Inbreeding and fitness in wild ungulates

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"Nothing happens in contradiction to nature; only in contradiction to what we know of it."

Dana Scully, The X Files 1997

Preface

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

Summary

Inbreeding and the effects of inbreeding on the fitness of individuals have been studied extensively for over a century. Surprisingly, there have been few studies of the fitness consequences of inbreeding in wild animal populations. In this thesis I examine inbreeding and its fitness consequences in two species of wild ungulates, red deer (*Cervus elaphus*) and Arabian oryx (*Oryx leucoryx*).

The long term study of red deer on the Isle of Rum has included profiling of individuals at polymorphic protein and microsatellite loci. I developed a likelihood-based method for large-scale paternity inference and implemented it via the computer program CERVUS. The system allowed genetic inference of paternity with 80% confidence for 536 red deer calves. I used these data to construct pedigrees and found that 11% of calves were the offspring of matings between paternal half sibs; however most inbreeding coefficients were zero. Microsatellite-based measures of inbreeding and outbreeding, individual heterozygosity and mean d^2, were calculated using data from nine microsatellite loci. Among stags born 1982-1986, neither individual heterozygosity nor mean d^2 influenced survival to reproductive age, while mean d^2 but not individual heterozygosity positively influenced stag lifetime mating success (LMS) among males which reproduced. Among hinds born 1982-1986, individual heterozygosity but not mean d^2 positively influenced survival to reproductive age and age at first breeding among hinds which reproduced, while mean d^2 (in an interaction with year of birth) but not individual heterozygosity influenced hind LMS.

Arabian oryx went extinct in the wild in 1972 and were reintroduced to Oman and Saudi Arabia after several generations of breeding in captivity. 343 Arabian oryx representing several major populations were typed at six microsatellite loci. Average heterozygosity and differentiation between groups were low. In reintroduced Arabian oryx in Oman no relationship was found between inbreeding coefficient and juvenile survival. However individual heterozygosity was positively related to juvenile survival while mean d^2 was negatively related to juvenile survival, implying simultaneous inbreeding and outbreeding depression. Genetic, environmental and anthropogenic factors all influence the survival of reintroduced Arabian oryx, but despite the joint influence of these factors, the Omani population is growing rapidly.

Acknowledgements

The work presented in this thesis would have been impossible without the help of many people. The Arabian oryx project in Oman was initiated by Ralph Daly with funding from Sultan Qaboos bin Said, and the project was run in the early years by Mark Stanley Price. I particularly thank Andrew Spalton for providing hospitality, samples and unpublished data from his thesis, and thanks are also due to Ralph and Elizabeth Daly, Robert and Anna Lewellyn-Smith and Stephen Brent for hospitality in Oman. Additional samples were provided by Mark Lawrence, Oliver Ryder and Helen Stanley, with grateful thanks to Paul Sunnucks for assembling such a large collection of samples and helping me to identify their origin, to Sarah Christie for supplying studbook data, to Georgina Mace for supplying the program FASTINB needed to analyse that data and to Rob Hammond for his gazelle primers. I also thank Jelle Boef and Khaldoon Kiwan (RSCN, Amman) for their hospitality in Jordan. Finally (in this first bit) Stéphane Ostrowski (NWRC Taif) and Jaime Samour (Fahad bin Sultan Falcon Centre, Riyadh) gave invaluable criticism on and additional information for Chapter 7, and I particularly thank Stéphane and all at NWRC for visa acquisition, their generous hospitality and in particular their surprisingly palatable brand of accidentally fermented grape juice.

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Thanks to Simon Goodman, David Bancroft and Bill Amos for program code which was incorporated into the CERVUS program, Nick Barton for another good idea which I wouldn't have thought of and Loeske Kruuk for her invaluable help with the use of likelihoods and with the deer work generally. Penny Allen, Elena Valsecchi, Judith Smith, Dave Coltman and Ashleigh Griffin offered up their data sets for butchery by early versions of CERVUS and gave me many insights into ways I could improve the program. I feel that Graeme Swanson, Steve Paterson, Ian Stevenson and Jill Pilkington should also appear somewhere in these acknowledgements and this seems like as good a place as any.

I would also like to thank the BBSRC for financial support, the DSS for liberal interpretation of the phrase "actively seeking work", my family for their continuous moral support and generous credit terms, and John Deag and Vernon French for offering me a ladder to liquidity as well as the opportunity to learn some statistics at the expense of the 1997-1998 Zoology Honours class.

The last-but-not-least final round of thanks go to all past and present members of the Molecular Ecology lab in Cambridge and Edinburgh for tolerating my endless questions and Fresh Air FM, to my flatmates Katrina Lythgoe and Amanda Voase for putting up with my nocturnal comings and goings over the last few months and most importantly to the head canary keeper, Josephine Pemberton, for encouraging and guiding me through this PhD and for fantastic criticism of the thesis itself, without which it would have been unimaginably worse than it is now.

I am sure I am guilty of many errors and omissions both in these acknowledgements and in the thesis as a whole. For these and everything else I apologise.

Right, I'm off to the pub.

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1 Introduction

1.1 In the beginning...

The Bible contains lucid instructions on the subject of inbreeding and how man should avoid it. In Leviticus 18 (Ebor 1970), God tells Moses:

> *"No man shall approach a blood-relation for intercourse. I am the Lord. You shall not bring shame on your father by intercourse with your mother... You shall not have intercourse with your sister, your father's daughter, or your mother's daughter, whether brought up in the family or in another home; you shall not bring shame upon them. You shall not have intercourse with your son's daughter or your daughter's daughter: that is to bring shame upon yourself. You shall not have intercourse with a daughter of your father's wife, begotten by your father: she is your sister, and you shall not bring shame upon her. You shall not have intercourse with your father's sister: she is a blood relation of your father. You shall not have intercourse with your mother's sister: she is a blood relation of your father."*

In this passage, a man is prohibited from pairing with most close female relatives: his mother, his sister or half-sister, granddaughter or aunt. The only first degree ($r = 0.5$) female relative omitted from this list is his daughter, perhaps because a man's daughter is so obviously his blood relative that no clarification is needed. Most female relatives with a coefficient of relationship $r = 0.25$ are also included, the obvious exception being his nieces. Although some non-incestuous and non-reproductive pairings are also proscribed in Leviticus Chapter 18, the emphasis given to identifying sibs and half sibs ("whether brought up in the family or another home") suggests that avoidance of inbreeding is a central theme of the text.

Analyses of contemporary human populations have consistently found an association between inbreeding and juvenile mortality, even among generally condoned cousin marriages (Morton *et al.* 1956; Seemanová 1971; Bittles *et al.* 1991; Bittles and Neel 1994). Despite this cost, a study of dispersal in humans (Clarke and Low 1992) identified its causes as resource and mate competition rather than inbreeding avoidance, providing an example of the hazards of assessing the potential costs of inbreeding by looking for inbreeding avoidance behaviour. The costs of inbreeding in plants and animals (particularly domestic animals) are also well-established (Darwin 1876; Abplanalp 1990; Falconer and Mackay 1996). More recently the consequences of inbreeding have become a major concern in conservation (Ralls *et al.* 1979, 1986).

Unfortunately, studies of inbreeding in humans, wild animals, captive animals, domestic livestock, plants and *Drosophila* all seem to be progressing with relatively little reference to one another. As a result, inbreeding has come to have a variety of interpretations (Hoogland 1992, Shields 1993, Waser 1993b), making the literature an unwieldy tangle of definitions and approaches. The following review outlines the theoretical basis of inbreeding, ways inbreeding can be measured, inbreeding depression and the avoidance of inbreeding, and then goes on to discuss what can be gleaned from existing empirical data on inbreeding and its effects across a wide range of higher taxa.

1.2 Theory and techniques

1.2.1 Inbreeding of individuals and populations

Early this century Sewall Wright described a coefficient of inbreeding, f, and a method for calculating it (Wright 1922). f represents the probability that both alleles at a given locus are descended from a single allele in a common ancestor (Frankel and Soulé 1981; Hartl and Clark 1989). Instead of this identity-by-descent definition, the inbreeding coefficient may be viewed as the genetic correlation between two uniting gametes (Michod 1993). The inbreeding coefficient is also equivalent to half the coefficient of relationship, r, of the parents, if there has been no inbreeding in preceding generations (May 1979).

To calculate the inbreeding coefficient of any individual, one needs a starting point (Ralls and Ballou 1986). Conventionally one begins at the first generation for which information is available by assuming that all individuals breeding at that point are not inbred or related in any way (Falconer and Mackay 1996). Inbreeding coefficients of descendants are then calculated relative to this point in time. This method relies on the idea that providing the population is large, relatedness between distant ancestors increases f only slightly, and so failure to include such information will not lead to large errors in later generations of the pedigree.

Inbreeding is a word that has more than one meaning (Hoogland 1992). On the one hand, individuals may be classed as inbred or outbred (the absolute perspective, Waser 1993b). On the other hand, inbreeding may be used to describe a population rather than an individual (Shields 1993). Inbreeding at the population level is defined as deviation from random mating (the relative perspective, Waser 1993b). For a series of subpopulations, inbreeding can be partitioned into different components:

$$(1-F_{IS})(1-F_{ST})=(1-F_{IT})$$

where F_{IT} is the correlation of uniting gametes relative to random gametes from the whole population, F_{IS} is the correlation of uniting gametes relative to gametes from each subpopulation (averaged over subpopulations) and F_{ST} is the correlation of uniting gametes within subpopulations relative to gametes from the whole population (Hartl and Clark 1989). F_{IS} represents the average inbreeding coefficient f of individuals within each subpopulation due to subdivision, while F_{ST} is commonly used to assess the degree of genetic separation of two or more subpopulations, as it can be expressed in terms of sums of allele frequencies at a series of neutral marker loci. F statistics can be written in terms of heterozygosities at different levels of the population, so average inbreeding is assessed at an empirical level by looking for deviations of gene frequencies at one or more loci from Hardy-Weinberg expectations (Hartl and Clark 1989; Falconer and Mackay 1996). An excess of homozygotes implies inbreeding, while an excess of heterozygotes implies outbreeding.

In most animal species, the closest inbreeding that can occur is between full sibs or parents and offspring, assuming no inbreeding in preceding generations. Plant geneticists often use the word inbreeding in a different way (Waser 1993b). The

striking contrast in plant mating systems is between selfing and outcrossing, and therefore inbreeding has come to be used interchangeably with selfing, and outbreeding with outcrossing (e.g. Lande and Schemske 1985). Plant geneticists therefore often define individuals as inbred if $f = 1$, and outbred if $f < 1$, a rather different dichotomy to that typically used by animal geneticists, where more often individuals with $f \geq x$ are classed as inbred and only those with $f < x$ are regarded as outbred, and x normally lies between 0.1 and 0.25. In reality, biparental inbreeding (e.g. sib mating or parent-offspring mating) is quite likely under outcrossing in the many plant species that show isolation-by-distance due to restricted pollen and seed dispersal (Waser 1993a) or are obligate outcrossers (e.g. Schierup 1998), and may be in itself an important force in mating system evolution (Waller 1993).

In practice the inbreeding coefficient may not be the ideal measure of inbreeding in an individual. There are two major problems. First, likely to be particularly acute in the study of natural populations, is that a low frequency of erroneous parent-offspring links in the pedigree, or a small number of unknown parent-offspring links, may change individual inbreeding coefficients greatly. Second, the assumption that each founder individual at the top of a pedigree is unrelated to every other means that any inbreeding prior to that point in time is not represented. Analysis of a thoroughbred horse studbook suggested that common ancestry deep in a pedigree can make a large contribution to the overall inbreeding coefficient (MacCluer *et al.* 1983), suggesting that truncation of a pedigree may lead to large errors in calculation of inbreeding coefficients. The inaccuracy caused by ignoring prior inbreeding is likely to be greatest for populations that have gone through a recent bottleneck event, or are currently at low numbers. Species of conservation interest are likely to fall into this category. The error is also greatest for individuals at the top of the pedigree: the founders and first generation descendants inevitably have calculated inbreeding coefficients of zero. In conservation, key genetic management decisions often have to be taken involving these individuals.

There are also two more minor problems with f as a measure. First, f is an average value, and in practice there will be a binomial assortment of blocks of linked genes (bounded by chromosome ends and recombination events) at each meiosis. The realised inbreeding coefficient of any particular inbred offspring will therefore vary

around the average value f (Christensen *et al.* 1996). This problem will be greatest for species with few chromosomes and low rates of recombination. Second, f conventionally represents only the inbreeding at autosomal loci in diploids. In particular, X chromosomes have a different pattern of inheritance (Bittles 1991; Werren 1993), and so f is a poor approximation of inbreeding for species such as *Drosophila* where the X chromosome makes up a large proportion of the genome, although X-specific formulae for f are available. Thus for several reasons, alternative measures of individual inbreeding have been sought.

1.2.2 Alternative measures of individual inbreeding

With the advent of techniques for characterising molecular variation at the individual level, an alternative approach to the assessment of inbreeding became possible. Given that at a population/sub-population level, average inbreeding is expected to be related to average heterozygosity, heterozygosity within an individual across a series of marker loci should likewise be correlated with the degree of inbreeding in the individual.

The first markers to become available were protein polymorphisms. Many studies have examined the association between protein heterozygosity and fitness (see reviews by Allendorf and Leary 1986, Ledig 1986 and Mitton 1993). Results have been mixed, and two general conclusions emerge. First, rather few proteins are polymorphic under typical assay conditions, so that the genome is poorly sampled. Second, polymorphic proteins tend to have few alleles, so that even outbred individuals tend to be homozygous at most loci. The lack of polymorphism and allelic diversity may be particularly acute in species of conservation interest (e.g. Bonnell and Selander 1974; Woodruff and Ryder 1986).

Where positive associations are found between heterozygosity and fitness, these may be due to selection favouring heterozygotes at particular expressed loci (David 1997). However to study the general implication of inbreeding, a genome-wide marker-based estimate of inbreeding is required. DNA markers such as microsatellites are better suited to assessing genomic heterozygosity, since to a first approximation they may be considered neutral markers (Jarne and Lagoda 1996). Microsatellites typically have much higher heterozygosity than polymorphic protein markers (Queller *et al.* 1993),

and therefore have greater power than protein loci, on a per locus basis, to detect homozygosity due to inbreeding. Chapter 5 contains a more detailed discussion of this topic. The equilibrium level of variation at neutral markers in a population depends on the mutation rate and the migration rate (respectively endogenous and exogenous sources of genetic variation) and the effective population size, N_e, which controls the rate of loss of genetic variation through genetic drift (Hartl and Clark 1989; Harris and Allendorf 1989). Effective population size is discussed in more detail in Chapter 9.

1.2.3 Mechanisms underlying inbreeding depression

Inbreeding depression, the loss of fitness resulting from inbreeding, may operate in two distinct ways. First, inbred individuals have an increased chance of expressing deleterious recessive alleles. This source of fitness reduction under inbreeding is referred to as the dominance hypothesis (East and Jones 1919) or the partial dominance hypothesis (Wright 1977; Charlesworth and Charlesworth 1987). Second, at some loci there is a fitness advantage associated with a heterozygous genotype. The cost of inbreeding due to loss of heterozygosity at such loci is referred to as the overdominance hypothesis (Wright 1977; Charlesworth and Charlesworth 1987).

Distinguishing the dominance and overdominance hypotheses has become something of a preoccupation of evolutionary biologists studying inbreeding. Most reviews (e.g. Charlesworth and Charlesworth 1987; see also Waser 1993b) reinforced by recent experimental work (e.g. Barrett and Charlesworth 1991) have concluded that the majority of inbreeding depression observed in natural populations can be explained by homozygosity of partially recessive deleterious mutations, but other studies (e.g. Connor and Bellucci 1979, Brewer *et al.* 1990) have suggested that overdominance may play as important a role as partial dominance in generating inbreeding depression. Furthermore Charlesworth and Charlesworth (1987) point out that the simple dichotomy between partial dominance and overdominance hypotheses does not account for genetic variation that is maintained by other forces such as frequency-dependent selection (e.g. Paterson *et al.* 1998) or environments varying in space or time (Hedrick 1986). In summary, it seems that both partial dominance or overdominance mechanisms may have a role to play, and may need to share the stage with other mechanisms (Michod 1993).

1.2.4 Measuring inbreeding depression

Conservation biologists, often observing at close hand the birth and death of individual animals, are likely to conclude that inbreeding depression is occurring if an animal with related parents dies at a young age. However the real concern of conservation is not viable individuals, but viable populations. The same parents may breed again, and produce a second offspring of higher than average fitness, that makes up for the loss of its sib by producing many grandchildren, so that overall reproductive success is as high for inbreeding as for non-inbreeding pairs, despite higher juvenile mortality among the offspring of inbreeding pairs (e.g. Van Noordwijk and Scharloo 1981, Bittles *et al.* 1991). In other words, inbreeding may be deleterious to a particular inbred individual without being detrimental to the net fitness of their parents, or to the population. A simple assessment of juvenile survival in this case would find elevated juvenile mortality, and this might be called inbreeding depression. Based only on this finding, it would be wrong to suggest that individuals should necessarily have evolved to avoid mating with relatives, even though the potential consequences of inbreeding for the inbred individual may be very serious.

The magnitude of inbreeding depression is expected to vary for a given trait between populations, depending on the history of each population (Shields 1993). A bottleneck, as occurred in captive Père David's deer (*Elaphurus davidianus*: Ralls *et al.* 1979, 1988) increases the frequency of inbreeding and hence homozygosity (Woodruff and Ryder 1986). However this may have the effect of purging lethal or sublethal recessive alleles from the population, as individuals homozygous for these alleles die young or fail to reproduce. In subsequent generations, fewer highly deleterious alleles segregate in the population, so inbreeding depression will tend to be less severe, if lethal or sublethal recessives are a major cause of inbreeding depression. A deliberate programme of inbreeding was carried out in captive Speke's gazelle (*Gazella spekei*) in order to rid the population, which had just three founders, of deleterious recessive alleles (Templeton and Read 1984, but see Ballou 1997 and Willis and Wiese 1997). On the other hand, mildly deleterious recessive alleles, which may represent a substantial proportion of the total genetic load, are less readily purged by inbreeding. Furthermore, overdominance effects are expected to be less labile to

short term changes, since overdominance cannot be purged in the same way as can recessive mutations.

In natural populations one can visualise periods of population stability during which the inbreeding load (in terms of deleterious recessive mutations) steadily increases (Lynch 1995), until some event reduces population size to a low level when inbreeding occurs, the population is to a greater or lesser extent purged of its inbreeding load, and then stability returns and the process begins once more. The average fitness of animals with inbreeding coefficient $f = 0.25$ will vary through this process. So the relationship between inbreeding and inbreeding depression may not be static, but fluid. Contemporary population dynamics as well as past history may affect the magnitude of inbreeding depression.

The magnitude of inbreeding depression is also likely to vary between traits within populations (Charlesworth and Hughes 1996; Husband and Schemske 1996). Individual fitness components may co-vary positively, negatively or not at all with total fitness when different levels of inbreeding are compared. For an evolutionary explanation of the observed balance between inbreeding depression and the lengths to which organisms go to avoid it, one needs to know the total inbreeding load across the whole life cycle. Easy-to-measure fitness components such as early survival cannot be used as surrogates for total fitness when assessing the evolutionary forces acting on inbreeding.

In summary, each species and population is expected to respond differently to inbreeding. Each has its own mating system, its own genetic history and its own environmental conditions (1.3.3), and inbreeding depression could act on any trait at any stage of life.

1.2.5 Inbreeding avoidance

If inbreeding tends to have deleterious effects, species may be expected to have evolved mechanisms for avoiding inbreeding (Moore and Ali 1984; Blouin and Blouin 1988; Pusey 1987; Haring *et al.* 1990). Indeed many plants have evolved sophisticated self-incompatibility mechanisms for inbreeding avoidance, and in these species, inbreeding can have strongly detrimental fitness consequences (Lande and Schemske

1985; Schemske and Lande 1985; Charlesworth and Charlesworth 1987; Haring *et al.* 1990; Waser 1993a). In animals, inbreeding avoidance may be mediated either by dispersal or some kind of active avoidance when breeding opportunities with close kin are available (Blouin and Blouin 1988). However there are several problems associated with probing inbreeding from the perspective of inbreeding avoidance.

First, it is very difficult to construct an appropriate null model against which to test inbreeding avoidance (Ralls *et al.* 1986; Shields 1987, 1993; Pärt 1996). Essentially, the problem is to define random mating in a real natural population. Dispersal is not infinite, and within the area of dispersal, there are a finite number of mates. Furthermore, these mates may vary in their probability of being selected for reasons other than relatedness. Quantifying the available choices is extremely difficult in most populations.

Second, if behaviours or mechanisms consistent with inbreeding avoidance can be demonstrated in natural populations, this is often taken as evidence that inbreeding, were it to occur, would be deleterious. However in many cases there may be alternative explanations for the evolution of mechanisms that apparently facilitate inbreeding avoidance. Most commonly these are intraspecific competition for resources or intrasexual competition for mates (Moore and Ali 1984; Clarke and Low 1992). In practice dispersal may result from a mixture of intraspecific, intrasexual and inbreeding avoidance forces, and it can be difficult to tease them apart.

Third, there is a paradoxical ascertainment bias. If close inbreeding is very deleterious in a given species, individuals of that species have reason to avoid inbreeding. A study of the species may identify inbreeding avoidance behaviour, but is unlikely to be able to demonstrate any inbreeding depression because inbreeding is rarely observed. On the other hand, if in another species close inbreeding is not so deleterious, inbreeding may not be avoided so assiduously, and thus may be detected at a statistically useful frequency. In a species of this type, a study will tend to focus on the results of observed inbreeding events rather than inbreeding avoidance behaviours, and since inbreeding is not so deleterious, the study will probably conclude that there is little inbreeding depression. In other words, there may be a bias towards study of inbreeding depression in those species where observable inbreeding depression is least likely to occur. Very few studies jointly examine both inbreeding avoidance and

inbreeding depression (empirical studies in animals are reviewed in section 1.3.2 below and summarised in Table 1.1).

Fourth, even when there is a detectable cost to inbreeding, there are several reasons why individuals might nevertheless not choose the most distantly related mate. If the mate is too genetically distant, there is a risk of outbreeding depression. This is the fitness loss resulting either from the breakdown of co-adapted gene complexes that have evolved in particular sub-species or populations (Templeton 1986) or from low fitness of individuals heterozygous for genes conferring adaptation to local environments (Waser 1993a). Furthermore, mates at a greater physical distance will often incur a greater cost of dispersal, and this cost may exceed the cost of moderate inbreeding (Bengtsson 1978). Finally, natural selection acts to maximise representation of an individual's genes in subsequent generations. An individual may mate with a relative despite the elevated risk of juvenile mortality, if this risk is compensated for by the increased representation of genes carried in surviving offspring (Smith 1979; Chesser and Ryman 1986), if those offspring that do survive are of better quality (Van Noordwijk and Scharloo 1981; Shields 1993), or if by inbreeding, the individual does not forfeit successful outbred matings (Waser *et al.* 1986). In other words, a species in which there is no apparent inbreeding avoidance behaviour may nevertheless suffer inbreeding depression.

In summary, inbreeding avoidance is an important aspect of the study of inbreeding, but may not be taken as a surrogate in place of measuring the effects of inbreeding depression directly. Careful study is required to distinguish inbreeding avoidance from dispersal due to competition for resources or mates, and from random mating.

1.3 Empirical data

1.3.1 Inbreeding in captive animals

In 1979, Ralls, Brugger and Ballou published a seminal paper which showed that in many captive ungulate species, there was a negative association between pedigree inbreeding coefficient and juvenile survival. In other words, inbreeding depression was widespread in zoo animals. This study was backed up by subsequent analyses and

reviews (Ballou and Ralls 1982; Ralls *et al.* 1988; Lacy *et al.* 1993) and the early findings were rapidly integrated into the manuals of conservation biology (Soulé 1980, 1986).

However it is not clear that what is true for captive animals should necessarily be true for those same animals in their natural habitat. Ralls *et al.* (1986) suggested that inbreeding depression is expected to be more severe in the natural habitat than in captivity, but there was little data available at the time to reinforce this view (Shields 1993). Since Ralls *et al.*'s (1986) review, there have been many more studies of inbreeding in wild animals published, in part prompted by the increased ability to estimate relatedness or ascertain parentage using molecular techniques. Here I attempt to integrate the findings of published empirical work on wild animals, reviewing both inbreeding avoidance studies and studies with significant data on inbreeding depression. I also review a series of experimental studies, mostly in plants, which highlight the growing awareness of the interaction between inbreeding depression and the environment in which it is measured.

1.3.2 Inbreeding avoidance and inbreeding depression in wild animals

There are a large number of published studies on inbreeding in wild mammals and birds, summarised in Table 1.1. The studies can be broadly grouped into those monitoring inbreeding by behavioural observation alone, common in the 1970s and 1980s, and those studies, mostly published in the last decade, which supplement behavioural observations with molecular analysis to confirm inferred relatedness or parentage. The following two subsections are a selective discussion of the studies listed in Table 1.1. I have focused on mammals and birds because these are the only groups of animals in which routine monitoring of individual life history is possible, and because many species of mammals and birds are of conservation interest.

Table 1.1 *(overleaf) 41 published studies on inbreeding avoidance (IA) and/or inbreeding depression in wild mammals and birds. Unless stated otherwise, inbreeding avoidance was inferred from behavioural observation alone and inbreeding depression was estimated using inbreeding coefficients calculated from behavioural parentage data.*

Latin name	Common name	Reference	Evidence for inbreeding avoidance (IA)	Effect of inbreeding
Acrocephalus arundinaceus	Great reed warbler	Bensch *et al.* 1994	Little close inbreeding (determined by DNA fingerprint band-sharing)	Reduced hatching
Antechinus spp.	-	Cockburn *et al.* 1985	IA occurs, not due to competition for resources or mates (but see Shields 1993)	Not assessed
Aphelocoma c. coerulescens	Florida scrub jay	Woolfenden and Fitzpatrick 1984	Low frequency of close inbreeding	Not assessed
A. ultramarina	Mexican jay	Brown and Brown 1998	Low frequency of close inbreeding	Reduced hatching and first year survival
Canis lupus	Grey wolf	Smith *et al.* 1997	Microsatellite evidence (lack of allele-sharing) for IA despite opportunity for inbreeding	Not assessed
C. simensis	Ethiopian wolf	Sillero-Zubiri *et al.* 1996	DNA fingerprint band-sharing evidence for IA by extra pack copulation	Not assessed
Cervus elaphus	Red deer	Coulson *et al.* 1998	Not assessed	Reduced birth weight and neonatal survival
Colobus badius rufomitratus	Red colobus	Marsh 1979	Dispersal of both sexes cannot be explained by IA	Not assessed
Cynomys ludovicianus	Black-tailed prarie dog	Hoogland 1982, 1992	IA for close relatives (dispersal and reproductive suppression); frequent moderate inbreeding (based on allozyme data)	No effect on litter size, juvenile survival or growth
Equus caballus	Horse	Duncan *et al.* 1984	IA arising from non-random mating, assessed using allozyme-based pedigree data	Not assessed
Falco naumanni	Lesser kestrel	Negro *et al.* 1997	No IA. Dispersal due to resource and mate competition	Not assessed

Latin name	Common name	Reference	Evidence for inbreeding avoidance (IA)	Effect of inbreeding
Ficedula albicollis	Collared flycatcher	Pärt 1996	IA not cause of sex-biased dispersal	Not assessed
Geospiza fortis	Medium ground finch	Gibbs and Grant 1989	No deviation from random pairing	No effect on clutch size, hatching, fledging or juvenile survival
G. magnirostris	Large ground finch	Grant and Grant 1995	Inbreeding inevitable due to small founder base	No effect on hatching or fledging
Helogale parvula	Dwarf mongoose	Keane *et al.* 1996	Little dispersal, random pairing with respect to relatedness (determined by behaviour and DNA fingerprint band-sharing)	No effect on litter size or adult survival
Heterocephalus glaber	Naked mole-rat	Reeve *et al.* 1990	Inbreeding very common within colonies (but occasional dispersal - see O'Riain *et al.* 1996)	Not assessed
Homo sapiens	Human	Clarke and Low 1992	Dispersal due to resource and mate competition	Not assessed
H. sapiens	Human	Morton *et al.* 1956	Not assessed	Reduced juvenile survival
H. sapiens	Human	Seemanová 1971	Not assessed	Frequent incidence of lethality and deleterious traits in inbred children
H. sapiens	Human	Bittles *et al.* 1991	Close and moderate inbreeding relatively common (dependent on religion)	Higher fertility for related pairs but reduced juvenile survival of offspring
H. sapiens	Human	Bittles and Neel 1994	Not assessed	Reduced juvenile survival
Malurus splendens	Splendid fairy wren	Brooker *et al.* 1990	IA by extra-pair copulation demonstrated using allozymes (cf. Rowley *et al.* 1986)	Not assessed

Latin name	Common name	Reference	Evidence for inbreeding avoidance (IA)	Effect of inbreeding
Melanerpes formicivorus	Acorn woodpecker	Koenig and Pitelka 1979	IA by dispersal and reproductive suppression	Not assessed
Melospiza melodia	Song sparrow	Arcese 1989	No IA. Dispersal due to resource competition	Not assessed
M. melodia	Song sparrow	Keller 1994, 1998	Not assessed	Reduced crash survival, first winter survival, adult survival and female lifetime reproductive success
Microtus pennsylvanicus	Meadow vole	Pugh and Tamarin 1988	No IA (but see Bollinger *et al.* 1993). Radionuclide/allozyme parentage analysis showed frequenct inbreeding	Not assessed
M. townsendii	Townsend's vole	Lambin 1994	Sex-biased dispersal, behavioural evidence for active IA	Not assessed
Ochotona princeps	Pika	Peacock and Smith 1997	No IA. DNA fingerprint band-sharing evidence for mating between intermediate relatives	Not assessed
Ondatra zibethicus	Muskrat	Caley 1987	Sex-biased dispersal only partially consistent with IA	Not assessed
Oryctolagus cuniculus	European wild rabbit	Webb *et al.* 1995	DNA fingerprint band-sharing and sex-biased dispersal consistent with IA	Not assessed
Pan troglodytes	Chimpanzee	Pusey 1980	Female dispersal/behaviour consistent with IA	Not assessed

Latin name	Common name	Reference	Evidence for inbreeding avoidance (IA)	Effect of inbreeding
Panthera leo	Lion	Packer and Pusey 1993	Male dispersal and breeding behaviour consistent with IA; DNA fingerprint band-sharing low between mated pairs	Productivity in Ngorongoro crater population declined with estimated allozyme heterozygosity
Papio anubis	Olive baboon	Packer 1979	Male dispersal consistent with IA	Reduced juvenile survival
P. cynocephalus	Chacma baboon	Bulger and Hamilton 1988	Females do not disperse; males often do not disperse either	No effect on juvenile survival
Parus caeruleus	Blue tit	Kempenaers *et al.* 1996	Low frequency of close inbreeding	Reduced hatching success (inbreeding based on DNA fingerprint band-sharing)
P. major	Great tit	Bulmer 1973	Relatively low frequency of close inbreeding	Reduced juvenile survival, adult survival and breeding success
P. major	Great tit	Greenwood *et al.* 1978	Relatively low frequency of close inbreeding	Reduced juvenile survival
P. major	Great tit	Van Noordwijk and Scharloo 1981	No evidence for IA	Reduced hatching but no net effect on recruitment
Peromyscus leucopus	White-footed mouse	Wolff *et al.* 1988	Radionuclide/allozyme parentage analysis showed infrequent inbreeding. Dispersal common, not due to mate competition	Not assessed (but see Jiménez *et al.* 1994)
Porphyrio p. melanotus	Pukeko	Craig and Jamieson 1988	Little dispersal or evidence for IA; some suggestion of reproductive suppression	Not assessed
Sorex araneus	Common shrew	Stockley *et al.* 1993	Frequent mating between first degree relatives shown by DNA fingerprint band sharing; multiple paternity may be IA strategy	Reduced juvenile survival and growth

1.3.2.1 Behavioural studies

Many studies of inbreeding focus on inbreeding avoidance, expressed as some combination of dispersal and active avoidance of mating with nearby kin. If dispersal is a function only of inbreeding avoidance, one or other sex should disperse, but not both, if there is a cost to dispersal. Olive baboons (Packer 1979) and chimpanzees (Pusey 1980) both show this pattern, and behavioural interactions between adult kin are suggestive of active avoidance of inbreeding. However in red colobus both sexes disperse (Marsh 1979) while in chacma baboons neither sex disperses (Bulger and Hamilton 1988). Packer (1979) was able to present limited data suggesting reduced juvenile survival for inbred offspring in the dispersing olive baboon, while Bulger and Hamilton (1988) showed no similar effect in the non-dispersing chacma baboon.

Because dispersal may also be driven by competition for resources or mates, the importance of inbreeding avoidance as a cause of primate dispersal has been very contentious (Moore and Ali 1984, 1985; Packer 1985; Pusey 1987, 1988; Moore 1988). A meta-analysis of dispersal in social mammals, concentrating largely on primates, suggested that patterns of sex-biased dispersal across species are broadly consistent with inbreeding avoidance (Clutton-Brock 1989), although some dispersal patterns (e.g. red colobus) cannot wholly be explained by inbreeding avoidance.

Three general problems with studying inbreeding in primates emerge from these studies and reviews. First, behaviour consistent with inbreeding avoidance may occur, but it is difficult to show that inbreeding avoidance is driving the behaviour. Second, it is beyond the scope of most studies to observe sufficient inbred matings to carry out a rigorous analysis of inbreeding depression. Third, even closely related species have different patterns of inbreeding avoidance and inbreeding depression, and therefore it is unwise to make predictions about one species based on findings from another.

The same difficulties apply to the study of inbreeding in many animal taxa. In birds, acorn woodpeckers (Koenig and Pitelka 1979) and Florida scrub jays (Woolfenden and Fitzpatrick 1984) show dispersal and active inbreeding avoidance, while collared flycatchers (Pärt 1996), lesser kestrels (Negro *et al.* 1997) and song sparrows (Arcese 1989) exhibit dispersal that is not due to inbreeding avoidance, and pukeko do not disperse or show inbreeding avoidance behaviour (Craig and Jamieson 1988). Great

tits (Bulmer 1973; Greenwood *et al.* 1978; Van Noordwijk and Scharloo 1981), great reed warblers (Bensch *et al.* 1994) and song sparrows (Keller *et al.* 1994; Keller 1998) show inbreeding depression for juvenile survival and in some cases for adult survival and reproductive success, while medium and large ground finches show no inbreeding depression for hatching or fledging (Gibbs and Grant 1995; Grant and Grant 1995). The power of the tests for both inbreeding avoidance and inbreeding depression was low in many of these studies.

For small mammals, the picture is equally varied. Townsend's voles show sex-biased dispersal and direct inbreeding avoidance behaviours (Lambin 1994), but in muskrats sex-biased dispersal can only be partially explained by inbreeding avoidance (Caley 1987). A well-known study of marsupial *Antechinus* species (Cockburn *et al.* 1985), appeared to lend very strong support to the idea that animals disperse in order to avoid inbreeding. All juvenile females remain on their natal territories, while all juvenile males disperse from their natal burrows. After the seasonal breeding period, all males die. Thus competition for breeding with older males cannot be a cause of dispersal, since male generations are discrete. The only reason for a mother to eject her sons from the natal group seems to be to precipitate an exchange of males to mate with her daughters (Ralls *et al.* 1986), in other words to avoid the occurrence of sib-mating. However, the mating system was not properly understood. More recent work has shown that the dispersing males aggregate in leks, and females leave their home ranges in order to solicit mating at these lek sites. Both related and unrelated males are present in the leks, and there is no evidence for inbreeding avoidance one way or the other (Shields 1993).

1.3.2.2 The role of molecular techniques in the study of inbreeding

A more extreme example of the dangers of making assumptions based on behavioural observations is provided by a study of splendid fairy wrens. Analysis using behavioural paternity assignment appeared to demonstrate a strikingly high (almost 20%) frequency of close inbreeding with no evidence of any inbreeding depression (Rowley *et al.* 1986). However subsequent protein electrophoresis showed at least 65% of offspring were fathered by extra-pair males, and based on the probability of detecting non-paternity by this technique, the authors estimated that the frequency of extra-pair paternity may be as high as 100% (Brooker *et al.* 1990). Thus the results of

the earlier study were spurious, and the species may in fact provide an example of strong inbreeding avoidance.

A number of other studies have used genetic techniques to study inbreeding. Wild horses (Duncan *et al.* 1984), white-footed mice (Wolff *et al.* 1988), American toads (*Bufo americanus*: Waldman and Rice 1992), European rabbits (Webb *et al.* 1995), Ethiopian wolves (Sillero-Zubiri *et al.* 1996) and grey wolves (Smith *et al.* 1996) have all been shown to avoid inbreeding. On the other hand meadow voles (Pugh and Tamarin 1988) and dwarf mongooses do not avoid inbreeding (Keane *et al.* 1996), common shrews engage in frequent sib mating despite inbreeding depression for juvenile survival and growth (Stockley *et al.* 1993) and pikas inbreed regularly, showing a preference for individuals of intermediate relatedness (Peacock and Smith 1996). The pika study is the first example in the wild showing a similar pattern to Bateson's (1982) experiment with Japanese quails (*Coturnix coturnix japonica*), in which he demonstrated a preference for cousins over sibs or unrelated individuals (see also Van den Berghe 1983 for a discussion of optimal outbreeding in humans).

Hoogland's 14-year study of the black-tailed prairie dog (Hoogland 1982, 1992) provides an example of the contradictory evidence which can emerge even within the study of a single population. A large ($n \approx 200$) study population of individually-marked animals was monitored intensively over several generations. Hoogland supplemented careful mating observations with electrophoretic data to determine paternity, and found a full range of inbreeding and outbreeding events. An early analysis suggested that prairie dogs avoid inbreeding with close relatives via a combination of dispersal and reproductive suppression (Hoogland 1982). However subsequent analysis showed that there is frequent inbreeding between moderately related individuals, and the distribution of inbreeding events is not significantly different from the pattern expected from random mating (Hoogland 1992).

Genetic techniques are a major step forward in the study of inbreeding in natural populations. Interestingly, while a number of studies used allozyme data to infer or confirm parentage so that pedigree inbreeding coefficients could be calculated, the more recent DNA-based techniques have for the most part instead been used to calculate band-sharing between parents as an estimate of their relatedness. The popularity of the band-sharing approach may arise from the fact that it allows

inbreeding avoidance and/or inbreeding depression to be evaluated from a single generation of reproduction. Another popular use of allozyme data has been to assess the relationship between individual heterozygosity and fitness (1.2.2). Given the inherent superiority of microsatellite markers over protein polymorphisms for this kind of study (1.2.2), it is surprising that there are only two published studies (Coulson *et al.* 1998b, discussed in section 6.1.2, and Coltman *et al.* 1998a) of the association between microsatellite heterozygosity and fitness in wild animals (see also Coltman *et al.* in prep., discussed in 1.3.3 below and Coulson *et al.* submitted, discussed in section 6.1.2).

1.3.3 Environmental dependency of inbreeding depression

There is a large body of literature emphasising the deleterious effects of inbreeding in captive populations of ungulates (Ralls *et al.* 1979; Ballou and Ralls 1982), carnivores (Laikre and Ryman 1991; Laikre *et al.* 1996), mammals in general (Ralls *et al.* 1988; Lacy *et al.* 1993) and in wild animals and plants in the lab (e.g. Sittmann 1966; Lynch 1977; Willis 1993; see also Charlesworth and Charlesworth 1987). However the effect of inbreeding in the lab or in captivity is of unknown relationship to the effect of inbreeding on those same species in the wild environment.

Several recent experimental studies in invertebrates and plants have shown that the detection or magnitude of inbreeding depression may depend on the environment in which inbreeding depression is measured (Table 1.2). In particular, Dudash (1990) and Chen (1993) compared directly inbreeding depression in the greenhouse/lab with inbreeding depression for the same species in a more natural setting, and both found that inbreeding depression is stronger in the more natural and presumably more stressful environment. Interestingly, Mitchell-Olds and Waller (1985) found a similar pattern for survival, but the opposite pattern for growth (i.e. inbreeding depression for growth was only detected in the greenhouse). The overall conclusion from Table 1.2 is that failure to detect inbreeding depression in one environment does not imply that inbreeding depression will not occur under a different set of environmental conditions. The fact that some other studies do not demonstrate a clear association between inbreeding depression and environment (e.g. Miller 1994; Dahlgaard *et al.* 1995; Dahlgaard and Loeschkhe 1997) does not alter this conclusion.

Latin name	Common name	Environmental factor(s)	Trait(s) showing IxE interaction	Reference
Anthoxanthum odoratum		Competition	Growth	Antonovics 1968
Arianta arbustorum	Land snail	Lab vs. garden	Survival	Chen 1993
Costus laevis *C. allenii* *C. guanaiensis*		Shade	Growth	Schemske 1983
Hydrophyllum appendiculatum		Density	Growth	Wolfe 1993
Impatiens capensis	Jewelweed	Field vs. greenhouse	Survival, growth	Mitchell-Olds and Waller 1985
Impatiens capensis	Jewelweed	Density, competition	Growth	Schmitt and Ehrhardt 1990
Ipomopsis aggregata	Scarlet gilia	Transplantation, simulated herbivory	Survival, growth	Heschel and Paige 1995
Lychnis flos-cuculi		Drought, density	Survival	Hauser and Loeschcke 1996
Peromyscus leucopus	White footed mouse	Lab vs. wild	Survival, growth	Jiménez *et al.* 1994
Sabatia angularis	Rose pink	Field vs. garden vs. greenhouse	Fitness	Dudash 1990
Tribolium castaneum	Red flour beetle	Humidity	Fecundity (competition)	Pray *et al.* 1994

Table 1.2 *Published experiments demonstrating environmental dependency of inbreeding depression. Competition denotes experiments in which inbred and outbred individuals were tested in direct competition with each other.*

The question of the environmental dependence of inbreeding depression has also been investigated in mammals and birds. An enlightening experiment involved taking *Peromyscus* mice into captivity, inbreeding a proportion of them, and then releasing the marked offspring back into the wild (Jiménez *et al.* 1994). After release, inbred mice lost body weight and had lower survival than initially similar outbred mice, and the calculated inbreeding load for the mice in the natural habitat was much greater than for the same mice in the laboratory, mirroring the findings of Dudash (1990) and Chen (1993).

A detailed long term study of song sparrows in British Columbia could find no evidence that dispersal was motivated by inbreeding avoidance, and identified competition for food resources as the cause of dispersal (Arcese 1989). However, the presence or absence of inbreeding avoidance is not necessarily related to the presence or absence of inbreeding depression (1.2.5). Subsequent study of the same population revealed that inbred birds were more likely to die in the severe population crash that occurred in 1989 (Keller *et al.* 1994). In this population, measurable inbreeding depression was only expressed in the face of an extreme environmental test.

Coltman *et al.* (in prep.) report a second example of environmentally-dependent inbreeding depression in Soay sheep (*Ovis aries*) on Hirta, St. Kilda, Scotland, a population which also suffers population crashes at irregular intervals. The population is parasitised by intestinal nematodes, primarily *Teladorsagia circumcinta*, which are known to negatively influence crash survival (Gulland 1992). Coltman *et al.* demonstrate that inbreeding also negatively influences crash survival, that the cost of inbreeding is mediated via parasite burden and that the relationship between parasite burden and inbreeding is dependent on population density.

The growing body of evidence on the environmental dependence of inbreeding depression shown both experimentally (Table 1.2) and in the wild (Keller *et al.* 1994; Coltman *et al.* in prep.; see also Coulson *et al.* submitted) demonstrates that genetic load is not a constant property of a species that can be measured in any experimentally convenient environment. In order to properly understand the long term selective forces acting on inbreeding and inbreeding depression, it is essential that inbreeding and its effects are evaluated in the species' natural setting (Shields 1993).

1.4 Outline of the thesis

Despite the fact that so much attention has been focused on the importance of inbreeding in captive ungulates (Ralls *et al.* 1979; Ballou and Ralls 1982), to date only Coulson *et al.* (1998b, based partly on data from this thesis) have published data on inbreeding in wild ungulates. The main objective of this study is to provide data on inbreeding and its fitness implications for two ungulate species, red deer (*Cervus elaphus*) and Arabian oryx (*Oryx leucoryx*), in their natural habitats. Chapter 2 describes the history of and previous work on red deer on the Isle of Rum. Chapter 3 reviews and develops the theoretical basis for paternity inference using data from co-dominant markers such as microsatellites, and Chapter 4 presents the results generated by applying these paternity inference techniques to Rum red deer. Chapter 5 compares different approaches to assessing inbreeding at the individual level, while Chapter 6 is a detailed analysis of inbreeding and its consequences in Rum red deer for both males and females. In Chapter 7 the focus shifts to Arabian oryx, beginning with a history and review of the current status of this charismatic species rescued from the brink of extinction. Chapter 8 is a microsatellite-based genetic analysis of Arabian oryx in the Middle East, focusing particularly on inbreeding and outbreeding in reintroduced Arabian oryx in Oman. The thesis concludes with Chapter 9, a critical perspective on the importance of genetics in conservation.

2 Red deer on Rum

2.1 Introduction

The red deer (*Cervus elaphus*) has a vast range across the temperate areas of Eurasia. The species is numerous in the UK, particularly in Scotland, where the estimated population is around 330,000 (Youngson 1998). Scottish red deer tend to be smaller than their mainland European counterparts, at least in part due to the harsh, mineral-poor environment in Scotland (Clutton-Brock *et al.* 1982). Eurasian red deer in turn are smaller than the closely related North American wapiti (*Cervus canadensis*). Adult male red deer are approximately 50% heavier than adult females, and the species is strongly polygynous.

The intensively studied population of the Isle of Rum (Clutton-Brock *et al.* 1982) is the focus of this work (Figure 2.1). The 10,600ha island of Rum (57°0'N, 6°20'W) lies south of Skye, 22km off the Scottish mainland (Figure 2.2), and apart from one large and several small fenced areas, is treeless. The red deer range across the whole island, with the highest densities in Kilmory Glen, on the north side of the island. The deer in Kilmory Glen, the west end of the adjoining Kinloch Glen and on the neighbouring hill Mulloch Mor down to the coast at Shamhnan Insir (collectively known as the North Block) form the study population. Regular culls take place across other parts of the island, but no culling has taken place in the study area since 1973 (Clutton-Brock *et al.* 1982) at which time there were approximately 60 hinds and 130 stags over one year of age in the study area. Between 1973 and 1979 hind densities increased dramatically in the study area, while between 1980 and 1982 stag densities decreased somewhat. Since 1982 the composition of the study area population has remained relatively constant, comprising around 130 adult hinds and 110 adult stags (Pemberton *et al.* 1996). The study area population accounts for approximately one quarter of the population across the island as a whole.

Hinds tend to remain in their natal areas, while stags tend to disperse before reaching reproductive age. One third of stags born in the study area before 1973 left the study population and adopted home ranges elsewhere before their fifth winter. Conversely

young stags born elsewhere tended to immigrate into the study population, and approximately 25% of all the stags between the ages of six and eight resident in the study area in 1979 were immigrants (Clutton-Brock *et al.* 1982). In addition, some stags temporarily move from their normal ranges to a rutting area in a different part of the island during the rut period (2.3). Given the high level of movement of stags around the island, the population can be considered panmictic.

