     LOCATE 7, 1: INPUT ; "Please specify a drive path for your datafiles ", drive$
     IF drive$ <> "a" AND drive$ <> "b" AND drive$ <> "c" AND drive$ <> "C" THEN GOTO 7000
     IF drive$ = "c" OR drive$ = "C" THEN drive$ = "c:\moilmate": GOTO 7075
     drive$ = LEFT$(drive$, 1): drive$ = drive$ + ":\"
7074 ON ERROR GOTO 7077
7075 CHDIR drive$: drive = 1
     IF drive$ = "c:\moilmate" THEN drive$ = "c:\moilmate\"
     ON ERROR GOTO 0
     GOTO 7080
7077 REM catch drive error
     PRINT : PRINT "Drive not Ready": RESUME 7050

7080 LOCATE 7, 1: PRINT "                                    "
7090 LOCATE 7, 10: PRINT "F I L E  M A N A G E R  M E N U"
     LOCATE 9, 10: PRINT "1.  List data files on drive "; drive$; ""
     LOCATE 10, 10: PRINT "2.  Rename a data file"
     LOCATE 11, 10: PRINT "3.  Create a new data file"
     LOCATE 12, 10: PRINT "4.  Delete a data file"
     LOCATE 13, 10: PRINT "5.  Load a data file"
     LOCATE 14, 10: PRINT "6.  Specify a new drive path"
     LOCATE 15, 10: PRINT "7.  Quit MOILMATE"
7150 a$ = INKEY$
     IF a$ = "1" THEN GOTO 7200
     IF a$ = "5" THEN load = 1: GOTO 7200
     IF a$ = "4" THEN del = 1: GOTO 7200
     IF a$ = "3" THEN new = 1: GOTO 4500
     IF a$ = "7" THEN GOTO 7300
     IF a$ = "6" THEN drive = 0: GOTO 7000
     IF a$ = "2" THEN ren = 1: GOTO 7200
     GOTO 7150

7200 CLS : d$ = drive$ + "*.dat": e$ = drive$ + "*.res"
     ON ERROR GOTO 7210
     FILES d$
7205 ON ERROR GOTO 7215
     PRINT : FILES e$
     ON ERROR GOTO 0
     IF del = 1 THEN GOTO 7260
     IF ren = 1 THEN GOTO 4000
     IF load = 1 THEN GOTO 7400

     GOTO 7220
```

```
7210 PRINT d$: PRINT "No files present": RESUME NEXT
7215 PRINT e$: PRINT "No files present": RESUME NEXT

7220 PRINT : PRINT "hit space bar to continue..."
     WHILE INKEY$ = ""
     WEND
     GOTO 7000

7260 PRINT : PRINT : INPUT "enter name of file to be deleted ", d$
     IF d$ = "" THEN del = 0: GOTO 7000
     d$ = drive$ + d$
     PRINT : PRINT "Are you sure you want to delete "; d$: INPUT "(y/n)"; k$
     IF k$ <> "y" AND k$ <> "N" THEN del = 0: GOTO 7000
     OPEN "R", #1, d$, 27: CLOSE : KILL d$
     del = 0: GOTO 7200

7300 PRINT : PRINT : PRINT : INPUT "Are you sure you want to quit (y/n)"; x$
     IF x$ = "Y" OR x$ = "y" THEN CLS : END
     IF x$ <> "Y" AND x$ <> "y" THEN GOTO 7000

7400 PRINT : INPUT ; "Name of file to be loaded "; file$
     IF file$ = "" THEN load = 0: GOTO 7000
     filename$ = drive$ + file$
     OPEN "R", #1, filename$, 108: FIELD #1, 5 AS fi$, 5 AS fm$, 5 AS ff$, 1 AS fl$, 1 AS fs$, 7 AS
fe$, 4 AS fd$, 3 AS fa1$, 3 AS fa2$, 64 AS fill$, 1 AS fg17$, 8 AS ffill$, 1 AS fend$
     code% = 1: GET #1, code%: form$ = fm$
     pointer = CVI(fi$): IF pointer >= 1 THEN load = 0: GOTO 7420
     PRINT : PRINT "*file not found*": PRINT "Hit <return> to get back to menu": CLOSE
     KILL filename$: GOTO 7400

7420 CLS : LOCATE 1, 1: PRINT "MOILMATE 93": LOCATE 1, 55: PRINT filename$
     LOCATE 7, 10: PRINT "C U R R E N T   M E N U"
     LOCATE 9, 10: PRINT "1. Display or edit the file"
     LOCATE 10, 10: PRINT "2. Add data to the file"
     LOCATE 11, 10: PRINT "3. Format the file"
     LOCATE 12, 10: PRINT "4. Output file data to a line printer"
     LOCATE 13, 10: PRINT "5. Calculate inbreeding coefficients"
     LOCATE 14, 10: PRINT "6. Calculate coefficients of coancestry (bestmate)"
     LOCATE 15, 10: PRINT "7. Founder calculations"
     LOCATE 16, 10: PRINT "8. Return to file manager"
     REM LOCATE 18, 10: PRINT "9. Data manipulation"
7540 a$ = INKEY$
     IF a$ = "2" THEN GOTO 4500
     IF a$ = "1" THEN GOTO 4930
     IF a$ = "3" THEN GOTO 5900
     IF a$ = "4" THEN GOTO 5600
     IF a$ = "5" THEN bm = 0: GOTO 7650
     IF a$ = "6" THEN bm = 1: GOTO 7650
     IF a$ = "7" THEN fou = 1: GOTO 7650
     IF a$ = "8" THEN GOTO 7000
     REM IF a$ = "9" THEN GOTO 16000
     GOTO 7540
```

```
7650 code% = 1: GET #1, code%: IF LEFT$(fm$, 1) <> "f" THEN GOTO 7670
     IF bm = 1 THEN bm = 0: GOTO 1820
     IF fou = 1 THEN fou = 0: GOTO 8000
     GOTO 200
7670 PRINT : PRINT "*file not correctly formatted - select format option*"
     GOTO 7540

8000 wat = 1: printo = 0
8001 CLS : PRINT "FOUNDER CALCULATIONS": LOCATE 1, 55: PRINT filename$
     IF wat = 0 THEN GOTO 8019
     bigfile$ = LEFT$(file$, LEN(file$) - 4)
     bigfile$ = drive$ + bigfile$ + ".fou"
     OPEN "R", #2, bigfile$, 214
     FIELD #2, 6 AS fg1$, 6 AS fg2$, 6 AS fg3$, 6 AS fg4$, 6 AS fg5$, 6 AS fg6$, 6 AS fg7$, 6 AS
fg8$, 6 AS fg9$, 6 AS fg10$, 6 AS fg11$, 6 AS fg12$, 6 AS fg13$, 6 AS fg14$, 6 AS fg15$, 6 AS fg16$,
6 AS ffre$, 6 AS fmoil$, 6 AS finbr$

8009 PRINT : INPUT "New (n), Continue (c) or View Results (r)"; new$
     IF new$ = "c" OR new$ = "C" THEN GOTO 15000
     IF new$ = "r" OR new$ = "R" THEN results = 1: GOTO 15000
     IF new$ = "" GOTO 7420
     IF new$ <> "n" AND new$ <> "N" THEN GOTO 8009
8012 PRINT : INPUT "Number of cycles "; cycles
     REM PRINT : INPUT "printout (y/n) "; copy$
     PRINT : PRINT "Hit any key to start"
     IF copy$ = "y" OR copy$ = "Y" THEN printo = 1
     WHILE INKEY$ = ""
     WEND

8018 IF wat = 1 THEN wat = 0: GOTO 8001
8019 code% = 1: GET #1, code%
     pointer = CVI(fi$)
     FOR q = 2 TO pointer
     code% = q: GET #2, code%
     LSET fg1$ = "     ": LSET fg2$ = "     ": LSET fg3$ = "     ": LSET fg4$ = "     ": LSET fg5$ = "
": LSET fg6$ = "     ": LSET fg7$ = "     ": LSET fg8$ = "     ": LSET fg9$ = "     ": LSET fg10$ = "
": LSET fg11$ = _
"     ": LSET fg12$ = "     ": LSET fg13$ = "     ": LSET fg14$ = "     ": LSET fg15$ = "     ": LSET
fg16$ = "     ": LSET ffre$ = "     ": LSET fmoil$ = "     ": LSET finbr$ = "     ": PUT #2, code%
     GET #1, code%: IF fg17$ = "f" THEN GOTO 8055
     LSET fa1$ = "  ": LSET fa2$ = "  ": PUT #1, code%
8055 NEXT q
     g1 = 0: g2 = 0: g3 = 0: g4 = 0: g5 = 0: g6 = 0: g7 = 0: g8 = 0: g9 = 0: g10 = 0
     g11 = 0: g12 = 0: g13 = 0: g14 = 0: g15 = 0: g16 = 0: fres = 0: moil = 0
     f1l = 0: f2l = 0: f3l = 0: f4l = 0: f5l = 0: f6l = 0: f7l = 0: f8l = 0
     f1d = 0: f2d = 0: f3d = 0: f4d = 0: f5d = 0: f6d = 0: f7d = 0: f8d = 0
     y = 0: upto = 1
8500 FOR b = upto TO cycles
     GOSUB 9030
     IF printo = 1 THEN GOSUB 12010

     GOSUB 14500
     NEXT b
     GOTO 10010
```

```
9030 x1 = 0: x2 = 0: x3 = 0: x4 = 0: x5 = 0: x6 = 0: x7 = 0: x8 = 0: x9 = 0
     x10 = 0: x11 = 0: x12 = 0: x13 = 0: x14 = 0: x15 = 0: x16 = 0
     FOR f = 2 TO pointer
     code% = f: GET #1, code%
     IF fa1$ <> "   " AND fa2$ <> "   " THEN GOTO 9080
     b$ = fi$
     GOSUB 9500
9080 NEXT f
     GOSUB 9909: RETURN

9500 REM processing
     individ$(1) = b$: master$ = b$
     LOCATE 4, 3: PRINT "Individ: "; master$

     x = 1: GOSUB 430
     IF m$(1) = "    " THEN GOTO 9564
     IF m$(1) = "M   " THEN GOTO 9566
     individ$(1) = m$(1)
     x = 1: GOSUB 430
     a = INT(RND * (1 + 1))
     IF a = 0 THEN GOTO 9560
     IF a = 1 THEN GOTO 9562
     STOP
9560 x$ = a1$(1): GOTO 9568
9562 x$ = a2$(1): GOTO 9568
9564 x$ = "x  ": GOTO 9568
9566 x$ = "m  "
9568 individ$(1) = master$: x = 1: GOSUB 430
     LSET fa1$ = x$: code% = item: PUT #1, code%
     LOCATE 6, 3: PRINT "Alleles: "; fa1$
     x = 1: GOSUB 430
     IF f$(1) = "    " THEN GOTO 9700
     IF f$(1) = "M   " THEN GOTO 9705
     individ$(1) = f$(1)
     x = 1: GOSUB 430
     a = INT(RND * (1 + 1))
     IF a = 0 THEN GOTO 9680
     IF a = 1 THEN GOTO 9690
     STOP
9680 x$ = a1$(1): GOTO 9710
9690 x$ = a2$(1): GOTO 9710
9700 x$ = "x  ": GOTO 9710
9705 x$ = "m  "
9710 individ$(1) = master$: x = 1: GOSUB 430
     LSET fa2$ = x$: code% = item: PUT #1, code%
     LOCATE 7, 3: PRINT "       "; fa2$
     IF fi$ = "n" THEN RETURN
     GET #2, code%
     IF fa1$ = "g1 " THEN g1 = g1 + 1: x1 = x1 + 1: LSET fg1$ = STR$((VAL(fg1$) + 1))
     IF fa2$ = "g1 " THEN g1 = g1 + 1: x1 = x1 + 1: LSET fg1$ = STR$((VAL(fg1$) + 1))
     IF fa1$ = "g2 " THEN g2 = g2 + 1: x2 = x2 + 1: LSET fg2$ = STR$((VAL(fg2$) + 1))
     IF fa2$ = "g2 " THEN g2 = g2 + 1: x2 = x2 + 1: LSET fg2$ = STR$((VAL(fg2$) + 1))
     IF fa1$ = "g3 " THEN g3 = g3 + 1: x3 = x3 + 1: LSET fg3$ = STR$((VAL(fg3$) + 1))
```

```
    IF fa2$ = "g3 " THEN g3 = g3 + 1: x3 = x3 + 1: LSET fg3$ = STR$((VAL(fg3$) + 1))
    IF fa1$ = "g4 " THEN g4 = g4 + 1: x4 = x4 + 1: LSET fg4$ = STR$((VAL(fg4$) + 1))
    IF fa2$ = "g4 " THEN g4 = g4 + 1: x4 = x4 + 1: LSET fg4$ = STR$((VAL(fg4$) + 1))
    IF fa1$ = "g5 " THEN g5 = g5 + 1: x5 = x5 + 1: LSET fg5$ = STR$((VAL(fg5$) + 1))
    IF fa2$ = "g5 " THEN g5 = g5 + 1: x5 = x5 + 1: LSET fg5$ = STR$((VAL(fg5$) + 1))
    IF fa1$ = "g6 " THEN g6 = g6 + 1: x6 = x6 + 1: LSET fg6$ = STR$((VAL(fg6$) + 1))
    IF fa2$ = "g6 " THEN g6 = g6 + 1: x6 = x6 + 1: LSET fg6$ = STR$((VAL(fg6$) + 1))
    IF fa1$ = "g7 " THEN g7 = g7 + 1: x7 = x7 + 1: LSET fg7$ = STR$((VAL(fg7$) + 1))
    IF fa2$ = "g7 " THEN g7 = g7 + 1: x7 = x7 + 1: LSET fg7$ = STR$((VAL(fg7$) + 1))
    IF fa1$ = "g8 " THEN g8 = g8 + 1: x8 = x8 + 1: LSET fg8$ = STR$((VAL(fg8$) + 1))
    IF fa2$ = "g8 " THEN g8 = g8 + 1: x8 = x8 + 1: LSET fg8$ = STR$((VAL(fg8$) + 1))
    IF fa1$ = "g9 " THEN g9 = g9 + 1: x9 = x9 + 1: LSET fg9$ = STR$((VAL(fg9$) + 1))
    IF fa2$ = "g9 " THEN g9 = g9 + 1: x9 = x9 + 1: LSET fg9$ = STR$((VAL(fg9$) + 1))
    IF fa1$ = "g10" THEN g10 = g10 + 1: x10 = x10 + 1: LSET fg10$ = STR$((VAL(fg10$) + 1))
    IF fa2$ = "g10" THEN g10 = g10 + 1: x10 = x10 + 1: LSET fg10$ = STR$((VAL(fg10$) + 1))
    IF fa1$ = "g11" THEN g11 = g11 + 1: x11 = x11 + 1: LSET fg11$ = STR$((VAL(fg11$) + 1))
    IF fa2$ = "g11" THEN g11 = g11 + 1: x11 = x11 + 1: LSET fg11$ = STR$((VAL(fg11$) + 1))
    IF fa1$ = "g12" THEN g12 = g12 + 1: x12 = x12 + 1: LSET fg12$ = STR$((VAL(fg12$) + 1))
    IF fa2$ = "g12" THEN g12 = g12 + 1: x12 = x12 + 1: LSET fg12$ = STR$((VAL(fg12$) + 1))
    IF fa1$ = "g13" THEN g13 = g13 + 1: x13 = x13 + 1: LSET fg13$ = STR$((VAL(fg13$) + 1))
    IF fa2$ = "g13" THEN g13 = g13 + 1: x13 = x13 + 1: LSET fg13$ = STR$((VAL(fg13$) + 1))
    IF fa1$ = "g14" THEN g14 = g14 + 1: x14 = x14 + 1: LSET fg14$ = STR$((VAL(fg14$) + 1))
    IF fa2$ = "g14" THEN g14 = g14 + 1: x14 = x14 + 1: LSET fg14$ = STR$((VAL(fg14$) + 1))
    IF fa1$ = "g15" THEN g15 = g15 + 1: x15 = x15 + 1: LSET fg15$ = STR$((VAL(fg15$) + 1))
    IF fa2$ = "g15" THEN g15 = g15 + 1: x15 = x15 + 1: LSET fg15$ = STR$((VAL(fg15$) + 1))
    IF fa1$ = "g16" THEN g16 = g16 + 1: x16 = x16 + 1: LSET fg16$ = STR$((VAL(fg16$) + 1))
    IF fa2$ = "g16" THEN g16 = g16 + 1: x16 = x16 + 1: LSET fg16$ = STR$((VAL(fg16$) + 1))

    IF fa1$ = "m  " THEN moil = moil + 1: LSET fmoil$ = STR$((VAL(fmoil$) + 1))
    IF fa2$ = "m  " THEN moil = moil + 1: LSET fmoil$ = STR$((VAL(fmoil$) + 1))
    IF fa1$ = "x  " THEN fres = fres + 1: LSET ffre$ = STR$((VAL(ffre$) + 1))
    IF fa2$ = "x  " THEN fres = fres + 1: LSET ffre$ = STR$((VAL(ffre$) + 1))
    IF fa1$ = fa2$ AND fa1$ <> "x  " THEN inbr = inbr + 1: LSET finbr$ = STR$((VAL(finbr$) + 1))
    code% = item: PUT #2, code%
9900 y = y + 2: p = p + 2
9901 IF stop$ = "yes" THEN stop$ = "": GOTO 15500
9902 IF b = 1 THEN GOTO 9908
9903 IF restart = 1 THEN restart = 0: GOTO 9908
9904 LOCATE 9, 3: PRINT "Calculation "; USING "###.###"; (y / (cycles * living)) * 100;
9905 PRINT " % complete"

9908 RETURN

9909 IF printo = 1 THEN GOSUB 14000
    FOR t = 2 TO pointer
    code% = t: GET #1, code%
    IF fg17$ = "f" THEN GOTO 9950
    LSET fa1$ = "  ": LSET fa2$ = "  ": PUT #1, code%
9950 NEXT t
    IF x1 = 0 THEN f11 = f11 + .5
    IF x2 = 0 THEN f11 = f11 + .5
    IF x3 = 0 THEN f21 = f21 + .5
    IF x4 = 0 THEN f21 = f21 + .5
    IF x5 = 0 THEN f31 = f31 + .5
```

```
    IF x6 = 0 THEN f3l = f3l + .5
    IF x7 = 0 THEN f4l = f4l + .5
    IF x8 = 0 THEN f4l = f4l + .5
    IF x9 = 0 THEN f5l = f5l + .5
    IF x10 = 0 THEN f5l = f5l + .5
    IF x11 = 0 THEN f6l = f6l + .5
    IF x12 = 0 THEN f6l = f6l + .5
    IF x13 = 0 THEN f7l = f7l + .5
    IF x14 = 0 THEN f7l = f7l + .5
    IF x15 = 0 THEN f8l = f8l + .5
    IF x16 = 0 THEN f8l = f8l + .5

    IF x1 <= .1 * p AND x1 <> 0 THEN f1d = f1d + .5
    IF x2 <= .1 * p AND x2 <> 0 THEN f1d = f1d + .5
    IF x3 <= .1 * p AND x3 <> 0 THEN f2d = f2d + .5
    IF x4 <= .1 * p AND x4 <> 0 THEN f2d = f2d + .5
    IF x5 <= .1 * p AND x5 <> 0 THEN f3d = f3d + .5
    IF x6 <= .1 * p AND x6 <> 0 THEN f3d = f3d + .5
    IF x7 <= .1 * p AND x7 <> 0 THEN f4d = f4d + .5
    IF x8 <= .1 * p AND x8 <> 0 THEN f4d = f4d + .5
    IF x9 <= .1 * p AND x9 <> 0 THEN f5d = f5d + .5
    IF x10 <= .1 * p AND x10 <> 0 THEN f5d = f5d + .5
    IF x11 <= .1 * p AND x11 <> 0 THEN f6d = f6d + .5
    IF x12 <= .1 * p AND x12 <> 0 THEN f6d = f6d + .5
    IF x13 <= .1 * p AND x13 <> 0 THEN f7d = f7d + .5
    IF x14 <= .1 * p AND x14 <> 0 THEN f7d = f7d + .5
    IF x15 <= .1 * p AND x15 <> 0 THEN f8d = f8d + .5
    IF x16 <= .1 * p AND x16 <> 0 THEN f8d = f8d + .5
    living = p: p = 0
10000 RETURN

10010 REM results menu
    PRINT "                          any key to continue..."
    WHILE INKEY$ = ""
    WEND

10028 CLS : LOCATE 1, 55: PRINT filename$
    LOCATE 7, 8: PRINT "F O U N D E R  R E S U L T S  M E N U"
    LOCATE 9, 8: PRINT "1. Percentage Contribution of Founders to Extant Population"
    LOCATE 10, 8: PRINT "2. Percentage of Each Founder Genome Lost / At Risk of Loss"
    LOCATE 11, 8: PRINT "3. Founder Composition of Animals (and inbreeding)"
    LOCATE 12, 8: PRINT "4. Information to Restart Analysis"
    LOCATE 13, 8: PRINT "5. Return to main menu"
10080 s$ = INKEY$
    IF s$ = "1" THEN GOTO 10200
    IF s$ = "2" THEN GOTO 10300
    IF s$ = "3" THEN GOTO 10500
    IF s$ = "5" THEN CLOSE #2: GOTO 7420
    IF s$ = "4" THEN GOTO 15500
    GOTO 10080
10200 CLS : PRINT "CONTRIBUTION OF FOUNDERS TO EXTANT HERD"
    PRINT : PRINT : PRINT "Founder      Contribution"
    PRINT "f1=        "; USING "###.###_%"; (g1 + g2) / y * 100
    PRINT "f2=        "; USING "###.###_%"; (g3 + g4) / y * 100
```

```
    PRINT "f3=        "; USING "###.###_%"; (g5 + g6) / y * 100
    PRINT "f4=        "; USING "###.###_%"; (g7 + g8) / y * 100
    PRINT "f5=        "; USING "###.###_%"; (g9 + g10) / y * 100
    PRINT "f6=        "; USING "###.###_%"; (g11 + g12) / y * 100
    PRINT "f7=        "; USING "###.###_%"; (g13 + g14) / y * 100
    PRINT "f8=        "; USING "###.###_%"; (g15 + g16) / y * 100

    PRINT "other      "; USING "###.###_%"; fres / y * 100
    PRINT "moiled     "; USING "###.###_%"; moil / y * 100

10270 PRINT : PRINT "any key to return to menu"
    WHILE INKEY$ = ""
    WEND: GOTO 10028

10300 CLS : PRINT "PERCENTAGE OF FOUNDER GENES LOST / AT RISK OF LOSS"
    PRINT : PRINT "Founder    % Lost"
    PRINT "f1         "; USING "###.###_%"; f1l / (b - 1) * 100
    PRINT "f2         "; USING "###.###_%"; f2l / (b - 1) * 100
    PRINT "f3         "; USING "###.###_%"; f3l / (b - 1) * 100
    PRINT "f4         "; USING "###.###_%"; f4l / (b - 1) * 100
    PRINT "f5         "; USING "###.###_%"; f5l / (b - 1) * 100
    PRINT "f6         "; USING "###.###_%"; f6l / (b - 1) * 100
    PRINT "f7         "; USING "###.###_%"; f7l / (b - 1) * 100
    PRINT "f8         "; USING "###.###_%"; f8l / (b - 1) * 100

    PRINT : PRINT "Founder    % At Risk"
    ON ERROR GOTO 10400
    PRINT "f1         "; USING "###.###_%"; f1d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f1d / (b - 1)) / (1 - (f1l / (b - 1))) * 100
    PRINT "f2         "; USING "###.###_%"; f2d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f2d / (b - 1)) / (1 - (f2l / (b - 1))) * 100
    PRINT "f3         "; USING "###.###_%"; f3d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f3d / (b - 1)) / (1 - (f3l / (b - 1))) * 100
    PRINT "f4         "; USING "###.###_%"; f4d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f4d / (b - 1)) / (1 - (f4l / (b - 1))) * 100
    PRINT "f5         "; USING "###.###_%"; f5d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f5d / (b - 1)) / (1 - (f5l / (b - 1))) * 100
    PRINT "f6         "; USING "###.###_%"; f6d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f6d / (b - 1)) / (1 - (f6l / (b - 1))) * 100
    PRINT "f7         "; USING "###.###_%"; f7d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f7d / (b - 1)) / (1 - (f7l / (b - 1))) * 100
    PRINT "f8         "; USING "###.###_%"; f8d / (b - 1) * 100;
    PRINT "   "; USING "_(###.##_%_)"; (f8d / (b - 1)) / (1 - (f8l / (b - 1))) * 100
    ON ERROR GOTO 0
    GOTO 10270

10400 REM trap division by zero error
    PRINT : RESUME NEXT

10500 CLS : PRINT "FOUNDER COMPOSITION OF INDIVIDUALS IN HERD"
    PRINT : PRINT "Enter herd book numbers of individuals"
    PRINT "(Type 'end', when done)"
    PRINT "or hit 'a' for all individuals"
    cowno = 1
```

```
11072 INPUT "individual "; x$
      IF x$ = "a" OR x$ = "A" THEN ret = 0: GOTO 11160
      IF x$ = "end" OR x$ = "END" THEN cowno = cowno - 1: GOTO 11088
      x$ = x$ + "     ": individ$(1) = LEFT$(x$, 5): x = 1: GOSUB 11400
      IF found = 0 THEN PRINT "no record of animal "; x$: GOTO 11072
      c(cowno) = item
      cowno = cowno + 1: GOTO 11072
11088 FOR f2 = 1 TO cowno
      f = c(f2)
      ret = 1: GOSUB 11170
      NEXT f2
      GOTO 10270

11160 FOR f = 2 TO pointer
11170 code% = f: GET #1, code%
      IF fg17$ = "f" THEN GOTO 11275
      IF fl$ = "n" THEN GOTO 11275
      GET #2, code%
      PRINT : PRINT "INDIVIDUAL : "; fi$; "     (inbreeding= "; USING "###.###_%_)"; VAL(finbr$) /
(b - 1) * 100;
      PRINT "  ="; finbr$
      PRINT "f1=        "; USING "###.###_%"; (VAL(fg1$) + VAL(fg2$)) / (2 * (b - 1)) * 100;
      PRINT "     g1: "; fg1$; "g2: "; fg2$
      PRINT "f2=        "; USING "###.###_%"; (VAL(fg3$) + VAL(fg4$)) / (2 * (b - 1)) * 100;
      PRINT "     g3: "; fg3$; "g4: "; fg4$
      PRINT "f3=        "; USING "###.###_%"; (VAL(fg5$) + VAL(fg6$)) / (2 * (b - 1)) * 100;
      PRINT "     g5: "; fg5$; "g6: "; fg6$
      PRINT "f4=        "; USING "###.###_%"; (VAL(fg7$) + VAL(fg8$)) / (2 * (b - 1)) * 100;
      PRINT "     g7: "; fg7$; "g8: "; fg8$
      PRINT "f5=        "; USING "###.###_%"; (VAL(fg9$) + VAL(fg10$)) / (2 * (b - 1)) * 100;
      PRINT "     g9: "; fg9$; "g10:"; fg10$
      PRINT "f6=        "; USING "###.###_%"; (VAL(fg11$) + VAL(fg12$)) / (2 * (b - 1)) * 100;
      PRINT "     g11:"; fg11$; "g12:"; fg12$
      PRINT "f7=        "; USING "###.###_%"; (VAL(fg13$) + VAL(fg14$)) / (2 * (b - 1)) * 100;
      PRINT "     g13:"; fg13$; "g14:"; fg14$
      PRINT "f8=        "; USING "###.###_%"; (VAL(fg15$) + VAL(fg16$)) / (2 * (b - 1)) * 100;
      PRINT "     g15:"; fg15$; "g16:"; fg16$

      PRINT "other      "; USING "###.###_%"; VAL(ffre$) / (2 * (b - 1)) * 100;
      PRINT "     ="; VAL(ffre$)
      PRINT "moiled     "; USING "###.###_%"; VAL(fmoil$) / (2 * (b - 1)) * 100;
      PRINT "     ="; VAL(fmoil$)
      IF f = pointer OR f2 = cowno THEN GOTO 11275
      WHILE INKEY$ = ""
      WEND
11275 IF ret = 1 THEN RETURN
      NEXT f
      GOTO 10270

11400 found = 0
      code% = 1: GET #1, code%
      pointer = CVI(fi$): form$ = fm$: f = 2
11430 code% = f: GET #1, code%
```

```
      IF individ$(x) = fi$ THEN item = f: i$(x) = fi$: m$(x) = fm$: f$(x) = ff$: l$(x) = fl$: s$(x) = fs$:
e$(x) = fe$: d$(x) = fd$: a1$(x) = fa1$: a2$(x) = fa2$: found = 1: RETURN
      IF f < pointer THEN f = f + 1: GOTO 11430
      RETURN

12005 REM printout option
12010 REM WHILE INKEY$ = ""
      REM WEND
      code% = 1: GET #1, code%
      pointer = CVI(fi$): form$ = fm$
      IF header = 1 THEN GOTO 5772
      LPRINT : LPRINT : LPRINT "number extant:"; living / 2
      IF header = 1 THEN RETURN
      FOR f = 2 TO pointer
      code% = f: GET #1, code%
      IF fl$ = "n" THEN GOTO 12160
      LPRINT fi$;
      code% = f: GET #2, code%
      f$ = STR$(f - 1): f$ = f$ + "    ": f$ = LEFT$(f$, 5)
      LPRINT "1"; fg1$; " 2"; fg2$; " 3"; fg3$; " 4"; fg4$; " 5"; fg5$; " 6"; fg6$; " 7"; fg7$; " 8"; fg8$
      LPRINT "    "; "9"; fg9$; " 10"; fg10$; "11"; fg11$; "12"; fg12$; "13"; fg13$; "14"; fg14$; "15";
fg15$; "16"; fg16$
      LPRINT "    "; "f"; ffre$; " m"; fmoil$; " i"; finbr$
12160 NEXT f
      LPRINT : LPRINT "total"
      LPRINT "g1    "; USING "#####"; g1;
      LPRINT "          g9     "; g9
      LPRINT "g2    "; USING "#####"; g2;
      LPRINT "          g10    "; g10
      LPRINT "g3    "; USING "#####"; g3;
      LPRINT "          g11    "; g11
      LPRINT "g4    "; USING "#####"; g4;
      LPRINT "          g12    "; g12
      LPRINT "g5    "; USING "#####"; g5;
      LPRINT "          g13    "; g13
      LPRINT "g6    "; USING "#####"; g6;
      LPRINT "          g14    "; g14
      LPRINT "g7    "; USING "#####"; g7;
      LPRINT "          g15    "; g15
      LPRINT "g8    "; USING "#####"; g8;
      LPRINT "          g16    "; g16
      LPRINT "Other "; USING "#####"; fres;
      LPRINT "          Moiled "; moil
      RETURN

14000 LPRINT "MOILMATE 93                              "; filename$
      LPRINT "Cycle: "; b
      FOR f = 2 TO pointer
      code% = f: GET #1, code%
      LPRINT fi$; fa1$; fa2$; "  ";
      NEXT f
      RETURN