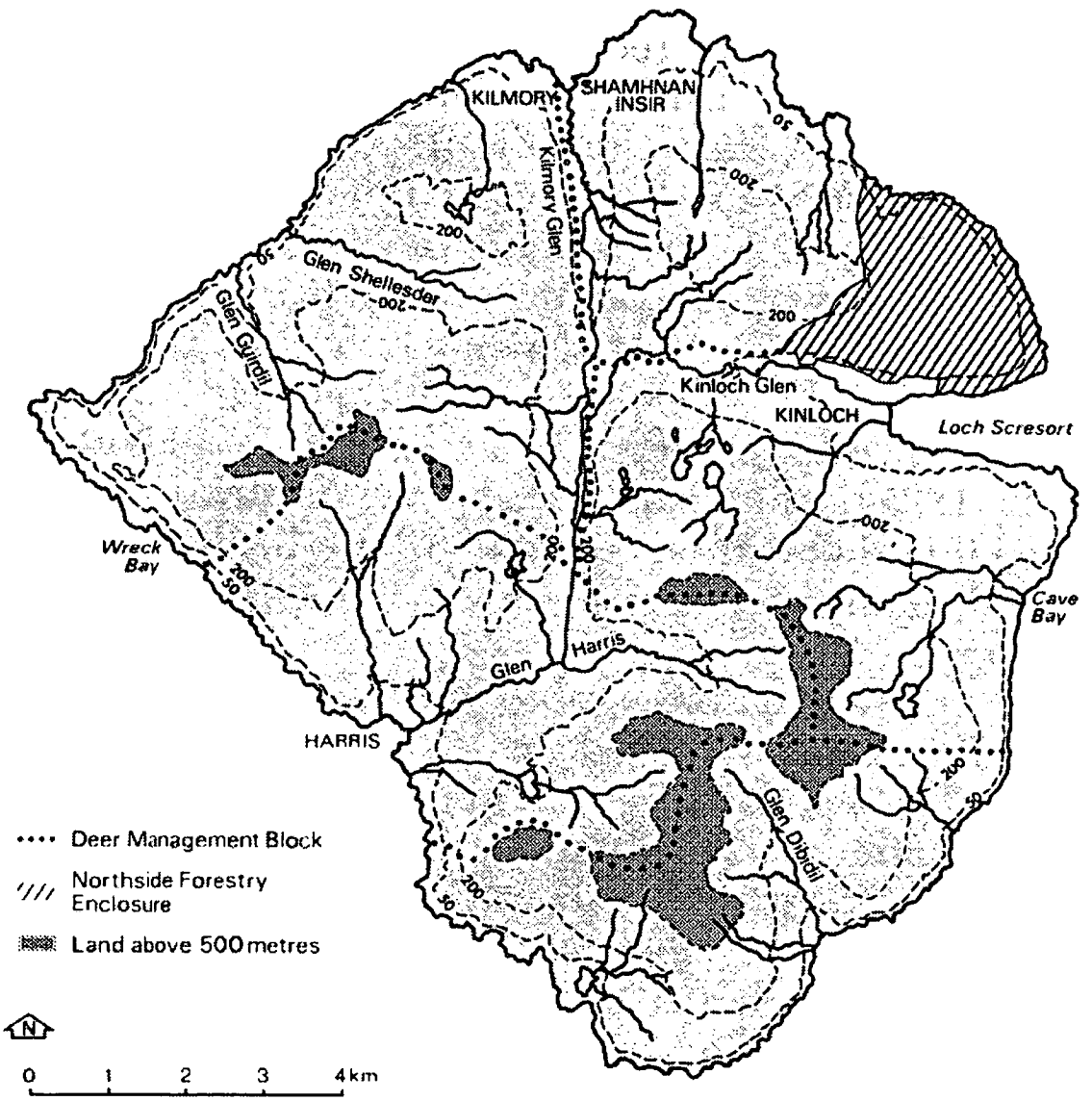

Figure 2.1 *Map of the Isle of Rum, reproduced from Clutton-Brock et al. (1982).*

Occasionally study area deer may be shot if they are present in other parts of the island during the cull. Unmarked animals, animals with home ranges on the fringes of the study area and animals that have emigrated from the study area are most vulnerable.

2.2 History of the population

Red deer were common on Rum in mid 16th century until at least the early 18th century (Clutton-Brock *et al.* 1982). It is not known whether they occurred naturally or were originally introduced by man. Although red deer are able to swim considerable distances (e.g. Rum deer are thought to have swum to the island of Eigg, 6km to the south-west (F. Guinness, pers. comm.) - see Figure 2.2), there is no record of any exchange with the red deer populations on Skye (11km) or the mainland (22km). The growth of the human population on Rum in the 18th century, the destruction of the forest and the concomitant rise in sheep farming led to a decline in deer numbers. By 1772 there were only 80 deer left, and around 1787 they went extinct (Clutton-Brock *et al.* 1982).

There were a large number of founders of the current Rum deer population, from a variety of sources. The island was first restocked by Lord Salisbury, who began importing red deer shortly after acquiring Rum in 1845 (Table 2.1). The initial source was the herd at Windsor Great Park in Berkshire, soon bolstered by animals from Knowsley Park in Lancashire, and quite possibly from other unrecorded sources. The changing size of the Rum deer population is charted in Figure 2.3. The island was taken over by the Campbells of Ballinaby in 1869, but the main effort to increase the deer population on Rum seems to have been undertaken by John Bullough, who bought Rum in 1886, and his son Sir George Bullough who took over the estate when his father died in 1891 (Love 1987). They brought hinds from their Meggernie estate in Perthshire, and stags from Windsor (J. Love, in prep.), presumably because well-fed Windsor stags tended to have impressive antlers compared with their nutritionally challenged Scottish counterparts. In the 1920s, a significant number of deer were brought to Rum from Warnham Park in Sussex, again probably because Warnham had a reputation for particularly well-endowed stags.

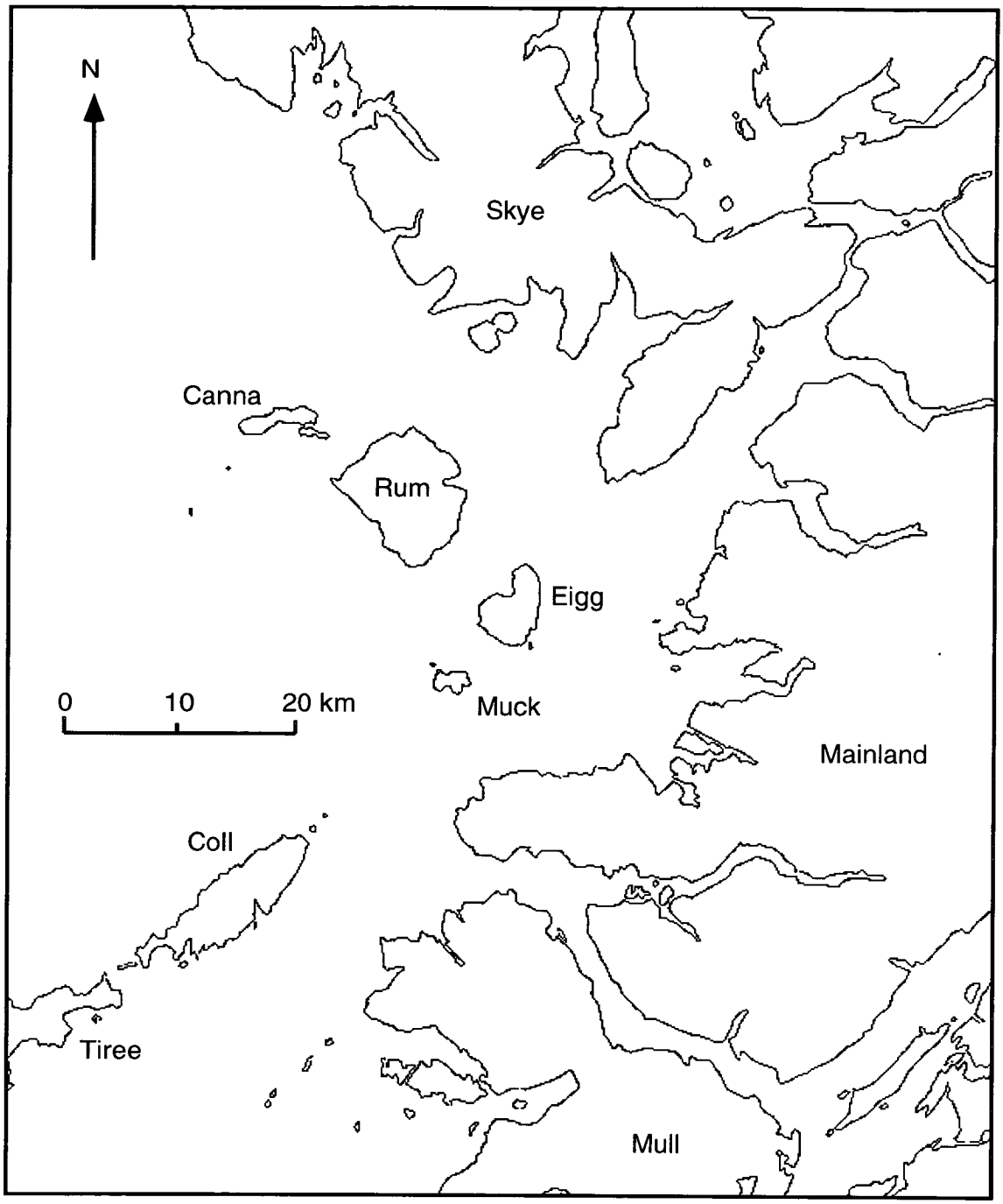

Figure 2.2 *Map of the Inner Hebrides around Rum.*

No deliberate introductions of deer to Rum took place after the Nature Conservancy Council took over the island in 1957. However one final introduction occurred by accident in 1972. A series of experiments exploring the inheritance of hummelism (the absence of antlers) took place in a fenced 14ha enclosure on Rum. In winter 1969-1970, a hummel from Braemar was crossed with several Rum hinds (Lincoln *et al.* 1971). In 1971 several male offspring of the hummel were released into the study

area population at Kilmory, having been vasectomised, and some further vasectomised male offspring of the hummel were released in 1972 and 1973 (Lincoln *et al.* 1973). One of these offspring, MAXI, went on to rut very successfully in the study area, holding a harem for several weeks in his first rut in 1975 aged 5 (Lincoln *et al.* 1976), and the vasectomy was clearly unsuccessful as dozens of calves born in the late 1970s and early 1980s have proved to be his offspring (Pemberton *et al.* 1992; Chapter 4). A few other stags of similar provenance also rutted in the study area in the 1970s, but in these cases the vasectomy operations are thought to have been successful.

Date	From	Importer	Number	Source
1845 →	Windsor Great Park, Berkshire	Marquis of Salisbury	Unknown	Banks (1977)
1850 - 1852	Knowsley Park, Lancashire	Marquis of Salisbury	Unknown	Love (in prep.)
1887 →	Meggernie, Glen Lyon, Perthshire	John Bullough	4-5 annually	Love (in prep.)
1887 →	Windsor Great Park, Berkshire	John Bullough	Unknown	Love (in prep.)
1926 - 1929	Warnham Park, Sussex	Sir George Bullough	19 stags, 26 hinds	Records from Warnham Park
1972	Mar Lodge Estate, Braemar	Gerald Lincoln and Roger Short	Stag MAXI, offspring of Rum hind and Braemar hummel	Lincoln and Short (1969)

Table 2.1 *Known introductions of red deer to Rum between 1845 and 1973. Almost certainly other introductions occurred prior to 1957 that are not listed here; however the 1920s introductions from Warnham Park are almost certainly the only importations from that source. An arrow is used to indicate a series of introductions from the same source beginning in the year specified, where details are not available. The Rum Game Book contains an entry on 4th October 1900 that a Captain Christie shot an animal referred to as "the Austrian stag". There is no record of importation of animals from Austria, and it may be that the stag was so named for its phenotypic features rather than its genetic origin. Details of UK deer parks are available in Hingston (1988).*

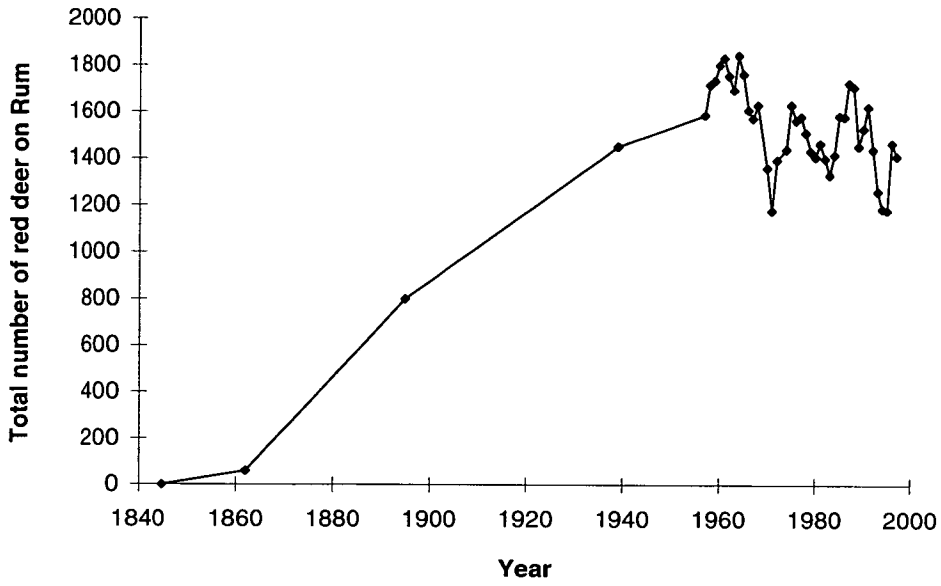

Figure 2.3 *Estimated numbers of deer on Rum between 1845 and 1997. Data 1957 - 1980 are a series of regular censuses by the Nature Conservancy Council (1957 - 1965) and the Deer Commission for Scotland (1966 - 1997). The 1957 - 1980 census data were taken from Appendix 2 of Clutton-Brock et al. (1982), while 1981 - 1997 census data were taken from Clutton-Brock et al. (1998). Other estimates were taken from Chapter 2 of Clutton-Brock et al. (1982), except the 1862 estimate of 60 (J. Love, in prep.). The population has been regulated by an annual cull for at least the last 40 years.*

2.3 Ecological and behavioural work

The book *Red Deer - Behavior and Ecology of Two Sexes* by Clutton-Brock *et al.* (1982), which focuses on the Rum population, is a seminal study of a large mammal species in its natural habitat. The book contains a wealth of information on the behaviour and ecology of male and female red deer, and here I will only summarise a few of the main findings from this and other studies on Rum which are relevant to the work presented in this thesis.

Since the early 1970s, 80% of calves born in the study area have been sexed, tagged, marked, measured and weighed, usually within 72 hours of birth but in some cases up to two weeks after birth. Markings consisted of a mixture of ear notches, ear tags,

coloured flashes, and additionally coloured and patterned Darvick (expandable) collars for females. The combination of markings allowed unique identification of all animals using the study area, the unmarked proportion being small enough that they could be recognised from individual phenotypic features by experienced field workers. Darting, using the drug Immobilon, was carried out on an opportunistic basis from 1982 onwards, avoiding rutting and calving seasons. Additional tagging and marking of these animals was carried out at the same time if their markings were deficient. Numbered plastic cattle collars supplemented with coloured blocks and slips were often fitted to sub-adults and adults to aid identification.

The annual rut takes place in autumn of each year. Dominance hierarchies are established among the stags during late August and September by roaring contests, supplemented by ritualised parallel walks and fights. By late September most hinds join one of the harems held by the dominant stags, and daily censuses of the study area are carried out throughout the rutting period to determine harem membership. The first hinds come into oestrus in late September, reaching a peak in mid-October. The rut continues at lower intensity through November and into December. Hinds are receptive during oestrus for only a few hours, particularly at the peak of the rut, and are generally mated only once. If they fail to conceive, hinds can come into oestrus again around 18 days later (Guinness *et al.* 1971).

Hinds probably join harems to avoid continual harassment by subordinate males. Most harems represent one or several matrilineally related hind and calf feeding groups. Hinds have to tolerate periodic chivvying by the dominant male in whose harem they reside, but otherwise are free to feed providing they do not wander too far. Males cannot physically prevent females from leaving a harem, though they always attempt to herd errant females back into the main group. Yearling and older males are not tolerated in these groups, but male calves are generally ignored by the dominant male.

Harem-holding males are typically aged 5-11, reaching their peak condition at age 8 (Figure 4.4). Non harem-holding males, particularly young males, tend to gather near large harems, and attempt to extract females from the harem when opportunities arise. Older males without harems may be more solitary, taking the opportunity to feed, in some cases challenging the tiring dominant males later in the rut.

Single calves are born after an average 235-day gestation from early June onwards (235 days is the median gestation length for 46 Rum hinds calculated from data presented in Guinness *et al.* (1978); Clutton-Brock *et al.* (1982) report means and standard deviations of gestation length as 236.1 ± 4.75 days for males and 234.2 ± 5.04 for females based on a total of $n = 70$ oestrus observations, but do not show that the sex difference is significant; the average value of 235 days was used in this study and in Clutton-Brock *et al.* (1997)). By backdating the average gestation length from the date of birth, the stag or stags seen holding the hind in the rut on the estimated date of conception and the five days either side of that date are noted. This period is known as the 11-day window, with five days representing approximately one standard deviation of the gestation length. Paternity may be assigned categorically to a stag if he held the hind for more days than any other during the 11-day window (the approach actually used by Clutton-Brock *et al.* (1982) and described in Rose (1995)) or fractionally according to the number of days each stag was seen with the hind during the 11-day window (the approach Clutton-Brock *et al.* (1982) alleged that they used; see also Pemberton *et al.* (1992) and Rose (1995)). These two behavioural approaches to paternity assignment, respectively called *longest hold* and *fractional* approaches (referred to as the 'whole calf' and 'subdivided calf' methods respectively in Rose (1995)), will be contrasted with genetic approaches to paternity assignment below (2.4.3) and in Chapter 4.

2.4 Genetic work

2.4.1 Sampling

Since 1982, captured calves have been sampled for genetic studies, samples typically including ear punches and capillary or jugular blood. All darted deer have also been sampled. Ear punches were stored at -20°C until analysis; blood samples, collected into tubes containing the anticoagulant heparin, were separated into plasma, white cell and red cell fractions before storage at -20°C (Pemberton *et al.* 1988). Mortality provided an additional opportunity for sampling. From 1982 onwards, ear, muscle and kidney samples were taken from all identifiable corpses in the study area. In cases of

significant decay, ear or other skin sample only was taken. Tissue was stored at -20°C until use.

2.4.2 Protein polymorphisms

Pemberton *et al.* (1988) surveyed 33 putative protein loci for polymorphism using a test panel of at least 40 individuals chosen at random. Seven loci (21%) were found to be polymorphic in at least one of the available tissues, but practical considerations (convenient tissue, high heterozygosity and clear Mendelian inheritance) meant that only three (isocitrate dehydrogenase, IDH-2, mannose phosphate isomerase, MPI and transferrin, TRF) were selected for large scale screening. Details of these loci can be found in Table 2.2.

For all three of these loci, Pemberton *et al.* (1988) showed differential survival for different genotypes. Pemberton *et al.* (1991) went on to show that differential survival at MPI and IDH-2 was opposed by increased fecundity of individuals with genotypes unfavourable for survival. Even though it is not certain that these loci, rather than linked loci, are responsible for the observed fitness differences, it seems that these protein loci cannot be regarded as neutral markers in Rum deer. For a detailed reanalysis of the fitness associations shown by Pemberton *et al.* (1988, 1991), see Coulson *et al.* (1998a).

Screening of protein loci was discontinued from 1994, given the much higher levels of polymorphism found at microsatellite loci (see 2.4.4 below). However, existing protein data were used in this work as they do contribute some useful information to paternity inference (Chapter 4).

2.4.3 DNA fingerprinting

The invention of multilocus DNA fingerprinting (Jeffreys *et al.* 1985a, 1985b), and the realisation that the technique could be applied to species other than humans (Burke and Bruford 1987) provided an opportunity to validate genetically the behavioural approaches to paternity determination described in Section 2.3 above (Pemberton *et al.* 1992). Paternity was determined by DNA fingerprinting for 80 calves, of which 74

(93%) were cases in which the stag had been seen holding the hind in his harem for at least one day during the 11-day window.

An important analysis in Pemberton *et al.* (1992) was illustrated by a graph showing the probability of paternity against the number of days for which the stag held the hind during the 11-day window (reproduced as Figure 2.4 below), which suggested that stags seen with a hind for 6 or more days of the 11-day window could be assigned paternity with complete certainty. This result was used to assign paternity for a large number of calves based only on behavioural data, and these paternity data were used in subsequent analyses (Rose 1995; Clutton-Brock *et al.* 1997; Rose *et al.* submitted-a, submitted-b). However the analysis in Pemberton *et al.* (1992) is problematic for at least three reasons. First, sample sizes in the upper portion of the graph are relatively small, and so there is insufficient statistical power to distinguish certainty (100% confidence) from, for example, 90% confidence. Second, the steepness of the slope may be exaggerated, since 227 data points are included for 76 calves. This implies that for every male that could have been the father, on average two others are also represented in the graph that could not have been the father. *A priori* these are most likely to be clustered in the left hand part of the graph. Third, the set of paternity tests carried out was not random with respect to behavioural observations. Any stag not recorded as holding a particular hind in his harem around the estimated date of her conception was not tested for paternity of her calf. If he were the true father (i.e. had obtained paternity by some kind of "sneaky" mating strategy, Brooker *et al.* 1990), paternity would remain unresolved and therefore the calf would be excluded from this analysis. Systematic exclusion of these cases, where the stag who held the hind for longest would not have been the true father, may lead to exaggeration of confidence which can be placed in behavioural inference of paternity. Given these criticisms, I repeated the analysis using a larger, unbiased data set derived by microsatellite-based paternity inference (4.3.5).

2.4.4 Microsatellites

Polymorphic simple sequence repeat (microsatellite) loci are ubiquitous in eukaryote genomes and can be amplified using the polymerase chain reaction (PCR) from small amounts of degraded DNA derived from almost any tissue (Schlötterer and Pemberton

1994). Microsatellites are single-locus Mendelian markers with unambiguous codominant alleles, often show high heterozygosity and yield data that can readily be computerised (Queller *et al.* 1993). Microsatellites were therefore chosen in order to carry out large-scale paternity analysis in Rum red deer. Initially $(CA)_n$ repeat microsatellites were cloned from red deer genomic DNA (J. Pemberton, unpublished data). Three primer pairs derived from these clones, CelJP15, CelJP27 and CelJP38, were found to reliably amplify loci showing high levels of polymorphism, and all sampled Rum deer were typed at these loci (Table 2.2).

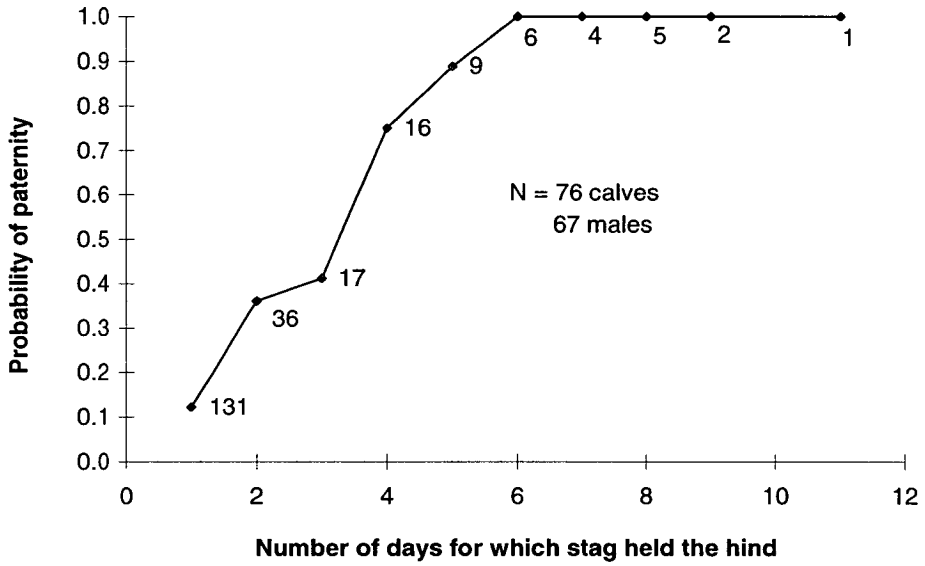

Figure 2.4 *Probability of paternity of Rum red deer calves, with respect to the number of days (of eleven around the estimated date of conception) during which a stag held the hind in question. Redrawn from Figure 3a in Pemberton et al. (1992).*

Another advantage of microsatellites is that primers cloned in one species can often be used to amplify the same locus in a related species (Moore *et al.* 1991; Schlötterer *et al.* 1991; Engel *et al.* 1996; Slate *et al.* in press). A series of primer pairs derived from microsatellite loci cloned in cattle and sheep were tested in Rum red deer (Pemberton and Slate 1994; J. Slate, unpublished data). A large proportion of primer pairs amplified polymorphic products in Rum red deer, and six loci, OarFCB193, OarFCB304, MAF35, MAF109, OarCP26 and TGLA94 were selected for typing in all sampled Rum deer (Table 2.2). These loci were selected on the basis of high

heterozygosity, reliability of amplification and ease of scoring. The allele frequencies are given in Table 2.3.

Locus name	Alleles	Number of genotypes	H_e	Reference
Mannose phosphate isomerase (MPI)	2	890	0.257	Pemberton *et al.* (1988)
Isocitrate dehydrogenase (IDH-2)	2	893	0.500	Pemberton *et al.* (1988)
Transferrin (TRF)	2	702	0.428	Pemberton *et al.* (1988)
CelJP15	10	1107	0.837	J. Pemberton, unpublished data
CelJP27	6	1059	0.691	J. Pemberton, unpublished data
CelJP38	8	999	0.786	J. Pemberton, unpublished data
OarFCB193	11	1044	0.761	Buchanan and Crawford (1993)
OarFCB304	9	1102	0.789	Buchanan and Crawford (1993)
MAF35	7	994	0.673	Swarbrick *et al.* (1991a)
MAF109	6	1056	0.751	Swarbrick and Crawford (1992)
OarCP26	13	1086	0.722	Ede *et al.* (1995)
TGLA94	9	1042	0.808	Georges and Massey (1992)

Table 2.2 *Polymorphic protein and microsatellite loci used in this study. Cel microsatellites are randomly-cloned sequences from red deer (Cervus elaphus); Oar and MAF microsatellites are randomly-cloned sequences from domestic sheep (Ovis aries); TGLA94 is a randomly-cloned microsatellite sequence from domestic cattle (Bos taurus). Expected heterozygosity (H_e) was calculated using the program* CERVUS.

A disadvantage of microsatellites is that segregating mutations in one or both primer binding sequences can give rise to non-amplifying or null alleles (Callen *et al.* 1993; Koorey *et al.* 1993; Phillips *et al.* 1993). Null alleles may occur more frequently when primers are used to amplify loci in species distantly related to the one in which the microsatellite sequence was originally cloned. A tenth microsatellite locus, RBP3 (MacHugh *et al.* 1997), was typed for all sampled Rum deer, but is not included in any of the analyses presented here since it was found to have a null allele segregating at a frequency of 6%. Screening at two further loci was discontinued at an earlier stage

when it became apparent that null alleles were segregating at high frequency (Pemberton *et al.* 1995: in this paper, RPB3 was called BOVIRBP).

MPI		IDH-2		TRF		OarFCB193	
Allele	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**
F	0.1517	F	0.4916	F	0.6902	99	0.1054
S	0.8483	S	0.5084	S	0.3098	101	0.1834
						103	0.0038
						105	0.0441
						107	0.0129
						111	0.4181
						115	0.0426
						117	0.0900
						119	0.0900
						125	0.0086
						135	0.0010

OarFCB304		CelJP15		CelJP27		CelJP38	
Allele	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**
129	0.0649	103	0.0804	183	0.4556	207	0.1136
131	0.3303	107	0.2127	187	0.0930	209	0.3248
133	0.2187	111	0.1256	191	0.2535	211	0.2127
135	0.0681	113	0.2254	193	0.1671	213	0.2067
137	0.0064	115	0.0014	195	0.0288	215	0.0671
139	0.0023	117	0.0208	197	0.0019	217	0.0536
143	0.1334	119	0.0217			221	0.0075
145	0.1665	121	0.0574			223	0.0140
147	0.0095	123	0.1906				
		131	0.0641				

MAF35		MAF109		OarCP26		TGLA94	
Allele	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**	**Allele**	**Frequency**
124	0.2777	161	0.2723	127	0.0009	128	0.1651
126	0.0005	177	0.0185	129	0.3416	130	0.0681
128	0.0277	181	0.2528	135	0.0005	134	0.0806
130	0.1378	183	0.1534	137	0.0336	140	0.0494
132	0.4738	185	0.2959	139	0.0364	148	0.1699
133	0.0760	189	0.0071	145	0.0953	152	0.3373
136	0.0065			147	0.0005	154	0.0566
				149	0.0005	156	0.0005
				157	0.0355	158	0.0725
				159	0.0322		
				163	0.3831		
				165	0.0373		
				171	0.0028		

Table 2.3 *Allele frequencies for three protein and nine microsatellite loci typed in Rum red deer (details in Table 2.2), calculated using the CERVUS program.*

2.5 Inbreeding and fitness in Rum red deer

The overall objective of my work on red deer was to assess the fitness consequences of inbreeding in the Rum population. In order to achieve this objective, it is necessary to calculate for each individual analysed a measure of inbreeding and a measure of fitness. The analyses presented in the following chapters (3-6) depend on the genetic data summarised in Table 2.2, which were obtained by J. Pemberton (proteins) and J. Slate (microsatellites). These data are used to assess individual inbreeding in several ways, and also to estimate individual fitness of male red deer.

One approach to the assessment of inbreeding is to calculate inbreeding coefficients from pedigree data. In Rum red deer, as in many polygynous populations, maternity can be derived by observation but paternity cannot. One obvious use of molecular data is to infer paternity for the calculation of inbreeding coefficients; the resulting paternity data can also be used to estimate male mating success. However the

statistical techniques required to infer paternity on such a large scale are far from obvious. In Chapter 3 I develop a technique for likelihood-based paternity inference using co-dominant marker data which allows assessment of statistical confidence in individual paternity assignments via computer simulation (see also Marshall *et al.* 1998, and associated Windows package CERVUS). In Chapter 4 I apply this technique to the Rum data set, and use the results to reassess previous DNA fingerprinting work (Pemberton *et al.* 1992), to validate behavioural approaches to paternity inference, and to estimate lifetime mating success of stags born 1982-1986. Data are also presented on hind lifetime mating success for the same cohorts.

In Chapter 5 I combine the paternity data presented in Chapter 4 with observational data on maternity in order to calculate pedigree-based inbreeding coefficients. I compare the utility of inbreeding coefficient data with two molecular measures of inbreeding, individual heterozygosity and a novel microsatellite-based measure, mean d^2, which was first suggested by Coulson *et al.* (1998b) in an analysis of the Rum data set. Coulson *et al.* (1998b) examined the relationship between measures of inbreeding and two early fitness measures, birth weight and neonatal survival, and in a subsequent paper examined the relationship between inbreeding and winter survival of juvenile red deer (Coulson *et al.* submitted). In Chapter 6 I assess the relationship of individual heterozygosity and mean d^2 with lifetime mating success of male and female red deer derived in Chapter 4, and synthesise the results with those of Coulson *et al.* (1998b, submitted).

3 Likelihood-based paternity inference using microsatellites

3.1 Introduction

In classical paternity inference one excludes as many as possible of the candidate males from paternity of a particular offspring using the available genetic data. If this procedure yields a single non-excluded male, paternity is assigned to that male. This is the basic methodology underlying human parentage testing (Chakraborty *et al.* 1974), and there are several examples of the approach in wild animal populations using microsatellite data (e.g. Morin *et al.* 1994; Hogg and Forbes 1997; Keane *et al.* 1997). However exclusion by itself may be insufficient to unambiguously resolve paternity in a considerable proportion of paternity tests, even using a series of very polymorphic co-dominant markers where the probability of excluding an arbitrary unrelated male is very high (Chakraborty *et al.* 1988; Prodöhl *et al.* 1998). While exclusion may be a useful starting point in paternity inference, a statistically-based method is needed to assign paternity when multiple males are non-excluded. The use of likelihood (Edwards 1972) for inference of relationships using genetic data was first explored in detail by Thompson (1975, 1976a), who showed that likelihood is an efficient approach to the evaluation of alternative relationships between a given pair of individuals in inference of human pedigrees. However the real problem of paternity inference in polygynous natural populations is subtly different. At issue is the evaluation of alternative pairs of individuals for a given relationship (father-offspring).

Meagher (1986) developed the likelihood approach of Thompson when he analysed allozyme data from a natural population of the lily *Chamaelirium luteum*, awarding paternity to the male with the highest log-likelihood ratio or *LOD score* (the likelihood ratio is the likelihood of paternity of a particular male compared with the likelihood of paternity of an arbitrary male). If two or more males were equally likely (usually because their genotypes were identical), paternity was left unassigned. However Meagher did not assess the statistical confidence in the paternities that he

awarded. Foltz and Hoogland (1981) carried out an analysis of paternity in prairie dogs (*Cynomys ludovicianus*), also using allozyme data. In each litter, to evaluate the likelihood of paternity for a series of males, Foltz and Hoogland used the difference in the log-likelihoods of the most-likely resident male and the most-likely non-resident male (ΔL), and chose an arbitrary threshold of 4 as a criterion for awarding paternity to a non-resident male. As in Meagher's (1986) study, Foltz and Hoogland were not able to assess the significance of their ΔL values, and they made *a priori* assumptions about the relative likelihood of paternity of resident and non-resident males. Furthermore Meagher (1986) criticised ΔL on the basis that it is not a valid likelihood ratio, since one hypothesis is not nested within the other.

An alternative to categorical assignment of paternity to the most likely male is to assign paternity fractionally to all non-excluded males based on their relative likelihoods of paternity (Devlin *et al.* 1988; Roeder *et al.* 1989; Smouse and Meagher 1994). This approach allows population-level patterns of paternity to be assessed, even when the discriminatory power of marker loci is low. Smouse and Meagher (1994) compared a maximum likelihood fractional method against the earlier categorical analysis of Meagher (1986) for the same *Chamaelirium* data set. The correlation between individual male success as measured by the two methods was significant but weak ($r = 0.32$). Smouse and Meagher argue that the fractional method is superior because it uses all the available data. However, the method will systematically underestimate variance in male reproductive success (Devlin *et al.* 1988; Smouse and Meagher 1994). I have therefore concentrated on the most likely or categorical approach to paternity assignment, which has much improved power with highly polymorphic DNA markers such as microsatellites, and has the additional advantage that the resulting paternity data can be used for analyses that require individual parent-offspring links, such as the calculation of inbreeding coefficients and heritabilities.

Published work on non-human paternity inference with co-dominant markers does not deal with the problems encountered when analysing the data typically obtained in large-scale genetic studies of natural populations. Paternity inference may be carried out with or without maternal genetic data, possibly within the same study; the two situations are statistically distinct and should be analysed appropriately. Statistical

analysis should take account of the number of candidate males and be able to resolve paternity with confidence when not all candidate males are sampled. At the genotypic level, individuals may not be typed at every locus and, perhaps most importantly, the analysis should be robust to errors in typing. Nearly all published studies of natural populations treat a mismatch between a male and a putative offspring as conclusive evidence for exclusion of that male from paternity. In practice, a mismatch could result either from a genuine non-relationship or from a laboratory typing error, a reality acknowledged for some time in human pedigree analysis (Thompson 1976b; Ashton 1980; Lathrop *et al.* 1983). When microsatellite markers are used, mutations (Queller *et al.* 1993) and null alleles (Callen *et al.* 1993; Koorey *et al.*; 1993; Phillips *et al.* 1993; Pemberton *et al.* 1995) may also generate mismatches between genuine relatives at measurable frequencies.

In this chapter, I develop the likelihood-based approach of Thompson (1975, 1976a) and Meagher (1986). Paternity is assigned to a particular male if his likelihood ratio is large relative to the likelihood ratios of alternative males. Like Meagher (1986) I express likelihood ratios as LOD scores (i.e. the logarithm of the likelihood ratio). Unfortunately, significance levels for LOD scores cannot conveniently be derived analytically (Edwards 1972; Meagher 1986). However, Thompson and Meagher (1987) show that the ratio of the likelihood ratios of two males is a true likelihood comparison of alternative father-offspring relationships. Considering the two most likely males, I define the logarithm of the ratio of likelihood ratios to be Δ (equal to the difference in LOD scores), and use computer simulation of paternity inference to generate criteria for Δ appropriate for paternity assignment in the study population. The use of simulation of paternity inference in order to evaluate the significance of LOD scores has also been suggested by Taylor *et al.* (1997).

A statistical pitfall of using conventional likelihood ratios to assess paternity has been pointed out by Thompson (1976a, 1976b) and Thompson and Meagher (1987). If there exist in the population full sibs of the offspring whose paternity is being tested, and no genetic data are available from the mother, non-excluded full-sibs on average have a higher likelihood of paternity than the true father. I examine the effect of relatives of the offspring on paternity inference with and without maternal genetic data, and compare this with the effect of relatives of the true father.

The simulation results presented in this chapter are derived with the computer program CERVUS using allele frequencies at three allozyme and nine microsatellite markers screened in the red deer population Rum (Table 2.3). In Chapter 3 the same approach is used to analyse paternity in Rum red deer, again using the CERVUS program. For convenience, I refer throughout to paternity inference; however the system is equally applicable to inference of maternity (e.g. Jones and Avise 1997).

3.2 Methods

3.2.1 Likelihood in paternity testing

Likelihood analysis takes data as a starting point, and evaluates hypotheses given that data (Edwards 1972). The likelihood L of a hypothesis H given data D can be written $L(H|D)$. The likelihood of one hypothesis (e.g. H_1) is always evaluated relative to another (e.g. H_2). This is the likelihood ratio, written as $L(H_1, H_2|D)$:

$$L(H_1, H_2|D) = \frac{P(D|H_1)}{P(D|H_2)} \qquad \textit{Equation 3.1}$$

where $P(D|H_i)$ is the probability of obtaining data D under hypothesis H_i. In the context of paternity inference, the data D are the genotypes of offspring, mother, and alleged father at a particular locus. The hypothesis of interest H_1 is that the alleged father is the true father, and this is tested against hypothesis H_2 that the alleged father is an unrelated individual selected at random from the population. The following interpretation (Equation 3.2 - Equation 3.4) is based on that of Meagher (1986), and assumes that the mother's genotype is known.

Let g_m, g_a and g_o represent the genotypes of mother, alleged father and offspring at a given locus. The likelihood that the mother and alleged father are the parents of the offspring can then be expressed:

$$L(H_1|g_m, g_a, g_o) = T(g_o|g_m, g_a).P(g_m).P(g_a) \qquad \textit{Equation 3.2}$$

Here $T(g_o|g_m,g_a)$, the probability of the offspring's genotype given the genotypes of the mother and alleged father, is the Mendelian segregation or transition probability. $P(g_m)$ and $P(g_a)$ are the frequencies of the mother's and alleged father's genotypes in

the population. The likelihood that the mother is the parent of the offspring and the father is a randomly-chosen individual from the population is expressed:

$$L(H_2|g_m, g_a, g_o) = T(g_o|g_m).P(g_m).P(g_a) \qquad \textit{Equation 3.3}$$

where $T(g_o|g_m)$ is the probability of the offspring's genotype given the mother's genotype. The likelihood ratio (Equation 3.2 divided by Equation 3.3), represents how much more likely it is that the alleged father, rather than an arbitrary male, passed his genes to the offspring (Aitkin 1995).

$$L(H_1, H_2|g_m, g_a, g_o) = \frac{T(g_o|g_m, g_a).P(g_m).P(g_a)}{T(g_o|g_m).P(g_m).P(g_a)} \qquad \textit{Equation 3.4}$$
$$= \frac{T(g_o|g_m, g_a)}{T(g_o|g_m)}$$

Note that the genotypic frequencies $P(g_x)$ cancel in Equation 3.4. The likelihood ratios for all compatible genotypic combinations at a co-dominant autosomal locus are presented in Table 3.1 (Equation 3.4 is referred to as the Paternity Index (PI) in human paternity testing (Pena and Chakraborty 1994)).

In cases where the mother's genotype is unknown, the likelihood ratio is different:

$$L(H_1, H_2|g_a, g_o) = \frac{T(g_o|g_a).P(g_a)}{P(g_o).P(g_a)} = \frac{T(g_o|g_a)}{P(g_o)} \qquad \textit{Equation 3.5}$$

Here the denominator, $P(g_o)$, is the frequency of the offspring's genotype. Allele frequencies may only be used to estimate genotype frequencies if Hardy-Weinberg equilibrium holds. The likelihood ratios for paternity inference without maternal genetic information, assuming Hardy-Weinberg equilibrium, are presented in Table 3.2.

When several unlinked marker loci are used in paternity inference, the likelihood ratios derived at each locus may be multiplied together and the natural ($\log_e$) logarithm taken. Meagher (1986) terms the natural logarithm of the combined likelihood ratio the LOD score. This definition of the LOD score differs from that used in genetic mapping, where the LOD score is defined as the common ($\log_{10}$) logarithm of the likelihood ratio. For consistency with standard likelihood analysis

(Edwards 1972) and with previous work in paternity inference (e.g. Thompson 1975), defining LOD as $\log_e$ seems appropriate.

A LOD score of zero implies that the alleged father is equally as likely to be the father of the offspring as a randomly selected male. A positive LOD score implies that the alleged father is more likely to be the father of the offspring than a randomly selected male; negative LOD scores may occur if the alleged father and offspring share a particularly common set of alleles.

Offspring's genotype (g_o)	Alleged father's genotype (g_a)	Mother's genotype (g_m)	$T(g_o \mid g_m, g_a)$	$T(g_o \mid g_m)$	$L(H_1, H_2)$
BB	*BB*	*BB*	1	*b*	1/*b*
BB	*BX*	*BB*	1/2	*b*	1/2*b*
BB	*BB*	*BX*	1/2	*b*/2	1/*b*
BB	*BX*	*BX*	1/4	*b*/2	1/2*b*
BC	*BB*	*CC*	1	*b*	1/*b*
BC	*BX*	*CC*	1/2	*b*	1/2*b*
BC	*BB*	*CY*	1/2	*b*/2	1/*b*
BC	*BX*	*CY*	1/4	*b*/2	1/2*b*
BC	*BB*	*BC*	1/2	(*b*+*c*)/2	1/(*b*+*c*)
BC	*BY*	*BC*	1/4	(*b*+*c*)/2	1/2(*b*+*c*)
BC	*BC*	*BC*	1/2	(*b*+*c*)/2	1/(*b*+*c*)

Table 3.1 *Likelihood ratios for all compatible mother – alleged father – offspring trios. X represents any allele other than B; Y represents any allele that is neither B nor C. The frequencies of alleles B and C are denoted b and c. The likelihood ratio, L (H_1,H_2), is the probability of the offspring's genotype given the mother's and alleged father's genotypes, T $(g_o \mid g_m, g_a)$, divided by the probability of the offspring's genotype given the mother's genotype, T $(g_o \mid g_m)$. A similar table is shown in more condensed form in Brenner (1997).*

Offspring's genotype (g_o)	Alleged father's genotype (g_a)	$T(g_o \mid g_a)$	$P(g_o)$	$L(H_1,H_2)$
BB	*BB*	b	b^2	$1/b$
BB	*BX*	$b/2$	b^2	$1/2b$
BC	*BB*	c	$2bc$	$1/2b$
BC	*BY*	$c/2$	$2bc$	$1/4b$
BC	*BC*	$(b+c)/2$	$2bc$	$(b+c)/4bc$

Table 3.2 *Likelihood ratios for all compatible alleged father – offspring pairs, in the absence of a genotyped mother. X represents any allele other than B; Y represents any allele that is neither B nor C. The frequencies of alleles B and C are denoted b and c. Likelihood ratios are calculated on the basis that Hardy-Weinberg equilibrium holds. The likelihood ratio, $L(H_1,H_2)$, is the probability of the offspring's genotype given the alleged father's genotype, $T(g_o \mid g_a)$, divided by the probability of the offspring's genotype, $P(g_o)$. A similar table is shown in more condensed form in Brenner (1997).*

3.2.2 Mismatches and typing errors

If genetic data are perfect, a mismatch at a single locus between alleged father and offspring can be logically treated as a paternity exclusion. However, data are often not perfect, and so it is unwise to exclude males entirely from paternity on this basis. A mismatch can result not only from non-paternity but also from erroneously typed paternal, maternal or offspring genotypes, or from marker mutation or null alleles (3.4). The use of a likelihood approach allows us to introduce a parameter, the *error rate*, which takes account of potential imperfections in the data. An error is defined as the replacement of the true genotype at a particular locus in an individual with a random genotype. Errors may occur in the genotypes of offspring, mother or alleged father, or in some combination of the three. The derivations of likelihood ratios incorporating errors are shown in Appendix A1. These likelihood ratios are used for all analyses presented in this chapter.

3.2.3 Assignment of paternity using LOD scores

In order to discriminate between non-excluded males, I define a statistic Δ as the difference in LOD scores between the most likely male and the next most likely male.

Let n be the number of candidate males with a LOD score greater than zero. The LOD score of male i is denoted LOD_i, and the males are ranked such that $LOD_i \geq LOD_{i+1}$ for $1 \leq i < n$ so that the LOD score of the most likely male is denoted LOD_1. Then Δ is defined as follows:

$$n \geq 2 \quad \Delta = LOD_1 - LOD_2$$

$$n = 1 \quad \Delta = LOD_1$$

$$n = 0 \quad \Delta \text{ undefined.}$$

Without a threshold LOD score of zero, Δ is sensitive to LOD_2. If LOD_2 is very negative (typically when all candidate males except the most likely male mismatch the offspring at several loci), Δ is large whatever the value of LOD_1. A threshold LOD score of zero stabilises Δ since it always lies between zero and LOD_1.

3.2.4 Simulating paternity inference

3.2.4.1 The simulation program

Simulations are used to assess the significance of Δ values. The simulation analysis of the program CERVUS (Figure 3.1) emulates the steps of paternity inference using allele frequencies at loci screened in a given study population. Parallel simulations are carried out for paternity inference with and without maternal genetic data.

Assuming Hardy-Weinberg equilibrium (Table 4.1), a maternal genotype and a paternal genotype are generated from allele frequencies observed in the study population, and an offspring genotype is derived by Mendelian sampling of the parental alleles. Genotypes are also generated for a number of unrelated candidate males. The genotypic data for all individuals are then altered to reflect the existence of unsampled males, missing loci and incorrectly typed loci, according to the values of the parameters described below. Next each candidate male is considered in turn as the alleged father, beginning with the true father. LOD scores are calculated for all males for whom genetic data exists. Once all males have been considered, the most likely and second most likely males are identified and the Δ score calculated (all males with LOD scores of 0 or less are ignored). The value of Δ is recorded along with the status of the most likely male (i.e. whether or not he is the true father).

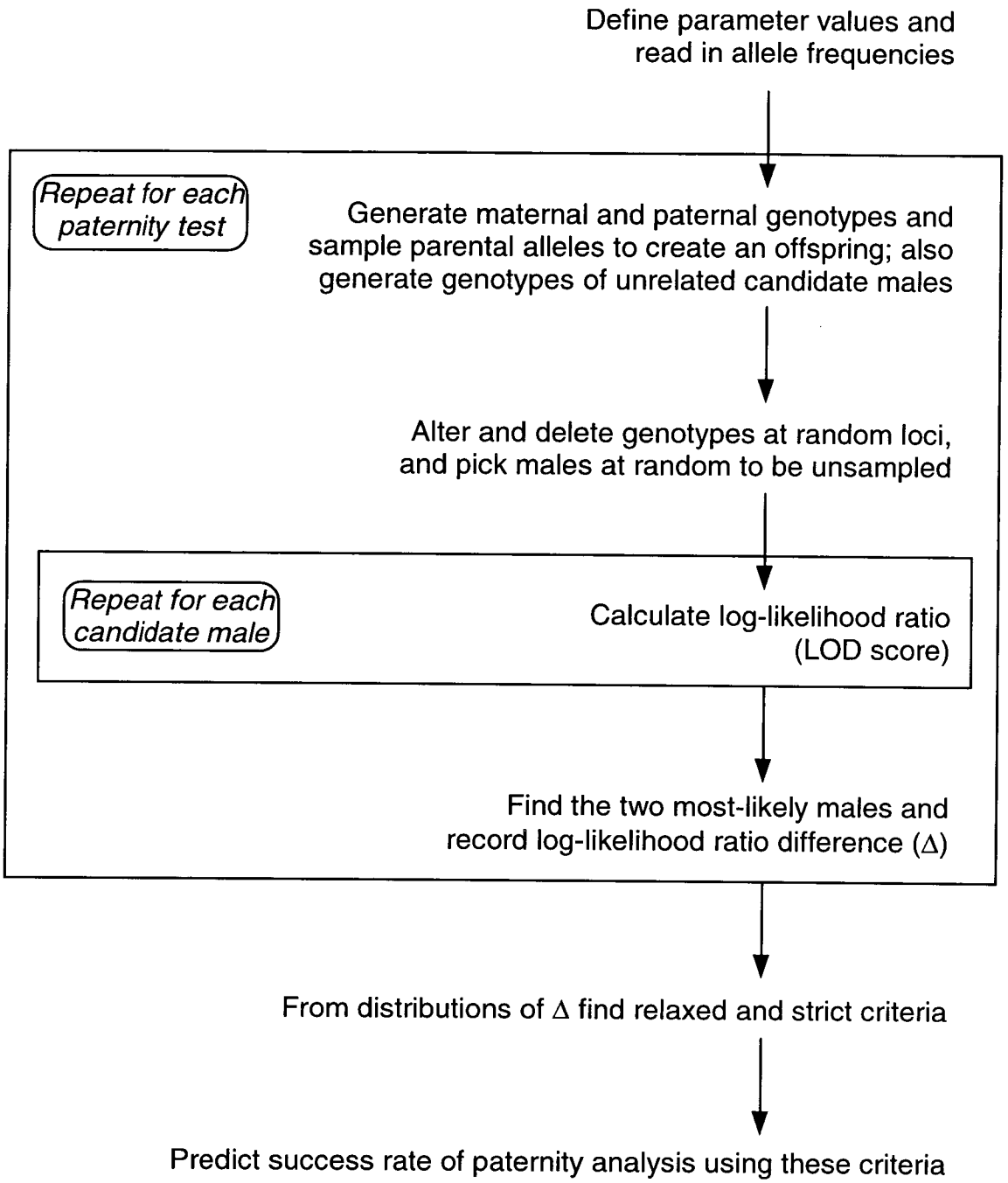

Figure 3.1 *A flow chart illustrating the operation of the* CERVUS *program's simulation of paternity inference. Boxes indicate repeated loops. Simulation of paternity inference where mothers are unsampled is carried out in a parallel simulation.*

3.2.4.2 Simulation parameters

Genetic data are generated and paternity tests carried out for a large number of simulated offspring in order to generate distributions of Δ (Figure 3.2). A total of

10,000 tests is sufficient in most cases (3.3.2). The following parameters (Table 3.3) are included in the simulation model in order to make simulated genetic data realistic:

Number of candidate males: the average number of males that are candidates for paternity of each offspring. The number of candidate males can be estimated from field data, and includes males that are not sampled. Candidate males other than the true father are assumed to be unrelated to the mother-father-offspring trio (the effect of relaxing this assumption is explored below in Section 3.3.5).