14500 LOCATE 10, 1: PRINT : PRINT "Cycle "; b
```

```
      PRINT "No. Extant "; living / 2
      PRINT "g1    "; USING "#####"; g1;
      PRINT "          g9     "; g9
      PRINT "g2    "; USING "#####"; g2;
      PRINT "          g10    "; g10
      PRINT "g3    "; USING "#####"; g3;
      PRINT "          g11    "; g11
      PRINT "g4    "; USING "#####"; g4;
      PRINT "          g12    "; g12
      PRINT "g5    "; USING "#####"; g5;
      PRINT "          g13    "; g13
      PRINT "g6    "; USING "#####"; g6;
      PRINT "          g14    "; g14
      PRINT "g7    "; USING "#####"; g7;
      PRINT "          g15    "; g15
      PRINT "g8    "; USING "#####"; g8;
      PRINT "          g16    "; g16
      PRINT "Other "; USING "#####"; fres;
      PRINT "          Moiled "; moil
      suma = g1 + g2 + g3 + g4 + g5 + g6 + g7 + g8 + g9
      sumb = g10 + g11 + g12 + g13 + g14 + g15 + g16 + fres + moil
      sumt = suma + sumb
      PRINT "Sum     "; sumt
      RETURN

14999 REM restart
15000 PRINT : INPUT "No. of Cycles Completed"; upto
      INPUT "Value of P"; p
      INPUT "Value of Y"; y
      FOR d = 1 TO 8
      LOCATE 9, 1: PRINT "input D "; d: INPUT dval
      IF d = 1 THEN f1d = dval
      IF d = 2 THEN f2d = dval
      IF d = 3 THEN f3d = dval
      IF d = 4 THEN f4d = dval
      IF d = 5 THEN f5d = dval
      IF d = 6 THEN f6d = dval
      IF d = 7 THEN f7d = dval
      IF d = 8 THEN f8d = dval
      LOCATE 10, 1: PRINT "          "
      NEXT d
      FOR l = 1 TO 8
      LOCATE 10, 1: PRINT "input L "; l: INPUT lval
      IF l = 1 THEN f1l = lval
      IF l = 2 THEN f2l = lval
      IF l = 3 THEN f3l = lval
      IF l = 4 THEN f4l = lval
      IF l = 5 THEN f5l = lval
      IF l = 6 THEN f6l = lval
      IF l = 7 THEN f7l = lval
      IF l = 8 THEN f8l = lval
      LOCATE 11, 1: PRINT "          "
      NEXT l
      INPUT "No. Extant"; extant: living = extant * 2
```

```
    IF results = 1 THEN b = upto: GOTO 15060

15050 PRINT : INPUT "No. of Cycles"; cycles
    IF cycles < upto THEN PRINT "Already completed"; upto - 1; "cycles": GOTO 15050
    REM INPUT "Printout (y/n)"; copy$
    REM IF copy$ = "Y" OR copy$ = "y" THEN printo = 1
15060 g1 = 0: g2 = 0: g3 = 0: g4 = 0: g5 = 0: g6 = 0: g7 = 0: g8 = 0: g9 = 0: g10 = 0
    g11 = 0: g12 = 0: g13 = 0: g14 = 0: g15 = 0: g16 = 0: fres = 0: moil = 0
    GET #1, 1
    pointer = CVI(fi$)
    FOR t = 2 TO pointer
    code% = t: GET #1, code%
    IF fg17$ = "f" THEN GOTO 15170
    GET #2, code%
    g1 = g1 + VAL(fg1$)
    g2 = g2 + VAL(fg2$)
    g3 = g3 + VAL(fg3$)
    g4 = g4 + VAL(fg4$)
    g5 = g5 + VAL(fg5$)
    g6 = g6 + VAL(fg6$)
    g7 = g7 + VAL(fg7$)
    g8 = g8 + VAL(fg8$)
    g9 = g9 + VAL(fg9$)
    g10 = g10 + VAL(fg10$)
    g11 = g11 + VAL(fg11$)
    g12 = g12 + VAL(fg12$)
    g13 = g13 + VAL(fg13$)
    g14 = g14 + VAL(fg14$)
    g15 = g15 + VAL(fg15$)
    g16 = g16 + VAL(fg16$)
    fres = fres + VAL(ffre$)
    moil = moil + VAL(fmoil$)
15170 NEXT t
    IF results = 1 THEN results = 0: GOTO 10010
    restart = 1
    CLS : PRINT "FOUNDER CALCULATIONS": LOCATE 1, 55: PRINT filename$
    GOTO 8500

15500 CLS : PRINT "FOUNDER CALCULATIONS": LOCATE 1, 55: PRINT filename$
    PRINT : PRINT "No. of Cycles Completed = "; b
    PRINT "Value of P = "; p; TAB(20); "  D.."; f1d; f2d; f3d; f4d; f5d; f6d; f7d; f8d
    PRINT "Value of Y = "; y; TAB(20); "  L.."; f1l; f2l; f3l; f4l; f5l; f6l; f7l; f8l
    PRINT "No. Extant = "; living / 2
    PRINT : PRINT "Keep a record of the above values, you'll need"
    PRINT "them to restart founder calculations";
    WHILE INKEY$ = ""
    WEND
    GOSUB 14500
    REM PRINT : INPUT "printout of data (y/n)"; copy$
    REM IF copy$ = "Y" OR copy$ = "y" THEN GOSUB 12010
    PRINT "Hit any key to continue"
    WHILE INKEY$ = ""
    WEND
    GOTO 10028
```

```
16000 CLS : PRINT "Are You Sure?": PRINT
      PRINT "This Will Remove ALL Stored Coefficient Data in This File  ("; filename$; ")"
      PRINT : PRINT "Hit 'w' to WIPE OUT your Precious Data"
      PRINT : PRINT "(or any other key to bottle out!)"
      PRINT : INPUT "The Choice is Yours"; a$
      IF a$ = "W" OR a$ = "w" THEN GOTO 16010
      IF a$ <> "W" AND a$ <> "w" THEN GOTO 7420

16010 code% = 1: GET #1, code%
      pointer = CVI(fi$): form$ = fm$
      FOR f = 2 TO pointer
      code% = f: GET #1, code%

      REM resets inbreeding coefficients to 0
      REM LSET fe$ = "?     ": PUT #1, code%

      REM sets all non-pure to dead
      REM IF fl$ = "n" THEN GOTO 16080
      REM IF LEFT$(fi$, 1) <> "a" AND LEFT$(fi$, 1) <> "b" AND LEFT$(fi$, 1) <> "c" THEN GOTO
16080
      REM LSET fl$ = "n": PUT #1, code%

      REM sets all pure to dead
      REM if fl$="n" then goto 16080
      REM IF LEFT$(fi$, 1) = "a" or LEFT$(fi$, 1) = "b" or LEFT$(fi$, 1) = "c" THEN GOTO 16080
      REM LSET fl$ = "n": PUT #1, code%

      REM alter individual animals
      REM IF LEFT$(fi$, 3) = "c75" OR LEFT$(fi$, 3) = "c77" OR LEFT$(fi$, 4) = "1056" THEN goto
16079
      REM IF LEFT$(fi$, 4) = "1055" OR LEFT$(fi$, 4) = "1049" OR LEFT$(fi$, 4) = "1048" THEN
goto 16079
      REM IF LEFT$(fi$, 3) = "c75" OR LEFT$(fi$, 3) = "c77" OR LEFT$(fi$, 4) = "1056" THEN goto
16079
      REM IF LEFT$(fi$, 3) = "c75" OR LEFT$(fi$, 3) = "c77" OR LEFT$(fi$, 4) = "1056" THEN goto
16079
      REM IF LEFT$(fi$, 3) = "c75" OR LEFT$(fi$, 3) = "c77" OR LEFT$(fi$, 4) = "1056" THEN goto
16079
      REM IF LEFT$(fi$, 3) = "c75" OR LEFT$(fi$, 3) = "c77" OR LEFT$(fi$, 4) = "1056" THEN goto
16079
      REM goto 16080
16079 REM LSET fl$ = "y": PUT #1, code%