Proportion of candidate males sampled: the average fraction of candidate males for which genotypic data are available. The proportion of sampled males can be estimated from field data. The true father falls into this category with the same probability as any other candidate male.

Proportion of loci typed: the fraction of loci typed, averaged across all loci and individuals. Missing genotypes are scattered at random across loci and individuals, including the mother, true father and offspring. The proportion of loci typed can readily be calculated from the genetic data used to estimate allele frequencies.

Parameter	Value used
Number of candidate males	75
Proportion of candidate males sampled	0.65
Proportion of loci typed	0.854
Rate of typing error	0.01
Number of tests	10,000
Relaxed confidence level	80%
Strict confidence level	95%

Table 3.3 *The parameters used in simulation of paternity inference with the CERVUS program, and the values used in simulations for Rum red deer presented in this chapter. The number of candidate males and the proportion of males sampled are average values from the ruts between 1981 and 1995 that gave rise to the calves born 1982-1996. The proportion of loci typed and the error rate are average values across the 12 loci screened (Table 2.2). See section 3.2.4.2 for details on choice of parameter values and section 3.2.4.3 for a description of confidence levels.*

Error rate: the fraction of loci typed incorrectly, averaged across all loci and individuals. An error is defined as the replacement of the true genotype at a given locus with a genotype selected at random under Hardy-Weinberg assumptions. Under this definition, an erroneous genotype will sometimes be the same as the true genotype. If mother-offspring pairs are known from field data, the error rate can be estimated from the frequency of mismatches (i.e. no alleles in common) between mothers and their offspring, bearing in mind that as defined not all errors alter the observed genotype, and that not all alterations of genotype can be detected as mother-offspring mismatches (see Appendix A1). Note that the error rate parameter is used in the simulation of paternity inference both to generate simulated genetic data and in the calculation of likelihoods (Appendix A1).

3.2.4.3 Identifying critical values of Δ

The final stage of the simulation is to find critical values of Δ so that the significance of Δ values found in paternity inference in the study population can be tested. The program compares the distribution of Δ scores for cases where the most-likely male was the true father with that for cases where the most-likely male was not the true father (Figure 3.2). Assuming, as an example, that a criterion is required for Δ which gives 95% confidence, the program identifies the value of Δ such that 95% of Δ scores exceeding this value are obtained by true fathers. If the program fails to find such a value of Δ (typically because the resolving power of the markers is insufficient), the critical value of Δ is set to an arbitrary high value of 99.99. When a male fulfilling the 95% confidence criterion is assigned paternity of an offspring, the father-offspring relationship is described as a 95% confident paternity.

Confidence levels can be thought of as levels of tolerance of "false positive" paternities, or paternities assigned to males who match by chance (Type I Error). For some purposes the number of paternities obtained may be most important, while in other situations very accurate paternity assignment may be required. The program therefore calculates separately *relaxed* and *strict* confidence levels. In this chapter I select relaxed confidence as 80% and strict confidence as 95%. For each level of confidence, the program shows the percentage of simulated paternity tests in which

the Δ score of the most likely male exceeded the critical value of Δ (i.e. the percentage of tests in which paternity was assigned), a statistic I refer to as the *success rate*.

3.3 Results

In this section, I explore the results generated by the simulation system of the CERVUS program. I examine in turn the statistical properties of Δ, the repeatability of simulation results, the importance of typing errors, the effect of varying simulation parameters on the predicted resolving power of markers and the impact of relatives on paternity inference. All simulations use the allele frequencies for Rum red deer screened at the three protein and nine microsatellite loci described in Table 2.2, along with the parameter values in Table 3.3 unless otherwise stated.

3.3.1 Distribution of Δ

The distributions of Δ scores, from a simulation of paternity inference using the parameter values listed in Table 3.3, are shown in Figure 3.2. Summarising observations of various distributions for different criteria and different populations, pairs of distributions of Δ for true fathers and non-fathers tend to fall into three categories, depending on the ratio of the number of true fathers, N_t (filled bars in Figure 3.2), to non-fathers, N_u (white bars):

1) $N_t > N_u$. The critical value is low, and success rate is high (e.g. Figure 3.2a). In the special case of $N_t / (N_t + N_u)$ being greater than or equal to the confidence level, the critical value is set to zero.

2) $N_t \approx N_u$. The critical value takes an intermediate value, but success rate is heavily dependent on the degree of overlap of the two distributions. If the overlap is narrow, more paternities can be assigned than if the overlap is wide.

3) $N_t < N_u$. The critical value is high, and the success rate is low (e.g. Figure 3.2b).

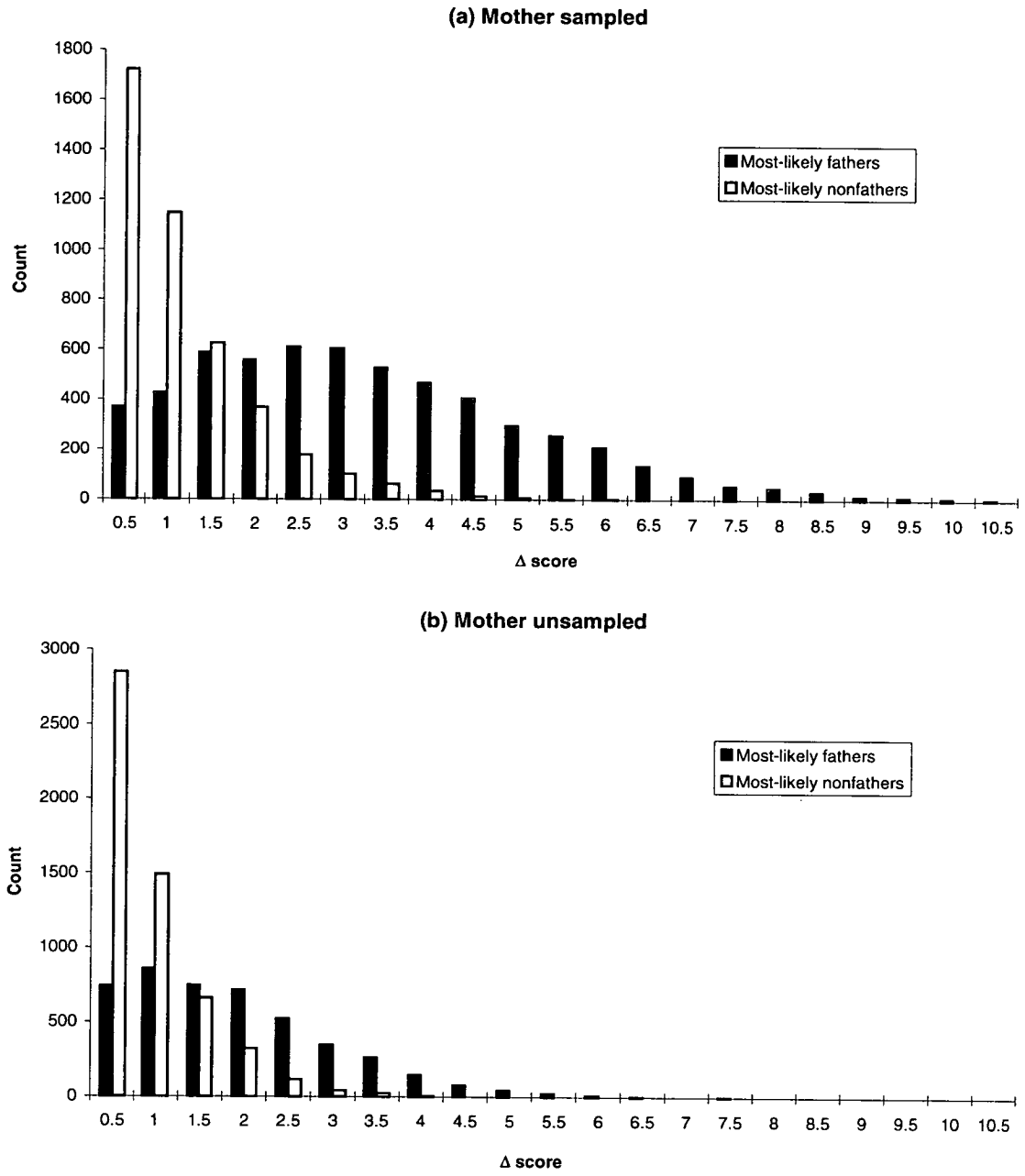

Figure 3.2 *Distributions of Δ scores generated by simulation of paternity inference using allele frequencies from Rum red deer and the parameter values shown in Table 3.3. In each plot the distribution of N_t cases where the most likely male is the true father (filled bars) is interleaved with the distribution of N_u cases where the most likely male is not the true father (white bars). Critical values of Δ are calculated from the degree of overlap of the two distributions shown in each plot. Critical values for 80% and 95% confidence are listed in Table 3.4. Plot (a) is from a simulation with sampled mothers, and plot (b) is from a simulation with unsampled mothers.*

Which of these three scenarios occurs varies according to whether or not the mother is sampled, the allele frequencies and the parameter values used in the simulation. $N_t + N_u$ may be less than the total number of paternity tests carried out, since under certain parameter conditions there may be a proportion of tests where no male has a LOD score greater than zero.

The distributions shown in Figure 3.2 were used to derive critical Δ scores and the corresponding predicted success rates (Table 3.4). Δ criteria were larger when mothers were not sampled and larger for higher confidence. Success rates were smaller when mothers were not sampled, and smaller for higher confidence.

Simulation results	**Mother sampled**		**Mother unsampled**	
(n = 10,000)	**80%**	**95%**	**80%**	**95%**
Critical value of Δ	1.13	2.78	1.49	3.15
Proportion of paternities	59.30%	28.86%	25.33%	4.35%

Table 3.4 *The critical Δ scores and number of red deer paternity tests predicted to be resolved by simulation using the program CERVUS. Parallel simulations were carried out for paternity inference with sampled mothers and unsampled mothers. Both relaxed (80% confidence) and strict (95% confidence) criteria are shown, along with the proportion of paternity tests (of 10,000) in which a male fulfilled the required criterion (i.e. was awarded paternity).*

3.3.2 Repeatability of simulation results

The simulation generates repeatable results with 10,000 paternity tests (Table 3.5). More than 95% of critical Δ values were within 10% of the mean critical Δ value, based on 16 runs using the parameter values shown in Table 3.3. From the same runs, 95% of predicted success rates were within 2% (expressed as a percentage of paternity tests resolved) of the mean predicted success rate. In other words, the variation in critical Δ values that did occur did not lead to large variations in predicted success rates. Critical Δ values at the lower end of the range did not give rise to many Type I Errors (incorrect assignment of paternity), and critical Δ values at the upper end of the range did not give rise to many Type II Errors (failure to assign paternity).

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Simulation results	Mother sampled		Mother unsampled	
(*n* = 16 runs)	80%	95%	80%	95%
Critical value of Δ	1.16 ± 0.03	2.80 ± 0.11	1.46 ± 0.03	2.80 ± 0.11
Proportion of paternities	59.0 ± 0.9%	29.3 ± 1.6%	26.6 ± 0.8%	7.1 ± 0.8%

Table 3.5 *The repeatability of simulation results from the program CERVUS. The mean ± one standard deviation of critical Δ and success rate from 16 runs is shown.*

In practice the simulation gives more accurate results at lower levels of confidence for a given length of run. If a very high level of accuracy is required, or if very high levels of confidence are demanded (e.g. 99% or more), simulations with more than 10,000 paternity tests may be carried out at a cost of increased computing time.

In the following sections describing simulation results, I concentrate on the comparison between paternity inference with and without sampled mothers at 80% confidence. In all cases, similar patterns were observed at 95% confidence.

3.3.3 Importance of typing errors

An error rate of zero ensures that any mismatch is treated as a paternity exclusion, and the likelihood equations then reduce to Equation 3.4 and Equation 3.5. Given that laboratory data are rarely error-free, is it satisfactory or prudent to ignore errors in paternity inference? I examined the impact of errors on confidence, and whether taking account of errors alters success rates.

If simulations and paternity inference in the study population are carried out on the basis that genetic data are error-free, then Figure 3.3 shows the true confidence in paternities allegedly assigned at 80% confidence for simulated genetic data including typing errors at various rates. In all cases, assuming genetic data to be error-free led to overestimates of the confidence in paternities assigned when data included errors. For example, when mothers were unsampled and the true error rate was 2%, paternities assigned with an apparent confidence of 80% (based on simulations assuming no errors) had a true confidence of 74%.

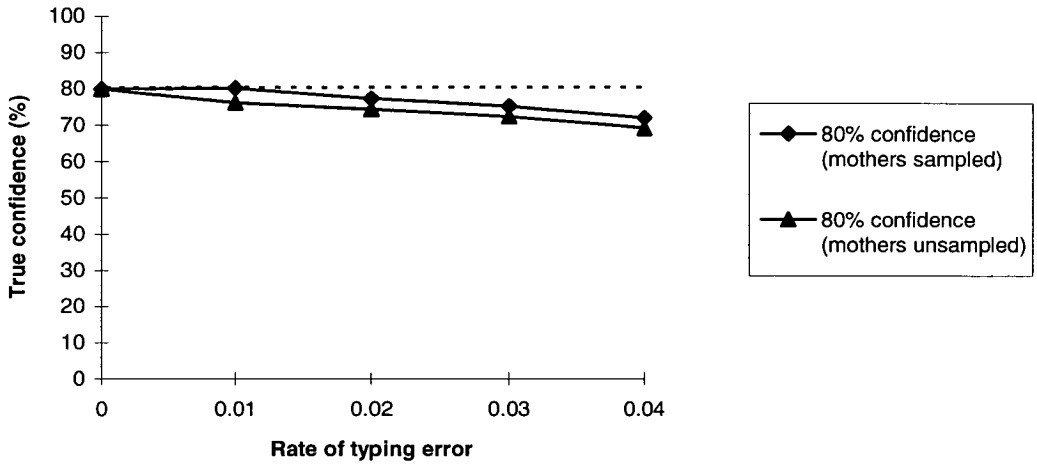

Figure 3.3 *The effect of ignoring typing error on confidence of paternity inference with error-prone genetic data. Simulation criteria from a simulation with an error rate of zero were applied to simulated data containing errors at rates from 1-4%; likelihood calculations in all cases used an error rate of zero. The true confidence was calculated as the ratio* $N_t / (N_t+N_u)$*. The dotted line represents the null hypothesis of no effect of ignoring typing error on confidence. Values used for other simulation parameters are shown in Table 3.3.*

Success rate may be improved by allowing for errors, because a true father that was previously excluded on account of typing error may now have LOD score sufficient for him to be identified as the father. On the other hand, allowing for typing errors may mean that unrelated candidates males that were previously excluded on account of mismatches at just one or two loci may now have similar LOD scores to the true father, meaning that the LOD score of the true father is no longer sufficiently large for him to be awarded paternity. The first of these effects predominates when there is redundancy of information across marker loci, while the second effect predominates when the resolving power of markers is limited. Figure 3.3 suggests that at least some of the additional paternities that may be awarded by ignoring errors are of inferior confidence. For example, "80% confident" paternities that were awarded by ignoring errors were not necessarily secure at 80% confidence when the correct simulation criteria were applied.

3.3.4 Success of paternity inference under various parameter conditions

The responses of success rates to parameter changes are shown in Figure 3.4, expressed as a percentage of paternity tests resolved with 80% confidence. In each case one parameter was varied while the others were held constant at the values shown in Table 3.3. Simulations were carried out assuming 65% of males were sampled (except Figure 3.4b where this parameter itself was varied). Thus success rates were unlikely to greatly exceed 65%, though false-positive paternities, especially at lower levels of confidence, can push the percentage a little higher.

Number of candidate males: Figure 3.4a shows the effect of varying the number of candidate males. Fewer paternity tests were resolved as the number of candidate males increased. Similar success rates were obtained when choosing between 100 candidate males with sampled mothers and choosing between 10 candidate males when mothers were unsampled, and this ratio is maintained across most of the chart. If the number of candidate males was large, maternal genetic data were essential to assignment of paternity with confidence of 80% using the set of markers screened in Rum red deer.

Proportion of candidate males sampled: Figure 3.4b shows the effect of varying the proportion of candidate males sampled. Success rates increased as the proportion of sampled candidates males increased.

Proportion of loci typed: Figure 3.4c shows the effect of varying the proportion of loci typed. Success rates were greater when genetic data were more complete.

Rate of typing error: Figure 3.4d shows the effect of varying the rate of typing error. Fewer paternity tests were resolved as the error rate increased. Note that the error rate parameter is included both in the generation of simulated genetic data and in the calculation of likelihoods.

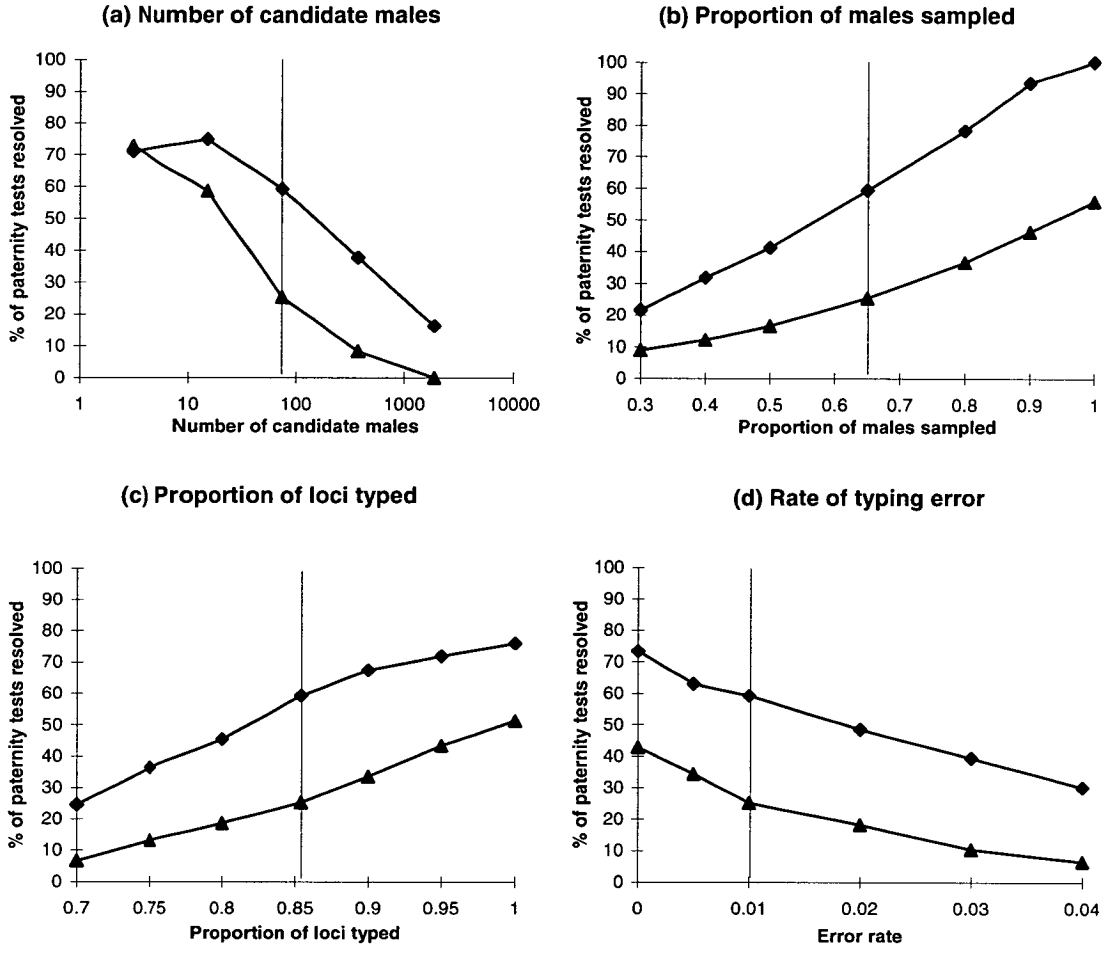

Figure 3.4 *The effect on the predicted success of paternity inference on varying the main parameters included in the model: (a) number of candidate males (log scale), (b) proportion of males sampled, (c) proportion of loci typed and (d) rate of typing error. In each case, the percentage of paternity tests resolved at 80% confidence is shown, with all other parameters being held at the values in Table 3.3. The values used for analysis of Rum red deer are indicated by a vertical line. Key as for Figure 3.3.*

3.3.5 The effect of relatives

Thus far, simulations have assumed that candidate males other than the true father are unrelated to the mother-father-offspring trio. I explored the effect of introducing relatives into the pool of candidate males, examining their effect on confidence in paternity assignments made assuming no relatives were present. I compared the effect of the degree of relatedness when candidate males were related to the offspring with

the effect when candidate males were related to the true parents. I also explored how the number of half sibs of the parents affects paternity inference.

Figure 3.5a shows the effect on confidence of introducing males related to the offspring, assessed by applying the Δ criteria in Table 3.4 to simulated paternity tests including five related males. Confidence in paternities assigned using these criteria declined with increasing relatedness. When mothers were sampled, the overestimate in confidence was small unless full sibs of the offspring (r = 0.5) were among the candidate males. When mothers were unsampled, the overestimate in confidence was large if males related as half sibs or more to the offspring ($r \geq 0.25$) were among the candidate males, suggesting that paternity cannot be resolved between the true father and his sons, especially sons by the same female (see also Thompson and Meagher 1987). Whether or not mothers were sampled, success rates declined only slowly with increasing relatedness (data not shown). In other words, as relatedness increases, a similar number of paternities can be assigned but with reduced confidence.

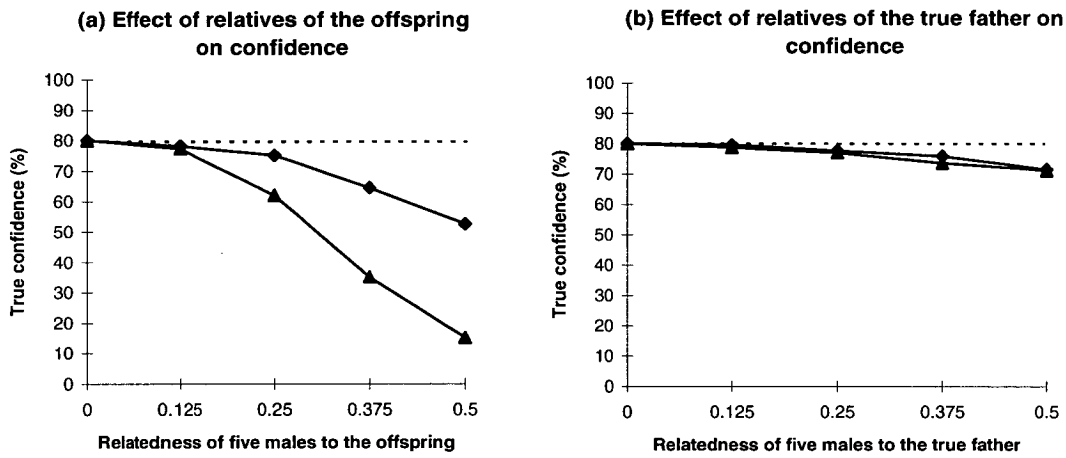

Figure 3.5 *The effect of including relatives among the candidate males on true confidence of parentage assignments, varying (a) relatedness of five males to the offspring, and (b) relatedness of five males to the true father. Simulation criteria from a simulation without relatives (Table 3.4) were applied to simulated genetic data containing five relatives of the specified level of relatedness. The true confidence was calculated as the ratio* $N_t / (N_t + N_u)$. *The dotted line represents the null hypothesis of no effect of relatives on confidence. Values used for simulation parameters are shown in Table 3. Key as for Figure 3.3.*

A more common relatedness problem is posed by the presence of male relatives of the true father. Figure 3.5b shows the effect on confidence when five males related to the true father were among the candidate males, assessed as for Figure 3.5a. Whether or not mothers were sampled, confidence declined only slowly with increasing relatedness, and even with five full sibs of the true father among the candidate males, true confidence was 72% when 80% confidence criteria from Table 5 were applied. Again, success rates declined only slowly with increasing relatedness (data not shown). Although I only considered relatives of the true father, note that when mothers are unsampled, male relatives of the mother have the same confounding effect on paternity inference as male relatives of the true father.

There is a straightforward explanation for why the impact of relatives of the offspring differs from the impact of relatives of the true father. Assuming no inbreeding, a full sib of the offspring and a full sib of the true father are both related to the true father with $r = 0.5$; a full sib of the offspring is also related to the mother with $r = 0.5$ whereas a full sib of the true father is unrelated to the mother. If the mother is unsampled, a candidate male that is a full sib of the offspring has a probability of 0.75 of carrying either the offspring's paternal or maternal allele or both due to relatedness, whereas a candidate male that is a full sib of the true father has a probability of 0.5 of carrying the offspring's paternal allele due to relatedness, and cannot carry the offspring's maternal allele due to relatedness since he is unrelated to the mother. This explains why Figure 3.5a and Figure 3.5b differ when mothers were unsampled. If the mother is sampled, only the offspring's paternal alleles are used in the paternity test. Providing that the paternal allele can always be unambiguously identified, full sibs of the offspring and full sibs of the true father have the same impact on paternity inference, at a given locus both having a probability of 0.5 of carrying the offspring's paternal allele due to relatedness. In practice there are always some loci where the paternal allele cannot be unambiguously identified, either because of missing maternal genetic data, or because mother and offspring share the same heterozygous genotype (most likely for loci with few alleles). In these cases, a candidate male that is a full sib of the offspring has a higher probability of bearing at least one parental allele than a full sib of the true father for the same reason as when the mother is unsampled. This explains why confidence also declines more steeply in Figure 3.5a than in Figure 3.5b

when mothers were sampled. Although some inbreeding is likely to occur in most natural populations, the qualitative conclusions of this argument are unchanged unless the level of inbreeding is very extreme (e.g. frequent self-fertilisation).

In polygynous species there may be many half sibs in each population, and half sibs of the true father may often be his closest relatives considered as candidate males in paternity inference. The likelihood system is insensitive to large numbers of half sibs of the true father. Even with 25 half sibs present (i.e. one third of all males), true confidence was 72% when 80% confidence criteria from Table 5 were applied (data not shown).

3.4 Discussion

In this chapter I extend standard likelihood-based paternity inference to deal with marker mistyping, and develop a likelihood-based statistic, Δ, for determining paternity using co-dominant molecular markers. Δ is tested against critical values derived by simulation of paternity inference using observed marker allele frequencies. The simulation system takes account of the number of candidate males, the proportion of them that are sampled, the completeness of genetic data and the rate of typing error in deriving these critical values.

The success of paternity inference using this approach is influenced by two major factors, aside from the number of candidate males and the quality of markers used: these are whether or not a sample is available from the mother, and the level of confidence required of paternities that are assigned. The results presented here show that there is a consistently large premium attached to obtaining the mother's genotype. Without this, many more loci are required to deliver a similar success rate at the same level of confidence (4.3.2). The results also show that there is a clear trade-off between the number of paternities assigned, and the confidence in those assignments. It is important to realise that paternities assigned with 80% confidence will be more accurate than can be achieved in most species by direct observation, and is also better than would be obtained in many studies by a purely exclusionary approach, where confidence in paternity of non-excluded males is generally unknown.

Previously published methods for paternity inference in natural populations assume, often implicitly, that the entire pool of candidate males has been sampled (e.g. Meagher 1986; Devlin *et al.* 1988; Smouse and Meagher 1994). For many studies this is not a satisfactory assumption. Complete sampling of natural populations is difficult, and it is unreasonable to exclude males from paternity purely on the basis that they are unsampled. Tackling the problems posed by unsampled candidate males has been one of the major motivations for developing this simulation approach. The key assumption of the simulation is that the distribution of reproductive success is the same for unsampled males as it is for sampled males. While this may not always be true, for example if alternative male mating strategies make some males easier to sample than others, any other assumption in the model would be hard to justify because appropriate data are unlikely to be available. If the proportion of candidate males sampled is low, or if there are *a priori* reasons for suspecting that sampled males and unsampled males have different distributions of reproductive success, caution should be exercised in extrapolating results of paternity inference using sampled individuals to the population as a whole. The simulation also assumes that the genotypes of sampled males are representative of the genotypes of unsampled males.

It is common practice in human paternity testing in some countries to use paternity likelihood ratios within a framework of Bayesian inference (Valentin 1980). Advocates of Bayesian inference point out that the approach allows prior information on paternity (the prior probability) to be combined with the genetic likelihood of paternity to derive a combined probability of paternity (the posterior probability). This leads immediately to the question: what is an appropriate prior probability? The simple answer is that nobody knows, as revealed by the furious debate that this question, and other questions on the appropriateness of Bayesian inference in paternity testing, have provoked in the literature (Walker 1983; Aickin 1984; Li and Chakravarti 1985, 1986; Elston 1986a, 1986b; Thompson 1986; Valentin 1986). I believe that the approach described above arrives at a statistically reasonable solution to the problem of evaluating confidence in paternity assignments without making unjustifiable assumptions about the prior probability of paternity of different males, and I therefore do not believe that a Bayesian framework is necessary or helpful.

Allowing for errors in likelihood calculations renders paternity inference relatively insensitive to typing errors. A system based on principles of exclusion may exclude the true father at relatively high frequency even when errors are infrequent, since a single typing error in any of the mother-father-offspring trio at any of the marker loci can lead to exclusion. In large-scale typing of allozymes, errors occur at a rate of the order of 1% (Lathrop *et al.* 1983), and if similar rates are true in large scale screening of microsatellites, typing errors are likely to be a major cause of mismatches between offspring and their true parents. Another recent study (SanCristobal and Chevalet 1997) examined the effect of mistyping offspring alleles on likelihood-based paternity inference, both analytically and by simulation. They found that allowing for errors was important, but that the choice of error rate (providing the value chosen was greater than zero) did not have a major impact on confidence or success rate, whether or not the true error rate was greater than zero. However I believe that SanCristobal and Chevalet's error model, where individual alleles were randomly replaced with non-identical alleles without reference to their respective frequencies, is not as realistic as the error model described above, where genotypes are randomly replaced by genotypes selected under Hardy-Weinberg assumptions. Switching of samples, leakage of samples between lanes during gel loading and misidentification of lanes on gels are all sources of the genotype-replacement error modelled here.

There are other possible causes of mismatches between parents and their offspring, aside from typing error. A commonly-encountered problem is the presence of null alleles. At high frequencies these leave a characteristic signature of repeated homozygote-homozygote mismatches between known parent-offspring dyads, and typing of the affected locus may be discontinued (Pemberton *et al.* 1995). Null alleles at low frequencies are harder to detect, but may be treated as typing errors. Although not statistically ideal, treating a mismatch generated by a null allele as an error is preferable to treating it as a basis for exclusion. Another possible cause of mismatches between offspring and their true parents is mutation. Although mutations are alterations of single alleles rather than pairs of alleles and may not be independent of previous allelic state (e.g. the stepwise mutation model for microsatellite markers, Valdes *et al.* 1993) treating a mutation as an error is preferable to using it as a basis for exclusion.

A statistic for paternity assignment that is insensitive to the relatedness structure of the population under scrutiny is very desirable, since it is the relatedness structure that the paternity inference is designed to reveal. Simulations suggest that paternity inference using Δ is in general robust to the presence of unknown close relatives of the parents among the candidate males (cf. paternity inference based on exclusion: Double *et al.* 1997). In other words, no prior knowledge of the relatedness structure of the population is needed before Δ can be used in paternity inference. Thompson (1976a, 1976b) and Thompson and Meagher (1987) express concern that non-excluded full sibs of the offspring on average have higher LOD scores than the true father. The simulations carried out here do show that full sibs of the offspring (and also half sibs of the offspring when mothers are unsampled) compromise confidence in paternity inference when included among the pool of candidate males. However, full sibs of the offspring (sons of the true father and the same mother) will in many populations not be considered as candidate males (e.g. because the reproductive lifespan of males is less than the time for development from conception to breeding status). Individuals related to one parent only do not lead to the same loss of confidence, and half sibs of the parents, probably the most common closest relative in polygynous species, have only modest effects on paternity inference even when present in large numbers. In conclusion, while close relatives do lead to overestimation of confidence, the overestimate is small under many commonly encountered conditions. The problem area is when males compete for paternity with their own sons, and is likely to be most acute when sampled sons can be assigned paternity of offspring in fact sired by their unsampled father.

The results of the simulation are useful for identifying ways to improve the success of paternity inference. For example, one can explore whether or not maternal sampling is important for successful paternity inference and whether gaps or errors in the data set are limiting factors. A preliminary screen of a small sample of the population (e.g. 25 individuals) at the chosen marker loci could also be used to predict whether or not paternity inference on the full population is likely to be productive. If the markers appear to be insufficiently informative, the simulation could be used to estimate how many additional markers would be needed to achieve a given level of success.

The primary purpose of this development is to aid parentage studies in wild animal and plant populations, though the method is equally applicable to captive or domestic animals where they are held in sizeable groups, and to cultivated plants, providing that populations are in Hardy-Weinberg equilibrium. The same approach to paternity inference has been applied in harbour seals (*Phoca vitulina*; Coltman *et al.* 1998b), and a similar approach, using an earlier version of the CERVUS program, has been used to infer paternity in Soay sheep (*Ovis aries*, Pemberton *et al.* 1996), grey seals (*Halichoerus grypus*, P. Allen unpublished data) and humpback whales (*Megaptera novaeangliae*, Valsecchi 1996). The method offers a flexible and practical framework for accurate assessment of paternity at the individual level, and I believe that it will be a useful tool in paternity inference for a wide range of species. Chapter 4 demonstrates the application of the system to the Rum red deer data set.

3.5 Summary

Paternity inference using highly polymorphic codominant markers is becoming common in the study of natural populations. However, multiple males are often found to be genetically compatible with each offspring tested, even when the probability of excluding an individual unrelated male is high. While various methods exist for evaluating the likelihood of paternity of each nonexcluded male, interpreting these likelihoods has hitherto been difficult, and no method takes account of the incomplete sampling and error-prone genetic data typical of large-scale studies of natural systems. I derive likelihood ratios for paternity inference with codominant markers taking account of typing error, and define a statistic Δ for resolving paternity. Using allele frequencies from the study population in question, a simulation program generates criteria for Δ that permit assignment of paternity to the most likely male with a known level of statistical confidence. The simulation takes account of the number of candidate males, the proportion of males that are sampled and gaps and errors in genetic data. I explore the potentially confounding effect of relatives and show that the method is robust to their presence under commonly-encountered conditions. The simulation is demonstrated using genetic data from the intensively studied red deer (*Cervus elaphus*) population on the island of Rum, Scotland. The Windows-based

computer program, CERVUS, described in this chapter is available from the author[1]. CERVUS can be used to calculate allele frequencies, run simulations and perform parentage analysis using data from all types of codominant markers.

[1] CERVUS 1.0 for Windows 95 can be downloaded from http://helios.bto.ed.ac.uk/evolgen. Note that not all analyses presented in this chapter can be carried out with the release version of the program.

4 Paternity analysis in Rum red deer

4.1 Introduction

The likelihood-based approach to paternity inference described in Chapter 3 provides a statistical framework for assigning paternity in polygynous species. The approach has been shown to be refractory to gaps and errors in genetic data, and to the presence of biologically plausible relatives among the pool of candidate males. In this chapter, I apply the likelihood approach to the analysis of paternity in Rum red deer using the program CERVUS with data from three protein and nine microsatellite loci. To my knowledge this is the largest molecular analysis of paternity in any wild animal population, and is the first to make any attempt to estimate the statistical confidence in inferred paternities.

I first summarise the results of the paternity analysis, making comparisons with the predictions of simulation, and assess the merits of enlarging the panel of microsatellite markers used in the analysis. Second, I compare these results with the results of an earlier study of paternity in Rum red deer based on DNA fingerprinting (Pemberton *et al.* 1992). Third, I explore to what extent the genetic paternity data justify the use of observations of rutting behaviour for paternity inference. Fourth, I examine the patterns of mating success (number of calves sired) and reproductive success (number of calves sired which survive to age two) across ages for stags that have died, with a view to being able to predict the lifetime mating success (LMS) and lifetime reproductive success (LRS) of stags that are still alive. Fifth, I calculate individual LMS/LRS for stags born 1982-1986, using both genetic and behavioural approaches to estimate LMS/LRS. Sixth, I briefly describe similar calculations of LMS and LRS for hinds of the same cohorts. Finally I calculate standardised variance (Sokal and Rohlf 1995) of LMS/LRS for stags and hinds and make comparisons with previous studies.

The paternity data are used in Chapter 5 to calculate pedigree-based inbreeding coefficients, and the LMS data are used in Chapter 6 to assess the impact of inbreeding on stag and hind reproduction.

4.2 Methods

4.2.1 Genetic data

Details of the red deer study on Rum can be found in Chapter 2. A total of 1168 individually-monitored deer were typed at up to 3 protein loci (by J. Pemberton) and 9 microsatellite loci (by J. Slate), and individuals were on average typed at 85% of loci (information on loci used appears in Table 2.2). Allele frequencies from the total Rum sample (Table 2.3) were used for all analyses presented below. Genotypic frequencies at all loci conform to Hardy-Weinberg expectations (Table 4.1).

Locus	*n*	X^2	d.f.	*p*-value
MPI	890	0.00	1	NS
IDH-2	893	0.00	1	NS
TRF	702	3.70	1	NS ($p < 0.1$)
OarFCB193	1044	24.08	15	NS ($p < 0.1$)
OarFCB304	1102	20.20	15	NS
CelJP15	1107	11.36	15	NS
CelJP27	1059	4.23	6	NS
CelJP38	999	11.57	10	NS
MAF35	994	11.69	6	NS ($p < 0.1$)
MAF109	1056	5.68	6	NS
OarCP26	1086	4.17	6	NS
TGLA94	1042	9.43	15	NS

Table 4.1 *Hardy-Weinberg tests for loci typed in Rum red deer (Table 2.2). Analysis was carried out using the* CERVUS *program. Note that the* X^2 *values for MPI and IDH-2 are correct to two decimal places.*

In likelihood-based paternity inference the multiplication of likelihood ratios over several loci assumes that the loci segregate independently. This assumption is violated if any pair of loci is in linkage disequilibrium. For example, high values of linkage disequilibrium are likely for a series of loci known to lie within a single gene cluster (e.g. the Major Histocompatibility Complex) or in zones of hybridisation between two

populations, sub-species or species (Barton and Gale 1993). In red deer, two pairs of microsatellite markers used in this analysis each share a linkage group. However neither pair is thought to be tightly linked (M. Tate, pers. comm.), and loose linkage of one or two pairs of markers should not seriously bias multi-locus likelihood calculations (Meagher 1986). When calculating LOD scores, all loci are therefore treated as if they segregate independently.

Using the CERVUS program paternity was analysed for all calves for which samples were available from 1975-1996 (the years for which samples were available from one or more candidate stags). All tests used the allele frequencies given in Table 2.3 to calculate likelihoods and the criteria given in Table 3.4 to assess confidence in individual paternities (these criteria were derived from a simulation using the CERVUS program with the same set of allele frequencies and the parameter values in Table 3.3). For some analyses, a subset of 875 calves born between 1982 and 1996 was used since neonatal sampling of calves began in 1982, or other subsets as specified.

4.2.2 Relationship data

Red deer have an annual rutting season in the autumn, and single calves are born the following spring. In each year, the set of candidate stags are all stags classified as behaviourally active at any time during the rut preceding the birth of that calf. Typically this includes any stag 3 years old or more observed in the study area at this time. Stags aged 2 or less have never been observed to obtain matings, and in practice mating opportunities for stags less than 5 years old are extremely infrequent (F. Guinness, unpublished data). The paternity inference procedure is thus conservative, in that it considers any stag potentially able to mate as a candidate stag. In ruts between 1981 and 1995 (giving rise to the calf cohorts 1982-1996), there were an average of 75 candidate stags, of which 65% were sampled. These values were used in the CERVUS simulation upon which paternity inference was based. Sampling was less complete for earlier ruts (1974-1980) but for consistency the same simulation criteria were applied.

Where maternal genetic data are used in inferring paternity, maternity is based on field observations. Inaccuracies in assignment of maternity are likely to show up as mismatches across multiple loci, and no such cases have been found (Pemberton *et al.*

1988, 1992, unpublished data). The sporadic mismatches between maternal and offspring genotypes that do occur are interpreted as errors in the typing process. The estimate for the overall rate of typing error, given that many errors will go undetected, is 1% (the method for deriving the typing error rate from the frequency of mother-offspring mismatches is given in Appendix A1).

4.2.3 Types of paternity analysis

A number of approaches to paternity analysis have been used in Rum red deer. In this chapter, many of these approaches are compared:

Locus-specific: paternity assignment based on protein/microsatellite data analysed using the CERVUS program as part of this study (see also Marshall *et al.* 1998). Paternity was assigned with 80% or 95% confidence, the latter data set being smaller but of greater accuracy.

DNA fingerprinting: paternity assignment based on multilocus minisatellite DNA profiles (Pemberton *et al.* 1992). These assignments are thought to be very accurate but are limited in number.

Longest hold: paternity assignment based on observations of rut behaviour (see 2.3). In order to estimate paternity from behavioural data, I wrote a dBASE IV program (BACKDATE) which identifies all stags seen with the hind on the estimated day of conception (235 days before parturition) and the five days either side of this date (±1 standard deviation of the gestation length), and counts the number of days for which each stag was seen with the hind during this period (known as the 11-day window). Not all hinds were seen for every day of their 11-day window. If one stag held the hind for a greater number of days than any other during the 11-day window, he was assigned longest hold paternity of the resulting calf. If the longest hold was tied by two or more stags, no assignment was made.

Fractional: an alternative approach to paternity assignment based on observations of rut behaviour (see 2.3). Instead of assigning a full paternity to the stag holding the hind for the longest period during the 11-day window, the fractional approach awards each male 1/11 of a paternity for each day he held a hind during her 11-day window.

4.2.4 Mating success and reproductive success

A number of cumbersome terms which appear in this chapter are abbreviated. Their definitions are given below:

Lifetime mating success (LMS): the number of offspring attributed to a stag or hind over its lifetime.

Lifetime reproductive success (LRS): the number of offspring attributed to a stag or hind over its lifetime which survived to 1st May in the second year after birth.

LRS may be expected to be an inferior measure of stag or hind quality since calf survival may be strongly influenced by systematic environmental effects, such as the part of the study area in which stags and hinds reproduced (Coulson *et al.* 1997), but is included for comparison with earlier studies (Clutton-Brock *et al.* 1982, 1988; Rose 1995).

Cumulative mating success (CMS) at age x: the number of offspring attributed to a stag which were conceived in ruts when the stag was aged x or less, or the number of offspring attributed to a hind born when the hind was aged x or less.

Cumulative reproductive success (CRS) at age x: the number of offspring surviving to May 1st of their second year attributed to a stag which were conceived in ruts when the stag was aged x or less, or attributed to a hind which were born when the hind was aged x or less.

4.3 Results

4.3.1 Paternity analysis using CERVUS

The critical values of Δ shown in Table 3.4 were applied in an analysis of paternity for 872 red deer calves born between 1982 and 1996, carried out using the CERVUS program (Table 4.2). All calves typed for 6 or more loci were tested against all candidate stags (4.2.2) typed at 6 or more loci. A hind was classified as sampled if it was typed at 6 or more loci. However if between one and five loci were typed in a hind, these data were used (this contrasts with Marshall *et al.* 1998 - see below).

Number of paternities	Mother sampled: n = 652		Mother unsampled: n = 220	
	80%	95%	80%	95%
Observed	382 (59%)	181 (28%)	80 (36%)	23 (10%)
Expected	387 (59%)	188 (29%)	56 (25%)	10 (4%)

Table 4.2 *The results of paternity inference using the program CERVUS for 872 Rum red deer calves born between 1982 and 1996. The criteria used to assign paternity are shown in Table 3.4. Cases where the mother was sampled were analysed separately from those where the mother was unsampled. The number of paternities obtained with 80% and 95% confidence (observed) is listed above the number of paternities predicted from success rates obtained in the simulation (expected - calculated by multiplying the success rates given in Table 3.4 by n, the number of calves tested).*

Paternity was determined with 80% confidence for 382 of the 652 calves tested with sampled mothers (59%). Less than half of the 80% confident paternities (28% of calves tested) were secure at 95% confidence. For the 220 paternity tests where the mother was unsampled, only 80 (36%) gave an 80% confident paternity, and less than one third of these (10% of calves tested) were secure at 95% confidence. A larger number of paternities could be assigned, or confidence in existing paternities increased, by sampling a larger number of candidate stags, adding missing genetic data or retyping existing samples to reduce the frequency of typing error (Figure 3.4). An alternative strategy would be to type additional loci (4.3.2). The observed numbers of paternities inferred were similar to the predictions of the simulation. This similarity suggests that the parameters and assumptions of the simulation reasonably reflected the situation in the field.

The results presented here differ slightly from Table 6 of Marshall *et al.* (1998). First, three calves were excluded from the current analysis as their mothers died in late pregnancy and so the calf was never born. All three had sampled mothers and had paternity assigned with 95% confidence. Second, in the current analysis any available genetic data from hinds classified as unsampled was used when their offspring were tested. The effect was to increase the success of paternity analysis when mothers were unsampled compared with the predictions of the simulation, with 13 additional paternities at 80% confidence of which 4 were secure at 95% confidence.

4.3.2 How many more markers?

With the current set of nine microsatellite and three protein loci, paternity cannot be inferred even at 80% confidence for a large proportion of calves tested. I examined how additional microsatellite markers might improve the success of paternity inference both in tests where the mothers were sampled and the mothers were unsampled (Figure 4.1). For simulations up to nine microsatellites, loci were used in the order shown in Table 2.3. Additional microsatellite loci were simulated using the allele frequencies from the same nine microsatellite loci as if they were a new set of markers unlinked to each other or to existing loci. The three biallelic protein loci are also included in all simulations, but contribute relatively little power to the analysis.

For paternity analysis with sampled mothers, there is a considerable benefit in typing three additional microsatellite loci, giving a total of twelve. After this point, the benefits of additional loci are relatively small. Three additional microsatellite loci would also improve paternity inference without sampled mothers very substantially, but in this case, an even greater number of loci would be beneficial. Eighteen loci are required to achieve the same success when no sample is available from the mother as can be achieved with just twelve loci when a sample is available from the mother.

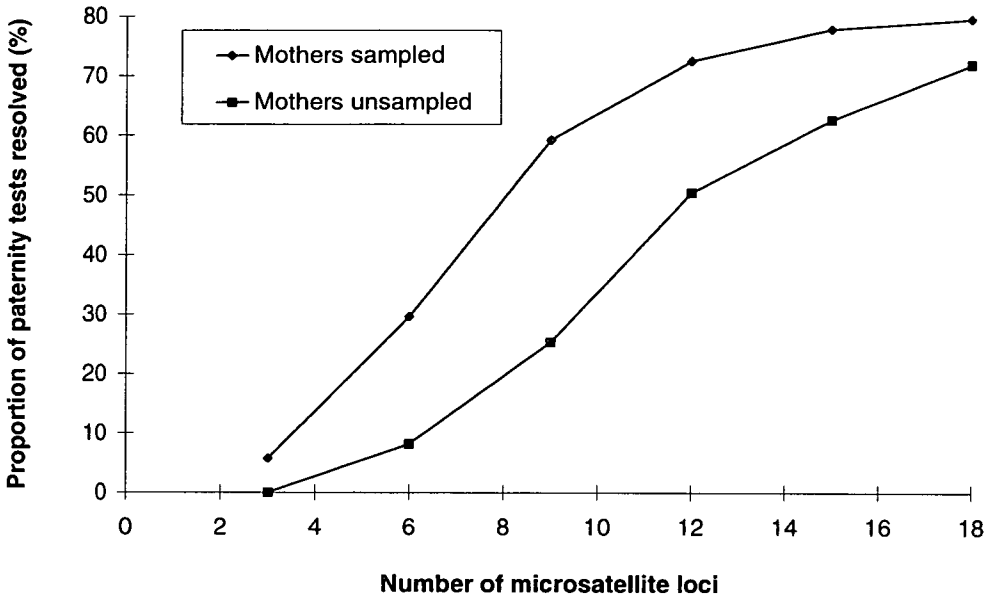

Figure 4.1 *Relationship between number of microsatellite markers and success of paternity inference predicted by the simulation module of the* CERVUS *program. Paternity inference where mothers were sampled is shown separately from paternity inference where mothers were unsampled. Microsatellite loci were added in the order shown in Table 2.2; additional loci were simulated by replicating the allele frequencies for the same nine microsatellite loci as if they were additional unlinked loci. All simulations also included the three protein loci. Parameter values are given in Table 3.3. Note that the proportion of paternity tests resolved can rise above 65% even though only 65% of stags are sampled, due to false-positive paternities.*

4.3.3 The full paternity analysis data set

The paternity analysis presented in Table 4.2 is for calves born 1982-1996. For certain analyses, such as the calculation of inbreeding coefficients (5.2.1) as many paternities as possible are required. I also analysed paternity for 247 calves born 1975-1981 applying the same criteria (Table 3.4), and a summary of the combined results is shown in Table 4.3.

Paternity analysis system	n tested	80% confidence	95% confidence
Proteins/microsatellites	1119	536†	246
DNA fingerprinting	114	80	80
Collectively	1121	554	283

Table 4.3 *Summary of the full paternity analysis data set for calves born 1975-1996. 71 of the tested offspring were typed only for proteins. In some cases, paternities that could not be inferred from locus-specific data had previously been assigned using DNA fingerprinting (Pemberton et al. 1992). Paternities assigned by DNA fingerprinting were assumed to have at least 95% confidence. †Includes two paternities which contradicted DNA fingerprinting data (see text).*

Some paternities that could not be inferred using locus-specific data had previously been assigned using DNA fingerprinting (Pemberton *et al.* 1992), and these paternities were assumed to have at least 95% confidence. In two cases paternities assigned with 80% confidence using locus-specific data contradicted the assignments made using DNA fingerprinting. In these cases, the DNA fingerprinting data were given precedence. The combined analyses gave a total of 554 calves for whom a father had been identified with 80% confidence, of which 283 were secure at 95% confidence.

4.3.4 Do locus specific and multilocus paternity inferences agree?

Pemberton *et al.* (1992) inferred paternity for 80 calves using DNA fingerprinting. These paternity assignments are thought to have a very high level of confidence, in excess of 95%. I used this earlier analysis as a benchmark against which to test results from the locus-specific analysis for the same set of calves (Table 4.4). None of the 42 paternities inferred with 95% confidence in the locus-specific analysis contradicted the earlier analysis. Only 2 of 61 (3%) of paternities inferred with 80% confidence contradicted the earlier analysis, significantly fewer than expected (12.2 of 61; $X^2 = 9.64$, d.f. = 1, $p < 0.01$). In fact most likely stags (i.e. using a critical $\Delta = 0$) had an actual confidence of 89% in this data set.

Simulated confidence	Same	Different	Total	Actual confidence	Success rate
Most likely	70	9	79	89%	99%
80% confidence	59	2	61	97%	76%
95% confidence	42	0	42	100%	53%

Table 4.4 *Locus-specific paternity inference for the 80 calves for which paternity had previously been assigned using multilocus DNA fingerprinting (Pemberton et al. 1992). The number of calves for which both paternity inference techniques implicated the same father (Same) is compared with the number of calves for which locus-specific paternity inference implicated a different father from DNA fingerprinting (Different). Actual confidence was calculated as Same divided by Total, assuming that paternity inferences based on DNA fingerprinting were correct. Success rate was calculated as Total divided by 80, expressed as a percentage. For one calf, no most likely stag was found because the sample was typed only for the three protein loci, and these were insufficient to identify a single most likely stag.*

Confidence was higher than expected because for all 80 calves the true father was sampled. In other words the major reason for lower confidence in the general data set was false assignments of stags to calves for whom the true father was not sampled. In all cases here, the true father was typed at 7 or more loci, and for 76, the true father was typed at 10 or more loci. The two cases where the locus-specific system identified the wrong father with 80% confidence both occurred when the true father was not typed for 4-5 microsatellite loci. This suggests that incomplete typing can compromise the analysis. Allowing for typing errors was also important. 7 of 42 95% confident paternities (17%) or 8 of 61 80% confident paternities (13%) would not have been assigned to the true father if an exclusionary approach had been adopted, as in these cases, the true father identified by DNA fingerprinting mismatched the offspring at one or more microsatellite loci (taking account of the mother's genotype if available).

The paternity inference system achieved a success rate of 76% at 80% confidence and 53% at 95% confidence among the set of calves for whom paternity had previously identified by DNA fingerprinting. These success rates are probably representative of cases when the true father is among the sampled candidate stags. Although the analysis at first sight suggests that DNA fingerprinting was more successful than the

locus-specific system, in practice the DNA fingerprinting technique is not practical without prior information on appropriate candidate stags from field data, and the locus-specific system is generally more amenable to large scale analysis.

4.3.5 Do genetic and behavioural estimates of paternity agree?

In Chapter 2, I discussed a comparison in Pemberton *et al.* (1992) between genetic assignment of paternity via DNA fingerprinting with behavioural assignment of paternity using harem-holding data. There were several sources of bias in that analysis (2.4.3). Here I repeat the analysis using the much larger and less biased data set of paternities inferred using the locus-specific paternity inference system: 246 paternities secure at 95% confidence.

Cases in which one stag held the hind for a greater number of days than any other were entered into the comparison of genetic and longest-hold paternity inference. In the ruts of 1971-72 and 1974-95, a single stag was identified for 1297 calves (Figure 4.2). For a further 132 offspring, behavioural data were available but two or more stags held the hind for an equal number of days. For 206 of 246 paternities assigned with 95% confidence using the locus-specific system, one stag held the hind for a greater number of days than any other during the 11-day window. These cases were used to evaluate the confidence in paternity assignment based on harem data.

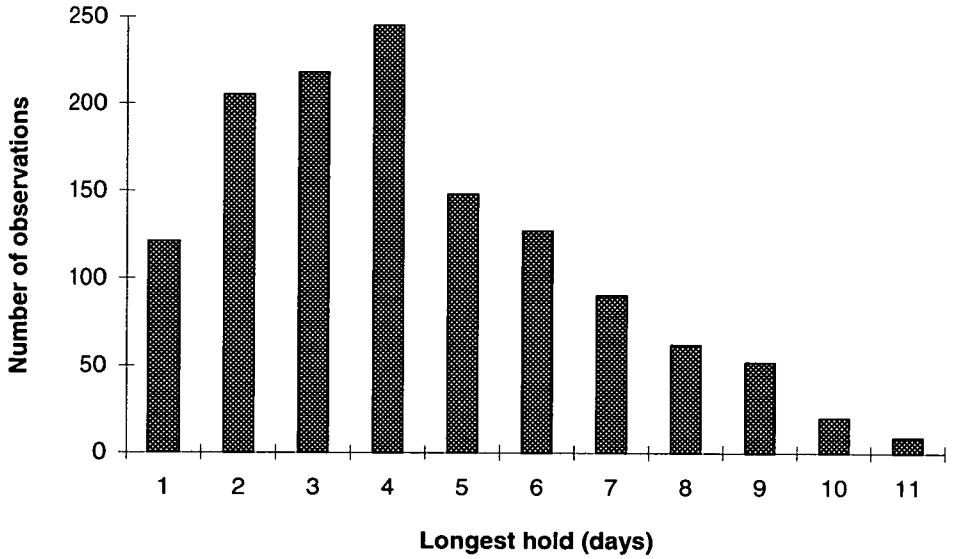

Figure 4.2 *The distribution of the longest number of days, of the 11 days closest to the estimated date of conception, for which each hind was held in one stag's harem during each rut (n = 1297 conceptions resulting in full-term pregnancy where the hind was seen with a stag for at least one day during her 11-day window). If two or more stags held the same hind in their respective harems for an equal number of days around her estimated conception date in a given year, no observation was included (an additional n = 132 conceptions). Rut census data was collected in years 1971 1972 and 1974-1995.*

Figure 4.3 is a cumulative probability chart, derived by comparing the stag identified using locus-specific paternity data with the longest hold stag indicated by the harem data for these 206 cases. For each number of days on the x-axis, the corresponding probability of paternity for a stag holding a hind for that number of days or longer is shown. This is analogous to asking the question: if a threshold number of x days was chosen for assignment of paternity, what would the overall confidence in assigned paternities be? If a criterion of just one day or more was chosen, paternity could assigned with 50% confidence in 1297 of 1429 cases (91%) (Figure 4.2). If a criterion of 5 days or more was chosen, paternity could be assigned with approximately 75% confidence for 508 of the 1429 cases (36%) based on behavioural data. 75%

confidence for a 5-day hold is considerably less than the 90% confidence suggested by Pemberton *et al.* (1992) (see Figure 2.4).

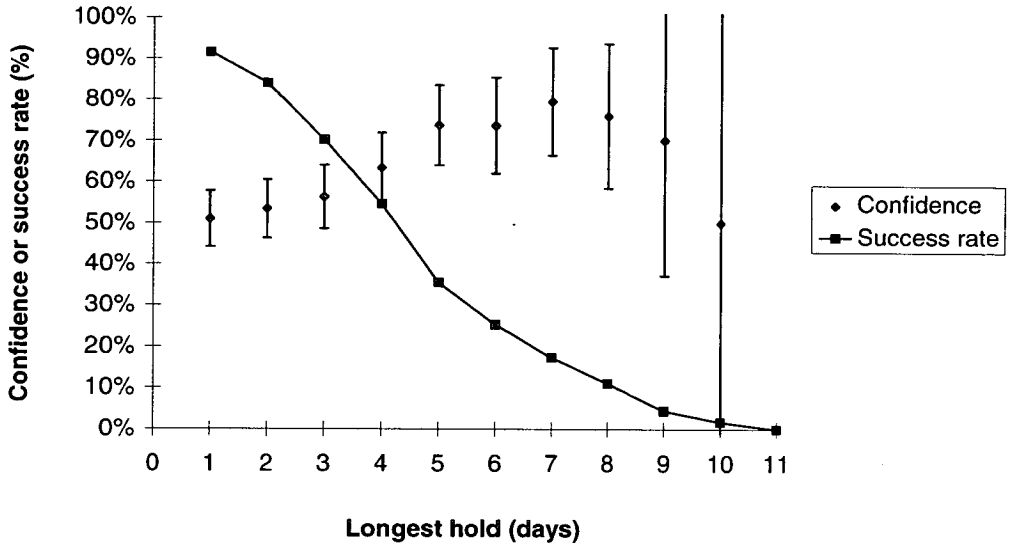

Figure 4.3 *The relationship between confidence in paternity inference and the longest number of days, of the 11 days closest to the estimated date of conception, for which each hind was held in one stag's harem during each rut. The corresponding success rate is also shown. The data are plotted in a cumulative manner, so that the confidence shown for each day is the proportion of paternities attributable to stags who held hinds for that number of days or longer. Error bars are 95% confidence intervals calculated by treating correctness of paternity inference as a binomial response, and bars are uncapped if their range extends down to 0% or up to 100% (note the distinction between confidence in paternity assignment and confidence interval).*

4.3.6 Mating success across age classes for natal and immigrant stags

In this analysis, I compared the distribution of mating success across age classes for stags born inside and outside the study area. The data set was 204 paternities inferred with 95% confidence for calf cohorts 1982-1996. Earlier cohorts were not used because sampling of rutting stags was insufficiently complete. Figure 4.4 shows the distribution of these paternities across stag age classes, with natal stags (54 stags with

153 calves between them) and immigrant stags (9 stags with 51 calves between them) plotted separately (natal stags are those known to have been born in the study area, and immigrants are all others).

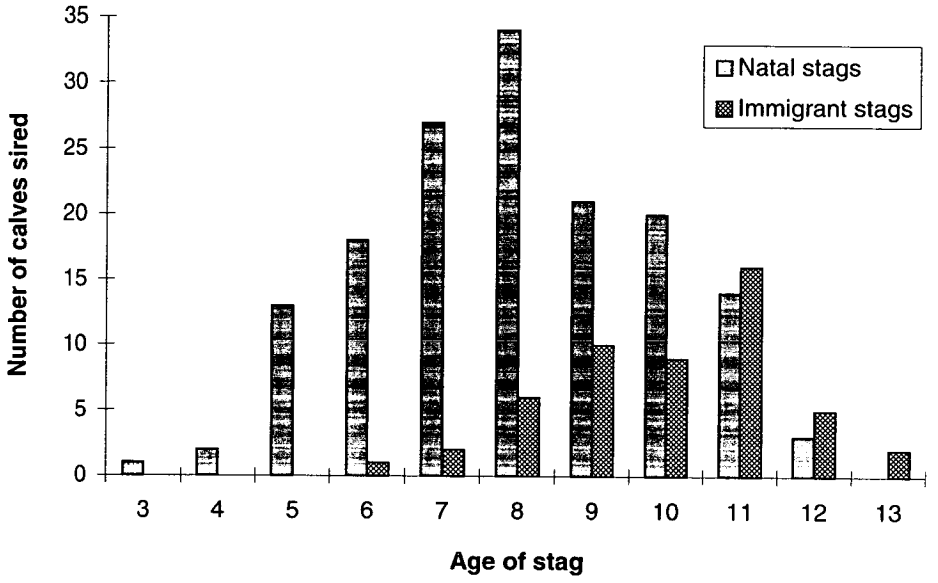

Figure 4.4 *Distribution of stag mating success across age classes, for 95% confident fathers of 204 calves born 1982-1996. Natal and immigrant stags are shown separately.*

The mean age at reproduction for natal stags was 8.0 years, while the mean age at reproduction for immigrant stags was 10.0 years. In 1982, one immigrant stag (GROW) had his most successful rut aged 11, fathering 9 calves born in 1983. This was the largest number of calves sired by any stag of any age in a single year. Excluding the 11 calves sired by GROW from the analysis reduced the mean age at reproduction for immigrant stags to 9.8. In other words, the difference in age between mean age at reproduction of natal and immigrant stags is not attributable to one particular stag, and there appears to be a systematic difference of approximately two years between the average age of natal stags and immigrant stags siring calves in the study area. One possible interpretation is that the ages of immigrant stags were not estimated accurately (see Discussion). Because accurate knowledge of stag age is important in subsequent analyses, I restricted the data set to natal stags only.

4.3.7 Stag lifetime mating success and lifetime reproductive success

4.3.7.1 Predicting the lifetime success of living stags

For this analysis, 31 stags were selected who were born in the study area from 1977 onwards (so that their offspring were born from 1982 onwards, when calf sampling became routine) and who had completed reproduction within the time frame of the paternity data set (i.e. had died before the rut of 1996, when the first cohort of calves too recent to be included in this study were sired). To avoid a bias towards stags that had died young, I selected only stags born in cohorts which had all died before the rut of 1996: in other words, stags born 1977-1981. Immigrant stags, stags that were shot during the cull on other parts of the island, and stags for which cause of death was not natural were excluded.

Genetic lifetime mating success (LMS) of a stag was assessed as the number of calves assigned with 80% confidence to that stag (80% confidence was used so that reasonable estimates of *individual* LMS could be obtained). In the sample of 31 stags, 12 was the oldest age at which any stag sired a calf. Genetic lifetime reproductive success (LRS) was assessed as the number of calves assigned with 80% confidence which survived to 1st May in the second year after birth. The same calculations were performed for LMS and LRS based on longest hold and fractional approaches to paternity inference.

To assess the predictive power of cumulative mating success (CMS) and cumulative reproductive success (CRS) at various ages on LMS/LRS, LMS/LRS was regressed on CMS/CRS at various ages (Table 4.5). Regressions were mostly significant and positive for CMS/CRS aged 6 or more. The key statistic is r^2, which can be interpreted as the proportion of variance in LMS/LRS explained by CMS/CRS at each age. For ages 6 and 7, the regression had low explanatory power (data not shown), and at age 8 it was intermediate. At age 9, the regression explained more than 50% of the variance (typically 70-90%), and at aged 10 and 11 more than 95% of the variance. On the basis of these results, age 9 was selected as the critical age from which LMS/LRS could be accurately estimated for individuals that were still living.

LMS/LRS could therefore be analysed for stag calves born between 1982 (when calf sampling began) and 1986 inclusive, since stags born in 1986 were aged 9 in the rut of

1995, when the 1996 calves (the last calf cohort analysed) were conceived. The gradients shown in Table 4.5 correspond to the scaling factors required to estimate LMS/LRS from CMS/CRS at each age for stags still alive in 1996.

4.3.7.2 Distribution of stag LMS and LRS

For cohorts 1982-1986, there were 105 sampled stags that did not permanently emigrate from the study area and did not die from unnatural causes (marking desertion as calves or shooting as adults). This analysis focuses on these stags, and implicitly assumes that those individuals included were a random sample of all calves.

Lifetime mating success (LMS) was estimated as the number of calves assigned to each stag with 80% confidence. Lifetime reproductive success (LRS) for each stag was estimated as the number of these calves which survived to 1st May in the second year after birth. Stags aged 12 or older in autumn 1995 were assumed to have completed reproduction by the end of that rut (4.3.7). For stags aged 11 or less in autumn 1995 who survived the following winter, their observed cumulative mating success (CMS) and cumulative reproductive success (CRS) was multiplied by the appropriate scaling factor from Table 4.5 to obtain their fitted LMS/LRS.

The genetic estimates of LMS and LRS were compared with two behavioural estimates. The first behavioural estimate was calculated from the number of calves for which the stag held its mother for more days than any other stag during the 11-day window (longest hold), and the second behavioural estimate was calculated from the total number of hind-days accruing to a stag divided by 11 (fractional). For all three measures, appropriate scaling factors shown in Table 4.5 were applied to estimate fitted LRS and LMS for stags that were still alive in 1996.

Paternity method	LMS/ LRS	Age	Mean CMS/ CRS (%)	r^2	*F*-ratio	d.f.	*p*-value	Gradient
Genetic	LMS	8	67%	0.539	26.94	1,23	<0.001	1.828
Genetic	LMS	9	82%	0.824	107.75	1,23	<0.001	1.399
Genetic	LMS	10	92%	0.968	692.55	1,23	<0.001	1.134
Genetic	LMS	11	99%	0.995	4659.05	1,23	<0.001	1.038
Genetic	LRS	8	57%	0.376	13.24	1,16	0.002	1.667
Genetic	LRS	9	80%	0.693	49.59	1,16	<0.001	1.296
Genetic	LRS	10	95%	0.962	550.46	1,16	<0.001	1.031
Genetic	LRS	11	100%	1	N/A	N/A	N/A	1
Longest hold	LMS	8	68%	0.418	10.78	1,15	0.005	1.415
Longest hold	LMS	9	83%	0.737	41.99	1,15	<0.001	1.353
Longest hold	LMS	10	93%	0.967	433.52	1,15	<0.001	1.109
Longest hold	LMS	11	100%	1	N/A	N/A	N/A	1
Longest hold	LRS	8	66%	0.209	3.97	1,15	0.066	1.333
Longest hold	LRS	9	82%	0.537	17.36	1,15	0.001	1.313
Longest hold	LRS	10	94%	0.963	393.83	1,15	<0.001	1.109
Longest hold	LRS	11	100%	1	N/A	N/A	N/A	1
Fractional	LMS	8	69%	0.732	60.15	1,22	<0.001	1.680
Fractional	LMS	9	83%	0.887	172.14	1,22	<0.001	1.475
Fractional	LMS	10	92%	0.976	894.81	1,22	<0.001	1.132
Fractional	LMS	11	99%	1	73878.33	1,22	<0.001	1.006
Fractional	LRS	8	61%	0.484	20.60	1,22	<0.001	1.526
Fractional	LRS	9	72%	0.716	55.36	1,22	<0.001	1.431
Fractional	LRS	10	84%	0.980	1079.54	1,22	<0.001	1.101
Fractional	LRS	11	91%	1	122954	1,22	<0.001	1.008

Table 4.5 *Regression of lifetime mating success (LMS) or lifetime reproductive success (LRS) against cumulative mating success (CMS) or cumulative reproductive success (CRS) at ages 6-11 for 31 stags. Regressions were carried out using EXCEL 5.0, with the fitted regression line forced through the origin. Only stags that had non-zero LMS/LRS were included in regressions; inclusion of others would not have changed the gradient as the regression line was forced through the origin.*

Figure 4.5 shows the distribution of the three estimates of LMS (data for LRS show a similar pattern). All three estimates are dominated by the fact that 77 of 105 stags (73%) did not reproduce at all because they failed to reach breeding age. The LMS and LRS estimates for the 28 stags that did reach breeding age are shown in Table 4.6. Only five stags surviving to breeding age (18% of 28) had estimated genetic LMS of zero (i.e. were not awarded any paternities at 80% confidence using the CERVUS program), and two of these (3ASP and 4CRE) died during their first rut.

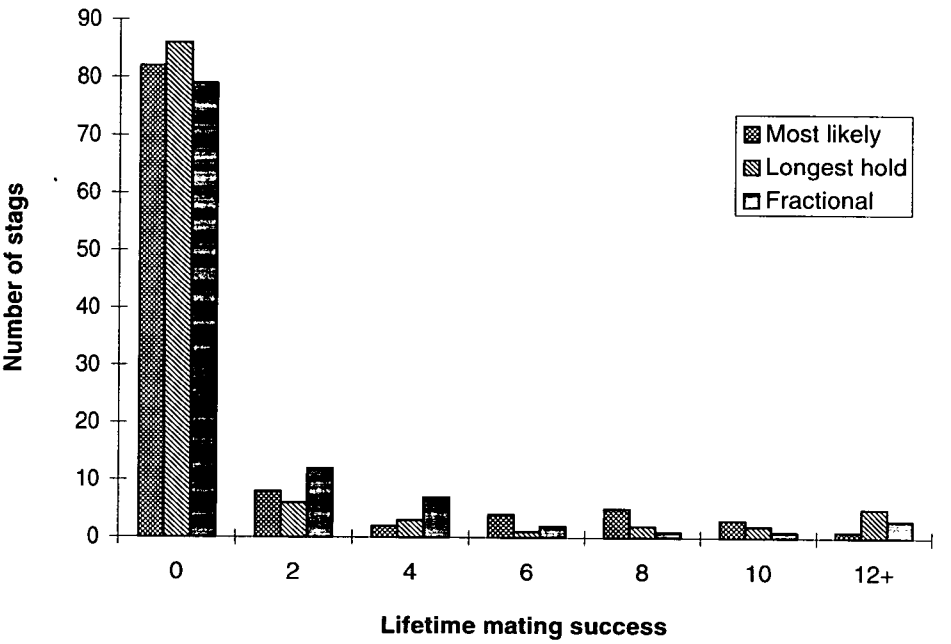

Figure 4.5 *Distribution of lifetime mating success (LMS) for 105 sampled stags born in the study area 1982-1986. One genetic (locus-specific) and two behavioural (longest hold and fractional) measures of LMS are shown. See text for details of paternity allocation procedures and the algorithm used to calculate fitted LMS for stags that were still alive.*

Code	Genetic LMS	Genetic LRS	Longest hold LMS	Longest hold LRS	Fractional LMS	Fractional LRS
2BAR	6.00	0.00	8.00	0.00	5.09	0.18
2PEC	0.00	0.00	0.00	0.00	0.27	0.09
2SAF	7.00	4.00	9.00	4.00	8.55	4.18
3ASP	0.00	0.00	0.00	0.00	0.00	0.00
3AVO	3.00	3.00	0.00	0.00	0.18	0.00
3BON	2.00	1.00	3.00	1.00	2.27	1.00
3LEN	6.00	2.00	10.00	1.00	5.64	1.00
3MEL	1.00	0.00	0.00	0.00	0.45	0.27
3OCH	9.00	4.00	23.00	9.00	14.27	5.73
3PRK	2.00	1.00	2.00	1.00	1.36	0.55
3SEP	3.00	1.00	4.00	3.00	3.82	2.36
3TRA	2.00	1.00	1.00	1.00	0.36	0.27
3TYS	10.00	3.00	21.00	11.00	14.64	7.45
4AVO	16.60	6.00	19.00	9.00	16.37	7.51
4CFU	1.04	1.00	0.00	0.00	0.09	0.09
4CRE	0.00	0.00	0.00	0.00	0.00	0.00
4CUR	0.00	0.00	0.00	0.00	0.45	0.36
4FOL	8.00	4.00	4.00	4.00	3.73	2.36
5AVO	0.00	0.00	1.11	0.00	1.85	1.10
5CUR	7.00	3.00	2.00	1.00	1.73	1.09
5DOR	1.13	1.03	0.00	0.00	0.31	0.20
5FAN	1.13	1.03	0.00	0.00	0.10	0.10
5INI	9.07	4.12	12.20	7.76	7.92	4.91
5SAF	6.80	5.15	11.09	7.76	2.98	2.20
5WEE	7.94	4.12	6.65	5.54	3.09	2.00
5WIG	1.13	1.03	1.11	0.00	1.65	0.50
6LAM	5.59	2.59	4.06	2.63	3.49	2.73
6MEL	5.59	2.59	1.35	1.31	3.22	1.69
Total	**122.02**	**55.66**	**143.57**	**70.00**	**103.88**	**49.92**

Table 4.6 *Lifetime mating success (LMS) and lifetime reproductive success (LRS) for the 28 stags born 1982-1986 who survived to reproductive age.*

4.3.7.3 The accuracy of individual behavioural estimates of LMS/LRS

To assess the predictive power of behavioural estimates of LMS/LRS, LMS/LRS estimated using genetic data was correlated separately with estimates derived using the two behavioural methods. Using data from the 28 stags who reached reproductive age, both longest hold ($r = 0.857$, $p < 0.001$) and fractional ($r = 0.835$, $p < 0.001$) estimates were highly correlated with genetic estimates of LMS (correlations used log-transformed data - see 6.2.1). The relationships were similar for LRS (longest hold: $r = 0.818$, $p < 0.001$; fractional: $r = 0.749$, $p < 0.001$; all correlations used log-transformed data). In other words, both behavioural estimates appear equally good at predicting individual LMS/LRS.

4.3.8 Hind lifetime mating success and lifetime reproductive success

4.3.8.1 Predicting the lifetime success of living hinds

The reproduction of hinds in the study area on Rum can be reliably monitored by observation. In order to estimate LMS/LRS of hinds that were still alive, I selected a group of 82 hinds from the cohorts 1975-1979, all of whom had died, and regressed CMS/CRS at various ages against LMS/LRS aged 17, the oldest age at which any hind in this group reproduced (Table 4.7). I excluded hinds that were shot or recorded as missing.

CMS/CRS explained more than 50% of the variance in LMS/LRS at age 8 or more, and by age 10 CMS/CRS explained more than 80% of the variance in LMS/LRS.

LMS/LRS	Age	Mean CMS/CRS (%)	r^2	*F*-ratio	d.f.	*p*-value	Gradient
LMS	6	39%	0.414	57.22	1,81	<0.001	2.534
LMS	7	49%	0.549	98.78	1,81	<0.001	2.064
LMS	8	59%	0.669	163.97	1,81	<0.001	1.699
LMS	9	71%	0.756	251.28	1,81	<0.001	1.438
LMS	10	78%	0.810	345.74	1,81	<0.001	1.300
LMS	11	85%	0.870	541.56	1,81	<0.001	1.204
LMS	12	91%	0.904	762.99	1,81	<0.001	1.112
LMS	13	94%	0.936	1190.70	1,81	<0.001	1.070
LMS	14	97%	0.970	2638.57	1,81	<0.001	1.037
LMS	15	98%	0.981	4259.75	1,81	<0.001	1.023
LMS	16	99%	0.996	18616.08	1,81	<0.001	1.011
LRS	6	40%	0.310	36.37	1,81	<0.001	2.075
LRS	7	51%	0.448	65.85	1,81	<0.001	1.726
LRS	8	64%	0.654	153.41	1,81	<0.001	1.492
LRS	9	75%	0.789	302.13	1,81	<0.001	1.317
LRS	10	84%	0.870	543.22	1,81	<0.001	1.201
LRS	11	89%	0.917	891.22	1,81	<0.001	1.138
LRS	12	94%	0.948	1481.94	1,81	<0.001	1.075
LRS	13	96%	0.969	2542.48	1,81	<0.001	1.052
LRS	14	100%	0.997	24911.02	1,81	<0.001	1.009
LRS	15	100%	1	N/A	N/A	N/A	1
LRS	16	100%	1	N/A	N/A	N/A	1

Table 4.7 *Regression of lifetime mating success (LMS) or lifetime reproductive success (LRS) against cumulative mating success (CMS) or cumulative reproductive success (CRS) at ages 6-16 for 82 hinds born 1975-1979. Regressions were carried out using EXCEL 5.0, with the fitted regression line forced through the origin.*

4.3.8.2 Distribution of hind LMS and LRS

To generate hind reproduction data directly comparable with the stag reproduction data just presented, I selected 85 sampled hinds from the cohorts 1982-1986, excluding hinds that were deserted as calves, shot or recorded as missing. Since reproduction data were available for hinds up to the calving season of 1996,

cumulative mating success and cumulative reproductive success were known for all hinds in the 1982-1986 cohorts at least up to the age of 10. The gradients shown in Table 4.7 were used to estimate LMS and LRS for hinds still alive in 1997 (Table 4.8, Figure 4.6). Fitting of LMS/LRS from age 10 upwards was imposed by choice of age 9 in the stag analysis, but appears justified by the regressions carried out for hinds.

Code	LMS	LRS	Code	LMS	LRS
2AVO	0.00	0.00	3SCO	2.00	1.00
2BUC	11.25	4.03	3TER	4.00	2.00
2CAL	6.00	3.00	3WHA	5.00	1.00
2CAN	3.00	2.00	4CAP	7.49	4.30
2HAR	7.00	1.00	4MUS	5.35	4.30
2KIL	6.00	4.00	4TER	4.00	1.00
2MOY	8.00	3.00	4THO	7.49	4.30
2PLO	12.28	7.06	5CAL	1.00	0.00
2RED	5.00	4.00	5CLA	7.78	3.41
2SIE	3.00	0.00	5FUL	3.00	0.00
2THO	8.18	7.06	5MAC	1.00	0.00
2UPT	4.00	1.00	5OCC	2.00	2.00
3BAR	5.00	4.00	5OCH	3.00	1.00
3CAN	1.00	0.00	5PET	7.78	3.41
3CUR	0.00	0.00	6EOL	2.00	0.00
3FUL	5.00	2.00	6FID	1.00	0.00
3LAM	8.30	5.26	6FUL	7.22	3.60
3MAW	9.33	4.21	6INI	9.63	4.81
3MOS	4.00	1.00	6LOB	4.82	0.00
3PET	4.00	1.00	6NON	1.00	0.00
3PGB	7.26	5.26	6THO	8.43	4.81
			Total	**212.60**	**99.83**

Table 4.8 *Lifetime mating success (LMS) and lifetime reproductive success (LRS) for the 42 hinds born 1982-1986 who survived to reproductive age. See text for details of the algorithm used to calculate fitted LMS and LRS for hinds that were still alive. Note that 2AVO, 3CUR, 5CAL and 5MAC died from dystocia with their first calf.*

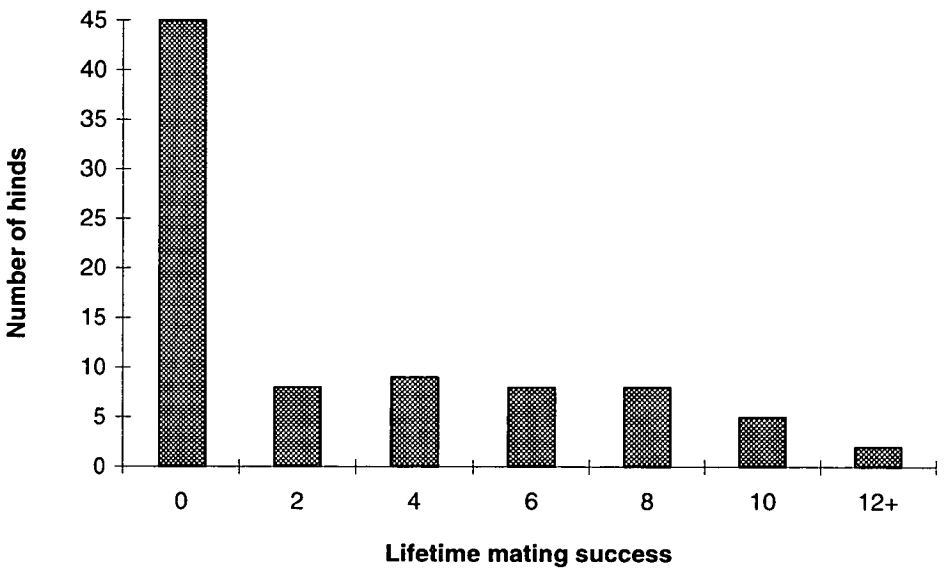

Figure 4.6 *Distribution of lifetime mating success (LMS) for 85 sampled hinds born in the study area 1982-1986. See text for details of the algorithm used to calculate fitted LMS for hinds that were still alive.*

4.3.9 Variance in LMS/LRS of stags and hinds

From the genetic and behavioural estimates of stag and hind LMS/LRS, it was possible to estimate the variance in stag and hind LMS/LRS according to each measure. The results of this analysis are shown in Table 4.9, along with several previous behavioural estimates of variance in stag and hind LMS/LRS. To control for differences in mean LMS/LRS derived by the various methods, the variance statistic that is compared is not the raw variance but the standardised variance, which is defined as the variance divided by the square of the mean (Sokal and Rohlf 1995). The mean, variance and standardised variance are shown for all stags and hinds and then separately for stags and hinds that reached breeding age, defined as 3 years for hinds and 5 years for stags (irrespective of whether they actually bred).

As in this study, Pemberton *et al.* (1992) derived standardised variance estimates for stags using genetic and behavioural (fractional) methods. However the data used were based on paternity of a non-random set of calves and the measures of stag success

used were not lifetime estimates. The estimates from Pemberton *et al.* (1992) are shown in Table 4.9, but are not equivalent to other estimates.

The estimates of standardised variance for all stags varied widely, and most of the variability in estimates was between studies rather than between methods. Surprisingly there was a strong positive association between the standardised variance and the number of stags included in each study, and this was not explained by variation in the proportion of stags failing to survive to breeding age (see Discussion).

The estimates of standardised variance for breeding stags were much smaller than those for all stags, and were relatively more consistent though by no means uniform. The estimates for breeding stags did not appear to vary systematically across studies but rather across methods. The four estimates of standardised variance based on behaviourally determined LRS were all more than twice as large as the estimate of standardised variance based on genetically determined LRS derived by the current study. This is the opposite of the pattern found by Pemberton *et al.* (1992) (see Discussion). Within the current study, estimates of standardised variance for stags were similar whether based on LMS or LRS.

Table 4.9 *(overleaf) Mean, variance and standardised variance of stag and hind lifetime mating success (LMS) and lifetime reproductive success (LRS). In each case, statistics are shown for all deer and then for breeders only, breeders being stags or hinds that survived to reproductive age irrespective of whether they actually bred. Note that Clutton-Brock et al. (1988) incorrectly states that the paternity assignment method used for the 1966-1972 cohorts was fractional (Rose 1995). Pemberton et al.'s (1992) estimates for stags were not based on lifetime estimates of mating success, and are therefore shown simply as MS, for mating success. The genetic paternity method in Pemberton et al.'s (1992) was multilocus DNA fingerprinting, shown as DNA FP. See text for details of statistical analysis for 1982-1986 cohorts. The standardised variance is the variance divided by the square of the mean (Sokal and Rohlf 1995).*

Stags born	Paternity method	LMS/ LRS	All stags				Breeders only				Data source
			n	Mean	Variance	Standardised variance	n	Mean	Variance	Standardised variance	
1964-1981	Fractional	MS	73	0.55	1.11	3.70	26	1.54	1.62	0.68	Pemberton *et al.* (1992)
1964-1981	DNA FP	MS	73	1.10	6.39	5.32	23	3.63	12.07	0.91	Pemberton *et al.* (1992)
1966-1972	Longest hold	LRS	48	3.73	35.00	2.51	33	5.41	41.90	1.43	Clutton-Brock *et al.* (1988)
1970-1980	Fractional	LRS	225	0.85	12.04	16.66	62	3.10	18.42	1.92	Rose (1995)
1982-1986	Longest hold	LRS	105	0.67	4.25	9.56	28	2.50	11.62	1.86	This study
1982-1986	Fractional	LRS	105	0.48	1.91	8.43	28	1.78	4.92	1.55	This study
1982-1986	Genetic	LRS	105	0.53	1.61	5.73	28	1.99	3.20	0.81	This study
1982-1986	Longest hold	LMS	105	1.37	16.99	9.08	28	5.13	45.43	1.73	This study
1982-1986	Fractional	LMS	105	0.99	8.30	8.48	28	3.71	21.49	1.56	This study
1982-1986	Genetic	LMS	105	1.16	8.07	5.97	28	4.36	16.62	0.88	This study
Hinds born	**Maternity method**	**LMS/ LRS**	**All hinds**				**Breeders only**				**Data source**
			n	Mean	Variance	Standardised variance	n	Mean	Variance	Standardised variance	
1966-1970	Observation	LRS	44	4.01	11.4	0.71	35	5.03	9.09	0.36	Clutton-Brock *et al.* (1988)
1982-1986	Observation	LRS	85	1.17	3.57	2.59	42	2.38	4.38	0.78	This study
1982-1986	Observation	LMS	85	2.50	11.19	1.79	42	5.06	9.66	0.38	This study

For hinds the same maternity assignment method, behavioural observation in the field, was used in both Clutton-Brock *et al.* (1988) and in this study. As for stags, estimates of standardised variance for all hinds varied widely between studies. When restricted to breeders only, standardised variance still varied considerably between the two studies. Indeed the current study found that standardised variance in LRS of hinds (0.78) was the same as standardised variance in LRS for stags based on genetic assignment of paternity (0.81). However the standardised variance in LMS of hinds (0.38) was less than half the standardised variance in LMS of stags based on genetic assignment of paternity (0.88). Possible reasons for the difference between LMS and LRS for estimating the standardised variance of hinds are explored in the Discussion.

4.4 Discussion

4.4.1 Locus-specific paternity inference using the CERVUS program

The success of paternity inference in Rum red deer is in close agreement with the predictions of the CERVUS simulation. Success at 80% confidence was relatively high when mothers are sampled, but low when mothers were unsampled, and in both cases, many fewer paternities were secure at 95% confidence than at 80% confidence. Differences between the predictions of the simulation and the outcome of the actual analysis could arise from between-cohort variation in number or sampling of candidate stags, locus-by-locus variation in frequency of missing data or error rates, inaccurate estimation of the error rate, unequal distribution of reproductive success between sampled and unsampled stags and relatives of the parents amongst the candidate stags. Despite these potentially confounding effects, the simulation appears to be a useful predictive tool, and simulation results suggest that typing of at least three further microsatellite loci would be a profitable enterprise.

4.4.2 Genetic and behavioural approaches to paternity inference

The genetic paternity data justify the use of behavioural methods for paternity inference in some circumstances. Behavioural approaches are most appropriate where a large sample size is of greater importance than the accuracy of the individual paternities. For instance for the cohorts 1971-1972 and 1974-1996, 1297 paternities

(69% of all calves born in the study area over these 25 years) can be assigned with 50% confidence based on behavioural data, whereas only 536 paternities (29% of all calves) can be assigned with 80% confidence using available locus-specific data. On the other hand the genetic paternity data derived in this study suggest that behavioural paternities, even when the most stringent criteria are applied, have a maximum confidence of around 75%, much less than the 100% suggested by Pemberton *et al.* (1992) (see Figure 2.4). A genetic approach is essential to generate paternity data with confidence greater than 75% in Rum red deer.

4.4.3 Age of natal and immigrant stags

The discrepancy of two years between the average age of natal stags and immigrant stags siring calves in the study area is interesting. There are two possible explanations. First, stags born in other parts of the island may take longer to attain dominant status than stags born in the study area due to, for example, differences in quality of grazing. Second, there may be no difference in maturation times across the island and instead those stags that successfully immigrate to the study area, where hind density (i.e. opportunity for mating) is higher than other parts of the island, are the largest stags in their cohorts, or alternatively were raised on a higher nutritional plane than study area stags (Clutton-Brock *et al.* 1997). Therefore when first seen in the study area, the age of these immigrant stags is systematically overestimated because they are on average larger (in terms of body size and/or antler growth) than their natal counterparts from the same cohort. In summary, it is unclear whether the discrepancy in average age of reproduction between natal and immigrant stags is genuine or an artefact arising from inaccurate estimation of the age of immigrant stags.

4.4.4 Variance in LMS/LRS of stags and hinds

4.4.4.1 Possible biases in estimates of standardised variance in stag LMS/LRS

The genetic estimates of stag LMS/LRS derived in this study are likely to be underestimates for two reasons. First an average of only 65% of calves were sampled. Second, for calves where the true father was sampled, only 75% of fathers were identified with 80% confidence (4.3.4). If these two effects are independent of each other and independent of the stags involved, total LMS/LRS estimated by the genetic

approach will be around 50% of the true value. In practice this means that successful stags sired more offspring than suggested by this analysis, and also that some stags shown as having no offspring did in fact leave one or more descendants. While the mean and raw variance in stag LMS/LRS are almost certainly underestimated, the estimate of standardised variance in stag LMS/LRS, one of the main objectives of the analyses presented in this chapter, is likely to be approximately correct since standardised variance is unaffected by scalar multiplication of the distribution from which it is calculated.

There is one possible sources of bias in the genetic estimates of standardised variance in stag LMS/LRS derived in this study. Given that the analysis was based on paternity inference at 80% confidence, on average one in five paternities are likely to be incorrect. These paternities will be assigned to other rutting stags at random with respect to those stags' true LMS/LRS. Because the variance in stag LMS/LRS is greater than the mean (Table 4.11), noise of this type means that less successful stags will overall tend to accrue false paternities at the expense of the most successful stags. The net effect is to reduce the variance in stag LMS/LRS relative to the mean, and hence to reduce the standardised variance in stag LMS/LRS. It seems unlikely that this bias would be sufficient to dramatically alter estimates of standardised variance in stag LMS/LRS.

Behavioural estimates of standardised variance may also be biased by false assignment. For the longest hold approach, false paternities will not be assigned to stags at random with respect to their true LMS/LRS; the potential for a large bias is greater given the higher proportion of false assignments (approximately 50%) but the direction of any bias is difficult to predict. Similarly the fractional approach leads to a non-random distribution of the "false" paternity fractions to stags with respect to their true LMS/LRS with an unpredictable bias. However the division of individual paternities in the fractional approach might be expected to bias standardised variances estimates from the fractional approach downwards with respect to estimates from the longest hold approach, and standardised variance was indeed lower when fractional paternity assignment was used in place of longest hold (categorical) paternity assignment.

All estimates of standardised variance in stag LMS/LRS may have been biased by the method used to estimate stag LMS/LRS for stags that were still alive. Cumulative stag mating success/reproductive success was multiplied by a scaling factor based on the stag's age. However for all approaches used to estimating stag LMS/LRS (genetic, longest hold and fractional), stags had completed an average of at least 70% of their LMS/LRS when scaling factors were applied (Table 4.5). It seems unlikely that the relatively modest scaling applied here could give rise to very inaccurate estimates of stag LMS/LRS, and the direction of any bias is not obvious. The same method was also used for estimating hind LMS/LRS for hinds that were still alive.

4.4.4.2 Which estimate of standardised variance in stag LMS/LRS is correct?

In this study the genetic approach to paternity assignment yielded very much lower standardised variance of stag LMS/LRS than both behavioural approaches. In contrast Pemberton *et al.* (1992) found that standardised variance of stag mating success was considerably higher when a genetic approach to paternity assignment was used compared with a fractional behavioural approach. There are three possible explanations for the contrast between Pemberton *et al.*'s (1992) results and those of this study. First, Pemberton *et al.*'s data were based on annual mating success (AMS) rather than lifetime mating/reproductive success. Standardised variance in AMS does not accurately estimate standardised variance in LMS if stags vary in the degree to which they devote all their reproductive effort to one or two ruts as opposed to rutting in a more sustained fashion over several years. Second, Pemberton *et al.*'s genetic approach used multilocus DNA fingerprinting rather than the series of single-locus protein and microsatellite polymorphisms used in the current study. False assignments are likely to be very rare using DNA fingerprinting, but one in five genetic paternities in the current study using the single-locus system are likely to be false. As discussed above false assignments may generate a downward bias in estimates of standardised variance with the single-locus system. However it seems unlikely that false assignments at a frequency of 20% could lead to such a large contrast between the findings of the two studies. Third, due to the technical limitations of the DNA fingerprinting technique Pemberton *et al.* tested only stags observed with a particular hind around her estimated date of conception. The mating success of stags following a "sneaky" mating strategy (Brooker *et al.* 1990) may have been underestimated by this

approach (2.4.3). I believe that the combined effect of a non-random set of stags and the fact that the annual rather than lifetime mating success of individual stags was assessed to be the primary reason for the discrepancy between the two studies. In summary, the results of the current study do not support Pemberton *et al.*'s suggestion that genetically-derived paternity data generate higher standardised variance in stag LMS/LRS than behaviourally-derived paternity data. Indeed the current study suggests that the opposite is true.

4.4.4.3 Does LMS or LRS give better estimates of variance for stags and hinds?

One of the key features of the polygynous mating system of red deer is thought to be that among breeding adults, stags have greater standardised variance in LRS than hinds (Clutton-Brock *et al.* 1982, 1988). However the current study does not support such a difference for LRS, if stag LRS is based on genetic paternity assignment. When LMS was used in place of LRS, the standardised variance of stags changed little but the standardised variance of hinds was halved (discussed below). Even so, the contrast between standardised variance of stag and hind LMS in the current study (stags 2.3 times greater) was not as great as the contrast found by Clutton-Brock *et al.* (1988) for LRS (stags 4.0 times greater). Interestingly, if longest hold LMS was used in place of genetic LMS for stags, standardised variance among stags was 4.6 times greater than standardised variance among hinds, similar to the ratio found by Clutton-Brock *et al.* (1988) for LRS.

Spatial substructure in the population influences calf survival, with calves born and raised in areas of high local population density more likely to die in their first winter (Coulson *et al.* 1997). Because of the restricted range of hinds, consistent differential mortality across the study area is likely to cause standardised variance in hind LRS to be considerably larger than standardised variance in hind LMS (such an effect may not influence stag LMS to the same extent as each stag tends to breed with a number of hinds over his lifetime, not all of which necessarily use the same part of the study area). Because study area density has increased since the hinds included Clutton-Brock *et al.*'s analysis completed most of their reproduction (Pemberton *et al.* 1996), juvenile mortality has also increased (proportion of calves surviving to age 2 born 1970-1979: 62%; born 1986-1995: 49%; two-sample $t = 2.17$, d.f. = 18, $p = 0.043$ two-tailed). The overall increase in juvenile mortality is likely to be reflected in an

increase in standardised variance in hind LRS due accentuation of mortality differences between parts of the study area. I therefore suggest that for hinds in the data set in this study, LMS is a more accurate measure of hind quality than LRS. For stags, because of incomplete sampling of calves only relative reproduction can be estimated. LMS is based on a larger sample of offspring than LRS and therefore can be more accurately estimated at the individual level.

For these reasons, LMS rather than LRS was used as a fitness measure for both stags and hinds in Chapter 6, where the fitness implications of inbreeding in Rum red deer are explored. Measures of inbreeding are explored in Chapter 5.

4.5 Summary

The paternity inference program CERVUS was used to infer the paternity of 536 red deer calves born between 1975 and 1996 with 80% confidence, given genetic data from three protein and nine microsatellite loci. 246 of these paternities were secure at 95% confidence. This is possibly the largest analysis of paternity ever undertaken in a natural population by molecular methods. The CERVUS simulation accurately predicted the proportion of paternities that would be resolved, and the results from the locus-specific analysis are in close agreement with an earlier DNA fingerprinting study. Comparison of the two data sets shows that allowing for typing errors was important in quite a number of cases. It is also shown that behavioural observations are reasonably predictive of paternity (50-75% accuracy), but they are not as accurate as suggested by a previous study.