16080 NEXT f
      code% = 0: GOTO 7420
```

APPENDIX III

IRISH MOILED BLOOD SAMPLES COLLECTED

SAMPLES TAKEN IN NORTHERN IRELAND
Springfield cattle

Herd Book No.	Name	Sex	d.o.b.	Gel	DNA Conc. (µg/ml)
916	Glenbrook Tulip 2nd	f	'74	gel 2	830
935	Glenbrook 1062	f	'80	gel 2	345
955	Laurelgrange Wisp	f	'80	gel 2	390
983	Maymore Ivor	m	'85	gel 9	435
986	Argory Eva	f	'86	gel 1	695
988	Argory Edith	f	'86	gel 9	285
989	Argory Ethel	f	'86	gel 2	1015
1014	Springfield Sparkle	f	'87	gel 2	650
1018	Argory 58	m	'88	gel 2	530
1023	Glencraig Daffodil	f	'88	gel 1	510
1027	Springfield Mistletoe	f	'88	gel 1	555
1047	Springfield Frolic	f	'89	gel 2	670
1053	Springfield Orchid	f	'89	gel 1	605
1061	Springfield Melody	f	'90	gel 3	390
1073	Springfield Fortune	f	'90	gel 2	425
1074	Springfield Holly	f	'90	gel 2	640
1076	Dal Ereman	m	'90	gel 1	445
1083	Springfield Promise	f	'90	gel 2	660
1091	Springfield Enchantment	f	'91	gel 9	230
1098	Derrymagone Timothy	m	'91	gel 3	650
1117	Springfield Ivy	f	'92	gel 2	455
1118	Springfield Ulsterman	m	'92	gel 2	425
1119	Springfield Snowdrop	f	'92	gel 3	495
1120	Springfield Snowman	m	'92	gel 2	805
C56	Glenbrook 1234	f	'86	gel 2	905
C67	Glenkeen Damson	f	'87	gel 1	580
C69	Springfield Sparkle	f	'88	gel 2	1965

1993 Calves

1018 × C67	gel 9	340 µg/ml
1076 × 916	gel 9	170 µg/ml

Tom Seeds Cattle

960	Glenbrook 1173	f	'84	gel 1	510
1033	Carin Lass	f	'88	gel 1	710

William Shields Cattle

A61	Conlig Buttercup	f	'92	gel 1	585
B43	Shankbridge Nora	f	'87	gel 1	345

Gordon Stockdales Cattle

973	Castledale Primrose	f	'85	gel 9	75
998	Castledale Rosebud	f	'87	gel 1	485
1112	Castledale Star	f	'91	gel 1	545
1133	Castledale Defender	m	'92	gel 1	350
A15	Glenbrook Perky	f	'78	gel 1	640
B70	Castledale Molly	f	'91	gel 1	495
B77	Castledale Dolly	f	'92	gel 9	280
C54	Castledale Snowflake	f	'86	gel 9	155

Belfast Zoo

929	Glenbrook 964	f	'79	gel 4	625
1042	Bellevue Iris	f	'89	gel 4	500

J. Mcfarlane

B62	Broughderg Heifer	f	'91	gel 4	345

Vaughn Byrne

1066	Bellvue Clover	f	'90	gel 4	435

J. Robinson

A45	Kilcoan Maeve	f	'89	gel 4	510
B37	Glenbrook 1245 (Molly)	f	'86	gel 4	380

Ulster Folk and Transport Museum

951	Bellevue Daisy	f	'82	gel 4	1000
1097	Cultra Eilis	f	'91	gel 5	655
1132	Cultra Kate	f	'92	gel 8	250
A5	Glenbrook 794	f	'75	gel 4	405

A. Irwin / A. Murray

1024	Springfield Feather	f	'88	gel 8	270
1121	Rosecorner Patricia	f	'92	gel 5	810
A40	Glenbrook Orange Lil	f	'87	gel 5	510

1993 calf

1018 × A40 gel 9 795 µg/ml

Donaldson

B47	Dungannon Fatima	f	'88	gel 5	325

1993 calf

1018 × B47 gel 9 435 µg/ml

Muckamore College

1043	Greenmount Isobel	f	'89	gel 5	450

Tom Jenkinson

A58	Conlig Josie Bell	f	'91	gel 5	870
B51	Shankbridge Honey	f	'89	gel 5	930

Shanes Castle Estate

B26	Glenbrook Tansy	f	'81	gel 7	310
B52	Laurelgrange Daisy 3rd	f	'89	gel 5	460

R. Swann

964	Glenbrook Tulip 5th	f	'84	gel 7	440
979	Glenbrook 1212	f	'85	gel 7	660
1002	Glenbrook 1274	f	'87	gel 8	240
A33	Glenbrook 1277	f	'87	gel 8	300
B35	Glenbrook 1209	f	'85	gel 5	520
C24	Glenbrook 965	f	'79	gel 5	450
C42	Glenbrook 1178	f	'84	gel 8	310

Laurence Moffatt

B53	Glasswater Rose	f	'90	gel 8	750
C45	Glasswater Una	f	'85	gel 5	1030

1993 calf

1009 × C45	gel 8	700 µg/ml

William McMaster

B46	Glenbrook 1299	f	'88	gel 5	720

Eddie Boyd

982	Beltany Tulip	f	'86	pat.	350
1034	Beltany Lilly	f	'88	gel 8	590
1058	Beltany Bull	f	'89	gel 8	365

1993 calves

1058 × C50	gel 8	555 µg/ml
limosin × 1034	pat.	220 µg/ml
limosin × 982	pat.	150 µg/ml

Eniskillen Agricultural College

1044	Derryhoney Victoria	f	'89	gel 5	810

Phoebe Warnock

1050	Laurelgrange Wych Elm	f	'89	gel 6	1160
1067	Bellevue Owen	m	'90	gel 6	1060
1070	Laurelgrange Aniseed	f	'90	gel 6	1540
1123	Laurelgrange Elm-nut	f	'92	gel 6	1020
A23	Laurelgrange Wych	f	'83	gel 6	280
A41	Laurelgrange Anise	f	'87	gel 6	940
B73	Laurelgrange Primrose	f	'92	gel 6	1650

(Highland bull × A23) gel 6 960 µg/ml

(1067 × Highland cross)	gel 6	540 µg/ml	
1993 calves			
1067 × A23	gel 6	820 µg/ml	
1000 × A23	gel 6	310 µg/ml	

Jack McKearney

944	Glenbrook Belinda	f	'81	gel 6	1100
1057	Derrymagone Pansy	f	'89	gel 8	500

J. and H. Osbourne

1035	Lisa of Knock	f	'88	gel 7	1000
1052	Derryhoney Victoria	f	'89	gel 6	700

S. Smiley

996	Shankbridge Gemini	f	'86	gel 7	1210
1026	Springfield Unique	m	'88	gel 7	410
A17	Glenbrook 1068	f	'80	gel 7	760
A42	Glenbrook Daisy 3rd	f	'86	gel 7	570
A56	Shankbridge Cherry	f	'91	gel 7	870
A62	Glassdrummond Bluebell	f	'92	gel 7	750
B44	Glenbrook 1286	f	'88	gel 7	1090

SAMPLES TAKEN IN ENGLAND

Temple Newsam, Leeds

918	Glenbrook 779	f	'74	gel 10	595
950	Glenbrook Catherine	f	'82	gel 11	1675
967	Glenbrook 1198	f	'84	gel 11	1060
976	Glenbrook 1219	f	'85	gel 11	2120
1036	Templeson Sorrel	f	'88	gel 10	895
1037	Templeson Coltsfoot	f	'88	gel 10	740
1039	Templeson Myrrhie	f	'89	gel 11	1925
1040	Templeson Tansy	f	'88	gel 10	1970
1046	Templeson Aughra	f	'89	gel 11	300
1054	Templeson Cornelian	m	'89	gel 12	730
1062	Templeson Grainne	f	'90	gel 11	2025
1063	Templeson Ciara	f	'90	gel 11	1285
1101	Templeson Serin	m	'91	gel 12	1000
1102	Templeson Grebe	f	'91	gel 10	1525
1125	Templeson Porcelain	f	'92	gel 9	575
1126	Templeson Cherry	f	'92	gel 10	1775
1127	Templeson Sienna	f	'92	gel 10	195(?)
1128	Templeson Turquoise	f	'92	gel 10	270
B27	Belle Vue Foxglove	f	'81	gel 11	1320
B56	Templeson Pandora	f	'90	gel 10	1635
B58	Templeson Aurora	f	'90	gel 12	1000
B66	Templeson Auklet	f	'91	gel 11	230
B72	Templeson Fawn	f	'92	gel 10	435
B74	Templeson Tawny	f	'92	gel 10	695
C43	Glenbrook Annie	f	'87	gel 13	1865
C62	Templeson Floss	f	'87	gel 12	750
C63	Templeson Poppy	f	'87	gel 13	845
C64	Templeson Autumn Rose	f	'87	gel 11	1825
C65	Templeson Rosehip	f	'87	gel 11	3050
C70	Templeson Lime	f	'88	gel 12	650
C74	Templeson Lavender	f	'88	gel 12	895
C76	Templeson Topaz	f	'89	gel 12	1140

1993 Calves (Sire: 1054)

T. Linden (f)	Dam: C70	gel 10	710 µg/ml
T. Ricicle (m)	Dam: C65	gel 10	2225 µg/ml
T. Brioche (f)	Dam: B27	gel 11	845 µg/ml
T .Cupcake(f)	Dam: 1063	gel 11	290 µg/ml
T. Granelli (m)	Dam: 1062	gel 12	1800 µg/ml
T. Polo (f)	Dam: 967	gel 12	670 µg/ml
T. Tiramisiu(f)	Dam: C67	gel 12	550 µg/ml
T. Soufle(f)	Dam: 976	gel 12	740 µg/ml
T. Caramac (m)	Dam: 1036	gel 12	340 µg/ml

Croxteth Country Park, Liverpool

928	Glenbrook Tulip 3rd	f	'78	gel 3	385
930	Glenbrook Tulip 4th	f	'79	gel 3	605
941	Glenbrook 1027	f	'81	gel 4	505
959	Bradfield Katerine	f	'83	gel 3	335
991	Croxteth Anne	f	'86	gel 3	1210
997	Myra Silken Thomas	m	'86	gel 9	245
1006	Croxteth Alison	f	'86	gel 3	525
1017	Croxteth Carol	f	'88	gel 13	1000
1092	Croxteth Fenella	f	'91	gel 4	1065
1093	Croxteth Forum	m	'91	gel 3	635
1131	Croxteth Gillian	f	'92	gel 4	335
A55	Croxteth Francesca	f	'91	gel 4	935
B30	Bellevue Cyclamen	f	'82	gel 3	420
B50	Croxteth Diana	f	'89	gel 4	375
B61	Croxteth Felicity	f	'91	gel 3	840
C57	Maymore Nimrod	m	'86	gel 13	1000
C79	Croxteth Daisy	f	'89	gel 3	490

1993 calves

997 × 991	gel 13	770 µg/ml
997 × c79	gel 13	515 µg/ml
997 × B30	gel 13	550 µg/ml
997 × 1006	gel 13	1000 µg/ml

Dorset Rare Breeds Centre

941	Glenbrook 1027	f	'81	gel 14	785
1012	Argory 52	f	'87	gel 14	585
1038	Dal Conchobar	m	'88	gel 14	1110

National Trust, Wimpole

1004	Argory Adeline	f	'87	gel 13	620
1065	Wimpole Abigail	f	'90	gel 13	1288
1108	Dal Finvarra	m	'91	gel 14	3160
B55	Croxteth Eileen	f	'90	gel 13	930

1993 calves

999 × 1065	gel 13	2885 µg/ml
999 × B55	gel 14	720 µg/ml
999 × 1004	gel 14	1390 µg/ml

J. Cloke

1077	Dal Eriu	f	'90	gel 15	1160
1111	Hallmark Patrick	m	'91	gel 15	2910

1993 calves

1141	gel 15	2740 µg/ml
1143	gel 15	720 µg/ml

B. Landshoff

B63	Templeson Linnet	f	'91	gel 15	970 µg/ml
B76	Sandhall Leila	f	'92	gel 15	820

1993 calf

B84	gel 15	680 µg/ml

APPENDIX IV

THE MOILED92.DAT DATA FILE

The moiled92.dat data file contains pedigree information for all Irish Moiled cattle listed in the 1992 Irish Moiled Herd Book. The inbreeding coefficients, calculated using the Moilmate computer programme, are also listed.

Item : This is the position in the data file at which an individual is listed. Individuals are listed in order of date of birth.

Name/Male Parent/Female Parent : Each individual and it's male and female parents are generally identified by their herd book number. In cases where the herd book number was unknown, animals were identified by ear tag numbers (eg item 19, "116SR"), name (eg item 29, "sue"), or a reference code (eg item 30, "pbc" - pure bred cow). A blank entry under male/female parent indicates that the parent was unknown or non-Irish Moiled. The entry "M" under male/female parent indicates that the parent was a pure registered, but unknown Irish Moiled animal.

Alive? : Animals from which a blood sample had been obtained, and all bulls for which semen straws were available, were listed as alive ("y"). All other animals were listed as dead ("n").

Sex : Sex was recorded as either male (m), or female (f).

Inbr. Coeff. : Calculated inbreeding coefficients for each animal are given. Blank entries for inbreeding coefficients occour where one or other of an individuals parents was unknown or non-Irish Moiled. A blank entry in this column indicates an inbreeding coefficient of zero.

Year Born : Dates of birth for animals born before 1958 were not given in the 1992 Irish Moiled Herd Book. Individuals born before this date were all listed as being born in 1950.

individuals in set: 476

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles	
1	783			n	f		1950	g1	g2
2	792			n	f		1950	g3	g4
3	762			n	m		1950	g5	g6
4	798			n	f		1950	g7	g8
5	788			n	f		1950	g9	g10
6	786			n	m		1950	g11	g12
7	790			n	f		1950	g13	g14
8	723			n	f		1950	g15	g16
9	731			n	f		1950		
10	751			n	f		1950		
11	768			n	f		1950		
12	766			n	f		1950		
13	820			n	m		1950		
14	777			n	m		1950		
15	804			n	f		1950		
16	802			n	f		1950		
17	814			n	m		1950		
18	836			n	f		1950		
19	116SR			n	f		1950		
20	114SR			n	f		1950		
21	99SR			n	f		1950		
22	808			n	f		1950		
23	810			n	f		1950		
24	795			n	f		1950		
25	98SR			n	f		1950		
26	112SR			n	f		1950		
27	799			n	f		1950		
28	g812			n	f		1950		
29	sue			n	f		1950		
30	pbc	M	723	n	f		1955		
31	878	786	790	n	m	.0000	1958		
32	877	786	723	n	m	.0000	1958		
33	857	762	751	n	f	.0000	1958		
34	867	777	804	n	f	.0000	1958		
35	863	762	802	n	f	.0000	1958		
36	862	814	836	n	f	.0000	1958		
37	860	762	116SR	n	f	.0000	1958		
38	859	762	114SR	n	f	.0000	1958		
39	858	762	798	n	f	.0000	1958		
40	845	814	768	n	f	.0000	1958		
41	856	814	99SR	n	f	.0000	1958		
42	844	814	808	n	f	.0000	1958		
43	842	777	783	n	f	.0000	1958		
44	840	814	810	n	f	.0000	1958		
45	866	762	98SR	n	m	.0000	1958		
46	864	762	795	n	m	.0000	1958		
47	861	762	112SR	n	m	.0000	1958		
48	843	777	112SR	n	m	.0000	1958		
49	841	777	799	n	m	.0000	1958		
50	879	786	788	n	f	.0000	1959		
51	884	762	798	n	m	.0000	1959		
52	882	877	pbc	n	f	.1250	1959		

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
53	889	786	792	n	f	.0000	1959	
54	881	820	766	n	m	.0000	1959	
55	886	762	783	n	m	.0000	1960	
56	880	786	792	n	f	.0000	1960	
57	887	762	768	n	m	.0000	1960	
58	885	762	731	n	m	.0000	1960	
59	c4	878		n	f		1961	
60	c3	878		n	f		1961	
61	c5	878		n	f		1961	
62	c6	878		n	f		1961	
63	c1	878		n	f		1961	
64	888	884	783	n	m	.0000	1961	
65	c2	878		n	f		1961	
66	890	884	783	n	m	.0000	1962	
67	892	877	882	n	m	.3125	1962	
68	891	884	798	n	m	.2500	1962	
69	899	878	798	n	f	.0000	1963	
70	900	878	857	n	f	.0000	1963	
71	b1	890	c3	n	f	.0000	1964	
72	902	878	798	n	f	.0000	1964	
73	894	886	792	n	m	.0000	1964	
74	b2	890	c1	n	f	.0000	1964	
75	901	878	783	n	m	.0000	1964	
76	c7	878		n	f		1964	
77	906	892	M	n	f		1965	
78	905	878	783	n	f	.0000	1965	
79	904	890	879	n	f	.0000	1965	
80	907	878	798	n	f	.0000	1965	
81	b5	890	c6	n	f	.0000	1965	
82	b3	890	c3	n	f	.0000	1965	
83	903	890	880	n	f	.0000	1965	
84	893	892	M	n	f		1965	
85	c8	878		n	f		1965	
86	b4	890	c2	n	f	.0000	1965	
87	b6	894	c4	n	f	.0000	1966	
88	908	894	783	n	m	.1250	1966	
89	895	892	M	n	m		1966	
90	c10	878		n	f		1966	
91	c9	894		n	f		1966	
92	909	894	798	n	m	.0000	1966	
93	(%M1)	M		n	m		1966	
94	(%M2)	M		n	m		1966	
95	b7	894	c5	n	f	.0000	1967	
96	c11		M	n	f		1967	
97	b8	(%M1)	M	n	f		1967	
98	b9	(%M2)	M	n	f		1967	
99	910	895	M	n	f		1969	
100	911	908	904	n	f	.0859	1969	
101	c12	894		n	f		1969	
102	a1	908	b3	n	f	.0859	1970	
103	914	908	902	n	m	.0000	1971	
104	913	908	904	n	f	.0859	1971	
105	b10	894	c10	n	f	.0000	1971	
106	b11	908	c9	n	f	.1563	1971	
107	912	908	899	n	f	.0000	1971	
108	915	908	879	n	f	.0000	1972	
109	b12	908	c12	n	f	.1563	1972	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
110	b13	908	c6	n	f	.0000	1973	
111	918	914	911	y	f	.1777	1974	
112	916	908	907	y	f	.0000	1974	
113	a4	914	b7	n	f	.1094	1974	
114	c47			n	f		1974	
115	a3	914	b2	n	f	.0898	1974	
116	917	914	903	n	f	.0898	1974	
117	a2	914	b3	n	f	.0898	1974	
118	a5	914	b5	y	f	.0898	1975	
119	920	914	a1	n	f	.1855	1975	
120	919	914	911	n	f	.1777	1975	
121	a6	914	b6	n	m	.1094	1976	
122	c15		M	n	m		1976	
123	a8	914	b1	n	f	.0898	1976	
124	923	914	905	n	f	.1406	1976	
125	922	914	907	y	m	.1250	1976	
126	a7	914	b7	n	f	.1094	1976	
127	c13	914		n	f		1976	
128	921	914	911	n	m	.1777	1976	
129	c14		M	n	m		1976	
130	b14	914	c7	n	f	.0625	1976	
131	a9	914	b6	n	f	.1094	1977	
132	a10	914	b7	n	f	.1094	1977	
133	a11	914	b11	n	f	.1797	1977	
134	924	914	907	n	m	.1250	1977	
135	c16	914		n	f		1977	
136	c17	914		n	f		1977	
137	c18	914		n	f		1977	
138	b15	914	c7	n	f	.0625	1977	
139	b16	c14	893	n	f	.0000	1977	
140	925	a6	906	y	m	.0117	1978	
141	c21	c15	a8	n	f	.0000	1978	
142	b19	c15	923	n	f	.0000	1978	
143	926	914	915	n	m	.1563	1978	
144	928	914	907	y	f	.1250	1978	
145	a13	914	b10	n	f	.1094	1978	
146	c20	c15	b2	n	f	.0000	1978	
147	a15	914	b6	y	f	.1094	1978	
148	927	914	913	n	m	.1777	1978	
149	c19	914		n	f		1978	
150	c22	c14	b9	n	f	.0000	1978	
151	c23	c14	c11	n	f	.0000	1978	
152	b17	a6	b8	n	m	.0000	1978	
153	b18	c15	923	n	m	.0000	1978	
154	a12	914	b1	n	f	.0898	1978	
155	929	914	918	y	f	.3389	1979	
156	930	914	907	y	f	.1250	1979	
157	931	914	a4	y	f	.3047	1979	
158	c24	922		y	f		1979	
159	c25	922		n	f		1979	
160	c26	922	g812	n	f	.0000	1979	
161	b20	c15	919	n	f	.0000	1979	
162	b21	922	c16	n	f	.1563	1979	
163	b22	a6	b9	n	f	.0000	1979	
164	a16	922	b12	n	m	.0898	1980	
165	934	922	918	n	f	.2085	1980	
166	935	922	923	y	f	.2227	1980	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
167	937	922	a7	n	f	.1992	1980	
168	a17	922	b2	y	f	.0918	1980	
169	b23	922	c13	n	f	.1563	1980	
170	c29	922		n	f		1980	
171	936	922	920	n	f	.2144	1980	
172	933	922	916	n	m	.2266	1980	
173	932	922	912	n	m	.1641	1980	
174	c27	922		n	f		1980	
175	c28	922	g812	n	f	.0000	1980	
176	c30	922		n	f		1980	
177	c31	922		n	f		1980	
178	944	922	a9	y	f	.1992	1981	
179	b27	926	c21	y	f	.1006	1981	
180	939	922	918	y	m	.2085	1981	
181	940	922	913	n	m	.1045	1981	
182	945	926	a7	n	f	.2148	1981	
183	943	926	928	n	f	.2031	1981	
184	941	926	a13	y	f	.2148	1981	
185	942	922	a11	n	f	.2012	1981	
186	b26	926	c20	y	f	.0371	1981	
187	947	926	a15	n	f	.2148	1981	
188	c48	922		n	f		1981	
189	946	922	a3	n	f	.2021	1981	
190	938	926	907	n	m	.0781	1981	
191	c32	922		n	f		1981	
192	c33	922	c47	n	f	.0000	1981	
193	c34	922		n	f		1981	
194	b24	925	c11	n	m	.0000	1981	
195	b25	925	c23	n	m	.0000	1981	
196	a18	922	b12	n	m	.0898	1981	
197	a19	922	b6	n	m	.0859	1981	
198	a20	925	b22	n	m	.1416	1981	
199	a31	925	b8	n	m	.0000	1981	
200	a21	922	b19	n	f	.1113	1982	
201	950	926	918	y	f	.2646	1982	
202	948	922	910	n	m	.0088	1982	
203	b30	a16	c21	y	f	.0798	1982	
204	951	922	a5	y	f	.2021	1982	
205	c49		c47	n	f		1982	
206	949	922	928	n	m	.3125	1982	
207	c35	926		n	f		1982	
208	c36	926		n	f		1982	
209	c37	b18	c17	n	f	.0801	1982	
210	b28	926	c24	n	f	.1016	1982	
211	b29	926	c16	n	f	.1641	1982	
212	957	922	913	y	f	.1045	1983	
213	952	939	937	n	m	.2979	1983	
214	959	a16	941	y	f	.1855	1983	
215	955	939	935	y	f	.3074	1983	
216	a23	925	b26	y	f	.0686	1983	
217	b31	939	c29	n	f	.1927	1983	
218	956	939	a17	n	f	.2411	1983	
219	958	940	934	n	f	.2797	1983	
220	c38	932		n	f		1983	
221	c39	922		n	f		1983	
222	c40		916	n	f		1983	
223	a22	939	b23	n	f	.2742	1983	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
224	a32	b24	910	n	f	.0220	1983	
225	966	939	a9	n	f	.2102	1984	
226	967	939	917	y	f	.2102	1984	
227	968	940	928	n	m	.2085	1984	
228	965	a16	941	n	m	.1855	1984	
229	962	925	931	n	f	.1367	1984	
230	994	939	942	n	f	.3096	1984	
231	a25	925	b26	n	m	.0686	1984	
232	c52	922		n	f		1984	
233	960	939	a13	y	f	.2102	1984	
234	964	939	916	y	f	.2085	1984	
235	c43	939		y	f		1984	
236	c42	939		y	f		1984	
237	c51	922		n	f		1984	
238	963	939	945	n	f	.2220	1984	
239	961	925	947	n	f	.1372	1984	
240	c41	939		n	f		1984	
241	a26	939	b14	n	f	.1960	1984	
242	996	925	994	y	f	.1131	1985	
243	985	925	944	y	m	.1333	1985	
244	a30	939	b27	n	f	.1697	1985	
245	a29	939	b19	n	m	.1146	1985	
246	976	940	945	y	f	.1853	1985	
247	969	925	928	y	m	.1084	1985	
248	983	922	910	y	m	.0088	1985	
249	971	940	930	n	f	.2085	1985	
250	973	925	931	y	f	.1367	1985	
251	975	940	929	n	f	.2355	1985	
252	974	925	931	y	f	.1367	1985	
253	972	925	943	n	f	.1123	1985	
254	977	968	a23	n	f	.1128	1985	
255	979	940	920	y	f	.2063	1985	
256	c45	952		y	f		1985	
257	c53	940		n	f		1985	
258	b35	940	c48	y	f	.1667	1985	
259	978	940	916	n	f	.2021	1985	
260	970	968	935	n	m	.2639	1985	
261	c46	952		n	f		1985	
262	c50	940		n	f		1985	
263	b36	940	c48	n	f	.1667	1985	
264	986	948	957	y	f	.1704	1986	
265	997	925	943	y	m	.1123	1986	
266	992	925	916	n	f	.0840	1986	
267	991	925	916	y	f	.0840	1986	
268	1006	a16	941	y	f	.1855	1986	
269	990	925	916	y	f	.0840	1986	
270	c57		910	n	m		1986	
271	a35	968	b30	n	m	.1575	1986	
272	988	948	923	y	f	.1157	1986	
273	989	948	907	y	f	.1621	1986	
274	993	a25	955	n	f	.1243	1986	
275	c56	940		y	f		1986	
276	b39	a25	c52	n	f	.0582	1986	
277	982	925	951	y	f	.1042	1986	
278	1007	948	962	n	m	.1035	1986	
279	c55		931	n	f		1986	
280	c54		931	y	f		1986	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
281	b37	940	c48	y	f	.1667	1986	
282	a42	940	b23	y	f	.2280	1986	
283	987	940	a13	n	f	.1685	1986	
284	981	940	956	n	f	.2488	1986	
285	980	940	920	n	f	.2063	1986	
286	b38	925	c38	n	f	.0481	1986	
287	1003	939	a30	n	m	.3870	1987	
288	c61	939		y	f		1987	
289	c62	939		y	f		1987	
290	c63	939		y	f		1987	
291	c64	939		y	f		1987	
292	c65	939		y	f		1987	
293	1005	985	944	y	m	.3665	1987	
294	1019	985	986	y	m	.1532	1987	
295	a34	939	b27	n	f	.1697	1987	
296	c67	a25		y	f		1987	
297	995	925	994	n	f	.1131	1987	
298	1014	925	951	y	f	.1042	1987	
299	1011	925	a15	y	m	.1582	1987	
300	1000	948	975	n	m	.1518	1987	
301	a41	969	b26	y	f	.0966	1987	
302	999	948	a21	y	m	.1718	1987	
303	998	948	974	y	f	.1035	1987	
304	1010	969	a23	n	m	.2018	1987	
305	1004	985	928	y	f	.1821	1987	
306	1012	985	957	y	f	.1713	1987	
307	1009	925	937	n	m	.1118	1987	
308	1020	a25	935	n	m	.1218	1987	
309	1013	985	934	y	f	.2051	1987	
310	1008	948	962	n	m	.1035	1987	
311	1002	969	a17	y	f	.1387	1987	
312	1001	969	964	n	m	.1709	1987	
313	c60	925		n	f		1987	
314	b43	925	c52	y	f	.0542	1987	
315	a40	969	b31	y	f	.1458	1987	
316	a33	969	b23	y	f	.1641	1987	
317	c66	925		n	f		1987	
318	a14	914	b13	n	f	.1719	1987	
319	1040	939	950	y	f	.3163	1988	
320	1036	939	976	y	f	.2509	1988	
321	1037	939	966	y	f	.4072	1988	
322	c73	939		n	f		1988	
323	c74	939		y	f		1988	
324	1026	983	989	y	m	.2161	1988	
325	1017	968	941	y	f	.1932	1988	
326	1038	948	962	y	m	.1035	1988	
327	1023	925	951	y	f	.1042	1988	
328	1018	985	934	y	m	.2501	1988	
329	1027	983	935	y	f	.2007	1988	
330	1024	983	986	y	f	.2202	1988	
331	c69		955	y	f		1988	
332	1025	948	929	n	f	.1332	1988	
333	1035	983	988	n	f	.1929	1988	
334	1034	948	982	y	f	.1355	1988	
335	1033	925	960	y	f	.1138	1988	
336	1032	925	994	n	m	.1131	1988	
337	1031	948	931	y	f	.1309	1988	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
338	1030	985	944	n	m	.3665	1988	
339	1029	983	977	n	m	.1181	1988	
340	1028	983	a23	n	m	.0707	1988	
341	1022	925	947	n	m	.1372	1988	
342	1021	969	a13	n	m	.1572	1988	
343	b47	925	c53	y	f	.0458	1988	
344	b46	969	c42	y	f	.0932	1988	
345	b44	969	c49	y	f	.0000	1988	
346	c68	925	sue	n	f	.0000	1988	
347	b45	969	c16	n	f	.1177	1988	
348	c70	939		y	f		1988	
349	c75	939		n	f		1989	
350	c76	939		y	f		1989	
351	c77	939		n	f		1989	
352	1054	939	950	y	m	.3163	1989	
353	1046	939	966	y	f	.4072	1989	
354	1039	939	a30	y	f	.3870	1989	
355	c79	c57	b30	y	f	.0018	1989	
356	b50	c57	991	y	f	.0125	1989	
357	1053	983	989	y	f	.2161	1989	
358	1047	983	986	y	f	.2202	1989	
359	1052	983	955	y	f	.1985	1989	
360	1050	999	a23	y	f	.0882	1989	
361	1044	939	947	y	f	.2220	1989	
362	1060	1010	962	y	f	.2343	1989	
363	1058	948	982	y	m	.1355	1989	
364	1057	939	944	y	f	.2979	1989	
365	1056	948	974	n	m	.1035	1989	
366	1055	939	988	n	m	.2128	1989	
367	1051	999	994	y	f	.2219	1989	
368	1049	999	a41	n	m	.1149	1989	
369	1048	1005	928	n	m	.2190	1989	
370	1045	1009	996	n	m	.2573	1989	
371	1043	948	972	y	f	.1047	1989	
372	1042	948	975	y	f	.1518	1989	
373	c80		977	y	f		1989	
374	b52	1010	c20	y	f	.0891	1989	
375	b51	948	c60	y	f	.0381	1989	
376	a45	939	b37	y	f	.2362	1989	
377	c71	948	sue	n	f	.0000	1989	
378	c72	968		n	f		1989	
379	c78	1009		n	f		1989	
380	1064	1019	976	y	f	.1820	1990	
381	b56	1019	c63	y	f	.1108	1990	
382	b57	1019	c62	n	f	.1108	1990	
383	b58	1019	c64	y	f	.1108	1990	
384	1067	948	929	y	m	.1332	1990	
385	1063	1019	950	y	f	.1622	1990	
386	1062	1019	966	y	f	.2116	1990	
387	c84	c57	b30	n	f	.0018	1990	
388	b55	c57	991	n	f	.0125	1990	
389	b54	c57	1006	n	f	.0029	1990	
390	1076	1038	995	y	m	.1960	1990	
391	1084	1000	1014	n	f	.1604	1990	
392	1083	983	916	y	f	.1162	1990	
393	1082	983	934	n	m	.1964	1990	
394	1074	1018	989	y	f	.1644	1990	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
395	1073	1018	986	y	f	.1956	1990	
396	1068	983	935	n	m	.2007	1990	
397	1061	983	934	y	f	.1964	1990	
398	a49	1018	b39	y	f	.1625	1990	