Mean lifetime mating success (LMS) for 105 stags born 1982-1986 was 1.16 and standardised variance was 5.97 based on the genetic paternities derived using CERVUS. Among the 28 stags which survived to reproductive age, mean LMS was 4.36 and standardised variance was 0.88. While the mean LMS is known to be an underestimate, the standardised variance is likely to be close to the true value. Two behavioural approaches to paternity assignment gave higher estimates of standardised variance than the genetic approach, contrasting with an earlier study which suggested that behavioural methods underestimate variance in stag mating success.

Mean LMS for 85 hinds born 1982-1986 was 2.50 and standardised variance was 1.79 based on maternity derived by behavioural observation. Among the 42 hinds which survived to reproductive age, mean LMS was 5.06 and standardised variance was 0.38. Relative to the standardised variance in hind LMS, standardised variance in stag LMS is not as large as previously suggested.

5 Measuring inbreeding within an individual

5.1 Introduction

Inbreeding at the level of the individual is traditionally measured using the inbreeding coefficient F (Wright 1922). The inbreeding coefficient is calculated from an individual's known pedigree, and represents the average probability of the two alleles at an autosomal locus being identical by descent due to inbreeding within the known pedigree:

$$F = \sum_{a=1}^{c} \left(\tfrac{1}{2}\right)^{m} \left(1 + F_a\right) \qquad \textit{Equation 5.1}$$

where m is the number of individuals in the path from one parent to the other via common ancestor a, F_a is the inbreeding coefficient of common ancestor a and the sum is taken over all c common ancestors (adapted from Equation 5.4 of Hartl and Clark 1989). Studies of inbreeding in captive animals, where pedigree records may be very extensive, have generally relied on the inbreeding coefficient (Chapters 7 and 8). However the measure captures no information on inbreeding due to common ancestors occurring prior to the start of the known pedigree.

The established alternative to the inbreeding coefficient is an empirical assessment of individual heterozygosity, traditionally based on protein polymorphisms (Mitton 1993). This is simply the proportion of loci typed that are heterozygous in an individual. Because the effect of inbreeding is to increase the proportion of loci that are homozygous, inbred individuals are expected to have lower individual heterozygosity than outbred individuals. This approach has the advantage that no pedigree information is required. However protein polymorphisms have the disadvantage that average heterozygosity at most loci is relatively low, and therefore the power to detect inbreeding as excess homozygosity is relatively low. On the other hand, given their typically large number of alleles, microsatellites offer much greater power to detect inbreeding.

In this chapter, I explore both pedigree-based and molecular approaches to assess inbreeding of individual Rum red deer, and also explore a recently described empirical measure of inbreeding, mean d^2 (Coulson *et al.* 1998b), which considers microsatellite heterozygosity in the light of the stepwise mutation hypothesis (Valdes *et al.* 1993).

5.2 Methods

5.2.1 Inbreeding coefficient

Since the ability to detect inbreeding in an individual is restricted by the number of its known common ancestors, it is important to have as deep a pedigree as possible for calculation of inbreeding coefficients. In Rum red deer, maternity was determined by observation for 2310 calves born 1965-1996 (of which 2289 were born 1970-1996), and paternity was determined with 80% confidence by molecular methods for 554 calves born 1975-1996 (Table 4.3). Non-zero inbreeding coefficients could only be calculated for individuals with known maternity and paternity, and therefore the number of calves that could have had non-zero inbreeding coefficients was 553, since for one calf with known paternity (QUIN), the mother was not known.

The calculation of inbreeding coefficients was performed using the Fortran program FASTINB (Boyce 1983) modified for pedigrees of more than 1,000 individuals. The algorithm used by the program is the iterative Quaas-Henderson method (described in Boyce 1983).

5.2.2 Individual heterozygosity

In Rum red deer both protein and microsatellite polymorphisms have been typed on a large scale (Table 2.2). However at least two of the three protein loci may be under selection (2.4.2). It is also of interest to compare individual heterozygosity with the microsatellite-specific measure mean d^2 (see 5.3.3 below), and the comparison is most meaningful if based on the same set of loci. Individual heterozygosity H was therefore calculated using genetic data from the nine microsatellite loci only, as:

$$H = \frac{1}{n}\sum_{i=1}^{n} h(i) \qquad \textit{Equation 5.2}$$

where $h(i)$ is a random variable with the value 0 when locus i is homozygous and the value 1 when locus i is heterozygous, for the n loci at which an individual was typed. Individual heterozygosity was not calculated for 38 individuals with fewer than five out of nine loci typed. Individual heterozygosity measures were therefore available for 1125 calves born 1964 - 1996 (of which 1113 were born 1970 - 1996), based on an average of 8.4 loci of 9 per individual (i.e. data were 93% complete).

5.2.3 Mean d^2

Recently, in an analysis of the Rum red deer data set, Coulson *et al.* (1998b) described a novel measure of inbreeding, mean d^2, which can be readily calculated from microsatellite data. Because of its relevance to much of the remainder of this thesis (Chapters 5, 6 and 8), the conceptual basis of mean d^2 is described below. The description is largely based on material contained in Coulson *et al.* (1998b) (see also Pemberton *et al.* 1998).

Microsatellite loci are thought to evolve by stepwise mutation (Valdes *et al.* 1993) or some variation of it (Di Rienzo *et al.* 1994; Zhivotovsky and Feldman 1995). The stepwise mutation model suggests that mutations do not occur from one allele to some completely random allele, but rather in a stepwise manner, plus or minus one repeat unit from the initial allele. Under this model alleles evolve apart by a random walk process, gradually diverging. Presuming that microsatellite allele lengths are bounded at both ends (clearly the repeat length cannot be less than zero, and at the other extreme genomes are of finite size), the random walk cannot be random for ever, but there is a period of time, several times the reciprocal of the mutation rate, for which the random walk approximation is likely to be true. Given that the mutation rate at microsatellite loci is of the order of 10^{-3} per locus per generation (Gyapay *et al.* 1994), the random walk approximation should hold for at least 10^4 generations.

This property of microsatellites has been used to estimate the degree of divergence between two genetically isolated populations, presuming that the set of alleles in each

population collectively follow the same random walk process. The genetic distance Δ_{ab} between two alleles a and b can be written as:

$$\Delta_{ab} = (a-b)^2$$

and $\overline{\Delta}$, the average of Δ across all alleles sampled, one from each population, is then an unbiased estimator of genetic distance between the two populations, linearly related to time (Goldstein *et al.* 1995). Note that Goldstein *et al.*'s use of Δ is unrelated to the log-likelihood difference Δ in paternity analysis (3.2.3).

What is true between populations should also be true between genomes represented in an individual. Assuming that there exists within a population variance in the degree of common ancestry of the two parental genomes represented in each individual, a distance measure equivalent to $\overline{\Delta}$ comparing alleles across loci within an individual should be an unbiased estimator of the distance between parental genomes. This measure is termed mean d^2, and is written:

$$\text{mean } d^2 = \frac{1}{n}\sum_{i=1}^{n}(i_a - i_b)^2 \qquad \textit{Equation 5.3}$$

where i_a and i_b are the length in repeat number of the two microsatellite alleles at locus i, for each of n typed loci (Coulson *et al.* 1998b). This measure was calculated for the same 1125 calves born between 1964 and 1996 as was individual heterozygosity (5.2.2). The interpretation of mean d^2 as a measure is considered in the Discussion.

Note that Coulson *et al.* (1998b) restricted calculation of individual heterozygosity and mean d^2 to individuals typed at seven or more loci rather than the threshold of five loci chosen in this study. The lower threshold was chosen because for some analyses in Chapter 6 sample size was in any case rather small.

5.3 Results

5.3.1 Inbreeding coefficient

5.3.1.1 Distribution of inbreeding coefficients

55 of the 553 calves (10%) for which both parents were known had non-zero inbreeding coefficients (Figure 5.1). However 110 of the 553 (20%) were sired by immigrant stags of unknown parentage, and therefore inbreeding in this group, although unlikely, could not have been detected were it to have occurred. The distribution of inbreeding coefficients was inevitably non-normal given both the inadequacy of the pedigree and the large influence of recent close inbreeding events on the overall inbreeding coefficient. Relationships between inbreeding coefficient and other measures of inbreeding were therefore assessed using non-parametric tests. Statistical analysis was carried out separately on the 553 calves for which inbreeding coefficients were calculated and on the subset of 55 calves for which the value of inbreeding coefficient was greater than zero.

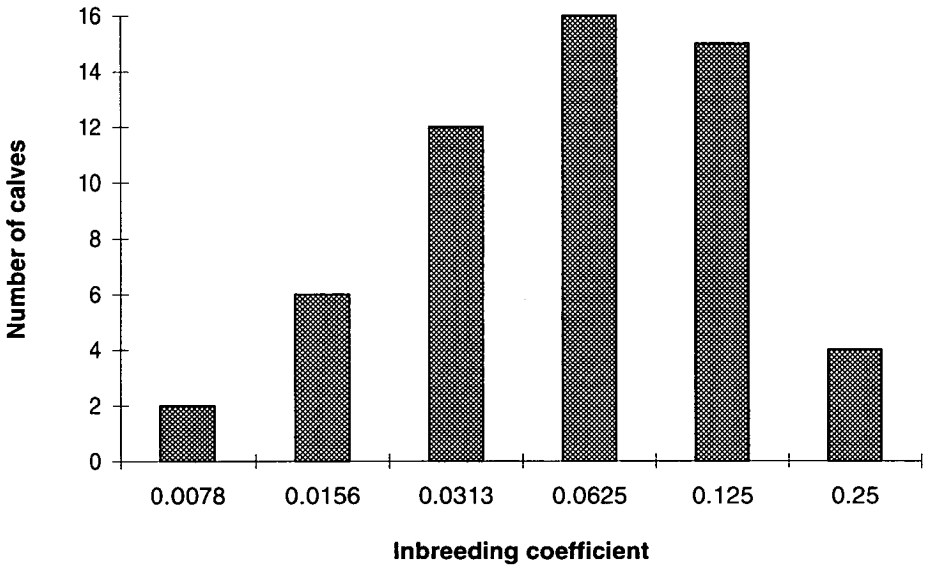

Figure 5.1 *Distribution of inbreeding coefficients (f) for 55 of 553 calves for which f was greater than zero. Note that the x-axis is a logarithmic rather than a linear scale, and bars are labelled with the upper boundary of the inbreeding coefficient category.*

5.3.1.2 Distribution of inbreeding over time

The percentage of calves of known parentage that were found to be inbred increased over the course of the study (Figure 5.2). This could be because inbreeding did become more common during the course of the study, or alternatively may be a consequence of the fact that the inbreeding coefficients are rooted at zero at the top of the pedigree, and inbreeding can only be detected in F2 generations onwards. There is no obvious reason why the true frequency of particular inbreeding events should have increased over time, and so it seems more likely that the apparently increasing frequency of detected inbreeding events over time is an artefact of the detection process.

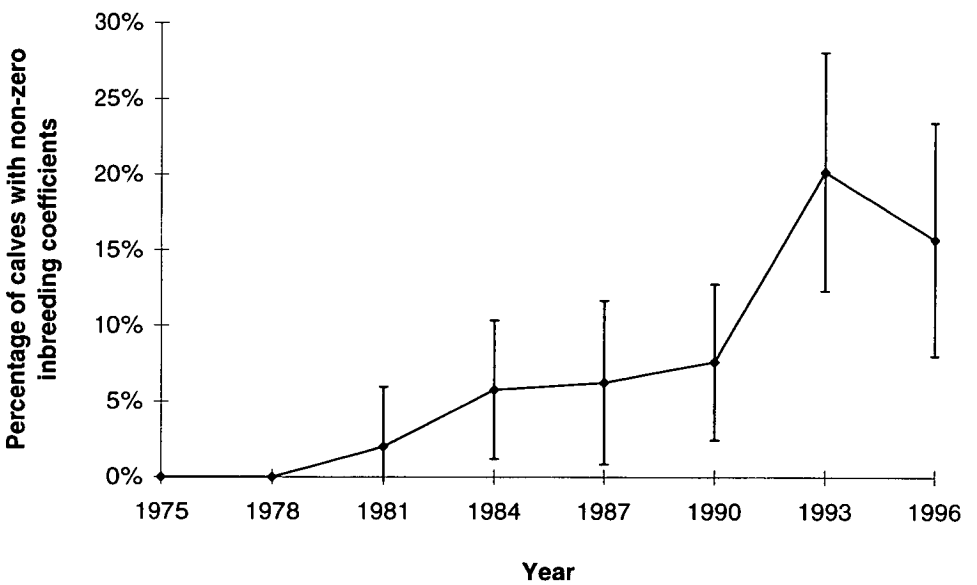

Figure 5.2 *The percentage of calves, of those with both parents known, with inbreeding coefficients greater than zero, over time. Years are grouped into threes, and each point represents the percentage of calves with non-zero inbreeding coefficients in the year shown and the two years preceding that year. Error bars are 95% confidence intervals calculated by assuming that inbreeding occurred as a binomial process with constant probability within each group of three years.*

5.3.1.3 Frequency of close and moderate inbreeding events

Defining a close inbreeding event as a calf born with a calculated inbreeding coefficient equal to or greater than 0.25 (Ralls *et al.* 1986) and a moderate inbreeding event as a calf born with $0.125 \leq f < 0.25$, there were 19 calves that were closely or moderately inbred (Table 5.1). Calves by paternal half sibs were significantly more frequent than calves by maternal half sibs ($X^2 = 25.3$, d.f. = 1, $p < 0.001$; this result is not dependent on small expected frequencies).

Male parent	Female parent	*f* of offspring	Number of calves tested	Number of inbred calves	Frequency of inbreeding event
Father	Daughter	0.25	195	3	1.5%
Son	Mother	0.25	440	0	0%
Full brother	Full sister	0.25	102	0	0%
Paternal half-brother	Paternal half-sister	0.125	103	11	10.7%
Maternal half-brother	Maternal half-sister	0.125	428	4	0.9%
Other	-	0.125	-	1	-
Total	**-**	**-**	**-**	**19**	**-**

Table 5.1 *Number of close and moderate inbreeding events of various types among 553 calves. The category Other includes uncle-niece, aunt-nephew, double first cousin and grandparent-grandchild pairings, and the observed case was a maternal grandmother-grandson pairing (MOMD with her grandson MDC5). The number tested in each case is the number of calves for which all the necessary relatives were known in order to infer whether or not a given inbreeding event had occurred.*

All the paternal half sib parents were the offspring of just three males, and 7 of the 11 were the offspring of a single male, MAXI. Two of the three maternal half sib pairs were the offspring of the same female, REDY. However none of the 19 closely or moderately inbred calves were the offspring of the same incestuous pairing, and indeed each of the 19 closely inbred calves was the offspring of a different stag. These inbreeding events can therefore reasonably be regarded as being independent of each other.

5.3.2 Individual heterozygosity

Individual heterozygosity was calculated as a simple arithmetic average across loci for 1125 calves typed at five or more microsatellite loci (Equation 5.2). The mean value was 0.75 and the standard deviation was 0.15, and these values did not change over time (data not shown). Heterozygosity is fairly similar (range 0.673 - 0.837) across the nine microsatellite loci screened (Table 2.2) giving a coefficient of variation of 0.11, and therefore missing data for particular loci should have little effect on the average across loci for each individual. Any error due to missing data should in any case be distributed at random with respect to phenotypic and environmental variables (Chapter 6). The distribution had an approximately normal form at a coarse scale (Figure 5.3) and arcsine transformation, the standard transformation for proportions (Sokal and Rohlf 1995), did not improve the normality of the distribution (raw data: Anderson-Darling $A^2 = 14.755$, $p < 0.001$; transformed data: $A^2 = 27.091$, $p < 0.001$). Plotted at a finer scale the distribution appeared more spiky due to the discrete nature of the data (Coulson *et al.* 1998b).

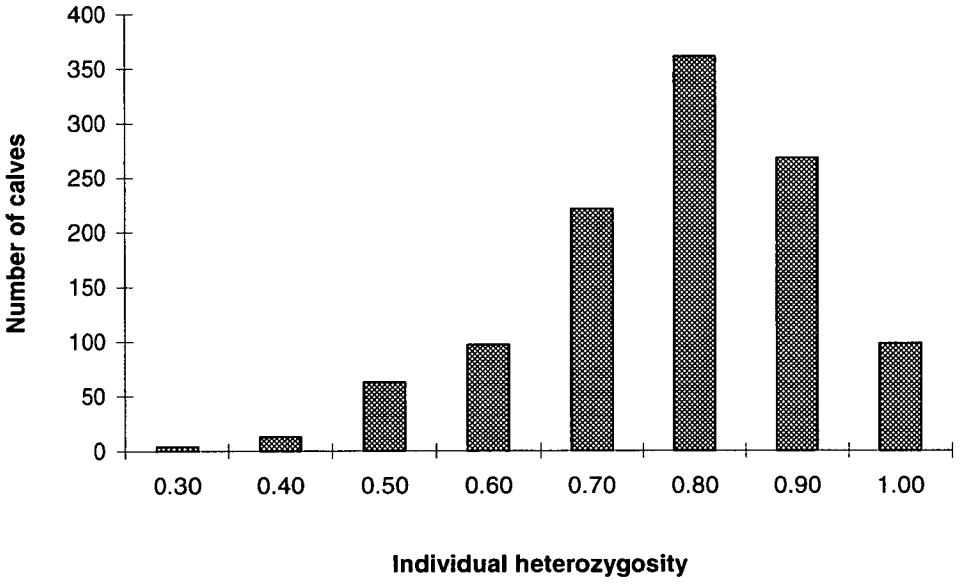

Figure 5.3 *Distribution of individual heterozygosity for 1125 red deer calves typed at seven or more microsatellite loci.*

5.3.3 Mean d^2

Mean d^2 was calculated as a simple arithmetic average across loci for 1125 calves typed at five or more microsatellite loci (Equation 5.3). The mean value was 35.5 and the standard deviation was 19.4, and these values did not change over time (data not shown). There was considerable variation in average d^2 scores between loci (range 5.0-121.8) giving a coefficient of variation of 0.55. Missing data at loci with largest average d^2 scores may therefore significantly reduce overall mean d^2, but such errors should be distributed at random with respect to phenotypic and environmental variables (Chapter 6). The distribution of mean d^2 scores was approximately normal (Figure 5.4), and logarithmic transformation (as used for mean d^2 in Coltman *et al.* 1998a) did not improve the normality of the distribution (raw data: Anderson-Darling $A^2 = 5.869$, $p < 0.001$; transformed data: $A^2 = 25.356$, $p < 0.001$). Plotted at a finer scale, the distribution still appears continuous (Coulson *et al.* 1998b).

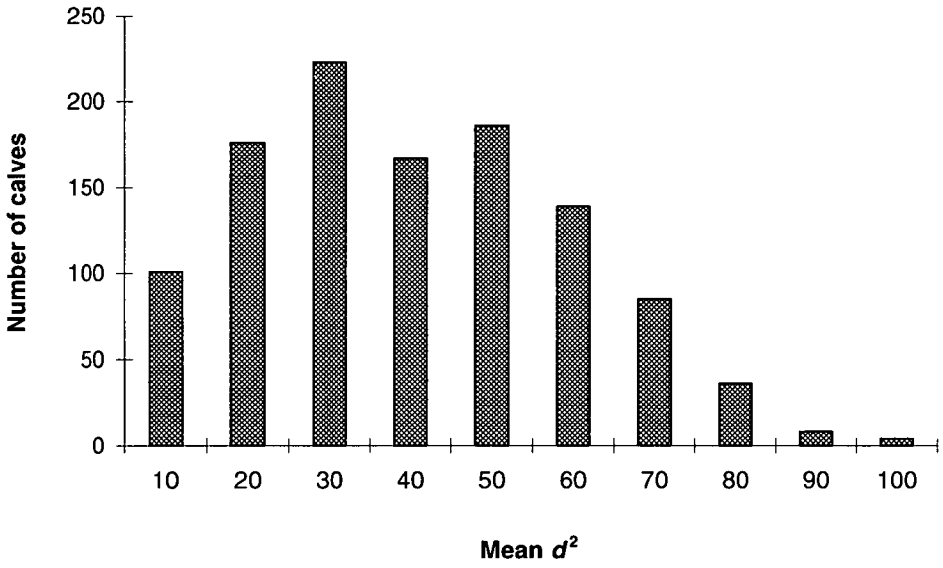

Figure 5.4 *Distribution of mean d^2 for 1125 red deer calves typed at five or more microsatellite loci.*

5.3.4 Relationships between measures of inbreeding

Among 546 calves with known parents, there was no difference in individual heterozygosity of inbred versus non-inbred calves ($F_{1,544}$ = 1.09, p = 0.296) and no difference in mean d^2 of inbred versus non-bred calves ($F_{1,544}$ = 0.56, p = 0.455); seven other calves, all of whom had inbreeding coefficients of zero, had known parents but were typed for fewer than five microsatellite loci. Coulson *et al.* (1998b) found similar results using inbreeding coefficients of calves born 1982-1996 calculated from a pedigree containing only paternities assigned with 95% confidence. Among the 55 calves with non-zero inbreeding coefficients, inbreeding coefficient was not significantly correlated with either individual heterozygosity (Spearman's rank correlation coefficient r_S = -0.177, p = 0.196) or with mean d^2 (r_S = -0.066, p = 0.633). Among the 1125 calves typed at five or more microsatellite loci, individual heterozygosity and mean d^2 were significantly correlated (Pearson's correlation coefficient r = 0.384, p < 0.001), as may be expected given that individual heterozygosity may be regarded as a component of mean d^2 (Coulson *et al.* 1998b).

5.4 Discussion

5.4.1 Measures of inbreeding

Inbreeding coefficients are sensitive both to the completeness and the correctness of the pedigree. In the Rum red deer pedigree, the data was neither complete nor particularly correct (paternities were assigned with 80% confidence), and inbreeding coefficients were therefore probably only weakly correlated with true inbreeding even within the time frame of the study. This may explain why inbreeding, as measured by inbreeding coefficient, was not significantly correlated with individual heterozygosity or with mean d^2. Mean d^2 was correlated with individual heterozygosity but the correlation was relatively weak.

Individual heterozygosity can be regarded as one component of the variation represented in mean d^2. Here I briefly discuss what else mean d^2 may be measuring. Given that mean d^2 is conceptually based on the stepwise mutation model for microsatellites (Valdes *et al.* 1993), at least some of the additional variation might be

some kind of deep inbreeding measure, assessing inbreeding arising from common ancestry hundreds or thousands of generations back in the pedigree. If so, population mixing events are likely to be especially well represented by mean d^2 (Coulson *et al.* 1998b), and the diverse and relatively recent origin of the Rum population (Table 2.1) makes it quite likely that mean d^2 could be picking up the ensuing mixing.

Pemberton *et al.* (1998) examined the contribution of individual loci to the association between mean d^2 and birth weight in Rum red deer (6.1.2) and found that d^2 at one locus, OarCP26, showed significant association with birth weight, while mean d^2 across the remaining eight loci did not. However mean d^2 calculated across all nine loci had a much stronger association with birth weight than OarCP26 alone, suggesting that other loci were contributing additional information. It would be possible to standardise d^2 scores (e.g. by dividing d^2 at each locus by the average for that locus) before obtaining mean d^2 over loci in order to reduce the variance in average d^2 between loci. However those loci at which average d^2 is largest (e.g. OarCP26) carry the most information under the stepwise mutation model, and so standardisation would lead to a loss of information. For this reason d^2 scores at each locus were not standardised prior to taking the mean across loci.

5.4.2 Frequency of inbreeding events

In the inbreeding that could be detected by pedigree analysis, paternal half-sib matings occurred significantly more frequently than maternal half-sib matings. Among stags born 1982-1986 and surviving to reproductive age, average paternal half-sibship size was 4.36 while average maternal half-sibship size among hinds of the same cohorts was 5.06 (Table 4.9). However true paternal half-sibship size is probably around twice as large (4.4.4.1), and so it seems likely that there is indeed greater opportunity for paternal half-sib mating than maternal half-sib mating. Whether this is sufficient to generate the observed pattern of inbreeding is not clear, and the detailed individual spatial data required to properly assess the opportunity for inbreeding is beyond the scope of this thesis.

Close inbreeding, resulting from full-sib or parent offspring matings (Ralls *et al.* 1986), was rare in Rum red deer. Father-daughter mating (3 calves of 195 where appropriate pedigree data were available) was the only class of close inbreeding

observed, giving a frequency of 1.5%. Given that no cases of mother-son or full-sib mating were observed, it is unlikely that the true frequency of these events exceed 1-2%. These analyses do make the assumption that inbred foetuses are no more prone to spontaneous abortion than outbred foetuses. If in fact inbreeding increases the chance of spontaneous abortion (e.g. due to homozygosity of recessive embryonic lethal genes), the true frequency of close inbreeding events (as distinct from birth of closely inbred calves) may be underestimated.

In contrast moderate inbreeding, in particular paternal half-sib mating, was relatively common. The frequency of paternity half-sib mating (10%) is similar to the frequency of moderate inbreeding in black-tailed prairie dogs (Hoogland 1992; note that I am using my definition of moderate inbreeding rather than Hoogland's). In emphasising the rarity of close inbreeding ($f \geq 0.25$) in nature, Ralls *et al.* (1986) are in danger of obscuring the fact that moderate inbreeding ($0.125 \leq f < 0.25$), considered illegal in most human societies (Chapter 1), may be relatively common in wild animals.

In Chapter 6, I go on to model a variety of fitness components in Rum red deer with respect to the molecular measures of inbreeding developed in this chapter, taking account of environmental factors which have previously been shown to influence these fitness components. I do not attempt to relate pedigree-based inbreeding coefficients to fitness because the variance in inbreeding coefficient is low (only 10% of calves had an inbreeding coefficient greater than zero).

5.5 Summary

Three measures of inbreeding were calculated for Rum red deer. Inbreeding coefficients were calculated for 553 calves from pedigree data, of which 55 were non-zero. The most common type of close or moderate inbreeding ($f = 0.125$ or greater) was half-sib mating, and paternal half-sibs paired significantly more frequently than maternal half-sibs. Paternal half-sib mating occurred at a frequency of around 10%. Two molecular-based measures of inbreeding, individual heterozygosity and mean d^2, were calculated for 1125 calves based on nine microsatellite loci. Both statistics were approximately normally distributed, and were positively correlated. Neither individual heterozygosity nor mean d^2 differed between inbred and non-inbred calves. Among

inbred calves there was no correlation between inbreeding coefficient and individual heterozygosity nor between inbreeding coefficient and mean d^2. Overall the statistical power of inbreeding coefficients calculated from the available pedigree data was weak, and the molecular-based measures of inbreeding are likely to be more useful for statistical modelling of fitness measures such as lifetime mating success in Rum red deer.

6 Inbreeding and fitness in Rum red deer

6.1 Introduction

6.1.1 Studying inbreeding in the wild

It is generally agreed that close inbreeding has negative fitness consequences in most species (Chapter 1). Following from this observation, it has been suggested that inbreeding depression has a major influence on the evolution of behaviour and mating systems of many species (Waller 1993). However there are relatively few studies examining the fitness consequences of inbreeding in natural populations, and no large scale studies in wild ruminants, carnivores or primates despite an extensive body of literature on inbreeding depression, measured in terms of juvenile survival, in captive populations of these groups (Ralls *et al.* 1979, 1988; Ballou and Ralls 1982; Lacy, Petric and Warnike 1993; Chapter 6).

There are major difficulties with extending the results of inbreeding studies on captive animals to wild populations for at least three reasons. First, the fitness consequences of inbreeding in captivity vary widely between even closely related species (Ralls *et al.* 1979), and so extrapolation across species is not appropriate. Second, the fitness consequences of inbreeding are not constant across environments (Table 1.2), and so it is not reasonable to extrapolate from one environment (e.g. captivity) to another environment (e.g. the wild). Third, the fitness consequences of inbreeding vary across life stages (Van Noordwijk and Scharloo 1981; Charlesworth and Hughes 1996), so it is not appropriate to extrapolate from the effect of inbreeding on, for example, juvenile survival to other fitness measures such as longevity or to lifetime mating success. To understand inbreeding in an evolutionary context, it is therefore essential to study animals in their natural habitat throughout their lifetime.

Although a number of studies of wild animals have examined the effect of inbreeding on juvenile traits (Table 1.1), to my knowledge there is only one published study of the relationship between inbreeding and lifetime reproductive success. Keller (1998) used behavioural observation to infer pedigree inbreeding coefficients for a population

of song sparrows on Mandarte Island. He found that inbred female song sparrows have significantly lower lifetime reproductive success than their outbred counterparts, while the lifetime reproductive success of male song sparrows is unaffected by inbreeding. Extra-pair paternity occurs at a frequency of approximately 15% in this population, but Keller (1998) argues that extra-pair paternities only make the analysis more conservative.

Previous work on the red deer on Rum has identified the major environmental factors affecting individual life history (Clutton-Brock *et al.* 1982, 1988). In this chapter, I combine the data from these studies with the paternity data obtained in Chapter 4 and the molecular measures of inbreeding derived in Chapter 5 in order to model the fitness consequences of inbreeding in Rum red deer. The analysis presented here is the first time that the effects of inbreeding on male lifetime mating success have been assessed using paternity data derived by molecular methods.

6.1.2 Previous work on inbreeding and fitness in Rum red deer

Using the inbreeding measure mean d^2, Coulson *et al.* (1998b) found that outbred Rum red deer calves have significantly higher birth weight than inbred calves (Table 6.1). Calf sex, mother's reproductive status, mother's age, mean April temperature and an interaction between April temperature and mean d^2 all significantly influence birth weight. Coulson *et al.* (1998b) also found that neonatal survival of outbred calves is significantly higher for calves with higher mean d^2 (Table 6.2a). This effect appears to be mediated via the effect of mean d^2 on birth weight, as mean d^2 does not explain variation in neonatal survival independent of birth weight when both are fitted in a model together (Table 6.2b).

First winter survival in Rum red deer follows a different pattern (Coulson *et al.* submitted). For hind calves, high mean d^2 is associated with higher survival as expected, but for stag calves the reverse is true. It is outbred stags that are at greater risk of dying. When cohorts are divided into years of low, intermediate and high mortality, the relationship between mean d^2 and survival of stag calves is only apparent in years of intermediate mortality. It is possible that a relationship with mean d^2 was not detectable in years of low and high mortality because there was little variance in survival (Coulson *et al.* submitted).

Term	*F*	d.f.	*p*-value	Direction of effect
Sex	19.4	1,634	< 0.001	Males heavier than females
Mother's reproductive status	2.88	4,637	0.022	Unknown
Mother's age (quadratic)	7.07	2,635	< 0.001	Low for young or old mothers
Mean d^2	4.13	1,635	0.042	Positive
Mean April temperature	4.67	1,635	0.031	Positive
Mean d^2.mean April temperature	7.2	1,634	0.007	Positive in cool Aprils

Table 6.1 *Full general linear model of birth weight in red deer calves (reproduced from Table 1 of Coulson et al. 1998b).*

Term	X^2	d.f.	*p*-value	Direction of effect
(a) Excluding birth weight				
Mother's age (linear)	5.6	1	< 0.05	Positive
Mean June temperature	5.1	1	< 0.05	Positive
Mean d^2	4.7	1	< 0.05	Positive
(b) Including birth weight				
Mother's age (linear)	5.1	1	< 0.05	Positive
Mean June temperature	6.5	1	< 0.05	Positive
Birth weight	12.9	1	< 0.01	Positive
Mean June temperature.birth weight	8.5	1	< 0.01	Positive in cool Junes

Table 6.2 *Full logistic regression of neonatal survival in red deer calves (a) excluding birth weight, and (b) including birth weight (reproduced from Table 2 of Coulson et al. 1998b). Mean d^2 could not be fitted to model (b).*

Three features of Coulson *et al.*'s (1998b, submitted) analyses should be noted. First, the sample sizes were large: for birth weight and neonatal survival, the analysis was based on 670 calves; for first winter survival, the analysis was based on 633 calves. Second, the statistical effects were relatively small, explaining only a few percent of the variation. Third, full models of birth weight, neonatal survival (without birth weight included) and first winter survival all featured mean d^2 but not individual

heterozygosity. The only significant relationship between individual heterozygosity and fitness was in a simple model of neonatal survival, in which calves with higher individual heterozygosity had higher survival (Coulson *et al.* 1998b).

The data set I use in this chapter is a set of stag and hind calves born in the years 1982 to 1986. By following a single set of individuals of the two sexes throughout their lifetime, it is possible to directly compare the effects of inbreeding between different life history stages and between the sexes. Analysing survival to reproductive age separately for stags and hinds, I show that for stags Coulson *et al.*'s (1998b, submitted) results are supported even within this relatively small data set, and more importantly that fitness differences between inbred and outbred deer persist into adulthood. Breeding stags with higher mean d^2 had higher lifetime mating success. For hinds, individual heterozygosity rather than mean d^2 influenced survival to reproductive age and also age at first breeding, while mean d^2 influenced lifetime mating success of breeding hinds.

6.2 Methods

In their analyses, Coulson *et al.* (1998b, submitted) conducted detailed investigations of birth weight, neonatal survival and first winter survival in relation to inbreeding measures, in large samples of calves born into the study population since routine genetic sampling began in 1982. In this chapter I analyse juvenile and adult fitness traits in much smaller samples of individuals for which complete, or nearly complete, lifetime mating success data are available (Chapter 1).

6.2.1 Response variables

Two true fitness components were modelled for males and females:

Survival to reproductive age: a binary response variable, with animals that reached reproductive age (3 for hinds, 5 for stags; Rose 1995) given the value one and animals that failed to survive to reproductive age given the value zero. This measure was modelled using logistic regression with a binomial error structure.

Lifetime mating success (LMS): a continuous response variable restricted to animals reaching reproductive age (3 for hinds, 5 for stags; Rose 1995). Genetic, longest

hold and fractional (4.2.3) fitted LMS estimates were available for stags (4.3.7). As all three distributions for stags were skewed to the right (n = 28; genetic: Anderson-Darling A^2 = 0.994, p = 0.011; longest hold: A^2 = 2.374, $p < 0.001$; fractional: A^2 = 2.428, $p < 0.001$), the fitted LMS data were log-transformed, so that the response variable was $\log_e$ (fitted LMS + 1) (genetic: A^2 = 0.876, p = 0.022; longest hold: A^2 = 0.991, p = 0.011; fractional: A^2 = 0.597, p = 0.110). Hind fitted LMS was modelled without transformation as the distribution was not significantly different from normal (n = 42; A^2 = 0.428, p = 0.297).

One other measure of deer quality was also assessed:

Age at first breeding (hinds only): modelled as a continuous response variable. It is thought that high-quality hinds are more likely to breed at the younger age. Although age at first breeding is a variable with discrete integer values (age 3: n = 4, age 4: n = 27, age 5: n = 8, age 6: n = 1), it was modelled as a continuous variable and inspection of the residuals did not appear to indicate any departure from normality.

6.2.2 Data sets

Fitted lifetime mating success was calculated for 105 stags and 85 hinds in the cohorts 1982 - 1986 which were sampled. Animals were excluded if they were deserted by their mothers after being marked as calves, shot, recorded as missing, emigrated or for one stag because it was the offspring of hand-reared hind.

Calves born before 1982 were not used because routine genetic sampling did not begin until 1982, and therefore the data set of sampled pre-1982 animals is biased very strongly towards deer which survived until 1982. At the other extreme, it was not possible to accurately estimate lifetime mating success for calves born after 1986 because those still alive have a substantial proportion of their reproductive lives ahead of them, the outcome of which could not be accurately predicted from their cumulative mating success to date (4.3). Unsampled calves born 1982 - 1986 were not included because their level of inbreeding could not be assessed using the molecular measures individual heterozygosity and mean d^2, the main focus of the analyses presented in this chapter.

6.2.3 Statistical analysis

Two types of analysis were carried out, with separate models constructed for stags and hinds. First, survival to reproductive age was modelled using the logistic regression procedure of SPSS 6.1. Models were constructed by testing each term in the model on its own. If one or more significant terms were found, the most significant term was added to the model, and then all other terms were retested. Interactions between genetic and non-genetic terms were also explored. This process was continued until no more significant terms could be added to the model.

Second, lifetime mating success and age at first breeding (hinds only) were modelled using the general linear model procedure of MINITAB 11.21. Models were constructed using a procedure similar to that used for logistic regression of survival to reproductive age. For both logistic regression and general linear models, initial simple models examined the effect of genetic variables singly. In more complex models where birth weight could be included, separate full models were constructed with and without birth weight, following the example of Coulson *et al.* (1998b) in an analysis of neonatal survival. This is because birth weight is itself associated with mean d^2 (Coulson *et al.* 1998b).

6.2.3.1 Independent variables

The following variables were investigated in models of survival to reproductive age, lifetime mating success and age at first breeding (females only):

Year of birth: categorical, with five levels, corresponding to each of the years 1982 - 1986. This variable takes account of a variety of temporal parameters such as weather and population density which influence birth weight, neonatal survival and first winter survival (Coulson *et al.* 1998b, submitted).

Birth weight: birth weight in kg was calculated from capture weight by regressing capture weight on age at capture. Since the gradients from separate regressions for stags and hinds were not significantly different (data not shown), the gradient from the combined data set, 0.0149 kg per hour, was used to calculate all birth weights (regression based on n = 1357 calves born 1961-1997). Birth weight data were not available for 25 stags (24%) and 14 hinds (16%) that were not caught as calves. Birth weight is known to be an important predictor of

neonatal (Coulson *et al.* 1998b) and first winter (Coulson *et al.* submitted) survival. Because birth weight is itself influenced by mean d^2 (Coulson *et al.* 1998b), separate models were constructed with and without birth weight in cases where birth weight was found to be significant.

Individual heterozygosity: continuous, a proportion between zero and one (see 5.2.2).

Mean d^2: continuous (see 5.2.3).

In the case of survival to reproductive age, the following additional variables relating to the mother were investigated:

Mother's age: continuous, in years, fitted as a linear or quadratic function. Mother's age is known to influence birth weight, neonatal survival and first winter survival (Coulson *et al.* 1998b, submitted).

Mother's reproductive status: categorical, with five levels:

first breeder - had not bred previously

true yeld - did not breed in the previous year

summer yeld - bred in previous year, but calf died before October 1st

winter yeld - bred in previous year, but calf died before May 1st in current year

milk - successfully reared a calf the previous year

Mother's reproductive status is known to influence birth weight (Coulson *et al.* 1998b).

Population density and weather variables (Coulson *et al.* 1997, 1998b, submitted) were not tested as it was unclear how temporally-varying measures should be included in models of survival to reproductive age, age at first breeding and lifetime mating success, all of which are likely to be influenced by temporal factors over a number of years. However the term year of birth may to some extent account for temporal fluctuations. Matriline (Coulson *et al.* submitted) was not included as a categorical term with dozens of degrees of freedom is unsuitable for the relatively modest data sets analysed here.

6.3 Results

6.3.1 Stags

I analysed the effect of inbreeding on survival and reproduction of 105 stags born 1982-1986. Separate analyses were carried out on survival to reproductive age (1st May in the fifth year after birth: 77 died, 28 survived), and among those surviving to reproductive age, lifetime mating success (LMS) assessed by genetic, longest hold and fractional paternity methods. The effect of inbreeding on LMS among stags with non-zero LMS only (genetic: n = 23; longest hold: n = 19; fractional: n = 26) was also assessed in each case. Finally, the effect of inbreeding on the ability of stags to win fights was assessed using a different data set: contests involving sampled stags observed in ruts between 1979 and 1997.

6.3.1.1 Survival to reproductive age

Survival to reproductive age was modelled for 105 stags by logistic regression. First, simple logistic regression models were constructed testing only genetic terms. Neither individual heterozygosity (X^2 = 2.45, d.f. = 1, p = 0.117) nor mean d^2 (X^2 = 0.04, d.f. = 1, p = 0.852) explained significant variation in survival to reproductive age, although there was a trend for higher survival among calves with higher individual heterozygosity.

Second, a model was constructed that excluded birth weight (known to be influenced by mean d^2, Coulson *et al.* 1998b). Year of birth was the only significant non-genetic term (X^2 = 14.80, d.f. = 4, p = 0.005). Neither individual heterozygosity (X^2 = 1.95, d.f. = 1, p = 0.163) nor mean d^2 (X^2 = 0.05, d.f. = 1, p = 0.831) explained independent variation in survival to reproductive age when year of birth was fitted. Interactions between genetic terms and year of birth were non-significant.

Third, a model was constructed including birth weight, based on the 80 stags which were weighed as neonates (Table 6.3). The non-genetic terms birth weight, year of birth, and mother's reproductive status all explained independent variation in survival to reproductive age. Stags with higher birth weight, stags born in 1983 and to a lesser extent 1985 or 1986 and stags born to true yelds or summer yelds and to a lesser extent to milk hinds or first breeders had higher survival than stags with low birth

weight, stags born in 1982 or 1984 and stags born to winter yelds. Among genetic terms, neither individual heterozygosity (X^2 = 0.63, d.f. = 1, p = 0.429) nor mean d^2 (X^2 = 0.08, d.f. = 1, p = 0.773) explained independent variation in survival to reproductive age, nor were there significant interactions between genetic and non-genetic terms. However it is notable that there was a non-significant interaction between mean d^2 and year of birth (X^2 = 9.67, d.f. = 5, p = 0.085: since mean d^2 was not significant on its own, the significance of the interaction was tested by adding the mean d^2 term and the interaction term simultaneously to the model).

Term	Coefficient	X^2	d.f.	p-value	% deviance explained
Model n = 80					
Constant	-12.491				
Birth weight	1.413	15.18	1	< 0.001	15.8
Year of birth		22.49	4	0.001	23.4
1982 (20)	-1.253				
1983 (16)	3.351				
1984 (19)	-0.955				
1985 (18)	0.137				
1986 (7)	0				
Mother's reproductive status		9.88	4	0.043	10.3
First breeder (13)	-0.668				
True yeld (28)	2.564				
Summer yeld (11)	2.296				
Winter yeld (6)	-6.196				
Milk (22)	0				

Table 6.3 *Full model of survival to reproductive age of 80 stags born 1982-1986. 23 of the 80 stags in the full model survived to reproductive age. Logistic regression was carried out using SPSS 6.1. Group sizes are shown in brackets for each level of factorial terms.*

6.3.1.2 Lifetime mating success

The effects of inbreeding on lifetime mating success (LMS) was assessed for the 28 stags born 1982 - 1986 surviving to reproductive age, using general linear models. Three measures of LMS were assessed in turn: genetic, longest hold and fractional (4.2.3).

Neither individual heterozygosity ($F_{1,26} = 2.16$, $p = 0.154$) nor mean d^2 ($F_{1,26} = 1.15$, $p = 0.293$) influenced genetic LMS among the 28 stags which survived to reproductive age. Among the 23 stags with non-zero genetic LMS, individual heterozygosity did not influence genetic LMS ($F_{1,21} = 1.25$, $p = 0.277$) but mean d^2 did influence genetic LMS ($F_{1,21} = 6.50$, $p = 0.019$). Among reproducing stags, those with higher mean d^2 had higher genetic LMS (Figure 6.1), and mean d^2 explained 24% of the variance in genetic LMS.

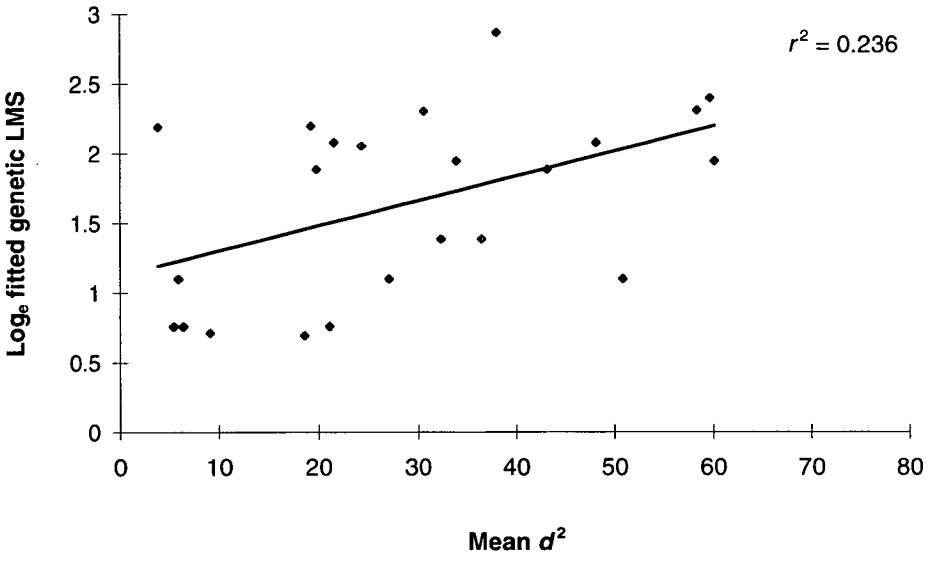

Figure 6.1 *Regression of log-transformed fitted genetic LMS on mean d^2 for 23 stags born 1982 - 1986 which had non-zero genetic LMS.*

Both individual heterozygosity ($F_{1,26} = 4.96$, $p = 0.035$) and mean d^2 ($F_{1,26} = 4.40$, $p = 0.046$) influenced longest hold LMS among the 28 stags which survived to reproductive age. Stags with higher individual heterozygosity or higher mean d^2 had higher longest hold LMS, and these terms explained 16% and 14% respectively of the

variance in longest hold LMS. Among the 19 stags with non-zero longest hold LMS, neither individual heterozygosity ($F_{1,17} = 0.36$, $p = 0.556$) nor mean d^2 ($F_{1,17} = 2.37$, $p = 0.142$) influenced longest hold LMS.

Both individual heterozygosity ($F_{1,26} = 6.98$, $p = 0.014$) and mean d^2 ($F_{1,26} = 7.77$, $p = 0.010$) influenced fractional LMS among the 28 stags which survived to reproductive age. Stags with higher individual heterozygosity or higher mean d^2 had higher fractional LMS, and these terms explained 21% and 23% respectively of the variance in fractional LMS. Among the 26 stags with non-zero fractional LMS, individual heterozygosity did not influence fractional LMS ($F_{1,24} = 3.53$, $p = 0.072$) but mean d^2 did influence fractional LMS ($F_{1,24} = 5.06$, $p = 0.034$). Among stags with non-zero fractional LMS, those with higher mean d^2 had higher fractional LMS (Figure 6.2), and mean d^2 explained 17% of the variance in fractional LMS.

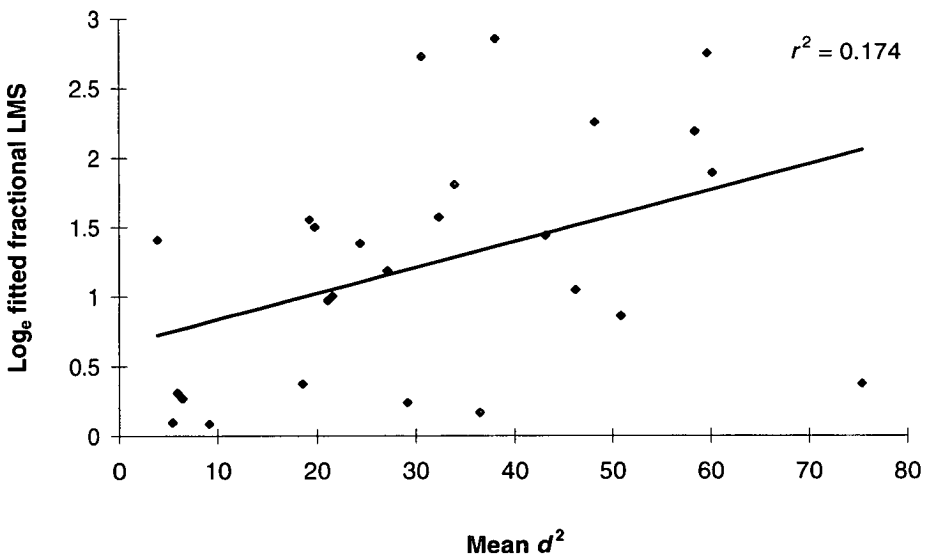

Figure 6.2 *Regression of log-transformed fitted fractional LMS on mean d^2 for 26 stags born 1982 - 1986 that had non-zero fractional LMS.*

Year of birth was tested in all models as a simple term and as an interaction with genetic terms, but did not influence LMS in any model. Birth weight (available for 23 of the 28 stags) was tested in all models as a simple term and as an interaction with genetic terms. Birth weight did not influence LMS in any model as a simple term, but

in most models there appeared to be a significant interaction between mean d^2 and birth weight. In all cases the significance of this interaction was entirely dependent on one stag, 4CUR, who had very high birth weight, high mean d^2 and low LMS. These models are not presented here as they did not appear to be robust.

6.3.1.3 Fighting ability

Fractional LMS was calculated from observations of stags holding harems during the rut (4.2.3), and was positively influenced by individual heterozygosity and by mean d^2 (Figure 6.2). To acquire harems, stags must be superior to other stags in terms of roaring, parallel walking and physical fighting (2.3). It is therefore possible that in any pairwise interaction between stags, the less inbred stag may be at an advantage. Suitable data on fights between stags were available (F. Guinness, unpublished data).

There were 269 fights observed between 1979 and 1997 in which one stag was a clear winner, where the winning stag chased the losing stag away from the area of the fight at high speed, usually followed by a bout of roaring by the winning stag in the direction of the losing stag. For 123 of these fights, samples were available from both stags. Although the same stag was frequently involved in several fights in this data set, only seven pairs fought twice and one pair fought three times. Each fight was therefore treated as an independent data point.

Neither individual heterozygosity (winners: 0.787; losers: 0.799; paired t = -0.76, d.f. = 122, p = 0.450, two-tailed) nor mean d^2 (winners: 37.78; losers: 35.79; paired t = 0.96, d.f. = 122, p = 0.357, two tailed) was greater for winners than for losers. Inbreeding and outbreeding did not influence the outcome of fights between stags.

6.3.2 Hinds

I analysed the effect of inbreeding on survival and reproduction of 85 hinds born 1982 - 1986. Separate analyses were carried out on survival to reproductive age (1st May in the third year after birth: 43 died, 42 survived), and among those reproducing (n = 40) on age at first breeding. I also analysed lifetime mating success (LMS) assessed by behavioural observation both including and excluding four hinds who died aged 3 or 4 from dystocia (calving complications) with their first calf.

6.3.2.1 Survival to reproductive age

Survival to reproductive age was modelled for 85 hinds by logistic regression. First, simple logistic regression models were constructed testing genetic terms individually. Individual heterozygosity explained significant variation in survival to reproductive age ($X^2 = 4.35$, d.f. = 1, $p = 0.037$) but mean d^2 did not ($X^2 = 1.13$, d.f. = 1, $p = 0.287$). Female calves with higher individual heterozygosity had higher survival.

No non-genetic terms significantly influenced survival to reproductive age, although there was a trend for female calves with higher birth weights to have higher survival ($n = 71$, $X^2 = 3.66$, d.f. = 1, $p = 0.056$; 14 hinds were not weighed at birth). Individual heterozygosity alone therefore represented the full model of survival to reproductive age; birth weight was non-significant in this model ($X^2 = 2.44$, d.f. = 1, $p = 0.118$). The full model explained 3.7% of the deviance in survival to reproductive age.

6.3.2.2 Age at first breeding

The effects of inbreeding on age at first breeding were assessed for the 40 hinds born 1982 - 1986 which reproduced using general linear models. When fitted as simple terms, individual heterozygosity ($F_{1,38} = 5.46$, $p = 0.025$, $r^2 = 0.13$) but not mean d^2 ($F_{1,38} = 0.13$, $p = 0.721$) influenced age at first breeding, with hinds with high individual heterozygosity breeding for the first time at a younger age. When fitted as simple terms, year of birth ($F_{4,35} = 4.16$, $p = 0.007$, $r^2 = 0.32$) and birth weight ($n = 36$, $F_{1,34} = 7.81$, $p = 0.008$, $r^2 = 0.19$; 4 hinds were not weighed at birth) also significantly influenced age at first breeding. Hinds with high birth weights bred for the first time at a younger age, and hinds born in 1985 bred for the first time at a younger age than hinds born in 1982 and 1984, while hinds born in 1983 and especially 1986 bred later than hinds born in other years. There were no significant interactions between genetic and non-genetic terms.

It was not possible to fit any of the terms simultaneously in a model of age at first breeding. In particular, neither individual heterozygosity ($F_{1,34} = 3.98$, $p = 0.054$) nor mean d^2 ($F_{1,34} = 0.00$, $p = 0.984$) significantly influenced age at first breeding in a model including year of birth, and neither individual heterozygosity ($F_{1,33} = 3.80$, $p = 0.060$) nor mean d^2 ($F_{1,33} = 0.03$, $p = 0.869$) significantly influenced age at first breeding in a model including birth weight. However in both cases there was a non-

significant trend for hinds with higher individual heterozygosity to breed for the first time at a younger age.

6.3.2.3 Lifetime mating success

The effect of inbreeding on lifetime mating success (LMS) was assessed for the 42 hinds born 1982 - 1986 surviving to reproductive age, using general linear models. Neither individual heterozygosity ($F_{1,40} = 0.66$, $p = 0.421$) nor mean d^2 ($F_{1,40} = 0.00$, $p = 0.968$) influenced lifetime mating success, nor were there significant non-genetic terms or interactions between genetic and non-genetic terms.

However it was observed that 4 of the 42 hinds (Table 4.8) died aged 3 or 4 of dystocia (calving complications) associated with their first calf; in two of these cases, the calf was never born. Since it appears that non-inbred hinds breed at a younger age (6.3.2.2), non-inbred hinds may be more susceptible to dystocia and hence to low LMS. When the data set was restricted to the remaining 38 hinds who completed their first calving without complications, once again neither individual heterozygosity ($F_{1,36} = 1.13$, $p = 0.295$) nor mean d^2 ($F_{1,36} = 0.00$, $p = 0.970$) influenced lifetime mating success. Birth weight ($F_{1,33} = 5.13$, $p = 0.030$, $r^2 = 0.135$) but not year of birth ($F_{4,33} = 0.77$, $p = 0.552$) did influence LMS, with hinds born heavier having higher LMS. No other terms, including interactions between genetic and non-genetic terms, could be fitted in a model including birth weight. However there was a significant interaction between mean d^2 and year of birth in a model excluding birth weight, and as this model had much greater explanatory power than the model including birth weight only, I considered it the full model (Table 6.4).

The main properties of this model were as follows. First, hinds born in 1982 and to a lesser extent 1984 had high LMS while hinds born in 1983 and particularly 1985 had low LMS. Second, hinds with high mean d^2 born in 1982 had lower LMS than hinds with low mean d^2 born in 1982, while hinds with high mean d^2 born in all other years had higher LMS than hinds with low mean d^2 born in those years. The terms in the full model shown in Table 6.4 involve a total of 9 degrees of freedom based on a sample size of 38 hinds, and explain 40% of the variance in hind LMS.

Term	Coefficient	*F*	d.f.	*p*-value
Model n = 38				
Constant	3.805			
Mean d^2	0.043	3.08	1,28	0.090
Year of birth		4.61	4,28	0.005
1982 (11)	5.686			
1983 (11)	-0.963			
1984 (4)	1.503			
1985 (5)	-0.723			
1986 (7)	0			
Mean d^2.year of birth		3.72	4,28	0.015
1982 (11)	-0.114			
1983 (11)	0.017			
1984 (4)	-0.025			
1985 (5)	-0.005			
1986 (7)	0			

Table 6.4 *Full model of lifetime mating success for 38 hinds born 1982 - 1986 which reproduced. General linear models were constructed using MINITAB 11. Group sizes are shown in brackets for each level of factorial terms. The full model accounts for 40% of the variance in lifetime mating success.*

6.4 Discussion

6.4.1 Individual heterozygosity and mean d^2

The two molecular measures of inbreeding, individual heterozygosity and mean d^2, have been extensively compared in this chapter for their efficacy in detecting the fitness consequences of inbreeding. For fitness characters modelled in stags, mean d^2 was at least as powerful as individual heterozygosity, and for lifetime mating success revealed significant fitness differences where individual heterozygosity did not. In hinds, the picture was less clear. For survival to reproductive age and age at first breeding, individual heterozygosity revealed significant fitness differences, and there

was no hint of any influence of mean d^2 (cf. Coulson *et al.* 1998, submitted). On the other hand hind lifetime mating success was influenced by mean d^2 (in an interaction with year of birth) but not by individual heterozygosity.

Individual heterozygosity and mean d^2 are correlated (5.3.4), and this can be most easily understood by dividing mean d^2 into two components: heterozygosity and microsatellite length information (Coulson *et al.* submitted). Obviously mean d^2 is a less good measure of individual heterozygosity than individual heterozygosity itself, and so if microsatellite heterozygosity alone is the better fitness correlate, individual heterozygosity is likely to show more significant fitness associations than mean d^2. Although individual heterozygosity was better for hind survival to reproductive age and age at first breeding, for other fitness measures mean d^2 showed equally significant or more significant fitness associations than individual heterozygosity. The implication is that in these analyses the microsatellite length component of mean d^2, unrelated to individual heterozygosity, was correlated with fitness (see also Coulson *et al.* submitted). The microsatellite length component of mean d^2 is thought to probe common ancestry much deeper in the pedigree than the heterozygosity component (5.4).

6.4.2 Is inbreeding always bad?

In most models where individual heterozygosity or mean d^2 had a significant influence, it was the less inbred individuals which had higher fitness. However the full model of hind lifetime mating success involved a mean d^2.year of birth interaction, and the coefficients indicated that more inbred hinds had higher fitness in certain years.

The analysis of survival to reproductive age conflates neonatal survival, first winter survival and survival of the remaining juvenile period up to the age of 5. Noting that mean d^2 positively influences neonatal survival (Coulson *et al.* 1998) but negatively influences first winter survival of stags, at least in years of intermediate mortality (Coulson *et al.* submitted), it is not surprising that mean d^2 does not have a net effect, positive or negative, on survival to reproductive age among stags born 1982-1986. The non-significant interaction between mean d^2 and year of birth hints at the environmentally-dependent influence of mean d^2 in stags found in Coulson *et al.*'s

(submitted) much larger analysis of first winter survival, the period accounting for the greatest stag mortality in the first five years of life.

In summary, the results of the analyses in this chapter and Coulson *et al.* (1998, submitted) largely support the theoretical and empirical dogma that inbreeding is deleterious (Chapter 1). However at a finer scale not all fitness associations favour outbreeding, supporting Bateson's (1983) notion of optimal outbreeding (see also Waser's (1993a) review of fitness effects of outcrossing distance in plants). In Chapter 8, I go on to show that there is a trade-off between inbreeding and outbreeding with an intermediate optimum in reintroduced Arabian oryx in Oman. For now it seems fair to conclude that inbreeding is *mostly* bad.

6.4.3 Stag lifetime mating success - genetics or behaviour?

It is striking that the behavioural LMS estimates (longest hold and fractional) showed similar associations with individual heterozygosity and mean d^2 as did the genetic LMS estimate, despite the fact that at least one of the behavioural paternity methods (longest hold) is clearly less accurate than the genetic approach (4.3.5). This may be explained in part by the fact that stags may be attributed paternity of any calf by behavioural observation, whereas the genetic assignment of paternity requires that the calf be sampled. Furthermore even where both calf and true father are sampled, genetic data may not be sufficient to permit confident assignment of paternity. This methodological problem with the genetic approach suggests that good behavioural observation, even if less accurate on an individual basis, may provide estimates of LMS that are as good or better than genetic estimates because of the larger number of calves contributing to the LMS estimate.

An analysis of the effect of inbreeding on a larger data set of 72 stags using the longest hold behavioural approach to estimate lifetime mating success supported the suggestion that mean d^2 is associated with higher LMS (L. Kruuk, unpublished data). In her analysis, Kruuk constructed a full model including not only mean d^2 but also total population density, local population density, birth weight and birth date (Kruuk *et al.* in prep.). Both population density measures exerted a negative influence on LMS, while birth weight had a positive influence and stags born later in the calving season tended to have lower LMS. Interestingly, Kruuk's analysis of the effect of

inbreeding on 68 hinds did not identify any influence of mean d^2 on LMS; total population density was the only factor in the full model, with a negative influence on LMS as for stags (Kruuk *et al.* in prep.).

6.4.4 Fitness of inbred and outbred deer

In this chapter I have shown that outbred red deer stags have significantly higher lifetime mating success than inbred stags. The fitness differences are large. Among reproducing stags, the most outbred stags on average have two and a half times the lifetime mating success of the most inbred stags, and mean d^2 explains 24% of the variance in stag LMS. The full model of hind LMS, which includes year of birth as well as mean d^2, explains 40% of the variance in hind LMS. Furthermore the fitness differences between inbred and outbred deer discussed in this chapter were based on samples of 105 stags and 80 hinds. In contrast Coulson *et al.* (1998b, submitted) analysed very large samples (670 and 633 calves respectively) and attributed a relatively small amount of the total variation in birth weight, neonatal survival and first winter survival to inbreeding, measured as mean d^2. The contrast between small effects of inbreeding in juvenile fitness traits and large effects of inbreeding on lifetime mating success favours the idea that inbreeding may be expressed with increasing strength through successive life history stages (Charlesworth and Hughes 1996).

The success of the relatively small-scale analysis in this chapter suggests that marker-based measures of inbreeding may prove useful in field studies of a more modest scale than the Rum red deer project. Furthermore a marker-based approach can be used to assess inbreeding without the need to construct the long term multi-generational pedigrees required to calculate inbreeding coefficients (Coulson *et al.* 1998b; Chapter 5). The limiting factor in the study of inbreeding using genetic markers is more likely to be the collection of appropriate individual fitness data.

6.5 Summary

For 105 red deer stags born 1982 - 1986, birth weight, year of birth and mother's reproductive status influenced stag survival to reproductive age, but neither the

inbreeding measure individual heterozygosity nor the outbreeding measure mean d^2 had a significant influence on survival. Among the 26 stags that reached reproductive age, lifetime mating success assessed by one genetic and two behavioural methods was positively related to mean d^2 and in some cases also to individual heterozygosity. In some cases effects were only significant when analysis was restricted to stags with non-zero lifetime mating success. Winners of fights between stags did not have higher individual heterozygosity nor higher mean d^2. For 85 hinds of the same cohorts, both survival to reproductive age and age at first breeding were positively influenced by individual heterozygosity but not by mean d^2. Lifetime mating success of hinds was influenced by mean d^2, with the direction of the effect depending on the year of birth of the hind. These results indicate that the effects of inbreeding and outbreeding pervade survival and reproduction of both hinds and stags. While inbreeding is generally detrimental, the fitness implications of inbreeding and outbreeding at individual life history stages are the result of a subtle interplay between genetic and environmental factors.

7 Arabian oryx in the Middle East

7.1 Introduction

The Arabian oryx (*Oryx leucoryx*) is a graceful desert antelope with a white coat and characteristic long straight horns. Viewed from the side, especially from a distance, the horns often appear as one, and the mythical unicorn is almost certainly based on the Arabian oryx. The species has a historical range extending right across the arid interior of the Arabian peninsula (Harrison and Bates 1991) (Figure 7.1). It is the smallest and most desert-adapted of the three oryx species (Table 7.1), and the only one found outside Africa (Stanley Price 1989).

The Arabian oryx has long been hunted at low intensity by the Bedouin tribes of Arabia, and its meat is highly prized (Stanley Price 1989). However, intensive hunting this century by wealthy Arabs in motorised hunting parties, especially in the 1950s and 1960s, led to severe contraction of its range. The motivation for hunting was in part sport and in part to capture live animals for private collections. As wild oryx became rare, the effort to capture live animals increased as sheikhs attempted to stock their captive groups as much as they could while wild oryx were still available. In this context, extinction of the species in the wild was inevitable, and the last group is thought to have been captured in 1972 (Henderson 1974). By this time a number of sheikhs around the Middle East had accrued large private collections of oryx and were breeding the animals successfully in captivity (Jones 1990; Harrison and Bates 1991).

Species	Subspecies	Common name	Range
Oryx leucoryx		Arabian oryx	Arabian peninsula
Oryx dammah		Scimitar-horned oryx	Sahara desert
Oryx gazella	*beisa*	Beisa oryx	East Africa (north of Tana river)
Oryx gazella	*callotis*	Fringe-eared oryx	East Africa (south of Tana river)
Oryx gazella	*gazella*	Gemsbok	Kalahari

Table 7.1 *Names and distributions of the three oryx species. There are three recognised subspecies of Oryx gazella. Details from Stanley Price (1989).*

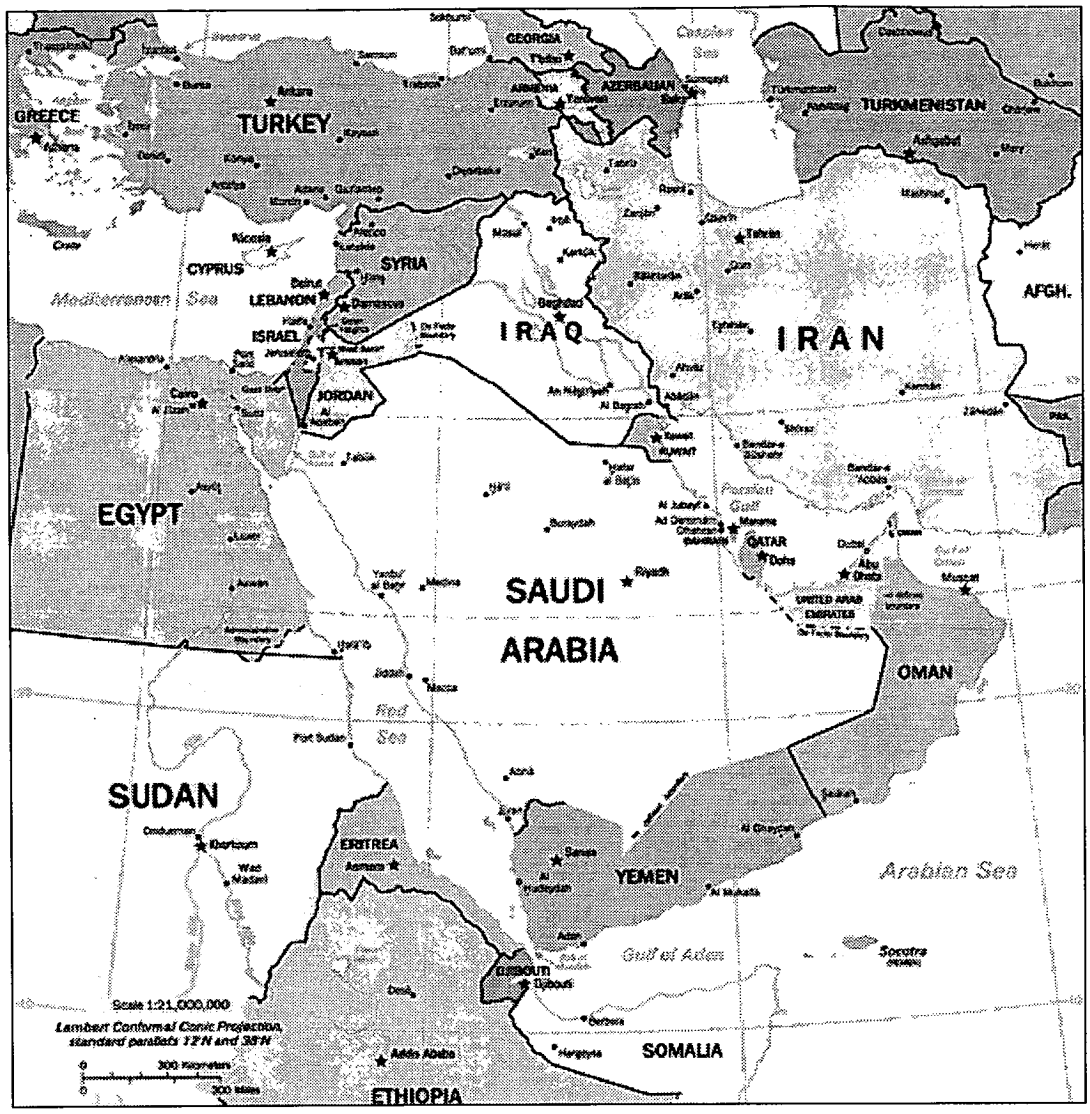

Figure 7.1 *Map of the Middle East region.*

Perhaps because of the charismatic nature of the species, much conservation effort has been devoted to the Arabian oryx. Captive breeding in the USA was initiated with the specific long term goal of reintroduction of the species to the wild (Homan 1988). In this chapter I first review the captive breeding and distribution of oryx from zoos in the USA, and the extent to which the breeding programme appears to have remained loyal to initial intentions. I then illustrate the genetic consequences of a small number of founders and lack of subsequent genetic management of the viability of captive Arabian oryx as a prelude to a detailed review of the history and current status of Arabian oryx groups throughout the Middle East region. Finally I describe the reintroduction of Arabian oryx to Oman, since the population there forms the basis of detailed genetic analysis in Chapter 8.

7.2 The role of zoos in captive breeding

7.2.1 Founding of the captive breeding programme in the USA

The imminent demise of the Arabian oryx in the wild did not go unnoticed in the West. Talbot (1960) reported that the Arabian oryx appeared to be extinct in all parts of its former range except along the southern edge of the Rub'al Khali. He believed that the few hundred animals that might remain would be exterminated within a few years, and recommended that a captive breeding programme be initiated to save the species. In response the Fauna Preservation Society (FPS) of Great Britain (now Fauna and Flora International) decided to mount an oryx capture expedition to the Eastern Aden Protectorate (now part of Yemen). The resulting *Operation Oryx* caught three males and a female in May 1962 (Grimwood 1962, 1967, 1988), but one of the males died shortly after capture. The remaining three oryx were taken for quarantine in Kenya.

It was clear that three animals was not a viable population for captive breeding (c.f. 7.3), and so additional animals were sought. The Zoological Society of London made available the female it possessed, which had been caught on the Jiddat-al-Harasis in Oman in 1960 (Woolley 1962). In June 1963 this animal was taken along with the three *Operation Oryx* animals, via further quarantine in New York, to Phoenix zoo in Arizona, selected for its arid climate and appropriate facilities. HH Sheikh Jaber bin Abdullah al-Sabah, ruler of Kuwait, offered the two female oryx he owned, but unfortunately one died soon afterwards (7.4.8). The remaining Kuwaiti animal arrived in Phoenix late in 1963. In spring 1964, two pairs of oryx were obtained as a gift from HM King Saud of Saudi Arabia, and were soon also taken to Phoenix. The group of Arabian oryx at Phoenix zoo was given the presumptuous title of the World Herd, and by summer 1964 contained nine founding individuals. Unfortunately the female from Kuwait and one of the males from Saudi Arabia never bred, and so the World Herd at Phoenix in practice had seven genetic founders from three distinct lineages. By the time the Saudi Arabian animals arrived, the World Herd already contained two calves and the grew steadily over the next few years, via a series of carefully controlled matings (Homan 1988) recorded in the newly established Arabian oryx studbook (Dolan 1976).

There was one further early importation of Arabian oryx to the USA, in 1967. A Dutch dealer obtained a pair of oryx from Riyadh zoo, quite possibly derived from the same group as the previous Saudi animals that went to Phoenix, and sold them to Los Angeles zoo (Jones 1988). While the trading of oryx antagonised the FPS and the International Union for Conservation of Nature (IUCN)'s Species Survival Commission (Fitter 1982), Los Angeles zoo nevertheless acquired valuable additional breeding stock. In fact the female arrived pregnant by a male other than the one she arrived with, so there were effectively two and a half founders in this group. Whatever Phoenix zoo's misgivings, pragmatic genetic management prevailed and an exchange of animals with Los Angeles zoo took place in May 1972. At this time, Los Angeles oryx were incorporated into the studbook, and their exclusion from the official World Herd was then but a political fiction. The origins of the World Herd founders are illustrated in Figure 7.2.

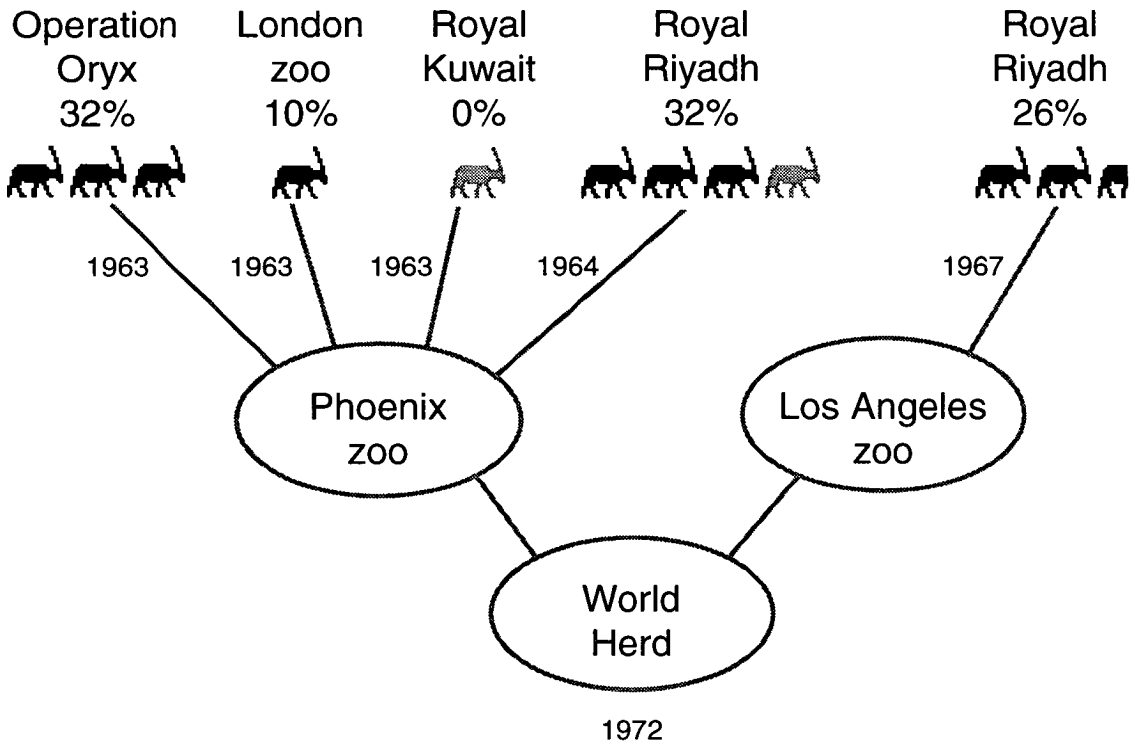

Figure 7.2 *Sources of Arabian oryx which founded the USA captive breeding programme. One oryx represents one founder. Oryx in grey failed to breed. A half oryx is used to represent the offspring of a founding female fathered by a male not amongst the founders. For each source, the percentage contribution of that source to the initial breeding stock is shown.*

7.2.2 Growth and distribution of the captive population from the USA

Although very little was known about the biology of Arabian oryx at the beginning of the captive breeding programme, they proved easy to manage in captivity and very fecund. An early problem was that the first seven calves born at Phoenix were all males (Homan 1988), so it was fortunate that female oryx also turned out to have a long breeding lifespan. By the end of 1971, Phoenix had 30 oryx, and six animals were transferred to San Diego Wild Animal Park (SDWAP). By the end of 1975, Phoenix had 45 oryx, and sent another 11 to SDWAP and two pairs to Gladys Porter Zoo at Brownsville, Texas. In the mean time, Los Angeles zoo had built up a group of around 20.

In the period 1963-1977, the number of oryx in the USA increased at an average rate of 17.2% per year (Stanley Price 1989). With over 100 oryx across the four institutions in 1977, space was becoming a problem. Oryx were sent to zoos in Europe (5 each to Berlin and Rotterdam and 6 to Zurich in 1979-1980), Morocco (2 to Rabat zoo in 1980 – see section 7.3) and to captive groups in Jordan and Israel (1978-1980), and later to Saudi Arabia and the United Arab Emirates (the history of these groups is discussed in more detail in section 7.4). Oryx were also provided for the reintroduction project in Oman (7.5). Known transfers of Arabian oryx from the USA to the Middle East are listed in Table 7.2.

Arabian oryx are still bred in a large number of zoos and wildlife parks in the USA and Europe. However it is unlikely that many of these will contribute to reintroduction of the species to the Middle East since there are several captive breeding facilities within the Middle East capable of supplying oryx in sufficient numbers to stock available reintroduction sites (7.4). The role of Arabian oryx in captivity in the Western World is no longer as a basis for future rehabilitation to the wild, but rather as an aesthetic and educational resource, a tangible reminder of the ease with which mankind can drive a species to the brink of extinction.

Transfer date	Males	Females	Destination
March 1978	4	0	Shaumari, Jordan
May 1978	4	4	Hai Bar, Israel
August 1979	0	4	Shaumari, Jordan
March 1980	3	2	Yalooni, Oman
September 1980	3	0	Hai Bar, Israel
December 1980	2	3	Yalooni, Oman
January 1981	0	1	Abu Dhabi, UAE
September 1981	3	1	Yalooni, Oman
March-October 1982	17	12	Thumamah, Saudi Arabia
October 1982	1	5	Abu Dhabi, UAE
July 1983	0	3	Yalooni, Oman
December 1983	0	4	Abu Dhabi, UAE
May 1984	1	1	Wildlife Research Centre, Dubai, UAE
April 1987	2	3	Abu Dhabi, UAE
January 1988	3	3	Yalooni, Oman
November 1988	5	4	Mahazat as-Sayd, Saudi Arabia
January 1989	3	3	Yalooni, Oman
May 1990	2	3	Mahazat as-Sayd, Saudi Arabia
June 1990	2	0	Shaumari, Jordan
February 1992	6	6	Mahazat as-Sayd, Saudi Arabia
September 1992	0	1	Hai Bar, Israel
January 1995	3	3	Yalooni, Oman
Total	64	66	130

Table 7.2 *Disposals of oryx from US zoos and parks to institutions in the Middle East 1978-1995. Data from Table 3.7 of Stanley Price (1989), Dolan and Sausman (1992) and A. Spalton (pers. comm.). Since an official studbook has not been published since 1992 there may be additional transfers from then onwards which are not listed.*

7.2.3 Did reintroduction motivate captive breeding in the USA in the 1980s?

Although the captive breeding programme in the USA was initiated for the specific purpose of reintroduction of the Arabian oryx to the wild (Homan 1988), it appears

that other pressures predominated during the 1980s. Significant numbers of oryx were transferred from the main breeding centres (Phoenix zoo, SDWAP, San Diego zoo and Gladys Porter zoo) to other establishments in the USA, rather than being sent for reintroduction to Oman, at a time when the reintroduction project there urgently required further animals (Stanley Price 1989). Some of the transfers were to mainstream zoological gardens and parks, but in the period 1980-1988, 159 oryx were sent to International Animal Exchange (IAE, Ferndale, Michigan) which is a commercial member of the American Zoo and Aquarium Association (AZA) described as an animal supplier. These oryx constituted 60% of the net production (births minus deaths) from the four breeding centres between 1980 and 1988. In contrast the reintroduction project in Oman, the only reintroduction project for the Arabian oryx at this time, received just 23 oryx (9% of net production).

IAE supplied two private collections in the Middle East: 16 oryx (of which the last 5 were bred at the neighbouring International Wildlife Park, IWP) to Sheikh Zayed in Abu Dhabi, and 29 to King Khalid in Saudi Arabia (Table 7.2). 11 oryx were also supplied to mainstream zoos outside the Middle East. The remaining 108 oryx which were transferred to IAE, plus 16 further animals which were bred at IWP, have no onward destination recorded in the Arabian oryx studbook (Dolan and Sausman 1992). 50 of the 108 died at IAE/IWP (39 of these within two years of arrival, despite the fact that most animals transferred were young), while the other 58 imported oryx plus the 16 bred there were presumably living at IAE/IWP at the end of 1988. How this collection of 74 oryx contributed to reintroduction of Arabian oryx to the Middle East is remarkably unclear, especially given that the studbook does not contain any record of deaths or transfers of IAE/IWP oryx from 1989 onwards.

7.3 Inbreeding depression in captive Arabian oryx

Inbreeding depression in small groups of captive ungulates has been acknowledged as a management problem for almost twenty years (Ralls *et al.* 1979; Ballou and Ralls 1982). Partly as a result of this discovery, international studbooks (e.g. Dolan and Sausman 1992) have been set up to coordinate captive breeding efforts in zoos around the world, and ensure that movements of animals between groups occur frequently enough that inbreeding is minimised. However the studbook system does not always

work as well as it might. I illustrate the problem with data from Rabat zoo in Morocco, which for the purposes of metapopulation management of Arabian oryx is grouped with European zoos.

The first animal to arrive at Rabat zoo was a male that came from Al Ain zoo in the United Arab Emirates in January 1975. Five years later, in September 1980, he was joined by a male from Phoenix and a female from SDWAP. These three animals were the basis of captive breeding at Rabat zoo. With such a small founder base, inbreeding was almost inevitable, especially given that the Phoenix zoo founders were themselves related. Figure 7.3 shows the inbreeding coefficients of calves born between 1981 and 1996. Excluding 1995-6 (see below), inbreeding coefficients rose over this period by an average of almost 2% per year. The highest inbreeding coefficient was 0.391, which is higher than the inbreeding coefficient from two generations of full-sib mating (0.375).

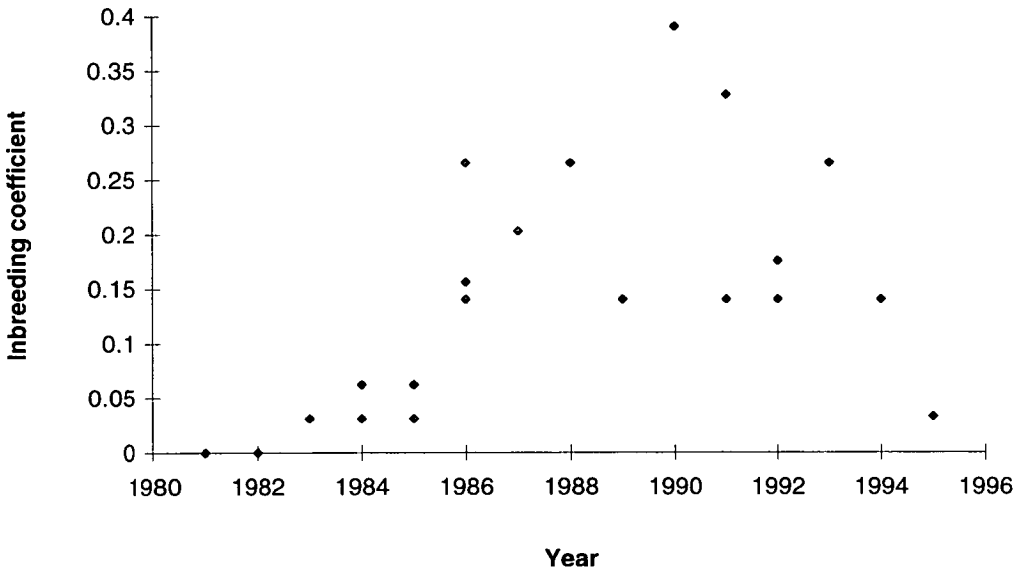

Figure 7.3 *Inbreeding coefficients of Arabian oryx calves born at Rabat zoo, Morocco 1981-1996. By the end of 1985 both male founders were dead. In January 1994, a male from Zurich zoo was introduced to the group at Rabat zoo.*

Not surprisingly, there is evidence for inbreeding depression in the Rabat group. The only fitness measure available from studbook data is survival, and survival to three

years was chosen for analysis since this approximately corresponds to survival to an age at which an oryx (male or female) can successfully reproduce (note that a female may calve at the age of two, but the calf is unlikely to survive if its mother does not survive until she is three). Oryx that died before they reached the age of three were significantly more inbred than those that survived their first three years (Table 7.3). Since inbred offspring tended to be born in later years, this analysis does not preclude the possibility that other time-dependent variables such as changes in herd size or herd management over time might be responsible for at least some juvenile deaths.

Age at death	*n*	Inbreeding coefficient			Mann-Whitney *W*	*p*-value
		Minimum	Median	Maximum		
< 3 years	11	0.031	0.176	0.391	151.0	0.0078
> 3 years	9	0	0.031	0.266		

Table 7.3 *Inbreeding depression in the Arabian oryx group at Rabat zoo, Morocco, for calves born 1981-1993, measured by comparing the inbreeding coefficients of individuals which died before reaching three years of age with the inbreeding coefficients of those that survived their first three years of life. Mann-Whitney comparison of medians was carried out using MINITAB 11.*

The number of births and deaths and the total number of oryx at Rabat zoo is shown in Figure 7.4 for the period 1980-1995. In the first few years, the group increased in the usual exponential manner (see for example Figure 7.6). However the group abruptly stopped increasing in 1987. In most years from then on deaths were equal to or exceeded births, and it is clear that inbreeding depression was not only affecting the viability of individuals but also the viability of the whole group. The population decline cannot be explained by a change in fecundity of females, which remained relatively constant at around 0.73 calves per female per year, or one calf every 16 months (gestation lasts around 9 months, Stanley Price 1989).

In 1994 a male was transferred from Zurich zoo to the herd at Rabat. His first offspring born in 1995 had the lowest inbreeding coefficient of any calf born for ten years at Rabat zoo. However the addition of fresh blood at this stage may be too late

to rescue the group from irreversible decline. Twice in the early 1990s the future breeding of the group rested entirely on a single female, and in November 1997 the group was represented by just one male and two females.

The history of the oryx group at Rabat zoo is only one example and is not necessarily representative of the fate of similarly sized groups of oryx elsewhere. However the Rabat example serves as a reminder that captive groups of oryx with only two or three founders may risk extinction due to inbreeding depression.

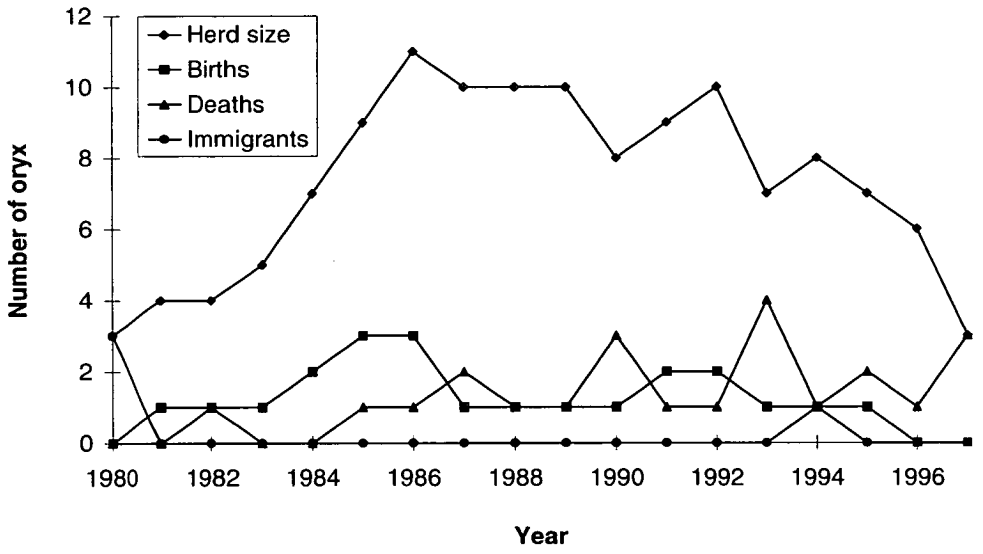

Figure 7.4 *Size of Arabian oryx group at Rabat zoo, Morocco 1980-1997. The three individuals in 1980 were the three founders. A male from Zurich was added to the group in January 1994. The herd size represents the number of individuals in the group on the 31st December of the year indicated, except for 1997 when data were only available for the year until 27th November.*

7.4 Arabian oryx in the Middle East

During the course of research into the genetic origin of groups from which I have obtained samples (8.2.1.1), it became apparent that there was no document describing in any detail all the groups of oryx in the Middle East region, and the origin of each. The only available summaries are by Jones (1988, 1989), but these often contradict

other sources such as the international Arabian oryx studbook (Dolan and Sausman 1992). The situation has also changed somewhat since Jones' work. Below I list all countries in the Middle East, and describe as completely as I have been able to determine the number of groups, the current number of oryx in each group, and the history of each group. I have also attempted to include relevant information of the status and facilities available to each group, and plans for their future, where known. In some cases I have quoted information from earlier paper copies of the Arabian oryx studbook published by J. Dolan at San Diego zoo, some of which contained useful information which was not carried forward to the current computerised version.

7.4.1 Israel

Talbot (1960) says that Arabian oryx were still found in Sinai and Palestine in about 1800, but there are no more recent reports of sightings. A captive breeding programme was founded at Hai Bar in 1978, with four pairs of oryx sent by Los Angeles zoo supplemented by three further males sent by SDWAP to Israel in 1980 (Stanley Price 1989; Dolan and Sausman 1992). In early 1997, oryx were released into the wild in Israel's southern Negev desert, and by late 1997, the population numbered 27. Three further releases are planned over the next decade using animals bred at Hai Bar, where there are currently around 65 oryx (D. Saltz, pers. comm.).

7.4.2 Egypt

The range of the scimitar-horned oryx (*Oryx dammah*) is thought to have been restricted to areas west of the Nile (Stanley Price 1989), while the Arabian oryx is unknown west of Suez (Harrison and Bates 1991). Although there are no Arabian oryx in Egypt today, the Israeli population reintroduced to the Negev desert may in time expand its range into the Sinai peninsula.

7.4.3 Qatar

7.4.3.1 Al Sulaimi

In the 1960s, Sheikh Qassim, then Education Minister built up a substantial group at his private farm, Al Sulaimi, starting with five wild-caught oryx from the southern

Rub'al Khali (Jones 1990). There were 15 oryx in a breeding herd there in 1966 (Grimwood 1967), 33 in 1976 (Jones 1990) when Sheikh Qassim died and a similar number in 1979 (Studbook 1979; Fitter 1982). This group was kept in a high-walled 1 ha enclosure which was always overcrowded, and subject to regular outbreaks of epidemic disease (Fitter 1982). The group eventually died out in 1989 (Jones 1990), and is now represented only at Al Areen (Bahrain) and in Saudi Arabia.

7.4.3.2 Al Wukayr and Al-Shahaniyah

At the time London zoo sent its female Arabian oryx to Phoenix to initiate the captive breeding programme in 1963 (Figure 7.2), there was also a male oryx at the zoo. This was sent by Sheikh Ahmad bin Ali al-Thani in 1961, but was in poor condition and apparently incapable of breeding (Stewart 1964). In 1967, Sheikh Ahmad captured four oryx in the southern Rub'al Khali (Jones 1990), and by 1974, Sheikh Ahmad's collection at his private farm, Al Wukayr, numbered 25 (Dolan 1976). In 1979, an impending state visit by Queen Elizabeth and Prince Philip led to the building of a farm at Al-Shahaniyah, stocked with 17 oryx from Al Wukayr (Studbook 1979; Jones 1990), so that the animals could be viewed more easily (Jones 1988). After the stocking of Al-Shahaniyah, there were still around 20 oryx at Al Wukayr. This group also provided oryx for collections in Saudi Arabia, United Arab Emirates (Abu Dhabi), Iraq, Kuwait and Jordan in the period 1978-1983, but now no longer exists as all remaining animals have been transferred to Al-Shahaniyah (Jones 1990).

The 1983 Studbook states that Al-Shahaniyah had 50 oryx at the end of that year. The studbook also lists causes of deaths for 23 oryx which died during 1983 either at Al-Shahaniyah or at one of two other smaller captive groups also owned by the Ministry of Agriculture. Four males and four females died of old age, three males and two females died fighting, and four males and two females died of foot-and-mouth disease. The fact that females were dying as well as males in fights suggests that density was extremely high, and it is clear that there were significant disease problems. By 1984, the population at Al-Shahaniyah had increased to 60 (Studbook 1984), and by the end of 1989, it was 108 (Jones 1990).

The first known importation to Qatar from any captive group outside the country is two males from American stock sent to Al-Shahaniyah from Taif in Saudi Arabia in

1990, in exchange from two males and a female from Al-Shahaniyah, which went to Taif in March 1992. In early 1997, an outbreak of foot-and-mouth disease killed nearly 40 oryx at Al-Shahaniyah (S. Ostrowski, pers. comm.). Overstocking is almost certainly the primary reason why such devastating disease outbreaks can occur. By the end of 1997, there were thought to be 150-200 oryx in Qatar in total, spread across Al-Shahaniyah, Doha zoo, two other reserves, Oshirig and Al-Mashabiyah, and three unnamed private collections (S. Ostrowski, pers. comm.).

7.4.4 Jordan

In 1978-9, four pairs of oryx were sent from the USA to the Royal Society for Conservation of Nature (RSCN)'s Shaumari Wildlife Reserve near Azraq in eastern Jordan, along with a male and two females from the Al Wukayr group in Qatar (Hatough and Al-Eisawi 1988). They were initially kept in a 3km^2 enclosure and given supplementary feed. By late 1983 there were 31 oryx, and the group was released into the current 22km^2 fenced reserve, where they feed on natural vegetation plus supplementary feed in the summer months, and have water *ad libitum* year-round (Abu Jafar and Hays-Shahin 1988; Ostrowski 1996). The group was augmented by three males imported from Zurich in 1984, and two more males from the USA in 1990.

In the benign conditions of the reserve the population increased rapidly, at 25.2% per year between 1979 and 1986 (Stanley Price 1989). Jones (1989) gives the population as 95, and at the end of September 1996 it was 205 (Boef 1996). This considerably exceeds some estimates of the carrying capacity of the reserve, and in 1991 the group was afflicted by an epizootic disease outbreak which reduced the population size considerably (Ostrowski 1996). Although the original management plan of the breeding programme at Shaumari included expansion of the reserve to 320km^2, subsequent human development in the Azraq area means that significant expansion of the current reserve is no longer possible (Ostrowski 1996). Three alternative reintroduction sites in more remote parts of eastern and south-eastern Jordan are currently being considered.

Shaumari provided oryx for the reintroduction projects in Oman and at Mahazat as-Sayd in Saudi Arabia, and has recently supplied animals to stock the new At Talila reserve in Syria (7.4.6). Shaumari also sent two pairs of oryx to Baghdad in 1987.

7.4.5 Lebanon

Lebanon is not known to be part of the ancestral range of Arabian oryx, though oryx did occur in neighbouring Syria (Harrison and Bates 1991). There are no oryx in captivity in Lebanon.

7.4.6 Syria

In December 1996, the At Talila nature reserve near Subkhat Muah and Palmyra took delivery of four pairs of oryx from the Shaumari reserve in Jordan. The animals are being kept in a 10km^2 fenced area which is part of a larger 200km^2 reserve (D. Williamson, pers. comm.). This is the only group in Syria, and is in an area where Arabian oryx are known to have occurred in historical times (Harrison and Bates 1991).

7.4.7 Iraq

Iraq which was, at least to the east of the Euphrates, part of the Arabian oryx's ancestral range (Harrison and Bates 1991). According to the 1980 Arabian oryx studbook, HE Ezzat Ibrahim al-Dori, then Minister of the Interior, had a pair of oryx, having received a male and two females from Qatar in 1979, probably from the Al Wukayr group (Jones 1990). Two pairs of oryx were shipped from Shaumari in Jordan (7.4.3) to Baghdad in 1987, supplementing the existing herd (Jones 1988) which may or may not have been the Qatari animals and their descendants. There is no record of any Arabian oryx being transferred to Iraq since 1987, and it is not known whether the oryx in Iraq survived the heavy bombing of Baghdad during the Gulf War in 1991.

7.4.8 Kuwait

The two females reported by Stewart (1963) in Kuwait belonged to HH Sheikh Jaber bin Abdullah al-Sabah. One died shortly afterwards giving birth to a calf sired by a

beisa oryx (*Oryx gazella beisa*) (Fitter 1982), a larger African species, and the other went to Phoenix zoo. This suggests that no oryx were left in Kuwait by 1964. However a pair from Al Wukayr in Qatar were given to Emir Sheikh Jaber al-Ahmed al-Sabah in 1980 (Jones 1990; Dolan and Sausman 1992). The female died in 1982, and the death date of the male is given as approximately September 1990, at the time of the Iraqi invasion of Kuwait. More recently, a pair of oryx were transferred from Phoenix zoo to Kuwait zoo. It is not known if these animals have bred.

7.4.9 United Arab Emirates (UAE)

The United Arab Emirates is made up of seven separate sheikhdoms, and of these, only Abu Dhabi and Dubai are known to have Arabian oryx. All groups are in private collections except the one at Al Ain zoo (in the emirate of Abu Dhabi) and in all cases accurate information is difficult to obtain. One of the captive groups in Abu Dhabi may have been founded from the last four oryx taken from the wild in 1972, as the hunting party that took the oryx was known to be from there (Henderson 1974).

7.4.9.1 Al Ain zoo, Abu Dhabi

Al Ain zoo collection itself was founded around 1975 with a male and two females, allegedly wild caught, and a male from Sheikh Hamdan's collection (see below) exchanged in 1980 for a male bred at Al Ain zoo. The studbook records (Dolan and Sausman 1992) show that there were two groups maintained separately at Al Ain zoo, and there was a high level of inbreeding, particularly in one of the groups (Figure 7.5). The unrelated male from Sheikh Hamdan dramatically reduced inbreeding in group one, but the effect was only temporary.

There were 53 oryx at the zoo in 1984 (Studbook 1984), 60 by 1987 (Jones 1988), 150 by 1989 (Jones 1989) and the same number in 1991 (G. Ramadan, pers. comm.). In 1990 Al Ain zoo took delivery of its first fresh blood since 1980 with the arrival of two males from US stock (G. Ramadan, pers. comm.), and by November 1997, there were three or four groups, with a total of 170 individuals (S. Ostrowski, pers. comm.)