399	1081	999	1004	y	f	.1678	1990	
400	1080	999	1004	y	f	.1678	1990	
401	1079	999	994	n	m	.2219	1990	
402	1078	1010	1025	n	m	.1357	1990	
403	1077	1010	962	y	f	.2343	1990	
404	1075	1011	998	y	f	.1775	1990	
405	1071	969	1012	y	f	.2004	1990	
406	1070	1029	a41	y	f	.1539	1990	
407	1069	922	996	y	f	.2460	1990	
408	1066	948	929	y	f	.1332	1990	
409	1065	1005	957	y	f	.2111	1990	
410	b53	1009	c45	y	f	.1402	1990	
411	1072	1019	967	n	m	.1828	1990	
412	b63	1019	c74	y	f	.1108	1991	
413	b64	1019	c61	n	f	.1108	1991	
414	b65	1019	c63	y	f	.1108	1991	
415	b66	1019	c64	y	f	.1108	1991	
416	1106	1019	918	n	m	.1661	1991	
417	1105	1019	950	y	f	.1622	1991	
418	1104	1019	1037	n	m	.2166	1991	
419	1103	1019	967	n	m	.1828	1991	
420	1102	1019	966	y	f	.2116	1991	
421	1101	1019	1036	y	m	.2018	1991	
422	1100	1019	1040	n	m	.1919	1991	
423	1099	1019	976	y	f	.1820	1991	
424	1093	925	992	y	m	.2949	1991	
425	1092	997	991	y	f	.2263	1991	
426	b61	997	c79	y	f	.0618	1991	
427	a55	997	b30	y	f	.1104	1991	
428	1098	939	944	y	m	.2979	1991	
429	1091	983	955	y	f	.1985	1991	
430	1113	922	1044	n	f	.2933	1991	
431	1112	1011	998	y	f	.1775	1991	
432	1111	1010	962	y	m	.2343	1991	
433	1109	997	990	y	f	.2263	1991	
434	1108	1010	1025	y	m	.1357	1991	
435	1107	1010	995	y	m	.2264	1991	
436	1097	939	951	y	f	.2983	1991	
437	1096	999	1004	y	f	.1678	1991	
438	1095	1018	1024	y	m	.1807	1991	
439	b62	939	c55	y	f	.1340	1991	
440	a56	922	b51	y	f	.1699	1991	
441	b70	1011	c54	y	f	.0996	1991	
442	a57	948		y	f		1991	
443	a58	1009	b43	y	f	.2156	1991	
444	b72	1005	c62	y	f	.1257	1992	
445	b74	1005	c64	y	f	.1257	1992	
446	b75	1005	c65	n	f	.1257	1992	
447	a64	1005	b56	y	f	.2265	1992	
448	1130	1005	966	y	f	.2869	1992	
449	1129	1005	966	n	m	.2869	1992	
450	1128	1005	1040	y	f	.2198	1992	
451	1127	1005	1036	y	f	.2249	1992	

Item	Name	Male Parent	Female Parent	Alive?	Sex	Inbr. Coeff.	Year Born	Founder Alleles
452	1126	1005	950	y	f	.1880	1992	
453	1125	1005	976	y	f	.1984	1992	
454	1124	1005	1037	n	m	.2692	1992	
455	1131	997	959	y	f	.1763	1992	
456	1136	1076	986	y	m	.1953	1992	
457	1135	1076	1023	y	f	.2235	1992	
458	1120	1018	988	y	m	.1816	1992	
459	1119	1018	1053	y	f	.1651	1992	
460	1118	1018	1047	y	m	.1807	1992	
461	1117	1018	1027	y	f	.2110	1992	
462	1134	1011	1023	y	f	.2405	1992	
463	1133	922	973	y	m	.1321	1992	
464	1132	939	951	y	f	.2983	1992	
465	1123	1000	1050	y	f	.1800	1992	
466	1122	1000	a41	y	f	.1269	1992	
467	1121	1018	1024	y	f	.1807	1992	
468	b77	1011	c51	y	f	.0769	1992	
469	b73	1000	c80	y	f	.0853	1992	
470	a63	1058	b62	n	f	.1409	1992	
471	a62	922	b44	y	f	.1052	1992	
472	a61	922	b43	y	f	.1948	1992	
473	b76	969	c74	y	f	.0932	1992	
474	1137	1011	916	y	f	.1201	1992	
475	1138	1076	916	y	m	.1352	1992	
476	1139	1076	989	y	m	.1719	1992	

APPENDIX V

FOUNDER COMPOSITION OF INDIVIDUAL ANIMALS

The following pages are a direct printout, from the moilmate program, of the founder composition results after 1000 cycles of gene-dropping simulation. The estimated percentage contribution of each of the eight founders to the 178 Irish Moiled animals listed as alive in the moiled92.dat data file is given. The inbreeding coefficient of the pure registered animals, estimated by gene-dropping, is also shown.

f1 =	Ballydugan Kat	783
f2 =	Miss Nugent	792
f3 =	Ballydugan Duke	762
f4 =	Ballydugan Mimosa	798
f5 =	Listerdonan	788
f6 =	Maymore VI	786
f7 =	Derylecka	790
f8 =	Derryboy Cyclamen	723
other =	non-Irish Moiled	
moiled =	pure registered but unknown Irish Moiled	

INDIVIDUAL : 918
(inbreeding= 17.200%)
f1= 37.550%
f2= 12.550%
f3= 8.750%
f4= 16.050%
f5= 5.450%
f6= 13.300%
f7= 6.350%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 916
(inbreeding= 0.000%)
f1= 31.250%
f2= 13.450%
f3= 5.300%
f4= 25.200%
f5= 0.000%
f6= 11.950%
f7= 12.850%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 922
(inbreeding= 12.400%)
f1= 15.200%
f2= 6.700%
f3= 2.750%
f4= 37.950%
f5= 0.000%
f6= 17.800%
f7= 19.600%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 925
(inbreeding= 0.800%)
f1= 10.150%
f2= 10.700%
f3= 4.500%
f4= 5.650%
f5= 0.000%
f6= 14.650%
f7= 6.050%
f8= 11.950%
other 6.700%
moiled 29.650%

INDIVIDUAL : 928
(inbreeding= 12.300%)
f1= 15.850%
f2= 6.100%
f3= 2.550%
f4= 36.550%
f5= 0.000%
f6= 19.600%
f7= 19.350%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 929
(inbreeding= 33.000%)
f1= 33.350%
f2= 12.250%
f3= 7.700%
f4= 20.450%
f5= 3.000%
f6= 13.750%
f7= 9.500%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 930
(inbreeding= 13.200%)
f1= 14.900%
f2= 7.450%
f3= 2.650%
f4= 36.950%
f5= 0.000%
f6= 19.100%
f7= 18.950%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 931
(inbreeding= 31.600%)
f1= 24.850%
f2= 15.600%
f3= 7.700%
f4= 19.950%
f5= 0.000%
f6= 13.050%
f7= 12.650%
f8= 0.000%
other 6.200%
moiled 0.000%

INDIVIDUAL : 935
(inbreeding= 24.400%)
f1= 28.000%
f2= 6.400%
f3= 2.750%
f4= 24.950%
f5= 0.000%
f6= 18.350%
f7= 19.550%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 944
(inbreeding= 21.000%)
f1= 18.200%
f2= 12.350%
f3= 6.750%
f4= 26.750%
f5= 0.000%
f6= 15.050%
f7= 15.200%
f8= 0.000%
other 5.700%
moiled 0.000%

INDIVIDUAL : 939
(inbreeding= 20.200%)
f1= 26.000%
f2= 9.700%
f3= 5.450%
f4= 27.950%
f5= 2.900%
f6= 15.500%
f7= 12.500%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 941
(inbreeding= 21.500%)
f1= 24.500%
f2= 16.700%
f3= 8.050%
f4= 11.850%
f5= 7.200%
f6= 16.400%
f7= 9.500%
f8= 0.000%
other 5.800%
moiled 0.000%

INDIVIDUAL : 950
(inbreeding= 24.700%)
f1= 32.300%
f2= 12.800%
f3= 7.950%
f4= 13.700%
f5= 9.750%
f6= 16.300%
f7= 7.200%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 951
(inbreeding= 19.000%)
f1= 20.350%
f2= 6.900%
f3= 6.750%
f4= 28.200%
f5= 0.000%
f6= 14.350%
f7= 16.450%
f8= 0.000%
other 7.000%
moiled 0.000%

INDIVIDUAL : 957
(inbreeding= 10.900%)
f1= 28.800%
f2= 10.200%
f3= 7.850%
f4= 21.800%
f5= 6.550%
f6= 14.650%
f7= 10.150%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 959
(inbreeding= 19.300%)
f1= 24.650%
f2= 17.650%
f3= 7.550%
f4= 14.900%
f5= 3.100%
f6= 12.800%
f7= 9.350%
f8= 0.000%
other 10.000%
moiled 0.000%

INDIVIDUAL : 955
(inbreeding= 31.800%)
f1= 27.350%
f2= 8.350%
f3= 3.850%
f4= 25.500%
f5= 1.850%
f6= 17.300%
f7= 15.800%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 967
(inbreeding= 20.400%)
f1= 26.650%
f2= 14.400%
f3= 7.500%
f4= 23.400%
f5= 1.500%
f6= 17.500%
f7= 9.050%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 960
(inbreeding= 22.500%)
f1= 23.500%
f2= 15.350%
f3= 7.500%
f4= 19.550%
f5= 1.400%
f6= 13.800%
f7= 12.950%
f8= 0.000%
other 5.950%
moiled 0.000%

INDIVIDUAL : 964
(inbreeding= 20.300%)
f1= 28.050%
f2= 12.500%
f3= 5.850%
f4= 26.000%
f5= 1.400%
f6= 13.200%
f7= 13.000%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 996
(inbreeding= 11.000%)
f1= 17.650%
f2= 11.800%
f3= 5.500%
f4= 16.150%
f5= 1.050%
f6= 13.200%
f7= 8.800%
f8= 6.100%
other 5.000%
moiled 14.750%

INDIVIDUAL : 985
(inbreeding= 11.000%)
f1= 14.200%
f2= 12.500%
f3= 5.050%
f4= 15.850%
f5= 0.000%
f6= 15.650%
f7= 10.050%
f8= 5.150%
other 6.350%
moiled 15.200%

INDIVIDUAL : 976
(inbreeding= 18.100%)
f1= 27.850%
f2= 14.100%
f3= 7.900%
f4= 15.700%
f5= 8.000%
f6= 14.000%
f7= 9.400%
f8= 0.000%
other 3.050%
moiled 0.000%

INDIVIDUAL : 969
(inbreeding= 11.800%)
f1= 13.050%
f2= 8.800%
f3= 3.800%
f4= 21.350%
f5= 0.000%
f6= 18.200%
f7= 11.200%
f8= 5.450%
other 2.900%
moiled 15.250%

INDIVIDUAL : 983
(inbreeding= 0.900%)
f1= 6.700%
f2= 3.450%
f3= 1.450%
f4= 18.950%
f5= 0.000%
f6= 14.200%
f7= 9.850%
f8= 5.800%
other 0.000%
moiled 39.600%

INDIVIDUAL : 973
(inbreeding= 15.000%)
f1= 16.450%
f2= 12.600%
f3= 6.300%
f4= 13.550%
f5= 0.000%
f6= 14.000%
f7= 9.600%
f8= 5.400%
other 6.800%
moiled 15.300%

INDIVIDUAL : 974
(inbreeding= 14.400%)
f1= 17.600%
f2= 12.700%
f3= 6.400%
f4= 12.600%
f5= 0.000%
f6= 14.350%
f7= 9.800%
f8= 6.150%
other 5.900%
moiled 14.500%

INDIVIDUAL : 979
(inbreeding= 20.300%)
f1= 32.700%
f2= 12.150%
f3= 8.700%
f4= 18.650%
f5= 3.350%
f6= 11.850%
f7= 9.200%
f8= 0.000%
other 3.400%
moiled 0.000%

INDIVIDUAL : 986
(inbreeding= 16.800%)
f1= 16.600%
f2= 7.150%
f3= 4.400%
f4= 20.450%
f5= 3.600%
f6= 14.500%
f7= 10.500%
f8= 2.800%
other 0.000%
moiled 20.000%

INDIVIDUAL : 997
(inbreeding= 11.000%)
f1= 16.600%
f2= 10.400%
f3= 4.700%
f4= 15.700%
f5= 2.450%
f6= 17.100%
f7= 9.000%
f8= 5.850%
other 3.400%
moiled 14.800%

INDIVIDUAL : 991
(inbreeding= 8.300%)
f1= 19.550%
f2= 12.450%
f3= 4.850%
f4= 16.200%
f5= 0.000%
f6= 13.900%
f7= 9.500%
f8= 5.900%
other 3.000%
moiled 14.650%

INDIVIDUAL : 1006
(inbreeding= 19.200%)
f1= 25.100%
f2= 17.450%
f3= 7.550%
f4= 14.800%
f5= 3.500%
f6= 12.600%
f7= 9.600%
f8= 0.000%
other 9.400%
moiled 0.000%

INDIVIDUAL : 990
(inbreeding= 7.400%)
f1= 20.450%
f2= 11.300%
f3= 4.950%
f4= 15.850%
f5= 0.000%
f6= 13.800%
f7= 9.900%
f8= 6.250%
other 3.200%
moiled 14.300%

INDIVIDUAL : 988
(inbreeding= 9.600%)
f1= 23.750%
f2= 5.000%
f3= 2.350%
f4= 16.650%
f5= 0.000%
f6= 15.950%
f7= 13.650%
f8= 2.900%
other 0.000%
moiled 19.750%

INDIVIDUAL : 989
(inbreeding= 16.500%)
f1= 3.300%
f2= 1.900%
f3= 0.750%
f4= 36.000%
f5= 0.000%
f6= 18.950%
f7= 17.150%
f8= 2.550%
other 0.000%
moiled 19.400%

INDIVIDUAL : 982
(inbreeding= 8.900%)
f1= 15.150%
f2= 8.400%
f3= 6.600%
f4= 16.550%
f5= 0.000%
f6= 14.350%
f7= 10.950%
f8= 6.250%
other 7.000%
moiled 14.750%

INDIVIDUAL : 1005
(inbreeding= 36.200%)
f1= 15.350%
f2= 12.850%
f3= 6.350%
f4= 21.750%
f5= 0.000%
f6= 15.800%
f7= 12.550%
f8= 2.550%
other 5.550%
moiled 7.250%

INDIVIDUAL : 1019
(inbreeding= 17.100%)
f1= 14.550%
f2= 10.050%
f3= 4.150%
f4= 18.500%
f5= 1.750%
f6= 15.050%
f7= 9.600%
f8= 4.500%
other 3.300%
moiled 18.550%

INDIVIDUAL : 1014
(inbreeding= 9.300%)
f1= 15.650%
f2= 9.450%
f3= 6.050%
f4= 16.550%
f5= 0.000%
f6= 14.200%
f7= 11.300%
f8= 5.900%
other 6.800%
moiled 14.100%

INDIVIDUAL : 1011
(inbreeding= 13.600%)
f1= 15.000%
f2= 14.650%
f3= 7.300%
f4= 8.650%
f5= 0.000%
f6= 14.550%
f7= 9.450%
f8= 6.350%
other 9.250%
moiled 14.800%

INDIVIDUAL : 999
(inbreeding= 22.400%)
f1= 10.850%
f2= 3.700%
f3= 1.500%
f4= 21.050%
f5= 0.000%
f6= 12.750%
f7= 12.850%
f8= 3.200%
other 7.350%
moiled 26.750%

INDIVIDUAL : 998
(inbreeding= 16.200%)
f1= 13.000%
f2= 7.500%
f3= 3.600%
f4= 16.300%
f5= 0.000%
f6= 14.500%
f7= 9.700%
f8= 6.100%
other 2.500%
moiled 26.800%

INDIVIDUAL : 1004
(inbreeding= 18.500%)
f1= 14.850%
f2= 9.550%
f3= 4.050%
f4= 25.400%
f5= 0.000%
f6= 16.550%
f7= 16.500%
f8= 2.800%
other 2.950%
moiled 7.350%

INDIVIDUAL : 1012
(inbreeding= 17.100%)
f1= 21.050%
f2= 11.350%
f3= 5.850%
f4= 18.850%
f5= 3.250%
f6= 16.100%
f7= 10.600%
f8= 2.800%
other 2.900%
moiled 7.250%

INDIVIDUAL : 1013
(inbreeding= 21.200%)
f1= 20.750%
f2= 10.700%
f3= 5.900%
f4= 21.100%
f5= 1.500%
f6= 15.900%
f7= 11.500%
f8= 2.100%
other 3.100%
moiled 7.450%

INDIVIDUAL : 1002
(inbreeding= 13.100%)
f1= 16.400%
f2= 5.850%
f3= 6.550%
f4= 23.850%
f5= 0.000%
f6= 15.800%
f7= 14.200%
f8= 2.800%
other 6.600%
moiled 7.950%