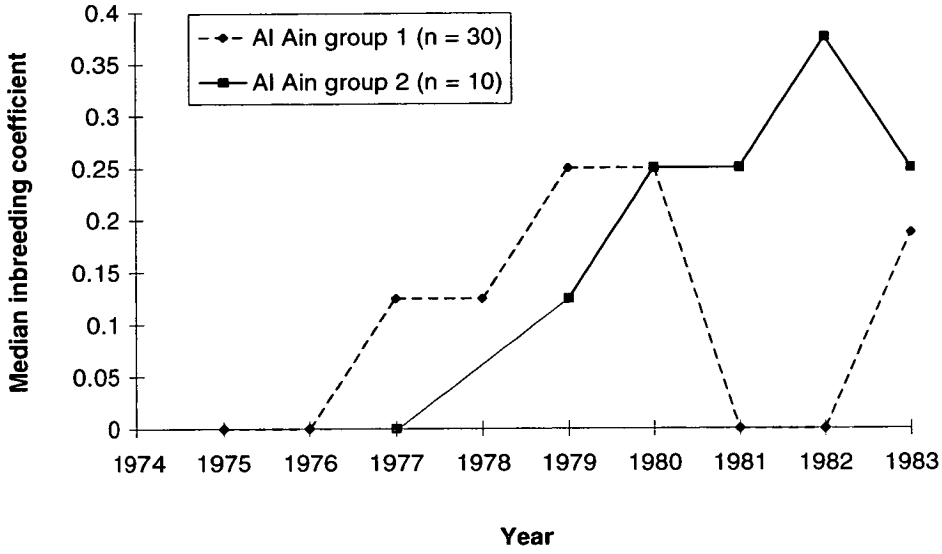

Figure 7.5 *Median inbreeding coefficients of calves born in two groups at Al Ain zoo, UAE 1975-1983. Each group at Al Ain was founded by a single unrelated pair. An unrelated sire from another lineage was introduced to group 1 in 1981. Inbreeding coefficients were calculated from studbook records (Dolan and Sausman 1992).*

The oryx at Al Ain zoo have pale brown or a complete lack of facial markings in place of the characteristic dark markings, and several individuals have pronounced scimitar-shaped horns, leading to the suggestion that hybridisation with scimitar-horned oryx has taken place (J. Samour, pers. comm.; P. Paillat, pers. comm.). However another observer believes that these unusual pelage and horn characteristics are a feature of inbreeding rather than hybridisation (A. Kitchener, pers. comm.). There is also a problem with disease in the cramped conditions at Al Ain zoo. In early 1992 five oryx died of foot-and-mouth disease, and in April 1998 a much larger outbreak killed 35-40 oryx (J. Samour, pers. comm.). The management of oryx at Al Ain zoo is of grave concern, particularly because this herd is regularly used as a source for stocking other herds in Abu Dhabi (see below).

7.4.9.2 Private collections in Abu Dhabi

Dolan (1976) reports that there were three male and two female oryx, three of which were captive bred, in an unnamed group in Abu Dhabi in 1974. The 1980 Arabian

oryx studbook says HH Sheikh Zayed bin Sultan al-Nahyan had 20 oryx in his private collection at Al Ain, and HE Sheikh Hamdan al-Nahyan had 17 in his private collection at Abu Mareica (Abu Dhabi); Sheikh Hamdan's collection is now owned by his son, Sheikh Sultan bin Hamdan al-Nahyan (J. Samour, pers. comm.). Both these groups are distinct from the one at Al Ain zoo, but it is not clear how the three groups are related to each other, if at all. It is also not clear to which Abu Dhabi group two pairs of oryx were sent from Al Wukayr, Qatar in 1980 (Jones 1990), nor is it known which was the recipient of 16 USA-bred oryx sent in four shipments to Abu Dhabi between 1981 and 1987 (Dolan and Sausman 1992), but it was probably not Al Ain zoo since there is no indication of these animals existing in the zoo's breeding records supplied to the international studbook up until the end of 1983. A likely destination for many of these animals is Sheikh Zayed's private island reserve, Sir Bani Yas (J. Samour, pers. comm.). Sheikh Zayed's Al Ain collection no longer exists and remaining stock are likely to have been transferred to Sir Bani Yas. This little-known reserve now holds a population of 270 oryx (J. Samour, pers. comm.).

Some other small private collections exist in Abu Dhabi. Those at Al Wagan and Abu Samra in 1997 had fewer than a dozen each, but that year 65 oryx were taken from Al Ain zoo to bolster these collections (J. Samour, pers. comm.). Sheikh Sultan bin Zayed al-Nehyan's collection at Al Ajban received 20 oryx, possibly from the USA, in January 1998 (J. Samour, pers. comm.), supplementing a small existing group of unknown origin. It is likely that there are additional small undocumented collections, probably stocked from Al Ain zoo, hidden in private residences around Abu Dhabi.

7.4.9.3 Dubai

The 1980 Arabian oryx studbook reports that HH Sheikh Mohammad bin Rashid al Maktoum had a male and two female oryx from an unknown source at that time. In 1984 a pair of oryx were sent to Dubai from SDWAP (Dolan and Sausman 1992), and probably joined Sheikh Mohammad's collection. No other transfers to or from this collection are known. This small group still exists, and is the only group in Dubai (M. Khan, pers. comm.). In 1996 it numbered just 3 oryx, but recently received additional animals from an unknown source (J. Samour, pers. comm.).

7.4.10 Bahrain

There is a single collection of oryx in Bahrain at Al Areen Wildlife Sanctuary. This group was founded by Crown Prince Sheikh Hamad bin Isa al-Khalifa in the mid 1970s with one male and two females from Sheikh Qassim's group at Al Sulaimi in Qatar (Jones 1990; Dolan and Sausman 1992). Sheikh Hamad's collection was moved from his palace to Al Areen in 1978. The following year, a second group was founded with a male from this group and two females from Sheikh Zayed's private collection in Abu Dhabi (Dolan and Sausman 1992; J. Samour, pers. comm.). Over the following few years, mixing was allowed between the two groups. Studbook records show that this mixing was efficient in ensuring that inbreeding was kept at a much lower level than in the comparable groups at Al Ain zoo (Figure 7.5). Indeed by 1983 the median inbreeding coefficient for calves born at Al Areen was still zero, and only a single calf among the 23 born between 1977 and 1983 had an inbreeding coefficient greater than 0.1. No studbook records are available beyond 1983. It is known that hybridisation between Arabian oryx and scimitar-horned oryx (*Oryx dammah*) occurred at Al Areen in 1985-1986. However all hybrids were destroyed in 1987 (J. Samour, pers. comm.), and so it is very unlikely that scimitar-horned oryx genes remain in the Arabian oryx population at Al Areen. By 1989, there were around 25 oryx in each group (Jones 1989), and by late 1997 there were a total of 124 at Al Areen (S. Ostrowski, pers. comm.).

The group at Al Areen is a valuable mixture of two lineages that are not readily available from any other source, and should be the source of choice for any group manager wishing to increase the genetic diversity of groups elsewhere. In 1991, four males from Al Areen Wildlife Sanctuary (Bahrain) were sent via Europe to the USA (J. Samour, pers. comm.). These animals, with ancestry entirely independent of the World Herd breeding programme, represented the first fresh blood to reach Western zoos for 24 years.

In early 1998 two males were sent to Al Areen from Al Ain zoo, representing yet another lineage. However given the chronic veterinary problems at Al Ain zoo (7.4.9.1), it is quite possible that the animals from there brought with them disease as well as diversity.

7.4.11 Saudi Arabia

Arabian oryx have been bred in captivity in Saudi Arabia for a long time, and Saudi Arabia now has one of the most vigorous captive breeding and reintroduction programmes anywhere in the Middle East. The oryx now in Saudi Arabia have an extremely diverse ancestry, built up largely by importing oryx from existing captive collections both in the Middle East and the USA.

Stewart (1963) reports around eight oryx in Riyadh, while the following year he reports six males and three females (Stewart 1964), after two pairs had been sent to the USA. Dolan (1976) reports three males and two females in Riyadh Zoological Gardens in March 1974, the same group from which the Los Angeles animals came in 1967. There is a great deal of confusion as to whether the Royal Riyadh line (from which animals were sent to Phoenix in 1963) and the Riyadh zoo line are the same line, or indeed the same group, and it is not clear how these groups relate to the current groups in Saudi Arabia.

In 1978 King Khalid built a palace at Thumamah, north of Riyadh. In the grounds, a large animal enclosure was built and stocked with a wide range of species. The first oryx to arrive at Thumamah came in 1978-1979 from the Al Wukayr group in Qatar (Williamson 1991), probably two groups of 10 (Jones 1990). An earlier shipment of 10 from Al Sulaimi, Qatar in 1975 or 1976 to Saudi Arabia (Jones 1990) must have therefore gone to a group other than the one at Thumamah, at least initially. A pair of oryx from Al Areen were added to the Thumamah group in May 1979 (descended from the Al Sulaimi group in Qatar), and by 1980 there were a total of 46 oryx at Thumamah (Studbook 1980). There was a further shipment of six from Al Wukayr to Thumamah in 1982 (Jones 1990; Studbook 1982), and 29 oryx were brought to Thumamah from the USA in the same year.

After King Khalid died in 1982, there was a lot of interest in obtaining oryx from Thumamah, and given that the keepers were unable to use the dart gun, they instead chased the oryx with vehicles and caught them with ropes, leading to many deaths (Williamson 1991). This implies many oryx were taken elsewhere in the period 1982-1986, but no records exist. It seems likely that a substantial proportion, if not all, would have been dispersed among the King's family within Saudi Arabia. It is known

that fifteen additional oryx were transferred from Al-Shahaniyah, Qatar to Saudi Arabia in 1983 (Studbook 1983); their actual destination is not recorded.

In early 1986, the Zoological Society of London took over the running of the farm at Thumamah, when it became known as the King Khalid Wildlife Research Centre (KKWRC). There were 72 Arabian oryx there at the time, kept in a large enclosure with hundreds of other ruminants of 20 species, including East African beisa oryx (*Oryx gazella beisa*) (KKWRC report, March 1987). 56 of the Arabian oryx were transported to a new captive breeding facility funded by Prince Saud Al-Faisal, the National Wildlife Research Centre (NWRC) at Taif. However, in summer 1986 there was tuberculosis epidemic at Taif, at the end of which only 35 oryx remained (Vassart *et al.* 1991). By a careful 3-generation segregation and hand-rearing programme, in time NWRC was able to produce tuberculosis-free oryx for reintroduction (Greth and Schwede 1993). In the mean time, animals for the reintroduction to the 2244km^2 fenced Mahazat as-Sayd reserve (22°N 41°E), northeast of Taif, had to be obtained from elsewhere. Reintroduction was initiated with oryx from the USA and Jordan in 1990 (Greth and Schwede 1993). Further US animals were brought in 1990 and 1992, supplemented by tuberculosis-free animals from the breeding centre in Taif.

During the early 1990s, NWRC Taif imported oryx from Al-Shahaniyah (Qatar), Al Areen (Bahrain) and Al Ain zoo (UAE). Some Al-Shahaniyah and Al Areen animals were later released in the Mahazat as-Sayd reserve. In December 1993, three European oryx were sent from Berlin and Zurich to Mahazat as-Sayd for reintroduction, but were in extremely poor condition when they arrived. One was blind and stunted, another died shortly after release, and the third, a female, produced only a single calf which died. Staff at NWRC Taif gained the impression that the European zoos were dumping poor quality stock on them, rather than making a genuine contribution to the reintroduction effort (S. Ostrowski, pers. comm.).

In 1995 reintroduction began at an unfenced reserve, 'Uruq Bani Ma'arid (19°N 44°E), in southern Saudi Arabia on the western edge of the Rub'al Khali (Empty Quarter), using stock bred at NWRC Taif. By March 1997 there were 100 oryx at liberty in the spectacular dune scenery. Reintroduction using Taif stock is also planned for the northern region of Saudi Arabia known as al-Khunfah (28°N 38°E), and several other sites in Saudi Arabia are being considered for reintroduction. A

comprehensive account of current reintroduction projects and future reintroduction plans in Saudi Arabia can be found in Ostrowski *et al.* (1998).

7.4.12 Oman

Two groups of oryx exist in Oman: the large reintroduced population at Yalooni on the Jiddat-al-Harasis in central Oman (this is described in detail in section 7.5), and a small captive group at the Bait Barakah Breeding Centre in Muscat. The latter group was founded in March 1985 with pair of American animals sent to Yalooni which were considered unfit for release. When it became clear that Kadil (studbook 40) was well past his prime, a male from Al Areen Wildlife Sanctuary in Bahrain was brought to Muscat in November 1985. He sired a total of seven oryx which were reintroduced at Yalooni as part of herds 4 and 5 (7.5.3). Three USA-bred oryx were added to the Muscat group in 1989, and a pair of wild-born females from Yalooni were also taken to Muscat in 1993. Breeding has continued at Bait Barakah, and the total number of oryx there in May 1998 was 22 (A. Spalton, pers. comm.).

7.4.13 Yemen

Yemen was part of the historical range of Arabian oryx (Harrison and Bates 1991), and indeed *Operation Oryx* captured its oryx in what is now eastern Yemen in 1962 (Grimwood 1962), prior to the extinction of oryx from the wild throughout Arabia. Stewart (1963) reports two oryx of unknown sex seen in the souqs of Taiz in August 1963, which later transpired to be the property of the municipal zoo (Grimwood 1988). They were said to be hornless and in poor condition. No animals are thought to exist in captivity in Yemen today. However, the population of oryx recently reintroduced to the unfenced reserve at 'Uruq Bani Ma'arid, Saudi Arabia could in time expand into Yemen.

7.4.14 Overall status of Arabian oryx in the Middle East

Table 7.4 shows the lineages of Arabian oryx now existing around the world. There are at the very least six separate lineages, three of which are collectively represented in the USA line (1-3). There are clearly two separate lineages originating from Qatar (4 and 5), and undoubtedly independent capture expeditions gave rise to some or all of

the groups in the UAE, where there are between one and four independent lineages (6-9).

Table 7.5 shows the number of Arabian oryx in the various groups in the Middle East, both captive and wild. Of the approximate total of 2,155, 1,376 (64%) are in captivity. Far from the original problem of near-extinction, the major management problem in the Middle East now is the large number of animals in captivity, a direct result of the high fecundity of Arabian oryx. Shaumari in Jordan, Al-Shahaniyah in Qatar, Al Areen in Bahrain and Sir Bani Yas and Al Ain zoo in UAE are all reported to be overstocked. Adult males frequently die in fights, and the risk of disease is high. Inbreeding is also a management problem in those groups with a narrow founder base and a long history of isolation, such as Al Ain zoo (see also 7.3).

Table 7.4 *(overleaf) Surviving lineages of Arabian oryx around the world. The number of founders is indicated where this information is available, and where possible divided into numbers of males and females respectively. Lineage 3 may represent two separate collections, one of which went to Phoenix (Royal Riyadh), and the other to Los Angeles (Riyadh Zoo). There is some confusion as to whether there were two separate groups in Riyadh, and if so, whether or not they originated from the same source. The situation in UAE (lines 6-9) is complex. Al Ain zoo maintained two partially related groups listed in the studbook 1976-1983, and these are almost certainly unrelated to any of lineages 1-6. Private collections of Sheikh Zayed and Sheikh Hamdan may each have been founded with capture expeditions independent of that which founded Al Ain zoo. Sheikh Mohammad in neighbouring Dubai may also have an independent wild-caught lineage, but this has certainly been mixed with American stock. For each lineage, I have indicated where descendants most related to that lineage may currently be found.*

Lineage	Name	Caught in	Caught by	Date	Taken to	Founders	Represented in	Reference
1	Operation oryx	Eastern Aden Protectorate (now Yemen)	Fauna Preservation Society	May 1962	Phoenix zoo	2.1	USA/Europe Oman Saudi Arabia	Grimwood 1962
2	Caroline	Jiddat-al-Harasis, Oman	Woolley	1960	London zoo → Phoenix zoo	0.1	USA/Europe Oman Saudi Arabia	Woolley 1962
3	Royal Riyadh	?	?	pre-1964	Riyadh → Phoenix zoo and Los Angeles zoo	4?	USA/Europe Oman Saudi Arabia	Grimwood 1988 Jones 1989
4	Al Sulaimi	Southern Rub'al Khali	Sheikh Qassim	1964-5	Al Sulaimi, Qatar	5	Al Areen, Bahrain Saudi Arabia	Jones 1990
5	Al Wukayr	Southern Rub'al Khali	Sheikh Ahmad	1967	Al Wukayr, Qatar	4	Shahaniyah, Qatar Saudi Arabia	Jones 1990
6	Al Ain zoo	?Jiddat-al-Harasis, Oman	Sheikh Zayed	Early 1970s	Al Ain zoo, Abu Dhabi	2.2	Al Ain zoo, UAE Rabat zoo, Morocco Saudi Arabia	Dolan and Sausman 1992
7	Sheikh Zayed	?	Sheikh Zayed	?	Al Ain, Abu Dhabi	?	Sir Bani Yas, UAE Al Areen, Bahrain	J. Samour, pers. comm.
8	Sheikh Hamdan	?	? Sheikh Hamdan	?	Abu Mareica, Abu Dhabi	?	Abu Mareica, UAE Al Ain zoo, UAE	Studbook 1980
9	Sheikh Mohammed	?	? Sheikh Mohammed	?	Wildlife Research Centre, Dubai	?	Wildlife Research Centre, Dubai, UAE	Studbook 1980

Reintroduction projects in Oman and Saudi Arabia are proceeding well. Including the fenced Mahazat as-Sayd reserve, there are 779 oryx in total the wild (Table 7.5). However the poaching that has occurred in Oman in 1996 and 1997 (7.5.4) is a great concern for all the reintroduction projects, especially those involving unfenced reserves. It is known that the Western-backed *Operation Oryx* capture expedition in 1962 stimulated sheikhs in the Middle East to mount similar expeditions in order to found captive groups (Fitter 1982), and so the precedent of poaching is as much a threat as the number of animals taken so far. Recently it has been suggested that it might be possible to reduce the demand for wild oryx by flooding the market with captive animals (Ostrowski *et al.* 1998), but the effectiveness of such a strategy is unclear. The other possible threats to the re-establishment of wild oryx are competition for grazing from domestic livestock, principally camels and goats, and development in reintroduction areas leading to degradation of habitat, such as the felling of shade trees (Lawrence 1996; Ostrowski *et al.* 1998). However neither of these factors appears to be limiting the rapid growth of the three reintroduced populations in Oman (see below) and Saudi Arabia (Ostrowski *et al.* 1998).

Table 7.5 *(overleaf) Groups of Arabian oryx in the Middle East. Some numbers, especially for Qatar and UAE, are only approximate. The origin column lists where the group was stocked from. Many of the sources themselves come from a mixture of sources. Where USA is shown, this implies a mixture of at least three independent lineages, from Operation Oryx, London zoo and Riyadh (7.2.1). The references indicate the source of the population estimate. The total is an approximate minimum estimate, using the lowest number of the range given in cases where the exact number was not known.*

Country	Population/Herd	Status/Area	Organisation/Owner	Origin	Date of count	Oryx	Source
Israel	Hai Bar	Captive	Nature Reserves Authority (NRA)	USA	November 1997	65	D. Saltz, NRA
	Negev desert	Wild	NRA	USA	November 1997	21	D. Saltz, NRA
Jordan	Shaumari Wildlife Reserve	22km^2 fenced reserve	Royal Society for Conservation of Nature (RSCN)	USA Al Wukayr, Qatar Europe	September 1996	205	J. Boef, RSCN
Saudi Arabia	King Khalid Wildlife Research Centre (KKWRC), Thumamah	Captive	National Commission for Wildlife Research and Development (NCWCD)	Riyadh zoo, Saudi Arabia Al Sulaimi, Qatar Al Wukayr, Qatar Al Areen, Bahrain USA	November 1997	15	S. Ostrowski, NWRC Taif
	National Wildlife Research Centre (NWRC), Taif	Captive	NCWCD	KKWRC, Saudi Arabia Al Areen, Bahrain Shahaniyah, Qatar Al Ain zoo, UAE USA	January 1997	245	Ostrowski *et al.* 1998
	Mahazat as-Sayd Reserve	2244km^2 fenced reserve	NCWCD	USA Shaumari, Jordan NWRC Taif, Saudi Arabia	January 1997	280	Ostrowski *et al.* 1998
	'Uruq Bani Ma'arid	Wild	NCWCD	NWRC Taif, Saudi Arabia	March 1997	100	Ostrowski *et al.* 1998

Country	Population/Herd	Status/Area	Organisation/Owner	Origin	Date of count	Oryx	Source
Saudi Arabia (continued)	-	Captive	Prince Abdullah bin Khaled, Jeddah	NWRC Taif, Saudi Arabia ? other Saudi Arabian stock	November 1997	4	S. Ostrowski, NWRC Taif
	-	Captive	Prince Bandar bin Mohammed bin AbdulRahman, Riyadh	NWRC Taif, Saudi Arab ? other Saudi Arabian stock	1996	3-6	S. Ostrowski, NWRC Taif
	-	Captive	Dr. Jammaz, Riyadh	NWRC Taif, Saudi Arabia ? other Saudi Arabian stock	1994	20	S. Ostrowski, NWRC Taif
	-	Captive	Prince Sultan bin Abdulaziz	NWRC Taif, Saudi Arabia Thumamah, Saudi Arabia	June 1997	3	S. Ostrowski, NWRC Taif
United Arab Emirates	Al Ain zoo, Abu Dhabi	Captive	Al Ain zoo	? Wild caught Abu Mareica, UAE	November 1997	170	S. Ostrowski, NWRC Taif
	Sir Bani Yas island, Abu Dhabi	Reserve	Sheikh Zayed bin Sultan al-Nahyan	? Wild caught ? USA	1997	270	J. Samour, pers. comm.
	Abu Mareica, Abu Dhabi	Captive	Sheikh Sultan bin Hamdan al-Nahyan	? Wild caught Al Ain zoo, UAE	1997	7	J. Samour, pers. comm.
	Al Wagan, Abu Dhabi	Captive	Sheikh Sultan bin Khalifa al-Nahyan	Al Ain zoo, UAE	1997	6+	J. Samour, pers. comm.
	Abu Samra, Abu Dhabi	Captive	?	Abu Mareica, Abu Dhabi, UAE	1997	8+	J. Samour, pers. comm.
	Al Ajban, Abu Dhabi	Captive	Sheikh Sultan bin Zayed al-Nahyan	USA	January 1998	20+	J. Samour, pers. comm.

Country	Population/Herd	Status/Area	Organisation/Owner	Origin	Date of count	Oryx	Source
UAE (continued)	Wildlife Research Centre, Dubai	Captive	Sheikh Mohammed	? Wild caught USA	1996	3+	J. Samour, pers. comm.
Qatar	Shahaniyah Oshirig al-Mashabiyah Doha zoo 3 private collections	Captive	Ministry of Agriculture	Al Wukayr, Qatar USA, via NWRC Taif	November 1997	150-200	S. Ostrowski, NWRC Taif
Bahrain	Al Areen Wildlife Park	Captive	Al Areen zoo	Al Sulaimi, Qatar Sheikh Zayed, UAE	November 1997	124	S. Ostrowski, NWRC Taif
Iraq	Baghdad	Captive	?HE Ezzat Ibrahim al-Dori	Al Wukayr, Qatar Shaumari, Jordan	1989	5	Jones 1989
Kuwait	Kuwait zoo	Captive	Kuwait zoo	USA	c. 1993	2	S. Ostrowski, NWRC Taif
Oman	Yalooni	Wild	White Oryx Project	USA Shaumari, Jordan Bait Barakah, Oman	December 1997	399	A. Spalton, White Oryx Project
	Bait Barakah Breeding Centre, Muscat	Captive	Sultan Qaboos bin Said	Yalooni, Oman Al Areen, Bahrain	May 1998	22	A. Spalton, White Oryx Project
Syria	At Talila Nature Reserve, Palmyra	200km^2 reserve	Food and Agriculture Organisation (FAO)	Shaumari, Jordan	January 1997	8	D. Williamson, FAO
Total						2155	

7.5 The reintroduction of Arabian oryx to Oman

7.5.1 Introduction

The reintroduced population of Arabian oryx in Oman is the main focus of my study of Arabian oryx, since this is the only population for which I have significant number of samples from animals in the wild environment with accompanying individual life history information. In Oman, the reintroduction is known as the White Oryx Project. A detailed description of early years of the project (1980-1986) and many more general issues about Arabian oryx and the process of captive breeding and reintroduction can be found in Mark Stanley Price's excellent book, *Animal Re-introductions: the Arabian Oryx in Oman* (1989).

7.5.2 The White Oryx Project

In 1974 Sultan Qaboos bin Said discussed the possibility of reintroduction with his Advisor for Conservation of the Environment, Ralph Daly, only two years after the last wild oryx were exterminated. The Sultan knew that Arabian oryx were being bred in the USA for eventual reintroduction to the Middle East, and wanted to ensure that Oman was at the forefront of the conservation effort. He also saw the potential of a reintroduction project to provide worthwhile, long-term employment to the desert dwelling Harasis tribes (Stanley Price 1989).

As a result, in 1977-8, two surveys took place that respectively identified the 27,500km^2 stony desert plateau of central Oman known as the Jiddat-al-Harasis (19°N 57°E) as a suitable area for reintroduction, and within the Jiddah, the area known as Yalooni as an appropriate site from which to coordinate the White Oryx Project. The Jiddah was the area from which the last wild oryx had been taken in 1972. Ralph Daly drew up detailed plans, and the Sultan agreed to fund the project late in 1978. Construction of a camp at Yalooni began in 1979 and was complete by early 1980.

7.5.3 Reintroduced oryx herds

Ten oryx for the reintroduction were provided by SDWAP in two consignments in 1980. The surviving eight of these were kept in the main enclosure at Yalooni where it was hoped that they would form a cohesive, natural herd unit, while additional animals that arrived in 1981 were kept in pens until the first herd was released into the desert in January 1982, so that the new arrivals could be the basis of a second herd.

Stanley Price (1989) is critical of the conduct of SDWAP during negotiations to obtain oryx for the reintroduction project, and in particular the fact that they sent two animals that were clearly unfit for release (a stunted male exhibiting stereotyped pacing behaviour and a female that was completely tame). There also appeared to be some reluctance on SDWAP's part to send animals of appropriate quality and in suitable quantity for reintroduction. The intervention of the World Wildlife Fund (one of the funding agencies which supported *Operation Oryx*) was necessary in order to obtain a shipment of three additional females from San Diego suitable for the second herd in 1983 (Stanley Price 1989). Supplemented by a male from Shaumari in Jordan, this herd was released in April 1984.

No further oryx were sent from the USA to Oman until 1988, a date which coincided with the cessation of disposals of oryx by SDWAP and other US captive breeding centres to International Animal Exchange (7.2.2). Jones (1988) suggests that the Omani reintroduction project began ten years too early, before a regular supply of animals for reintroduction from the USA could be assured. However, it is abundantly clear from studbook records that US zoos were generating the requisite stock during the mid 1980s, but chose not to send oryx to Oman. Further herds were released at Yalooni in 1988, 1989, 1992 and 1995 (Table 7.6; Spalton 1993), using a mixture of American animals and oryx bred in the Bait Barakah breeding centre in Muscat (7.4.12).

Herd	Release date	Males	Females	Source
1	31st January 1982	4	4	USA
2	4th April 1984	2	3	USA
		1	0	Jordan
3	6th October 1988	3	2	USA
		2	2	Jordan
4	20th September 1989	2	2	USA
		2	1	Muscat
5	7th October 1992	2	2	Muscat
6	9th April 1995	3	3	USA
Total		21	19	40

Table 7.6 *Herds released to the Jiddat-al-Harasis, Oman from Yalooni 1980-1997. Oryx from Jordan were bred at the Shaumari wildlife reserve, and oryx from Muscat were bred at the Bait Barakah Breeding Centre (7.4.12). Herd 5 also included two adult females and three calves (two of which were hand-reared) brought into the enclosure at Yalooni during the drought of 1991. Data from Stanley Price (1989), Spalton (1993) and A. Spalton (pers. comm.).*

7.5.4 Population growth and the threat of poaching

The population in Oman grew relatively slowly at first (Figure 7.6), the main constraint being a prolonged drought (Spalton 1993). Supplementary feed and water was provided from late 1984 until abundant rains fell in June 1986. An additional impediment to reproduction was that the dominant male in herd 2, Mustafan, turned out to be infertile (Stanley Price 1989). Productivity from this herd was very low until the herd split into smaller dispersed units, allowing other males access to some of the females. A second drought in 1991 slowed population growth in 1991 and 1992. During the drought more than 50% of calves did not survive their first year of life (Spalton 1993).

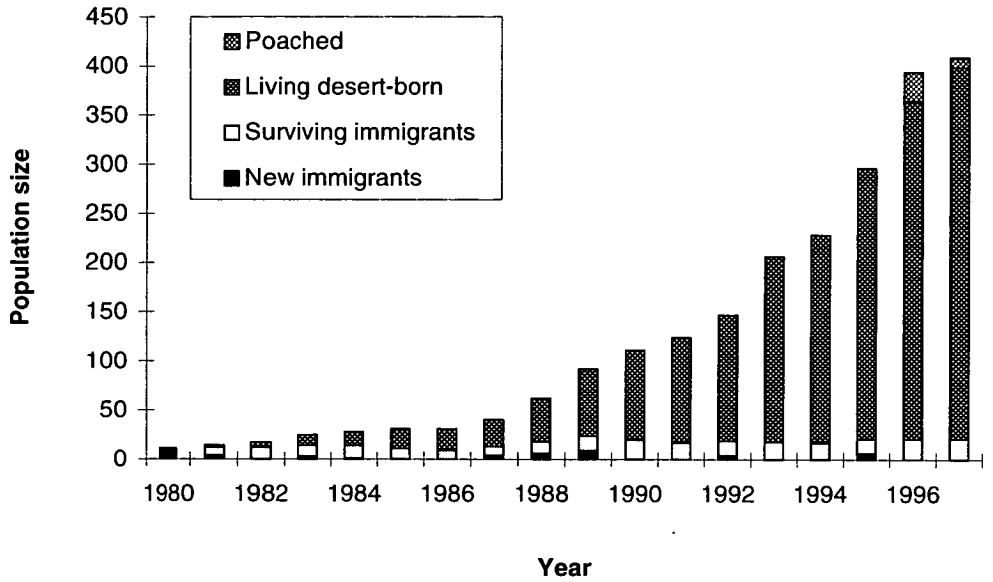

Figure 7.6 *The population size of the Arabian oryx reintroduced to Oman 1980-1997. Each year is divided into new immigrants, surviving immigrants, desert-born animals and poached animals. The poached animals category includes both animals killed by poachers and those caught alive and smuggled out of Oman. Population sizes from 1993 onwards are estimates, since at that time it became impossible to keep track of all oryx at an individual level. Modified from Lawrence (1996), with additional data from Rowe (1997) and A. Spalton (pers. comm.).*

Overall, during the period 1980-1995 the population grew at a rate of 22.0% per year, a doubling time of 3 years and 2 months (including immigrant animals as part of the population). The rate of growth compares favourably with the rate of increase of oryx in captivity in the USA 1963-1978 of 17.2% (Stanley Price 1989).

In 1996 population growth was again retarded, but on this occasion the cause was not drought but poaching. Despite the fact that the legally protected and patrolled Arabian Oryx Sanctuary is now a UNESCO World Heritage Site, poachers entered the Sanctuary on a number of occasions, and an estimated 30 oryx were killed or captured during 1996. The poaching is taking place because of a demand for captive oryx outside Oman (Lawrence 1996), precisely the reason why Arabian oryx disappeared from the Jiddat-al-Harasis in the first place. After an assertive anti-poaching drive in Autumn 1996 the poaching appeared to have stopped (Lawrence 1996), but further

poaching incidents occurred in spring 1997 (Rowe 1997). Poaching undoubtedly represents the greatest threat to the reintroduction in Oman, which has otherwise been extremely successful.

A key component of the success of the project has been the close involvement, from the outset, of the local Harasis tribespeople (Stanley Price 1989; see also Lawrence 1996). The White Oryx Project provides high status employment that makes good use of the exceptional tracking skills of these traditionally nomadic herders. Since release of the first oryx in 1982, Harasis rangers have carried out daily motorised patrols of the Jiddah, and collecting data on births, deaths, herd structures, ranging behaviour and so on. In addition meteorological monitoring is carried out at the camp at Yalooni, and in the period January 1987 - May 1993, there were regular surveys of the quantity and the quality of the vegetation available to the oryx (Spalton 1995). Together these data form the backbone of much of the analysis of inbreeding and outbreeding presented in Chapter 8.

8 A genetic analysis of an endangered species - the Arabian oryx

8.1 Introduction

For some time there has been a consensus among most conservation biologists that conservation programmes should incorporate some form of genetic management. The aims of genetic management are typically the avoidance of inbreeding and the maintenance of genetic variation. The potential costs of inbreeding are well known (Ralls *et al.* 1979; Ballou and Ralls 1982; 7.3), but the need to maintain genetic variation is motivated only by a vague Darwinian notion that genetic variation is beneficial rather than by empirical demonstrations of negative consequences of lack of variation on species viability. In practice it is very difficult to disentangle the diffuse, long term benefits of genetic variation from the strong, short term effects of inbreeding. It has also been shown that excessive outbreeding may incur a fitness cost (Grosberg 1987; Lacy *et al.* 1993; Waser 1993a), and that this may have implications for conservation (Templeton 1986). Taking these suggestions together, it is possible that there is some intermediate optimum between inbreeding and outbreeding (Bateson 1983), where the trade-off between maintaining adaptive potential and preserving coadaptation of genes reaches some kind of equilibrium.

Genetic variation of selective importance generally occurs in the form of quantitative traits, influenced by many loci across the genome as well as by environmental circumstances. The ideal measure of genetic variation would encompass these quantitative trait loci directly. However there are many loci and many traits, and characterising even a small proportion of loci is extremely impractical. Instead genetic variation may be measured at marker loci, on the basis that variation at these loci may be at least loosely correlated with variation at selectively important quantitative trait loci. Marker variation may be assessed both at the population and at the individual level. Marker variation at the population level may be considered as a measure of the adaptive potential of the population, while marker variation at the individual level amounts to a measure of inbreeding and/or outbreeding.

An alternative measure of variation at the individual level may be generated by inferring pedigree relationships of individuals, either by observation or using these same molecular markers, and calculating inbreeding coefficients for each individual from the pedigree (5.2.1). In many ways the pedigree-based approach is less satisfactory because it measures variation with respect to the variation present in the founders of the pedigree whereas marker-based measures of variation have no such restriction. Nevertheless, the calculation of inbreeding coefficients has been the dominant approach in the literature, primarily because it is often more convenient, and in any case highly informative DNA-based markers have become available only recently.

The Arabian oryx (Chapter 7) provides an opportunity to compare the effects of a narrow population bottleneck on different classes of molecular markers, and also to observe how well a species which has come through a bottleneck of known size can survive and adapt to its natural habitat, the deserts of the Arabian peninsula, after several generations in captivity. In particular I examine how drought, inbreeding and outbreeding influence juvenile survival of Arabian oryx reintroduced to Oman, using both pedigree-based and molecular measures of inbreeding and outbreeding.

8.1.1 Previous genetic work on Arabian oryx

Genetic variation in individuals and populations of Arabian oryx has previously been assessed both by simulation and empirically. Simulation was used to assess loss of genetic variation through generations of the Arabian oryx pedigree, while at the empirical level genetic variation has been assayed using polymorphic protein markers, by karyotyping and using multilocus DNA fingerprints probing minisatellite variation. Below I summarise these studies, and my reasons for choosing microsatellites as molecular markers for my work on Arabian oryx.

8.1.1.1 Simulation

Mace (1988) carried out a genedrop analysis on the pedigree data contained in the Arabian oryx studbook available at the end of 1985. Genedropping is a simulation technique that allows assessment of the degree to which the genomes of different founders have been lost over the generations represented in the pedigree (MacCluer *et*

al. 1986). Mace's (1988) results suggested that less than 25% of the genome had been lost from 7 of the 9 founders of the USA breeding programme, and for 5 founders, less than 4% of the genome had been lost. One female founder arrived pregnant at Los Angeles zoo by a male not represented among the founders and almost 50% of his genome was retained in the breeding programme via this single offspring (the "half oryx" in Figure 7.2). Mace concluded that early management of the Arabian oryx in captivity in the USA, particularly at Phoenix zoo, had been remarkably efficient in maintaining neutral genetic variation, although some loss had inevitably occurred.

Mace's (1988) analysis suggested that the genomes of founders of groups in Jordan, Bahrain and Abu Dhabi (7.4) were less well represented in the 1985 studbook. Since then animals from these groups have been dispersed around the Middle East and more recently a few have reached Western zoos, so these non-USA founders are now rather better represented world-wide than Mace's analysis suggested, and especially in Middle Eastern groups (7.4) which are poorly documented in the studbook.

8.1.1.2 Polymorphic proteins

Woodruff and Ryder (1986) surveyed 24 protein loci in 46 individuals from the USA breeding programme and found only one clearly polymorphic locus with two alleles, haemoglobin. Excluding malate dehydrogenase, for which Woodruff and Ryder describe the results as ambiguous, the proportion of protein loci polymorphic in Arabian oryx was just 0.04 and average heterozygosity (including monomorphic loci) was less than 0.01.

Vassart *et al.* (1991), using samples from 85 oryx of predominantly Middle Eastern ancestry found a higher level of variation among the 18 protein loci they surveyed. In addition to haemoglobin, superoxide dismutase and lactate dehydrogenase were also found to be polymorphic with two alleles each, giving a proportion of polymorphic loci of 0.16 and an average heterozygosity, including monomorphic loci, of 0.05. These values are similar to those found by Mace *et al.* (1992) for captive scimitar-horned oryx (five of 27 loci polymorphic (19%), with average heterozygosity of 0.025), and representative of ruminants in general (Vassart *et al.* 1991). However even among the more variable Middle Eastern Arabian oryx, the power of polymorphic protein data for inference of relationships between individuals or populations is low.

8.1.1.3 Karyotypes

In 1989 Cribiu *et al.* (1990) identified a chromosomal polymorphism in Arabian oryx in Saudi Arabia. Of 62 Arabian oryx tested, 54 had the expected chromosomal complement of $2n = 58$ (Wurster and Benirschke 1968), seven had $2n = 57$ and one had $2n = 56$. The oryx with fewer than 58 chromosomes all appeared to carry the same fusion between acrocentric chromosomes 17 and 19, a rearrangement called a Robertsonian translocation. Robertsonian translocations are commonly found in Bovoidea (Wurster and Benirschke 1968) and are compatible with normal chromosomal pairing at meiosis (Short *et al.* 1974). However in some cases heterozygous translocation carriers may suffer reduced fitness (Gustavsson 1969).

Asmodé *et al.* (1990) compared age at first calving and mean birth interval of females heterozygous for the translocation ($2n = 57$) with females without the translocation ($2n = 58$) and found no significant differences among oryx in Saudi Arabia. Male reproductive function was not assessed. Spalton (1992) carried out a similar analysis on reintroduced Arabian oryx in Oman, with some karyotypes inferred by assuming Mendelian inheritance of the translocation. Sample sizes were insufficient for formal statistical tests, but one female translocation carrier appear to be reproducing normally while two male carriers appear to have depressed reproductive function, with one not thought to have sired any calves by the age of seven. Spalton (1992) suggests that if male carriers do suffer reduced fertility, natural selection in the desert environment is likely to prevent the translocation from becoming common.

All karyotypic data are consistent with a single origin of the Robertsonian translocation from the Arabian oryx kept at Al Wukayr, Qatar in the 1970s (7.4.10) (Cribiu *et al.* 1991; Spalton 1992). It is unknown whether the polymorphism arose in captivity or was present in one or more of the wild-caught oryx that founded this group in the 1960s (Cribiu *et al.* 1991).

Although the Robertsonian translocation in Arabian oryx can be regarded as a biallelic co-dominant genetic marker (Cribiu *et al.* 1990) it is not a convenient marker for the analysis of population genetic structure, because karyotyping requires fresh tissue, a rare resource in Arabian oryx genetics, and because the polymorphism is likely to occur only in Arabian oryx with Qatari ancestry.

8.1.1.4 Minisatellites

Greth *et al.* (1991), using the same set of samples as Vassart *et al.* (1991), examined variability at the DNA level using multilocus DNA fingerprints generated with the probe 33.15 (Jeffreys *et al.* 1985a). A mean of 28 bands was scored for each individual, with average band sharing among unrelated individuals approximately 0.45. Greth *et al.* demonstrate that the DNA fingerprinting data in principle can be used to sort pairs of individuals into relatedness classes. However there are methodological problems with multilocus DNA fingerprinting, principally the requirement for large quantities of high molecular weight DNA and the difficulty of comparing individual fingerprints across gels. Furthermore the high level of background band-sharing, which has also been found in captive populations of other species of endangered ruminants (e.g. scimitar-horned oryx, Mace *et al.* 1992), mean that the technique is not efficient for assessing relationships between individuals or populations of Arabian oryx.

8.1.1.5 Microsatellites

Given the shortcomings of protein, karyotype and minisatellite polymorphisms, I chose microsatellite markers (2.4.4) to assay genetic variation in Arabian oryx. Although cloning of microsatellite sequences can be a time-consuming process (Queller *et al.* 1993), PCR primers derived from microsatellite sequences cloned in one species are often found to amplify polymorphic microsatellite loci in related species (Moore *et al.* 1991; Schlötterer *et al.* 1991; Engel *et al.* 1996; Slate *et al.* in press). At the beginning of the study around 200 PCR primer pairs that amplified microsatellite sequences from other ruminants were published, and many more became available in the course of the study. As a substantial proportion of these loci proved to be polymorphic in Arabian oryx, there was no need to clone microsatellites in Arabian oryx and all microsatellite typing was carried out using PCR primers cloned in sheep, cattle, red deer or gazelles.

The microsatellite data were used to compare levels of variation among populations of Arabian oryx around the world, to look for evidence of population differentiation, to verify pedigree data and to assess evidence for inbreeding depression and outbreeding depression in the reintroduced Arabian oryx in Oman.

8.1.2 Previous ecological work on Arabian oryx in Oman

The Arabian oryx reintroduced at Yalooni, Oman have been intensively studied throughout the reintroduction project (7.5). Early work by Stanley Price (1986, 1989) has been continued by Spalton (1993, 1995), who was able to carry out detailed statistical analyses of the effects of rainfall on reproduction and mortality of Arabian oryx using data in the larger population existing during the late 1980s and early 1990s. Because Spalton's (1995) data were the starting point for my analysis of inbreeding and outbreeding, Spalton's findings are summarised below.

Spalton (1995) analysed the factors affecting survival of Arabian oryx calves to one year of age (Table 8.1), using logistic regression similar to that described for neonatal and winter survival in red deer (Chapter 1). The crude protein content of *Stipagrostis* grasses available to the oryx for the 30 days before and 60 days after parturition positively influenced survival. Crude protein around the date of parturition provides an estimate of the quality and quantity of milk available to the growing calf, and is mostly dependent on the time since rain last fell. Two other factors also significantly affected juvenile survival independently of crude protein. These were the reproductive status of the mother, with the first calf of each female more likely to survive than subsequent calves, and origin of the calf's grandmother, with calves of desert-born grandmothers more likely to survive. Since females often conceive on a post-partum oestrus, multiparous females may have just weaned a calf before calving again and may be in poorer condition than primiparous females who have not been burdened by recent lactation. This difference is likely to show up most clearly when nutrition is poor, and it does appear that primiparous females have the greatest advantage over multiparous females in calf survival when crude protein is low (Spalton 1995). It is not clear why calves with desert-born grandmothers have higher survival, but given that a calf is often born into the herd containing its grandmother, it seems reasonable to suppose that the desert-born grandmothers are better adapted to the desert environment than captive bred animals, and can influence their herd in some way that improves its nutritional status.

Term	Coefficient	X^2	d.f.	*p*-value	% deviance explained
Model n = 173					
Constant	-5.16				
Crude protein	1.116	16.0	1	< 0.001	10.2
Mother		4.3	1	< 0.05	2.7
Primiparous	1.413				
Multiparous	0				
Status of grandmother		3.9	1	< 0.05	2.5
Immigrant	-1.033				
Desert-born	0				

Table 8.1 *Non-genetic model of survival to one year of 173 Arabian oryx calves at Yalooni (reproduced from Table 3 in Chapter 7 of Spalton 1995). Logistic regression was carried out using GENSTAT, with the significance of terms assessed by dropping them from the full model. Spalton (1995) tested a series of other terms in this model, (year of birth, sex, matriline, mother's origin, mother's age, survival of previous calf and interactions between significant terms in the model) but none had a significant influence on juvenile survival.*

Given that the data from Spalton's (1995) analysis were available, I was able to model the relationship between inbreeding and juvenile survival in Arabian oryx in Oman taking account of the factors shown to be important in Spalton's analysis. This approach allows comparison of the results with similar analyses in red deer (Chapter 6).

8.2 Methods

8.2.1 Genetic analysis

8.2.1.1 Sampling

Samples used in genetic analysis of Arabian oryx were obtained from a wide variety of sources. Samples from USA-based animals were in the form of whole blood supplied by Prof. O. Ryder at San Diego Zoo (Woodruff and Ryder 1986). A few blood and tissue samples from Arabian oryx in Europe were obtained by Dr. P.

Sunnucks, formerly of the Institute of Zoology, London from the Regent's Park Zoo veterinary hospital. He and co-workers also gathered many samples from the Arabian oryx breeding programme at the National Wildlife Research Centre in Taif, Saudi Arabia (mostly whole blood, plus a small number of skin samples) (Vassart *et al.* 1991; Greth *et al.* 1991) and from the White Oryx Project at Yalooni, Oman (a mixture of blood and skin samples), and all these samples were made available for this study. Additional skin, blood, bone and horn samples from the Omani reintroduction project were supplied direct by Dr. A. Spalton at the Office of the Advisor for Conservation of the Environment in Muscat, while two DNA samples were provided by Dr. M. Stanley Price at the African Wildlife Foundation in Nairobi, where material had been used for karyotyping (8.1.1.3). The numbers of samples available from all populations is shown in Table 8.2.

Area	n	Origin
Abu Dhabi	4	From USA
Dubai	2	From USA
Europe	6	Includes 2 from USA
Israel	2	From USA
Jordan	3	Includes 1 from USA
Morocco	1	From USA
Oman	108	Includes 4 from Jordan, 22 from USA
Qatar	1	From USA
Saudi Arabia	160	Includes 4 from Al Ain (UAE), 3 from Bahrain, 1 from Europe, 4 from Qatar, 12 from USA, 1 from Jordan
USA	60	F1 generation of captive breeding programme onwards
Total	347	

Table 8.2 *Number of samples obtained from Arabian oryx herds around the world. Animals that moved between groups are listed in the country where they spent the majority of their adult life, with the origin column showing the group in which they were born. Samples from four oryx did not yield amplifiable DNA. In addition three horn samples were obtained from the pre-extinction population in Oman, one of which yielded amplifiable DNA.*

Blood or skin samples (in the form of ear punches) were obtained whenever possible during routine handling of animals in captivity. In the case of reintroduced oryx in Oman, additional skin samples were obtained post mortem (in varying states of decay), and also when live animals were immobilised by darting. Biopsy darting was attempted at Yalooni but the trial was abandoned when an adult female died as a result of injuries sustained from the biopsy dart (A. Spalton, pers. comm.) In total, samples were obtained for 67 Arabian oryx born in the wild 1982-1992 in Oman, with a bias in favour of sampling animals born in the earlier years. In addition three horn samples collected on the Jiddat-al-Harasis which are believed to have come from oryx resident on the Jiddah prior to extinction of the original wild population in the early 1970s.

8.2.1.2 Identifying polymorphic microsatellite loci

DNA was extracted from all samples using a standard phenol-chloroform method (see Appendix A2). DNA concentration was checked by running samples on a 0.7% agarose gel, and samples were diluted according to the strength of bands visible under ultraviolet light after staining with ethidium bromide.

66 primer pairs amplifying polymorphic microsatellite sequences in sheep, cattle, red deer or gazelle were tested (Table 8.3). The test panels consisted of 3-7 Arabian oryx samples that were not close relatives of each other. Where possible the test panels included samples from multiple herds plus a control sample from the species in which the microsatellite sequence was originally.