INDIVIDUAL : 1040
(inbreeding= 31.800%)
f1= 28.900%
f2= 10.500%
f3= 6.150%
f4= 21.550%
f5= 6.200%
f6= 16.850%
f7= 9.850%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 1036
(inbreeding= 24.900%)
f1= 26.850%
f2= 11.350%
f3= 6.350%
f4= 22.600%
f5= 5.450%
f6= 15.150%
f7= 10.950%
f8= 0.000%
other 1.300%
moiled 0.000%

INDIVIDUAL : 1037
(inbreeding= 37.900%)
f1= 25.500%
f2= 12.100%
f3= 6.100%
f4= 24.550%
f5= 2.500%
f6= 14.950%
f7= 11.250%
f8= 0.000%
other 3.050%
moiled 0.000%

INDIVIDUAL : 1026
(inbreeding= 24.600%)
f1= 5.250%
f2= 2.750%
f3= 1.300%
f4= 27.300%
f5= 0.000%
f6= 16.700%
f7= 14.400%
f8= 3.950%
other 0.000%
moiled 28.350%

INDIVIDUAL : 1017
(inbreeding= 20.700%)
f1= 24.650%
f2= 12.000%
f3= 6.550%
f4= 20.500%
f5= 5.350%
f6= 15.800%
f7= 12.350%
f8= 0.000%
other 2.800%
moiled 0.000%

INDIVIDUAL : 1038
(inbreeding= 12.900%)
f1= 12.500%
f2= 8.500%
f3= 3.950%
f4= 16.050%
f5= 0.000%
f6= 13.550%
f7= 9.900%
f8= 6.700%
other 3.700%
moiled 25.150%

INDIVIDUAL : 1023
(inbreeding= 9.200%)
f1= 15.050%
f2= 9.200%
f3= 5.250%
f4= 16.900%
f5= 0.000%
f6= 14.650%
f7= 11.300%
f8= 5.950%
other 7.150%
moiled 14.550%

INDIVIDUAL : 1018
(inbreeding= 19.600%)
f1= 19.850%
f2= 10.150%
f3= 5.350%
f4= 21.300%
f5= 1.400%
f6= 16.250%
f7= 12.450%
f8= 2.550%
other 3.000%
moiled 7.700%

INDIVIDUAL : 1027
(inbreeding= 19.500%)
f1= 16.900%
f2= 5.100%
f3= 2.250%
f4= 22.450%
f5= 0.000%
f6= 16.450%
f7= 15.100%
f8= 2.750%
other 0.000%
moiled 19.000%

INDIVIDUAL : 1024
(inbreeding= 27.900%)
f1= 11.700%
f2= 5.450%
f3= 2.800%
f4= 19.000%
f5= 1.900%
f6= 14.750%
f7= 9.350%
f8= 4.150%
other 0.000%
moiled 30.900%

INDIVIDUAL : 1034
(inbreeding= 17.300%)
f1= 11.450%
f2= 5.450%
f3= 4.200%
f4= 17.050%
f5= 0.000%
f6= 14.800%
f7= 10.150%
f8= 6.000%
other 3.550%
moiled 27.350%

INDIVIDUAL : 1033
(inbreeding= 11.700%)
f1= 15.750%
f2= 13.700%
f3= 6.050%
f4= 11.500%
f5= 0.700%
f6= 14.850%
f7= 9.750%
f8= 6.500%
other 6.700%
moiled 14.500%

INDIVIDUAL : 1031
(inbreeding= 13.100%)
f1= 14.850%
f2= 9.750%
f3= 4.350%
f4= 19.150%
f5= 0.000%
f6= 14.250%
f7= 10.800%
f8= 3.250%
other 3.750%
moiled 19.850%

INDIVIDUAL : 1054
(inbreeding= 32.600%)
f1= 28.600%
f2= 10.650%
f3= 6.550%
f4= 22.050%
f5= 6.500%
f6= 15.800%
f7= 9.850%
f8= 0.000%
other 0.000%
moiled 0.000%

INDIVIDUAL : 1046
(inbreeding= 42.400%)
f1= 26.450%
f2= 11.100%
f3= 6.750%
f4= 24.400%
f5= 2.050%
f6= 14.400%
f7= 12.150%
f8= 0.000%
other 2.700%
moiled 0.000%

INDIVIDUAL : 1039
(inbreeding= 40.600%)
f1= 24.400%
f2= 9.000%
f3= 4.900%
f4= 23.650%
f5= 4.250%
f6= 15.000%
f7= 11.450%
f8= 0.000%
other 3.450%
moiled 3.900%

INDIVIDUAL : 1053
(inbreeding= 23.700%)
f1= 4.700%
f2= 2.800%
f3= 1.100%
f4= 28.200%
f5= 0.000%
f6= 15.900%
f7= 13.250%
f8= 4.150%
other 0.000%
moiled 29.900%

INDIVIDUAL : 1047
(inbreeding= 26.500%)
f1= 10.650%
f2= 5.450%
f3= 2.900%
f4= 19.350%
f5= 1.300%
f6= 15.150%
f7= 10.700%
f8= 4.300%
other 0.000%
moiled 30.200%

INDIVIDUAL :	1052
(inbreeding=	18.400%)
f1=	16.450%
f2=	6.000%
f3=	2.750%
f4=	21.650%
f5=	1.000%
f6=	16.350%
f7=	13.100%
f8=	2.500%
other	0.000%
moiled	20.200%

INDIVIDUAL :	1050
(inbreeding=	12.100%)
f1=	12.900%
f2=	5.900%
f3=	3.700%
f4=	14.100%
f5=	1.950%
f6=	14.300%
f7=	8.850%
f8=	4.700%
other	10.700%
moiled	22.900%

INDIVIDUAL :	1044
(inbreeding=	21.200%)
f1=	25.600%
f2=	12.900%
f3=	5.650%
f4=	20.150%
f5=	5.050%
f6=	16.050%
f7=	11.250%
f8=	0.000%
other	3.350%
moiled	0.000%

INDIVIDUAL :	1060
(inbreeding=	21.000%)
f1=	16.650%
f2=	10.800%
f3=	5.950%
f4=	12.700%
f5=	0.900%
f6=	15.000%
f7=	8.900%
f8=	5.850%
other	7.350%
moiled	15.900%

INDIVIDUAL :	1058
(inbreeding=	19.500%)
f1=	10.250%
f2=	5.550%
f3=	3.650%
f4=	17.500%
f5=	0.000%
f6=	14.650%
f7=	10.300%
f8=	5.700%
other	4.100%
moiled	28.300%

INDIVIDUAL :	1057
(inbreeding=	30.100%)
f1=	22.000%
f2=	10.500%
f3=	5.750%
f4=	27.650%
f5=	1.550%
f6=	15.300%
f7=	14.000%
f8=	0.000%
other	3.250%
moiled	0.000%

INDIVIDUAL :	1051
(inbreeding=	19.900%)
f1=	18.400%
f2=	7.450%
f3=	3.800%
f4=	23.350%
f5=	0.850%
f6=	13.500%
f7=	12.050%
f8=	1.450%
other	6.200%
moiled	12.950%

INDIVIDUAL :	1043
(inbreeding=	14.800%)
f1=	11.700%
f2=	6.900%
f3=	2.500%
f4=	18.600%
f5=	1.650%
f6=	15.650%
f7=	9.800%
f8=	6.400%
other	1.700%
moiled	25.100%

INDIVIDUAL : 1042
(inbreeding= 14.200%)
f1= 18.300%
f2= 7.600%
f3= 4.600%
f4= 20.650%
f5= 2.150%
f6= 14.300%
f7= 9.550%
f8= 3.150%
other 0.000%
moiled 19.700%

INDIVIDUAL : 1064
(inbreeding= 16.700%)
f1= 20.700%
f2= 12.250%
f3= 6.450%
f4= 16.450%
f5= 5.100%
f6= 15.350%
f7= 9.250%
f8= 2.150%
other 3.350%
moiled 8.950%

INDIVIDUAL : 1067
(inbreeding= 16.000%)
f1= 20.800%
f2= 7.700%
f3= 4.100%
f4= 20.750%
f5= 1.450%
f6= 14.100%
f7= 9.950%
f8= 3.100%
other 0.000%
moiled 18.050%

INDIVIDUAL : 1063
(inbreeding= 16.100%)
f1= 25.150%
f2= 11.900%
f3= 5.850%
f4= 15.100%
f5= 5.250%
f6= 15.300%
f7= 7.900%
f8= 2.350%
other 1.400%
moiled 9.800%

INDIVIDUAL : 1062
(inbreeding= 20.400%)
f1= 19.500%
f2= 12.300%
f3= 6.300%
f4= 20.450%
f5= 1.550%
f6= 13.600%
f7= 10.050%
f8= 2.100%
other 4.450%
moiled 9.700%

INDIVIDUAL : 1076
(inbreeding= 20.400%)
f1= 14.000%
f2= 9.200%
f3= 4.500%
f4= 16.850%
f5= 0.300%
f6= 14.200%
f7= 10.300%
f8= 5.350%
other 4.850%
moiled 20.450%

INDIVIDUAL : 1083
(inbreeding= 13.600%)
f1= 18.550%
f2= 7.600%
f3= 3.200%
f4= 23.800%
f5= 0.000%
f6= 14.050%
f7= 11.300%
f8= 2.600%
other 0.000%
moiled 18.900%

INDIVIDUAL : 1074
(inbreeding= 19.500%)
f1= 11.700%
f2= 5.850%
f3= 2.900%
f4= 27.550%
f5= 0.750%
f6= 18.850%
f7= 15.650%
f8= 2.250%
other 1.500%
moiled 13.000%

INDIVIDUAL : 1073
(inbreeding= 18.900%)
f1= 18.250%
f2= 8.650%
f3= 4.950%
f4= 20.200%
f5= 2.500%
f6= 14.900%
f7= 11.750%
f8= 2.700%
other 1.500%
moiled 14.600%

INDIVIDUAL : 1061
(inbreeding= 18.100%)
f1= 17.000%
f2= 7.300%
f3= 3.550%
f4= 22.400%
f5= 1.300%
f6= 15.050%
f7= 10.950%
f8= 2.800%
other 0.000%
moiled 19.650%

INDIVIDUAL : 1081
(inbreeding= 18.000%)
f1= 12.650%
f2= 7.100%
f3= 2.750%
f4= 23.950%
f5= 0.000%
f6= 13.800%
f7= 15.000%
f8= 2.700%
other 5.150%
moiled 16.900%

INDIVIDUAL : 1080
(inbreeding= 17.700%)
f1= 12.650%
f2= 6.300%
f3= 2.800%
f4= 22.800%
f5= 0.000%
f6= 14.600%
f7= 14.300%
f8= 3.550%
other 5.300%
moiled 17.700%

INDIVIDUAL : 1077
(inbreeding= 24.500%)
f1= 14.850%
f2= 11.300%
f3= 6.000%
f4= 13.100%
f5= 0.850%
f6= 14.750%
f7= 8.900%
f8= 5.600%
other 7.050%
moiled 17.600%

INDIVIDUAL : 1075
(inbreeding= 20.300%)
f1= 13.100%
f2= 10.950%
f3= 5.550%
f4= 13.050%
f5= 0.000%
f6= 15.050%
f7= 9.850%
f8= 6.300%
other 5.750%
moiled 20.400%

INDIVIDUAL : 1071
(inbreeding= 18.500%)
f1= 17.150%
f2= 9.300%
f3= 4.650%
f4= 20.350%
f5= 1.700%
f6= 16.750%
f7= 10.750%
f8= 4.500%
other 3.300%
moiled 11.550%

INDIVIDUAL : 1070
(inbreeding= 16.200%)
f1= 15.200%
f2= 7.050%
f3= 4.250%
f4= 16.800%
f5= 3.150%
f6= 14.100%
f7= 8.600%
f8= 4.400%
other 7.000%
moiled 19.450%

INDIVIDUAL : 1069
(inbreeding= 23.300%)
f1= 16.250%
f2= 9.600%
f3= 3.900%
f4= 27.950%
f5= 0.650%
f6= 15.450%
f7= 14.200%
f8= 2.950%
other 2.350%
moiled 6.700%

INDIVIDUAL : 1066
(inbreeding= 12.500%)
f1= 19.500%
f2= 7.200%
f3= 4.050%
f4= 20.250%
f5= 1.800%
f6= 13.900%
f7= 10.400%
f8= 3.000%
other 0.000%
moiled 19.900%

INDIVIDUAL : 1065
(inbreeding= 20.900%)
f1= 22.000%
f2= 11.050%
f3= 6.750%
f4= 21.900%
f5= 3.150%
f6= 15.300%
f7= 11.650%
f8= 1.250%
other 2.850%
moiled 4.100%

INDIVIDUAL : 1105
(inbreeding= 16.500%)
f1= 23.350%
f2= 11.350%
f3= 6.250%
f4= 16.400%
f5= 5.550%
f6= 15.350%
f7= 8.350%
f8= 2.500%
other 1.500%
moiled 9.400%

INDIVIDUAL : 1102
(inbreeding= 20.700%)
f1= 20.750%
f2= 11.850%
f3= 5.950%
f4= 20.100%
f5= 1.650%
f6= 13.900%
f7= 10.100%
f8= 2.500%
other 4.850%
moiled 8.350%

INDIVIDUAL : 1101
(inbreeding= 18.600%)
f1= 21.400%
f2= 10.600%
f3= 5.400%
f4= 20.300%
f5= 3.250%
f6= 15.500%
f7= 10.350%
f8= 2.300%
other 2.300%
moiled 8.600%

INDIVIDUAL : 1099
(inbreeding= 18.900%)
f1= 21.300%
f2= 12.550%
f3= 6.300%
f4= 17.250%
f5= 4.800%
f6= 13.850%
f7= 9.250%
f8= 2.200%
other 3.100%
moiled 9.400%

INDIVIDUAL : 1093
(inbreeding= 26.200%)
f1= 16.200%
f2= 11.350%
f3= 4.600%
f4= 10.100%
f5= 0.000%
f6= 13.800%
f7= 8.500%
f8= 8.950%
other 5.100%
moiled 21.400%

INDIVIDUAL : 1092
(inbreeding= 23.900%)
f1= 16.950%
f2= 11.800%
f3= 3.900%
f4= 15.800%
f5= 1.450%
f6= 16.050%
f7= 9.350%
f8= 6.250%
other 2.900%
moiled 15.550%

INDIVIDUAL : 1098
(inbreeding= 30.600%)
f1= 21.700%
f2= 10.450%
f3= 6.200%
f4= 28.000%
f5= 1.300%
f6= 16.050%
f7= 13.200%
f8= 0.000%
other 3.100%
moiled 0.000%

INDIVIDUAL : 1091
(inbreeding= 18.300%)
f1= 17.050%
f2= 5.600%
f3= 2.500%
f4= 22.200%
f5= 0.950%
f6= 15.650%
f7= 12.800%
f8= 2.950%
other 0.000%
moiled 20.300%

INDIVIDUAL : 1112
(inbreeding= 18.900%)
f1= 14.000%
f2= 12.000%
f3= 5.300%
f4= 12.150%
f5= 0.000%
f6= 15.500%
f7= 9.300%
f8= 5.900%
other 5.800%
moiled 20.050%

INDIVIDUAL : 1111
(inbreeding= 24.000%)
f1= 16.200%
f2= 11.200%
f3= 5.150%
f4= 13.800%
f5= 0.950%
f6= 15.350%
f7= 8.600%
f8= 5.700%
other 7.200%
moiled 15.850%

INDIVIDUAL : 1109
(inbreeding= 20.700%)
f1= 17.100%
f2= 10.350%
f3= 4.000%
f4= 16.500%
f5= 1.150%
f6= 16.900%
f7= 10.200%
f8= 6.050%
other 3.100%
moiled 14.650%

INDIVIDUAL : 1108
(inbreeding= 16.900%)
f1= 16.150%
f2= 8.250%
f3= 4.650%
f4= 17.400%
f5= 1.850%
f6= 15.650%
f7= 8.950%
f8= 3.850%
other 4.100%
moiled 19.150%

INDIVIDUAL : 1107
(inbreeding= 21.900%)
f1= 16.800%
f2= 9.650%
f3= 4.700%
f4= 14.700%
f5= 1.200%
f6= 15.650%
f7= 8.850%
f8= 6.350%
other 6.150%
moiled 15.950%

INDIVIDUAL : 1097	
(inbreeding=	29.500%)
f1=	23.150%
f2=	8.200%
f3=	6.050%
f4=	28.150%
f5=	1.400%
f6=	14.850%
f7=	14.900%
f8=	0.000%
other	3.300%
moiled	0.000%

INDIVIDUAL : 1096	
(inbreeding=	17.000%)
f1=	13.400%
f2=	6.650%
f3=	2.700%
f4=	23.700%
f5=	0.000%
f6=	15.150%
f7=	14.950%
f8=	2.400%
other	4.800%
moiled	16.250%

INDIVIDUAL : 1095	
(inbreeding=	19.500%)
f1=	15.700%
f2=	7.350%
f3=	3.900%
f4=	20.450%
f5=	1.600%
f6=	15.700%
f7=	10.600%
f8=	3.200%
other	1.500%
moiled	20.000%

INDIVIDUAL : 1130	
(inbreeding=	26.100%)
f1=	19.900%
f2=	13.900%
f3=	6.600%
f4=	21.350%
f5=	0.650%
f6=	14.550%
f7=	12.050%
f8=	1.150%
other	5.800%
moiled	4.050%

INDIVIDUAL : 1128	
(inbreeding=	19.100%)
f1=	22.350%
f2=	11.050%
f3=	5.900%
f4=	22.050%
f5=	3.550%
f6=	16.250%
f7=	11.000%
f8=	1.200%
other	3.150%
moiled	3.500%

INDIVIDUAL : 1127	
(inbreeding=	20.800%)
f1=	21.100%
f2=	12.550%
f3=	6.350%
f4=	21.350%
f5=	2.800%
f6=	15.900%
f7=	11.500%
f8=	1.300%
other	3.650%
moiled	3.500%

INDIVIDUAL : 1126	
(inbreeding=	19.200%)
f1=	23.500%
f2=	12.100%
f3=	6.750%
f4=	17.400%
f5=	4.900%
f6=	16.950%
f7=	10.600%
f8=	1.000%
other	2.850%
moiled	3.950%

INDIVIDUAL : 1125	
(inbreeding=	18.600%)
f1=	20.600%
f2=	13.350%
f3=	7.800%
f4=	18.350%
f5=	4.100%
f6=	14.850%
f7=	11.400%
f8=	1.250%
other	4.400%
moiled	3.900%

INDIVIDUAL : 1131
(inbreeding= 16.600%)
f1= 20.300%
f2= 14.250%
f3= 6.150%
f4= 15.250%
f5= 2.900%
f6= 14.350%
f7= 9.400%
f8= 3.200%
other 6.450%
moiled 7.750%

INDIVIDUAL : 1136
(inbreeding= 21.100%)
f1= 15.300%
f2= 7.650%
f3= 4.750%
f4= 18.750%
f5= 2.200%
f6= 15.000%
f7= 10.300%
f8= 3.900%
other 2.150%
moiled 20.000%

INDIVIDUAL : 1135
(inbreeding= 22.600%)
f1= 14.700%
f2= 9.250%
f3= 4.800%
f4= 16.850%
f5= 0.200%
f6= 14.800%
f7= 10.650%
f8= 5.150%
other 5.650%
moiled 17.950%

INDIVIDUAL : 1120
(inbreeding= 19.100%)
f1= 23.100%
f2= 8.300%
f3= 3.500%
f4= 18.650%
f5= 0.500%
f6= 15.450%
f7= 12.750%
f8= 2.900%
other 1.650%
moiled 13.200%

INDIVIDUAL : 1119
(inbreeding= 19.700%)
f1= 10.900%
f2= 6.500%
f3= 3.450%
f4= 25.300%
f5= 0.600%
f6= 16.300%
f7= 12.450%
f8= 3.600%
other 1.400%
moiled 19.500%

INDIVIDUAL : 1118
(inbreeding= 21.000%)
f1= 15.850%
f2= 8.450%
f3= 4.250%
f4= 20.200%
f5= 1.300%
f6= 14.900%
f7= 11.800%
f8= 3.700%
other 1.650%
moiled 17.900%

INDIVIDUAL : 1117
(inbreeding= 21.100%)
f1= 18.850%
f2= 7.000%
f3= 3.750%
f4= 22.200%
f5= 0.850%
f6= 16.900%
f7= 13.650%
f8= 2.600%
other 1.250%
moiled 12.950%

INDIVIDUAL : 1134
(inbreeding= 22.900%)
f1= 16.500%
f2= 11.650%
f3= 5.850%
f4= 12.300%
f5= 0.000%
f6= 15.050%
f7= 10.550%
f8= 6.500%
other 8.450%
moiled 13.150%

INDIVIDUAL : 1133
(inbreeding= 18.200%)
f1= 16.500%
f2= 9.550%
f3= 4.650%
f4= 26.650%
f5= 0.000%
f6= 15.550%
f7= 15.000%
f8= 2.550%
other 2.950%
moiled 6.600%

INDIVIDUAL : 1132
(inbreeding= 30.600%)
f1= 22.450%
f2= 8.400%
f3= 5.800%
f4= 29.200%
f5= 1.350%
f6= 15.100%
f7= 14.750%
f8= 0.000%
other 2.950%
moiled 0.000%

INDIVIDUAL : 1123
(inbreeding= 19.700%)
f1= 15.700%
f2= 6.150%
f3= 4.200%
f4= 17.400%
f5= 2.250%
f6= 14.100%
f7= 8.900%
f8= 4.650%
other 5.550%
moiled 21.100%

INDIVIDUAL : 1122
(inbreeding= 15.200%)
f1= 18.200%
f2= 6.550%
f3= 4.550%
f4= 20.050%
f5= 3.000%
f6= 14.300%
f7= 8.600%
f8= 3.400%
other 5.350%
moiled 16.000%

INDIVIDUAL : 1121
(inbreeding= 19.100%)
f1= 15.100%
f2= 8.000%
f3= 4.650%
f4= 20.200%
f5= 1.550%
f6= 15.500%
f7= 10.900%
f8= 3.500%
other 1.200%
moiled 19.400%

INDIVIDUAL : 1137
(inbreeding= 10.600%)
f1= 22.350%
f2= 13.850%
f3= 5.300%
f4= 18.100%
f5= 0.000%
f6= 12.550%
f7= 11.800%
f8= 3.250%
other 5.500%
moiled 7.300%

INDIVIDUAL : 1138
(inbreeding= 13.200%)
f1= 23.000%
f2= 11.200%
f3= 5.150%
f4= 20.950%
f5= 0.100%
f6= 11.950%
f7= 12.200%
f8= 2.600%
other 2.450%
moiled 10.400%

INDIVIDUAL : 1139
(inbreeding= 18.400%)
f1= 8.700%
f2= 5.300%
f3= 2.800%
f4= 26.900%
f5= 0.200%
f6= 16.200%
f7= 13.650%
f8= 4.000%
other 2.650%
moiled 19.600%

INDIVIDUAL : a5

f1=	28.100%
f2=	7.150%
f3=	9.800%
f4=	19.000%
f5=	0.000%
f6=	10.650%
f7=	12.300%
f8=	0.000%
other	13.000%
moiled	0.000%

INDIVIDUAL : a15

f1=	21.100%
f2=	19.200%
f3=	9.000%
f4=	13.250%
f5=	0.000%
f6=	12.700%
f7=	12.250%
f8=	0.000%
other	12.500%
moiled	0.000%

INDIVIDUAL : c24

f1=	6.500%
f2=	3.000%
f3=	1.650%
f4=	19.500%
f5=	0.000%
f6=	9.350%
f7=	10.000%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : a17

f1=	20.200%
f2=	3.150%
f3=	8.300%
f4=	23.950%
f5=	0.000%
f6=	16.850%
f7=	16.450%
f8=	0.000%
other	11.100%
moiled	0.000%

INDIVIDUAL : b27

f1=	21.450%
f2=	7.600%
f3=	5.350%
f4=	9.950%
f5=	7.000%
f6=	12.600%
f7=	5.850%
f8=	0.000%
other	30.200%
moiled	0.000%

INDIVIDUAL : b26

f1=	22.400%
f2=	8.100%
f3=	5.850%
f4=	10.250%
f5=	6.700%
f6=	12.050%
f7=	6.200%
f8=	0.000%
other	28.450%
moiled	0.000%

INDIVIDUAL : b30

f1=	20.550%
f2=	10.300%
f3=	6.350%
f4=	14.200%
f5=	0.000%
f6=	7.750%
f7=	7.200%
f8=	0.000%
other	33.650%
moiled	0.000%

INDIVIDUAL : a23

f1=	15.600%
f2=	9.050%
f3=	4.650%
f4=	8.150%
f5=	3.700%
f6=	14.750%
f7=	5.900%
f8=	6.100%
other	17.650%
moiled	14.450%

INDIVIDUAL : c43

f1=	12.350%
f2=	4.950%
f3=	3.150%
f4=	13.500%
f5=	1.350%
f6=	7.450%
f7=	7.250%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c42

f1=	12.850%
f2=	3.650%
f3=	3.550%
f4=	14.000%
f5=	1.700%
f6=	7.400%
f7=	6.850%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c45	
f1=	13.800%
f2=	3.950%
f3=	3.600%
f4=	11.300%
f5=	0.600%
f6=	6.400%
f7=	7.000%
f8=	0.000%
other	53.350%
moiled	0.000%

INDIVIDUAL : a42	
f1=	23.850%
f2=	7.900%
f3=	4.650%
f4=	22.500%
f5=	3.650%
f6=	13.050%
f7=	11.200%
f8=	0.000%
other	13.200%
moiled	0.000%

INDIVIDUAL : b35	
f1=	18.250%
f2=	6.750%
f3=	4.500%
f4=	20.100%
f5=	3.150%
f6=	12.400%
f7=	10.450%
f8=	0.000%
other	24.400%
moiled	0.000%

INDIVIDUAL : c61	
f1=	12.450%
f2=	5.000%
f3=	3.250%
f4=	13.000%
f5=	1.350%
f6=	8.050%
f7=	6.900%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c56	
f1=	14.850%
f2=	4.700%
f3=	3.700%
f4=	11.500%
f5=	2.800%
f6=	7.800%
f7=	4.650%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c62	
f1=	12.400%
f2=	5.250%
f3=	3.200%
f4=	12.550%
f5=	1.600%
f6=	8.250%
f7=	6.750%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c54	
f1=	13.250%
f2=	9.050%
f3=	4.050%
f4=	8.950%
f5=	0.000%
f6=	5.600%
f7=	6.000%
f8=	0.000%
other	53.100%
moiled	0.000%

INDIVIDUAL : c63	
f1=	12.150%
f2=	4.200%
f3=	3.100%
f4=	12.900%
f5=	1.750%
f6=	8.600%
f7=	7.300%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : b37	
f1=	18.800%
f2=	6.400%
f3=	4.150%
f4=	21.400%
f5=	2.950%
f6=	11.900%
f7=	9.900%
f8=	0.000%
other	24.500%
moiled	0.000%

INDIVIDUAL : c64	
f1=	12.500%
f2=	4.600%
f3=	3.050%
f4=	14.550%
f5=	1.150%
f6=	7.450%
f7=	6.700%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c65

f1=	11.950%
f2=	5.000%
f3=	3.400%
f4=	12.950%
f5=	1.200%
f6=	8.000%
f7=	7.500%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c67

f1=	8.250%
f2=	4.450%
f3=	2.950%
f4=	4.250%
f5=	1.800%
f6=	7.450%
f7=	3.200%
f8=	3.200%
other	57.550%
moiled	6.900%

INDIVIDUAL : a41

f1=	18.050%
f2=	7.850%
f3=	4.500%
f4=	16.600%
f5=	3.250%
f6=	14.600%
f7=	9.150%
f8=	2.950%
other	16.050%
moiled	7.000%

INDIVIDUAL : b43

f1=	10.000%
f2=	6.950%
f3=	2.850%
f4=	13.600%
f5=	0.000%
f6=	12.850%
f7=	8.000%
f8=	5.600%
other	25.550%
moiled	14.600%

INDIVIDUAL : a40

f1=	14.250%
f2=	7.000%
f3=	3.300%
f4=	22.400%
f5=	0.700%
f6=	15.150%
f7=	13.400%
f8=	2.800%
other	13.550%
moiled	7.450%

INDIVIDUAL : a33

f1=	15.850%
f2=	7.200%
f3=	2.550%
f4=	21.650%
f5=	0.000%
f6=	15.300%
f7=	12.700%
f8=	3.000%
other	14.700%
moiled	7.050%

INDIVIDUAL : c74

f1=	13.150%
f2=	4.450%
f3=	3.250%
f4=	12.350%
f5=	1.600%
f6=	8.300%
f7=	6.900%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c69

f1=	13.500%
f2=	4.050%
f3=	2.200%
f4=	12.850%
f5=	0.800%
f6=	8.300%
f7=	8.300%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : b47

f1=	11.900%
f2=	6.150%
f3=	3.600%
f4=	9.100%
f5=	1.400%
f6=	13.150%
f7=	5.100%
f8=	6.750%
other	28.600%
moiled	14.250%

INDIVIDUAL : b76

f1=	12.800%
f2=	6.400%
f3=	3.400%
f4=	17.300%
f5=	0.900%
f6=	13.550%
f7=	10.450%
f8=	2.750%
other	25.600%
moiled	6.850%

INDIVIDUAL : b44

f1=	9.950%
f2=	5.600%
f3=	2.250%
f4=	13.450%
f5=	0.000%
f6=	10.200%
f7=	9.050%
f8=	3.100%
other	39.000%
moiled	7.400%

INDIVIDUAL : c70

f1=	12.300%
f2=	4.700%
f3=	3.350%
f4=	13.100%
f5=	1.200%
f6=	8.300%
f7=	7.050%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c76

f1=	11.650%
f2=	4.300%
f3=	3.750%
f4=	13.500%
f5=	1.650%
f6=	8.300%
f7=	6.850%
f8=	0.000%
other	50.000%
moiled	0.000%

INDIVIDUAL : c79

f1=	10.200%
f2=	5.400%
f3=	3.050%
f4=	6.900%
f5=	0.000%
f6=	6.750%
f7=	3.700%
f8=	3.250%
other	40.950%
moiled	19.800%

INDIVIDUAL : b50

f1=	11.500%
f2=	5.950%
f3=	2.400%
f4=	7.000%
f5=	0.000%
f6=	8.900%
f7=	5.450%
f8=	5.300%
other	27.650%
moiled	25.850%

INDIVIDUAL : b52

f1=	14.250%
f2=	4.700%
f3=	5.600%
f4=	9.950%
f5=	1.000%
f6=	10.850%
f7=	7.800%
f8=	2.100%
other	37.000%
moiled	6.50%

INDIVIDUAL : b51

f1=	6.200%
f2=	4.000%
f3=	1.950%
f4=	11.200%
f5=	0.000%
f6=	11.0p0%
f7=	6.300%
f8=	5.700%
other	27.100%
moiled	26.550%

INDIVIDUAL : a45

f1=	22.450%
f2=	7.450%
f3=	5.400%
f4=	23.650%
f5=	2.550%
f6=	13.300%
f7=	12.300%
f8=	0.000%
other	12.900%
moiled	0.000%

INDIVIDUAL : c80

f1=	9.000%
f2=	3.450%
f3=	2.200%
f4=	10.000%
f5=	1.650%
f6=	8.900%
f7=	5.350%
f8=	2.000%
other	53.900%
moiled	3.550%

INDIVIDUAL : b56

f1=	15.300%
f2=	6.950%
f3=	3.800%
f4=	14.950%
f5=	2.050%
f6=	12.050%
f7=	10.250%
f8=	1.650%
other	25.850%
moiled	7.150%

INDIVIDUAL : b58

f1=	14.800%
f2=	6.900%
f3=	4.000%
f4=	16.000%
f5=	1.400%
f6=	10.600%
f7=	8.400%
f8=	2.000%
other	28.050%
moiled	7.850%

INDIVIDUAL : a49

f1=	18.100%
f2=	9.900%
f3=	5.050%
f4=	17.500%
f5=	2.300%
f6=	14.900%
f7=	11.250%
f8=	2.800%
other	12.050%
moiled	6.150%

INDIVIDUAL : b53

f1=	14.350%
f2=	7.350%
f3=	4.75%
f4=	13.400%
f5=	0.400%
f6=	10.800%
f7=	9.150%
f8=	3.200%
other	29.500%
moiled	7n100%

INDIVIDUAL : b62

f1=	19.200%
f2=	9.150%
f3=	5.250%
f4=	18.20%
f5=	1.300%
f6=	10.900%
f7=	10.050%
f8=	0.000%
other	25.900%
moiled	0.000%

INDIVIDUAL : b63

f1=	14.150%
f2=	6.600%
f3=	5.000%
f4=	16.050%
f5=	2.050%
f6=	12.550%
f7=	9.350%
f8=	2.150%
other	25.150%
moiled	6.950%

INDIVIDUAL : b65

f1=	13.850%
f2=	6.000%
f3=	4.400%
f4=	16.050%
f5=	2.250%
f6=	11.400%
f7=	8.950%
f8=	1.900%
other	27.550%
moiled	7.650%

INDIVIDUAL : b66

f1=	15.250%
f2=	7.000%
f3=	4.450%
f4=	17.050%
f5=	1.450%
f6=	10.250%
f7=	9.700%
f8=	1.650%
other	25.900%
moiled	7.300%

INDIVIDUAL : b61

f1=	13.950%
f2=	8.350%
f3=	3.400%
f4=	11.350%
f5=	1.500%
f6=	11.250%
f7=	6.650%
f8=	3.800%
other	22.450%
moiled	17.300%

INDIVIDUAL : a55

f1=	18.750%
f2=	10.150%
f3=	5.750%
f4=	15.050%
f5=	1.750%
f6=	13.000%
f7=	7.650%
f8=	2.400%
other	18.400%
moiled	7.100%

INDIVIDUAL : a56

f1=	10.750%
f2=	4.900%
f3=	2.550%
f4=	24.550%
f5=	0.000%
f6=	14.650%
f7=	13.200%
f8=	2.200%
other	12.800%
moiled	14.400%

INDIVIDUAL : b70

f1=	13.000%
f2=	12.800%
f3=	4.450%
f4=	8.950%
f5=	0.000%
f6=	10.300%
f7=	8.400%
f8=	3.200%
other	32.450%
moiled	6.450%

INDIVIDUAL : a57

f1=	4.200%
f2=	1.500%
f3=	1.150%
f4=	8.950%
f5=	0.000%
f6=	6.750%
f7=	4.950%
f8=	3.600%
other	50.000%
moiled	18.900%

INDIVIDUAL : a58

f1=	11.650%
f2=	9.500%
f3=	3.950%
f4=	14.850%
f5=	0.000%
f6=	13.550%
f7=	9.600%
f8=	5.950%
other	15.950%
moiled	15.000%

INDIVIDUAL : b72

f1=	14.300%
f2=	9.550%
f3=	4.850%
f4=	17.100%
f5=	0.900%
f6=	10.850%
f7=	11.600%
f8=	1.250%
other	26.300%
moiled	3.300%

INDIVIDUAL : b74

f1=	14.450%
f2=	9.500%
f3=	4.650%
f4=	18.250%
f5=	0.550%
f6=	10.350%
f7=	9.950%
f8=	1.250%
other	28.000%
moiled	3.050%

INDIVIDUAL : a64

f1=	16.200%
f2=	10.050%
f3=	4.700%
f4=	18.050%
f5=	0.950%
f6=	12.400%
f7=	12.700%
f8=	2.000%
other	15.700%
moiled	7.250%

INDIVIDUAL : b77

f1=	11.500%
f2=	9.450%
f3=	3.850%
f4=	13.800%
f5=	0.000%
f6=	12.550%
f7=	8.950%
f8=	2.950%
other	29.700%
moiled	7.250%

INDIVIDUAL : b73

f1=	12.850%
f2=	5.100%
f3=	3.900%
f4=	15.200%
f5=	2.200%
f6=	11.950%
f7=	8.250%
f8=	2.300%
other	27.500%
moiled	10.750%

INDIVIDUAL : a62

f1=	12.300%
f2=	5.950%
f3=	2.350%
f4=	25.700%
f5=	0.000%
f6=	13.950%
f7=	14.400%
f8=	1.850%
other	19.700%
moiled	3.800%

INDIVIDUAL : a61

f1=	11.800%
f2=	6.350%
f3=	3.700%
f4=	25.800%
f5=	0.000%
f6=	15.100%
f7=	14.350%
f8=	2.900%
other	13.250%
moiled	6.750%

INDIVIDUAL	:	b46
f1=		14.300%
f2=		5.850%
f3=		2.700%
f4=		17.400%
f5=		0.800%
f6=		12.500%
f7=		10.450%
f8=		3.400%
other		26.600%
moiled		6.000%