Table 8.3 *(overleaf) Microsatellite loci tested for polymorphism in Arabian oryx. One control sample was included from the species in which the microsatellite locus was originally cloned, except for the six gazelle loci (where no gazelle DNA was available) and three cattle loci (all of which could be amplified in Arabian oryx).*

Locus name	Source species	Control amplified	*n* oryx tested	Alleles in oryx	Primer sequences obtained from
BM1233	Cattle	No	4	1?	Bishop *et al.* (1994)
BM1258	Cattle	Yes	5	1	Bishop *et al.* (1994)
BM1815	Cattle	Yes	4	1	Bishop *et al.* (1994)
BM1818	Cattle	Yes	5	2	Bishop *et al.* (1994)
BMC1013	Cattle	Yes	0	N/A	Bishop *et al.* (1994)
BoLADRBP1	Cattle	No	4	1	Fries *et al.* (1993)
BoLADRBP2	Cattle	Yes	6	1	Fries *et al.* (1993)
CSSM65	Cattle	Yes	4	1	Moore *et al.* (1994)
ETH185	Cattle	No	0	N/A	Steffen *et al.* (1993)
ILST030	Cattle	No	2	1?	Kemp *et al.* (1995)
INRA11	Cattle	Yes	3	3	Vaiman *et al.* (1992)
MAP2C	Cattle	Yes	3	2?	Moore *et al.* (1992)
RBP3	Cattle	Yes	264	2	MacHugh *et al.* (1997)
RM90	Cattle	ND	3	1	McGraw *et al.* (1997)
RM95	Cattle	ND	3	1	Kossarek *et al.* (1994)
RM106	Cattle	Yes	6	2	Kossarek *et al.* (1993)
RME25	Cattle	Yes	2	1?	Grosse *et al.* (1995)
TGLA94	Cattle	ND	3	1	Georges and Massey (1992)
TGLA322	Cattle	No	0	N/A	Georges and Massey (1992)
pGazac1	Gazelle	N/A	0	N/A	R. Hammond, pers. comm.
pGazac2	Gazelle	N/A	22	3	R. Hammond, pers. comm.
pGazac3	Gazelle	N/A	4	1?	R. Hammond, pers. comm.
pGazac4	Gazelle	N/A	0	N/A	R. Hammond, pers. comm.
pGazac6	Gazelle	N/A	4	1?	R. Hammond, pers. comm.
pGazagc2	Gazelle	N/A	6	1?	R. Hammond, pers. comm.
CelJP1	Red deer	No	5	1?	J. Pemberton, pers. comm.
CelJP6	Red deer	No	0	N/A	J. Pemberton, pers. comm.
CelJP14	Red deer	Yes	5	1	J. Pemberton, pers. comm.
CelJP15	Red deer	Yes	47	2	J. Pemberton, pers. comm.
CelJP18	Red deer	No	0	N/A	J. Pemberton, pers. comm.
CelJP23	Red deer	Yes	1	2?	J. Pemberton, pers. comm.
CelJP27	Red deer	Yes	154	6	J. Pemberton, pers. comm.
CelJP30	Red deer	?	5	1?	J. Pemberton, pers. comm.

Locus name	Source species	Control amplified	*n* oryx tested	Alleles in oryx	Primer sequences obtained from
CelJP31	Red deer	No	0	N/A	J. Pemberton, pers. comm.
CelJP38	Red deer	Yes	0	N/A	J. Pemberton, pers. comm.
MAF4	Sheep	?	6	1?	Buchanan *et al.* (1992a)
MAF18	Sheep	Yes	6	1	Crawford *et al.* (1990)
MAF23	Sheep	Yes	6	1	Swarbrick *et al.* (1990)
MAF35	Sheep	Yes	6	2	Swarbrick *et al.* (1991a)
MAF36	Sheep	No	0	N/A	Swarbrick *et al.* (1991b)
MAF45	Sheep	Yes	6	1	Swarbrick *et al.* (1992c)
MAF46	Sheep	Yes	312	5	Swarbrick *et al.* (1992a)
MAF48	Sheep	Yes	6	1	Buchanan *et al.* (1992b)
MAF50	Sheep	Yes	290	6	Swarbrick *et al.* (1992b)
MAF64	Sheep	Yes	47	2	Swarbrick *et al.* (1991c)
MAF65	Sheep	Yes	5	1	Buchanan *et al.* (1992c)
MAF92	Sheep	Yes	5	1	Crawford *et al.* (1991)
MAF109	Sheep	Yes	47	2	Swarbrick and Crawford (1992)
OarAE119	Sheep	Yes	45	2	Penty *et al.* (1993)
OarCP16	Sheep	Yes	0	N/A	Ede *et al.* (1995)
OarCP26	Sheep	Yes	298	6	Ede *et al.* (1995)
OarFCB11	Sheep	Yes	6	1	Buchanan and Crawford (1993)
OarFCB128	Sheep	Yes	6	2	Buchanan and Crawford (1993)
OarFCB193	Sheep	Yes	6	1	Buchanan and Crawford (1993)
OarFCB266	Sheep	Yes	4	1?	Buchanan and Crawford (1993)
OarFCB304	Sheep	Yes	302	2	Buchanan and Crawford (1993)
OarHH41	Sheep	No	0	N/A	Henry *et al.* (1993)
OarHH56	Sheep	Yes	6	1?	Ede *et al.* (1994)
OarHH64	Sheep	Yes	266	7	Henry *et al.* (1993)
OarVH34	Sheep	Yes	6	2	Pierson *et al.* (1993)
OarVH54	Sheep	Yes	0	N/A	Pierson *et al.* (1994)
OarVH116	Sheep	Yes	5	1	Hanrahan *et al.* (1993)
OLADRB	Sheep	Yes	5	2	Schwaiger *et al.* (1993)
OLADRBps	Sheep	No	0	N/A	Blattman and Beh (1992)
OMHC1	Sheep	No	5	1?	Groth and Wetherall (1994)
OPACAP	Sheep	Yes	6	1	Bancroft (1994)

The PCR protocol is described in Appendix A2. ^{32}P-labelled dCTP or fluorescently-tagged PCR primer was included in the reaction according to the detection method used. Radiolabelled PCR products were separated on a manual polyacrylamide sequencing gel and the products visualised using autoradiography. Fluorescently-tagged PCR products were separated on a polyacrylamide gel in an ABI377 automatic sequencer system and the products were visualised using GENESCAN and GENOTYPER software. Details of both detection methods can be found in Appendix A2.

Primers may amplify random sequences but amplification of the target microsatellite locus is usually readily distinguished by a strong band accompanied by the characteristic stutter bands arising from slippage during PCR replication of the microsatellite sequence (Murray *et al.* 1993). Target products were also expected to occur in a similar size range as the allele(s) observed in the control sample from the source species. 38 primer pairs (58% of those tested) amplified a product that appeared to be a microsatellite sequence in at least one of the test samples. 19 primer pairs (29% of those tested) generated unambiguously polymorphic products, while only 7 (11% of those tested) amplified loci with more than two alleles among the oryx samples tested.

8.2.1.3 Large-scale typing of polymorphic microsatellite loci

Seven polymorphic microsatellite loci were chosen for large-scale screening (Table 8.4). These loci were selected on the basis of high heterozygosity, reliability of amplification and ease of scoring, although with Arabian oryx it proved difficult to identify loci which fulfilled all these requirements simultaneously. In particular, RBP3 had only two alleles, had low heterozygosity in some populations and did not always amplify. CelJP27 proved very difficult to amplify from many samples and among samples from which the locus could be amplified, individual alleles were amplified inconsistently. As a result CelJP27 was dropped from later analysis, with reluctance given that its was one of the most polymorphic loci identified in this study. Across the remaining six loci samples were typed at an average of 85% of loci.

Locus	Source species	Annealing temp. (°C)	Size range (bp)	Alleles
RBP3	Cattle	44/46°C	144-146	2
OarFCB304	Sheep	51/53°C	119-121	2
MAF50	Sheep	48/50°C	149-167	6
MAF46	Sheep	54/56°C	108-118	5
OarCP26	Sheep	54/56°C	139-151	6
OarHH64	Sheep	54/56°C	121-139	7
CelJP27	Red deer	54/56°C	176-202	6

Table 8.4 *Microsatellite loci used in large-scale microsatellite typing in Arabian oryx. Annealing temperature refers to the temperature of the annealing phase of the PCR reaction (see Appendix A2). The allele numbers and size ranges are those found across all contemporary Arabian oryx typed during the course of this study. Additional alleles were observed at RBP3, OarMAF46 and OarCP26 in a sample from the pre-extinction wild population in Oman (8.4.2).*

8.2.2 Statistical analysis

8.2.2.1 Levels of variation

Four groups of Arabian oryx were sufficiently well represented in the sample for detailed genetic analysis. These were the herd at San Diego Wild Animal Park (SDWAP), USA, the reintroduced population at Yalooni, Oman, the captive herd at Thumamah, Saudi Arabia and the captive herd at Taif, Saudi Arabia (7.4.5). Allele frequencies and expected heterozygosities (Nei 1987) were calculated for each of these populations using the program CERVUS 1.0 (Chapter 3; Marshall *et al.* 1998), and also for all sampled Arabian oryx (n = 343 from which amplifiable DNA could be extracted).

Deviations from Hardy-Weinberg equilibrium were assessed at each locus for each of the four main populations using chi-square values supplied by CERVUS. Expected heterozygosity was calculated at each locus for each population using CERVUS, and compared using unreplicated two-way analysis of variance (ANOVA).

8.2.2.2 Population differentiation

Population differentiation was assessed using the microsatellite-based measure of differentiation, R_{ST}, which takes account of the stepwise mutation dynamics of microsatellite sequences (Valdes *et al.* 1993; Slatkin 1995b). R_{ST} estimates were calculated using the program RSTCALC (Goodman 1997), which can calculate pairwise R_{ST} for populations of unequal size and evaluate the significance of R_{ST} estimates via a bootstrap procedure. Although there is some debate as to the degree to which microsatellites follow a strict single-step mutation model (Di Rienzo *et al.* 1994; Jarne and Lagoda 1996; Ortí *et al.* 1997), R_{ST}, which assumes strict stepwise mutation, is likely to give less biased estimates of differentiation with microsatellite data than F_{ST} (1.2.1), which assumes every mutation event generates a novel allele with a length unrelated to its ancestor.

8.2.2.3 Pedigree data

The pedigree data in the Arabian oryx studbook (Dolan and Sausman 1992), all of which has been derived by behavioural observation, plus supplementary studbook information supplied by NWRC Taif, was assessed using CERVUS 1.0 (Chapter 3; Marshall *et al.* 1998). For this analysis, genetic data from all contemporary oryx from which useful samples were obtained (n = 343) were pooled in order to calculate allele frequencies (Table 8.8, last column). Pooled allele frequencies were used to ensure that all sampled individuals could be tested, irrespective of whether they were in one of the four main populations or one of the less well represented populations for which accurate calculation of allele frequencies in isolation would be impossible.

Using the pooled allele frequencies, various simulations were run and it quickly became apparent that parentage inference, as carried out for Rum red deer (Chapter 4), would not be possible for Arabian oryx since the six microsatellite markers did not have sufficient power to resolve parentage even at 80% confidence in more than a few percent of simulated parentage tests. Parentage analysis was therefore restricted to validation of existing behaviourally-determined parent-offspring relationships.

For simulations of validation of existing relationships, the minimum number of candidate parents is two (one candidate parent would imply no uncertainty), of which one is the sampled putative parent indicated in the pedigree and the other is an

unknown individual. The proportion of sampled candidate parents is therefore 0.5. Simulations were run using the parameters shown in Table 8.5, with the results shown in Table 8.6. The key finding is that a critical Δ of 0 gives approximately 80% confidence whether or not a sample is available from the mother. In other words, if only one candidate parent is tested and the LOD score is positive, parentage can be assigned with 80% confidence.

Parameter	Value used
Number of candidate parents	2
Proportion of candidate parents sampled	0.5
Proportion of loci typed	0.85
Rate of typing error	0.01
Number of tests	10,000
Relaxed confidence level	80%
Strict confidence level	95%

Table 8.5 *Values of CERVUS simulation parameters used for simulation of paternity inference with Arabian oryx microsatellite data.*

Simulation results	Mother sampled		Mother unsampled	
(n = 10,000)	80%	95%	80%	95%
Critical value of Δ	0.00	1.31	0.01	1.63
Proportion of paternities	53.50%	31.26%	54.00%	14.91%

Table 8.6 *Results of simulation of paternity analysis in Arabian oryx with the CERVUS program using the parameters shown in Table 8.5 and the allele frequencies shown in the last column of Table 8.8.*

8.2.2.4 Inbreeding and fitness

Detailed life history records were available for 173 Arabian oryx born in Oman between March 1987 and May 1993 (Spalton 1995). Samples were available from 57 of these animals (33%). Samples were always collected post-mortem if possible, and

animals that died as juveniles were systematically more likely to be sampled that those that did not (Table 8.7; $X^2 = 22.85$, d.f. = 1, $p < 0.001$).

Survival to one year	Died	Survived	Total
Sampled	16	41	57
Unsampled	3	113	116
Total	19	154	173

Table 8.7 *Number of sampled and unsampled calves Arabian oryx calves born at Yalooni, Oman that survived to one year.*

Individual heterozygosity and mean d^2 (5.2; Coulson *et al.* 1998b) were calculated for 57 sampled oryx, all of which were typed for six microsatellite loci. Individual heterozygosity was not normally distributed (Figure 8.1a; Kolmogorov-Smirnov Z = 1.593, p = 0.013) and arcsine transformation, appropriate for proportions, did not improve its normality (Kolmogorov-Smirnov Z = 1.589, p = 0.013). However the distribution was unimodal and reasonably symmetrical and departure from normality appeared to be due to the approximation of a discrete variable as continuous. I therefore analysed individual heterozygosity as if it were a normally distributed continuous variable. As the distribution of mean d^2 was right-skewed (Figure 8.1b; Kolmogorov-Smirnov Z = 1.867, p = 0.002), the data were log-transformed prior to analysis yielding a distribution that did not depart significantly from normality (Kolmogorov-Smirnov Z = 1.153, p = 0.14).

Inbreeding coefficients (5.1) were calculated with the existing studbook data using the program FASTINB (Boyce 1983) modified for large pedigrees. Each unknown parent was assumed to be unrelated to all other individuals in the pedigree. Among the 173 Arabian oryx studied by Spalton (1995), inbreeding coefficients were calculated for 123 oryx for which both parents were known. As inbreeding coefficient is a proportion and the data were right-skewed (Figure 8.2; Kolmogorov-Smirnov Z = 1.940, p = 0.001), the data were arcsine transformed prior to analysis yielding a distribution that did not depart significantly from normality (Kolmogorov-Smirnov Z = 1.145, p = 0.15).

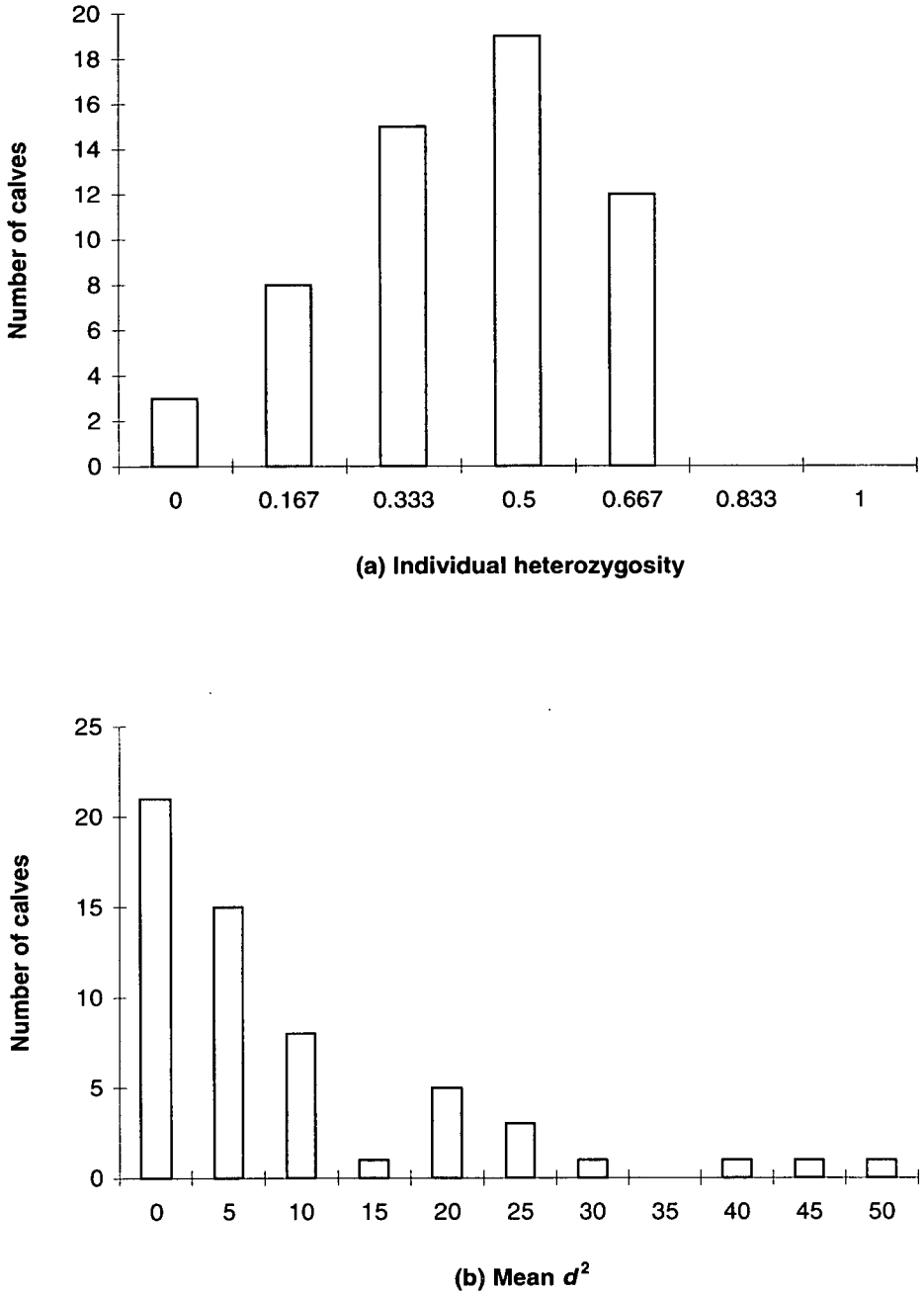

Figure 8.1 *Distribution of (a) individual heterozygosity and (b) mean d^2 scores for 57 Arabian oryx calves.*

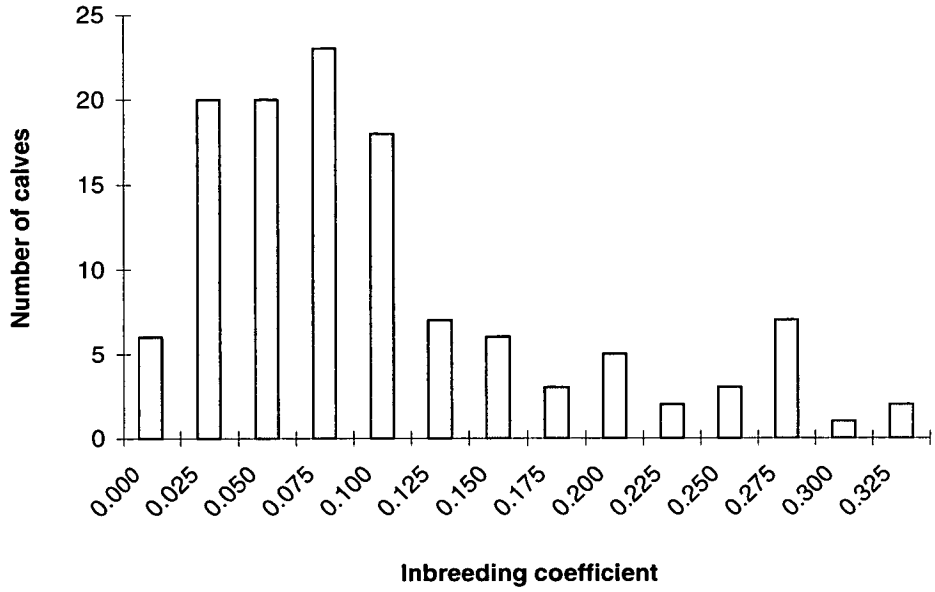

Figure 8.2 *Distribution of inbreeding coefficients among 123 calves born 1987-1993 in the reintroduced population of Arabian oryx at Yalooni, Oman.*

Logistic regression of juvenile survival to one year was carried out using SPSS 6.1. The following variables were investigated:

Mother's reproductive status: categorical, with two levels:

primiparous - had not previously bred

multiparous - had previously bred

Grandmother's origin: categorical, immigrant or desert-born

Crude protein: continuous, expressed as a percentage. Although a proportion, the distribution of crude protein did not significantly depart from normality (Kolmogorov-Smirnov $Z = 0.861$, $n = 136$, $p = 0.45$), and was therefore not transformed prior to analysis.

Individual heterozygosity: continuous, $n = 57$

Mean d^2: continuous, log-transformed, $n = 57$

Inbreeding coefficient: continuous, arcsine transformed, $n = 123$

8.3 Results

8.3.1 Levels of variation

Table 8.8 shows the allele frequencies and heterozygosities at six microsatellite loci for four populations of Arabian oryx, and also combined allele frequencies for all samples (n = 343). There is no very clear pattern in allelic diversity across populations, but at all loci with more than two alleles there are alleles present in some populations that are absent in others. All such alleles occur at a frequency of less than 10% in the population in which they are most common. This suggests that some microsatellite alleles that were initially rare were lost during the founding of individual captive populations.

Where possible deviations from Hardy-Weinberg equilibrium were assessed for each locus using chi-square goodness-of-fit tests separately for the four main populations (Table 8.9). There were no consistent patterns of deviations from Hardy-Weinberg equilibrium across populations for particular loci nor across loci within populations. However some modest deviations from Hardy-Weinberg equilibrium were found for specific locus-population combinations. Some of these deviations were due to an excess of heterozygotes, while others were due to an excess of homozygotes. Cases where there was an excess of homozygotes (OarFCB304 at Thumamah, OarCP26 at Yalooni and a similar trend for MAF50 at Yalooni) could not be explained by segregating null alleles, since subsequent analysis with CERVUS (8.3.3) did not reveal repeated homozygote-homozygote mismatches between mothers and their offspring for any locus in any population.

Table 8.8 *(overleaf) Allele frequencies at six loci calculated using the CERVUS program for 298 Arabian oryx from four populations, and for all 343 contemporary oryx combined, including those born in herds other than the four shown. San Diego Wild Animal Park is abbreviated to SDWAP. Oryx that were transferred between populations were categorised by the population in which they were born.*

Locus	Allele	Population				
		SDWAP (*n* = 90)	Yalooni (*n* = 77)	Thumamah (n = 34)	Taif (*n* = 97)	All (*n* = 343)
RBP3	144	0.877	0.954	0.704	0.747	0.833
	146	0.123	0.046	0.296	0.253	0.167
OarFCB304	119	0.746	0.644	0.483	0.395	0.575
	121	0.254	0.356	0.517	0.605	0.423
MAF50	149	0	0	0	0.006	0.002
	153	0.020	0.014	0.109	0.089	0.050
	155	0.574	0.356	0.478	0.506	0.507
	157	0.007	0.069	0.326	0.247	0.123
	158	0.399	0.562	0.087	0.146	0.317
	167	0	0	0	0.006	0.002
MAF46	108	0.077	0.034	0.259	0.222	0.131
	110	0	0	0	0.017	0.005
	114	0	0.007	0	0	0.002
	116	0.622	0.411	0.482	0.500	0.500
	118	0.301	0.548	0.259	0.261	0.363
OarCP26	139	0	0	0.065	0.013	0.010
	141	0.469	0.662	0.413	0.644	0.588
	143	0.031	0.088	0	0	0.037
	147	0.188	0.027	0.130	0.063	0.093
	149	0.156	0.155	0.196	0.094	0.136
	151	0.156	0.068	0.196	0.188	0.136
OarHH64	121	0	0	0.044	0.029	0.015
	123	0.115	0.062	0.022	0.069	0.083
	131	0	0.021	0	0	0.006
	133	0.354	0.295	0.261	0.184	0.269
	135	0.354	0.493	0.348	0.402	0.417
	137	0.021	0.089	0.152	0.167	0.103
	139	0.156	0.041	0.174	0.149	0.107

Locus	Population			
	SDWAP	Yalooni	Thumamah	Taif
RBP3	N/A	N/A	N/A	N/A
OarFCB304	N/A	0.02 ↓	4.03 ↓ *	1.76 ↑
MAF50	0.06 ↓	3.74 ↓	0.69 ↑	4.58 ↑ *
OarCP26	5.20 ↑ *	5.43 ↓ *	N/A	0.10 ↑
MAF46	0.64 ↑	0.02 ↓	0.01 ↑	2.48 ↑ (3 d.f.)
OarHH64	0.21 ↓	0.06 ↑	N/A	0.55 ↓

Table 8.9 *Chi-square values testing for deviations from Hardy-Weinberg equilibrium at six microsatellite loci calculated using the program CERVUS (expected genotype frequencies based on Equation 7.8 of Nei 1987) separately for each of the four populations. All chi-square values have one degree of freedom except where otherwise indicated, and are marked with * if they were significant ($p < 0.05$). The direction of each deviation is indicated with an arrow: ↑ indicates an excess of heterozygotes while ↓ indicates a deficit of heterozygotes. N/A is shown in cases where it was not possible to combine allelic classes so that all expected genotype frequencies exceeded five.*

Because there did not appear to be significant substructure in any of the four populations, nor significant deviations from equilibrium at any particular loci across all populations, I carried out subsequent analysis on the basis that Hardy-Weinberg equilibrium prevailed.

Expected heterozygosity varied significantly between populations having taken account of differences in heterozygosity between loci (two-way ANOVA: $F_{3,15} = 6.20$, $p = 0.006$). Heterozygosity was higher in the Saudi Arabian populations (Taif and Thumamah) than in USA (SDWAP) and Omani (Yalooni) populations, presumably reflecting the more diverse ancestry of Saudi Arabian oryx (7.4.5). These results concur with the higher level of variation found at allozyme loci in Saudi Arabian oryx (Vassart *et al.* 1991) compared with USA-bred oryx (Woodruff and Ryder 1986).

Locus	Population			
	SDWAP	Yalooni	Thumamah	Taif
RBP3	0.217	0.088	0.425	0.381
OarFCB304	0.381	0.462	0.508	0.481
MAF50	0.514	0.557	0.660	0.658
OarCP26	0.520	0.533	0.646	0.636
MAF46	0.700	0.528	0.748	0.541
OarHH64	0.719	0.661	0.772	0.753
Mean expected heterozygosity	0.509	0.472	0.627	0.575

Table 8.10 *Expected heterozygosity assuming Hardy-Weinberg equilibrium at six microsatellite loci calculated using the program* CERVUS *(based on Equation 8.4 of Nei 1987) separately for each of four populations.*

8.3.2 Population differentiation

Table 8.11 shows pairwise R_{ST} values between populations, with a large value of R_{ST} implying a high level of differentiation between a pair of populations. R_{ST} values were significantly different from zero for all pairs of populations apart from the pair of Saudi Arabian populations (Thumamah and Taif). Differentiation is intermediate between the American (SDWAP) and the three Middle Eastern populations, and highest between Omani (Yalooni) and the two Saudi Arabian populations.

Population	SDWAP	Yalooni	Thumamah
Yalooni	0.047	-	-
Thumamah	0.025	0.103	-
Taif	0.033	0.067	0.001

Table 8.11 *Pairwise R_{ST} values (averaging over variance components) calculated using Goodman's (1997) program* RSTCALC. *All R_{ST} values are significantly different from zero apart from the Thumamah - Taif comparison (bootstrap data not shown).*

Assumptions of Slatkin's (1995b) R_{ST} model include an island model of population structure, random mating and a population in mutation-drift equilibrium (Goodman 1997). It is unlikely that the Arabian oryx populations in this study fulfil these assumptions. In particular mating at Taif has been strictly controlled to avoid inbreeding and is in no sense random. However the rank order of R_{ST} estimates derived above is likely to be at least loosely correlated to true relative pairwise differentiation, and it is reassuring the two Saudi Arabian populations which have essentially identical ancestry (7.4.5) show no significant differentiation.

8.3.3 Verification of the pedigree

Maternity and paternity analysis was carried out using the CERVUS program. The objective was to verify the pedigree, and so for each maternity test, only the putative mother was tested, and for each paternity test, only the putative father was tested.

From an exclusionary perspective (i.e. ignoring the possibility of typing errors giving rise to mismatches), there were 13 of 121 maternities (11%) that were excluded at one or more loci of which 6 (5%) were excluded at two or more loci. All but one pair of the 13 involved different mothers. Given that the average probability of excluding an unrelated individual from maternity at one or more loci was 0.707, the estimated frequency of non-maternity based on all observed mismatches is 15%. This calculation assumes that no typing or sample labelling errors occurred (violation would lead to overestimation of the rate of non-maternity) and that there were no missing data (violation would lead to underestimation). The average probability of excluding an unrelated individual from maternity at two or more loci was 0.296. Assuming that only mismatches at two or more loci represent definite non-maternities, the estimated frequency of non-maternity is 17%, similar to the figure obtained above.

Carrying out paternity analysis ignoring maternal genotypes, there were 27 of 177 paternities (15%) which were excluded at one or more loci, of which 3 (2%) were excluded at two or more loci. 17 different males were involved in the 41 mismatches and so not all exclusions were necessarily independent. Treating all mismatches as non-paternities, these data suggest a rate of non-paternity of 22%. Treating only mismatches at two or more loci as definite non-paternities, the estimated frequency of non-paternity is 6%.

Carrying out paternity analysis making use of maternal genotypes in the 83 cases where sufficient data were available, and assuming that mothers were correctly identified, there were 22 paternities (27%) which were excluded at one or more loci, of which 8 (10%) were excluded at two or more loci. Treating all mismatches as non-paternities, these data suggest a rate of non-paternity of 29%, given that the average probability of exclusion at one or more loci was 0.904. Treating only mismatches at two or more loci as definite non-paternities, the estimated frequency of non-paternity is 16%, given that the average probability of exclusion at two or more loci was 0.614.

Parentage was also assessed via LOD scores generated by the CERVUS program (Figure 8.3). Unlike the exclusionary approach, LOD scores statistically take account of possible typing errors. In the case of the Arabian oryx pedigree, allowing for typing errors at a frequency of 1%, a LOD score of greater than zero implied that parentage could be assigned with approximately 80% confidence, whether or not the genotype of the other parent was known (Table 8.6). 18 of 121 maternities tested (15%) yielded a LOD score of less than zero, while 33 of 177 paternities tested (19%) yielded a LOD score of less than zero. Some of these low LOD scores may have been the result of a particularly common set of shared alleles occurring by chance, while others may indicate genuine non-relationship.

The analysis of the Arabian oryx studbook presented here suggests that the frequency of errors in the pedigree may be quite substantial, and is almost certainly sufficient to cause considerable error in the calculation of inbreeding coefficients. However there was insufficient power in the available molecular data to allow correction of the pedigree. The following calculation of inbreeding coefficients therefore assumed that the pedigree is correct, in the knowledge that pedigree errors may have contributed noise to analyses involving inbreeding coefficient data.

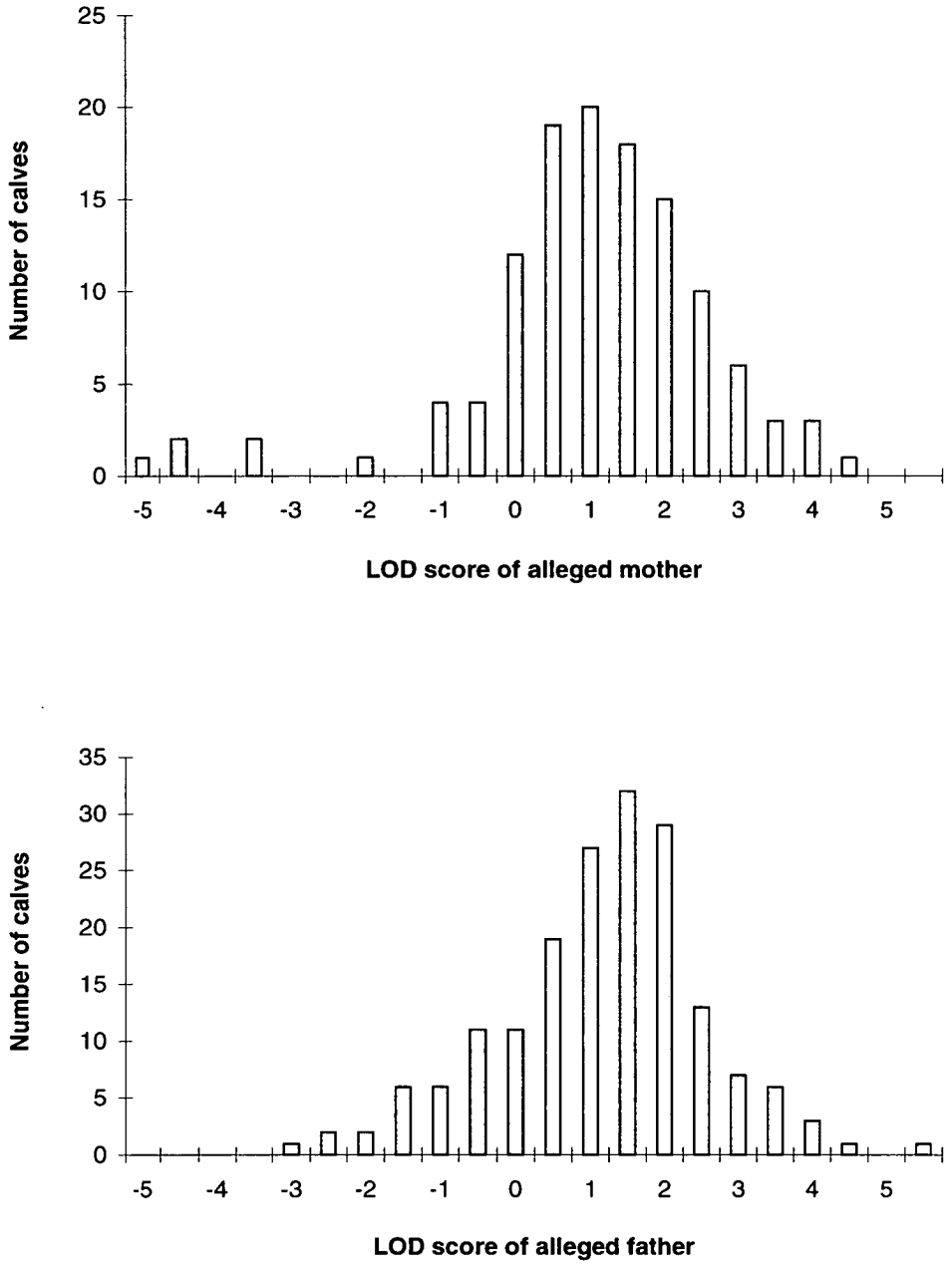

Figure 8.3 *LOD scores for (a) mothers (n = 121), and (b) fathers (n = 177) in the Arabian oryx pedigree. Data are shown for oryx calves for which maternity and paternity respectively could be tested at a minimum of three loci. Paternity analysis was carried out making use of available maternal genotypes.*

8.3.4 Inbreeding coefficients and inbreeding events

In reintroduced Arabian oryx in Oman, inbreeding was common (Figure 8.2) but the level of inbreeding did not change significantly over time (regression $F_{1,121} = 0.442$; $p = 0.51$). The mean inbreeding coefficient among the 123 calves born 1987-1993 for which both parents are known was 0.087 (based on transformed data), with 31 calves (25%) having inbreeding coefficients of 0.125 or more and 13 calves (11%) having inbreeding coefficients of 0.25 or more. These figures are strikingly higher than for red deer on Rum (Table 5.1), where only 3% of calves had inbreeding coefficients of 0.125 or more and less than 1% had inbreeding coefficients of 0.25 or more.

Male parent	Female parent	*F* of offspring	Number of calves tested	Number of inbred calves	Frequency of inbreeding event
Father	Daughter	0.25	114	3	2.6%
Son	Mother	0.25	123	3	2.4%
Full brother	Full sister	0.25	105	5	4.8%
Paternal half-brother	Paternal half-sister	0.125	105	7	6.7%
Maternal half-brother	Maternal half-sister	0.125	123	3	2.4%
Other	-	0.125	-	10	-
Total	-	-	-	31	-

Table 8.12 *Number of close inbreeding events of various types among 123 reintroduced Arabian oryx at Yalooni. The category Other includes uncle-niece, aunt-nephew, double first cousin and grandparent-grandchild pairings. In many cases the overall inbreeding coefficient resulting from a particular mating was higher than the value shown because of additional shared ancestry of the parents further back in the pedigree.*

However, the apparent difference between the species in the frequency of close inbreeding may arise from the fact that the Omani Arabian oryx pedigree is very much more complete than the Rum red deer pedigree. In order to make direct comparisons, it is important to calculate the number of cases in which each type of inbreeding event

could have been detected. Table 8.12 shows the frequency of various inbreeding events, analogous to Table 5.1 showing similar data for Rum red deer. In contrast to Rum red deer, calves by paternal half sibs are not significantly more common than calves by maternal half sibs in Omani Arabian oryx ($X^2 = 1.51$, d.f. = 1, $p > 0.05$). In Arabian oryx, 11 full-sib or parent-offspring pairings occurred (10%) among 105 cases where all four grandparents were known, whereas in red deer no such pairings occurred among the 102 cases where all four grandparents were known (the three father-daughter matings observed in Rum red deer all involved stags with one or both parents unknown). The implication is that inbreeding is genuinely more common in Arabian oryx in Oman than in red deer on Rum (5.3.1.3), and may be a consequence of the smaller pool of unrelated mates in the Yalooni population.

8.3.5 Relationship between measures of inbreeding

Most of the recent common ancestors of contemporary Arabian oryx in Oman are contained within the pedigree documented in the Arabian oryx studbook (Dolan and Sausman 1992). Acknowledging that the pedigree almost certainly contains errors, inbreeding coefficients calculated using studbook data may nevertheless estimate the level of homozygosity arising from recent common ancestry at selectively neutral loci.

Among the calves born at Yalooni for which appropriate data were available, individual heterozygosity was negatively correlated with inbreeding coefficient (r = -0.280, n = 59, p = 0.037), but the relationship was not particularly strong. Mean d^2 was not significantly correlated with inbreeding coefficient (r = -0.252, n = 59, p = 0.059), but showed a trend in the same direction as individual heterozygosity, which can be explained by the strong positive correlation between mean d^2 and individual heterozygosity ($r = 0.584$, $n = 73$, $p < 0.001$).

The poor association between individual heterozygosity and inbreeding coefficient has three possible explanations. First, the errors in the pedigree may make the inbreeding coefficients very inaccurate estimators of true inbreeding. Second, individual heterozygosity based on only six microsatellite loci may have a large binomial sampling variance and hence be a poor estimator of inbreeding. Third, individual heterozygosity may incorporate information on common ancestry between studbook founders which is not reflected in the inbreeding coefficients.

8.3.6 Inbreeding and juvenile survival

8.3.6.1 Inbreeding coefficient

Among 123 Arabian oryx calves born 1987-1993 for which inbreeding coefficients could be calculated, there was no significant difference in mean inbreeding coefficient between calves that died (n = 18) and calves that survived their first year of life (t = 1.21, d.f. = 20, two-tailed p = 0.241). A logistic regression model of juvenile survival, first testing non-genetic factors previously shown to influence juvenile survival in this population (Table 8.1), also showed no significant effect of inbreeding coefficient on survival (Table 8.13). There was a weak trend for the inbreeding coefficient of individuals that died to be higher than the inbreeding coefficient of those that survived.

Term	Coefficient	X^2	d.f.	p-value	% deviance explained
Model n = 123					
Constant	0.911				
Crude protein	0.371	5.21	1	0.023	5.1
Mother		8.86	1	0.003	8.7
Primiparous (28)	4.286				
Multiparous (95)	0				
Rejected terms					
Status of grandmother		0.68	1	0.409	
Immigrant (77)	Negative				
Desert born (46)	0				
Inbreeding coefficient (arcsine transformed)	Negative	1.83	1	0.177	

Table 8.13 *Model of survival to one year of 123 Arabian oryx calves at Yalooni Logistic regression was carried out using SPSS 6.1, with the significance of terms included assessed by dropping them from the full model and the significance of rejected terms assessed by adding them to the full model.*

8.3.6.2 Molecular measures of inbreeding

Among 57 Arabian oryx calves for which individual heterozygosity and mean d^2 could be calculated, there was no significant difference in individual heterozygosity between calves that died (n = 16) and calves that survived their first year of life (t = 1.08, d.f. = 55, two-tailed p = 0.286) nor any significant difference in mean d^2 between calves that died and calves that survived (t = 1.26, d.f. = 55, two-tailed p = 0.214). However a logistic regression model of juvenile survival, first testing non-genetic factors previously shown to influence juvenile survival in this population (Table 8.1), showed that individual heterozygosity positively influenced survival while mean d^2 negatively influenced survival when fitted together (Table 8.14). In other words inbreeding depression (illustrated in Figure 8.4a) and outbreeding depression (illustrated in Figure 8.4b) appear to be occurring simultaneously in Arabian oryx in Oman.

Term	Coefficient	X^2	d.f.	p-value	% deviance explained
Model n = 57					
Constant	-8.289				
Crude protein	0.678	11.28	1	< 0.001	16.7
Individual heterozygosity	6.822	7.79	1	0.005	11.5
Mean d^2 (log transformed)	-1.073	6.83	1	0.009	10.1
Rejected terms					
Mother		0.28	1	0.594	
Primiparous (9)	Positive				
Multiparous (48)	0				
Status of grandmother		3.57	1	0.059	
Immigrant (39)	Negative				
Desert born (18)	0				

Table 8.14 *Model of survival to one year of 57 Arabian oryx calves at Yalooni Logistic regression was carried out using SPSS 6.1, with the significance of terms included assessed by dropping them from the full model and the significance of rejected terms assessed by adding them to the full model.*

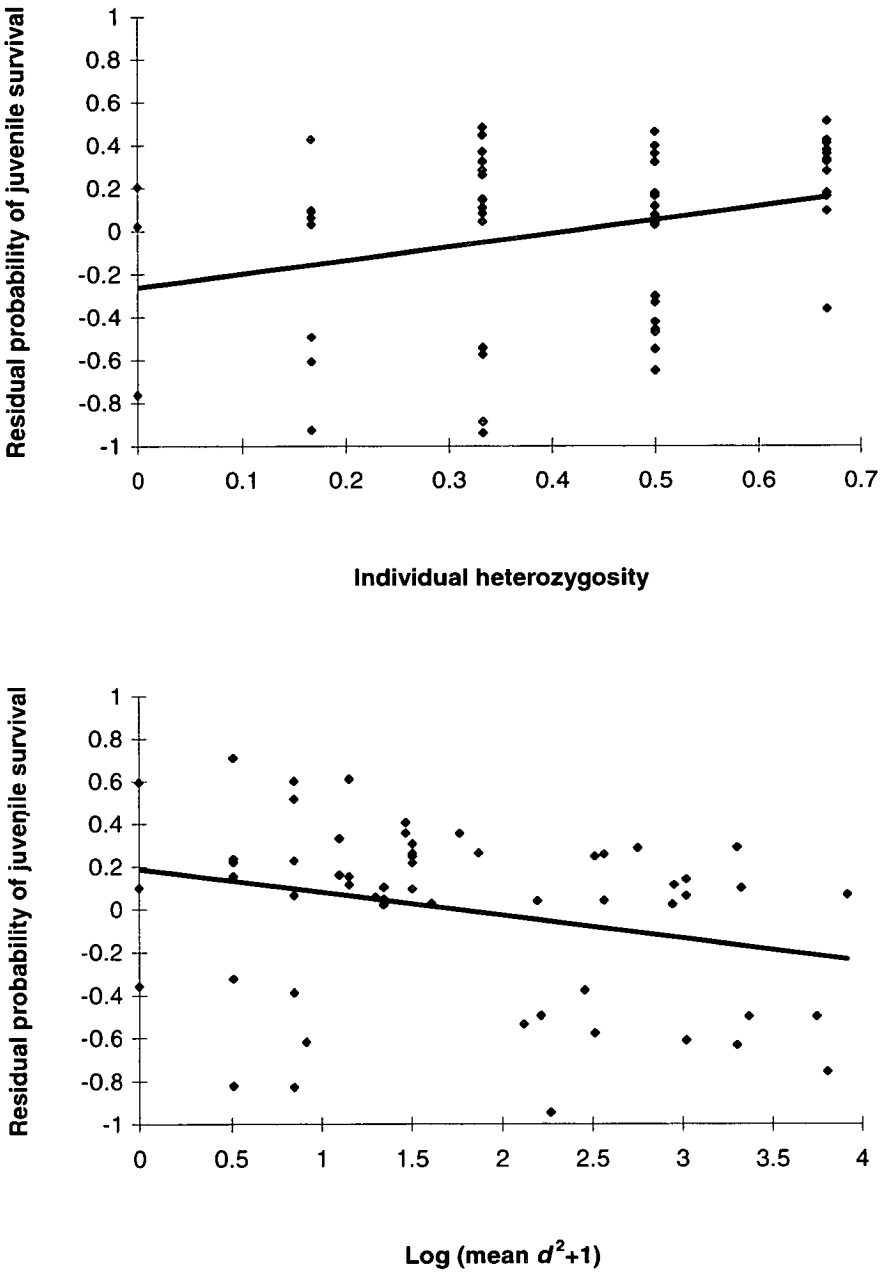

Figure 8.4 *Plot of residual probability of juvenile survival (a) against individual heterozygosity in a model with crude protein and mean d^2 fitted, and (b) against mean d^2 in a model with crude protein and individual heterozygosity already fitted.*

Both the effect of inbreeding and the effect of outbreeding are of similar magnitude to the effect of crude protein content of the vegetation, the principal environmental determinant of juvenile survival identified by Spalton (1995). There is no evidence for

interactions between crude protein and individual heterozygosity or mean d^2 (cf. environmental dependency of inbreeding depression discussed in section 1.3.3).

Mother's reproductive status and grandmother's origin (two other factors identified by Spalton (1995) – see Table 8.1) did not explain independent variation in this model, but there was a trend for calves with immigrant grandmothers to have lower survival. The fact that these terms were not significant in this analysis may be explained by the smaller sample size in this study (n = 57) compared with the sample size in Spalton's (1995) analysis (n = 173).

8.4 Discussion

8.4.1 Hybridisation of Arabian oryx with other oryx species

The finding of a 17-19 Robertsonian translocation in Arabian oryx (Cribiu *et al.* 1990) ignited speculation that hybridisation of Arabian oryx with other oryx species may have occurred, and that hybrid genes had introgressed into the herds destined for reintroduction in Saudi Arabia and Oman. Speculation was fuelled by the fact that North African scimitar-horned oryx, which also have a normal chromosomal complement of $2n$ = 58 (Cribiu *et al.* 1990), carry coincidental rearrangement involving chromosome 17 (Spalton 1992). However Robertsonian translocations are the primary mode of chromosomal evolution in Bovoidea (Wurster and Benirschke 1968) and hybridisation is not necessary for their formation. Furthermore all Arabian oryx carrying the 17-19 Robertsonian translocation can be traced back to the herds of Arabian oryx in Qatar in the 1970s (Cribiu *et al.* 1991), and there is no evidence that any other oryx species were present in Qatar at that time (Jones 1990).

Hybridisations of Arabian oryx with other oryx species are known to have occurred in other parts of the Middle East. Hybridisation between a male beisa oryx (*Oryx gazella beisa*) and a female Arabian oryx in Kuwait in the 1960s led to the death of the female at parturition (7.4.8). Hybridisation between beisa oryx and Arabian oryx also occurred at Thumamah, Saudi Arabia some time after the founding of the farm in the late 1970s. At least some of the hybrids were viable (KKWRC Report, March 1987). First or second generation hybrids would have been phenotypically distinguishable

from the Arabian oryx, and it is very unlikely that any such animals would have been left among the Arabian oryx when intensive management of the farm at Thumamah began in 1986. Hybridisation has also occurred between North African scimitar-horned oryx and Arabian oryx at Al Areen Wildlife Sanctuary in 1985-6, but these were all put down in 1987 (J. Samour, pers. comm.). Recent reports of hybridisation at Al Ain zoo in the UAE (7.4.9) have been discounted by another observer, who suggested that pale markings and curving horns were a result of inbreeding rather than cross-breeding.

In summary, hybridisation of Arabian oryx with both beisa oryx and scimitar-horned oryx has occurred on several occasions in the Middle East. However fertile hybrids have never been explicitly reported, and on current evidence it seems unlikely that genetic material from other oryx species has introgressed into any of the documented captive populations of Arabian oryx in the Middle East.

8.4.2 Population differentiation and loss of variation

The magnitude of R_{ST} values is consistent with known ancestries of the herds. The Taif population in the sample was founded almost entirely from animals born at Thumamah (the offspring of one male from Bahrain and one male from San Diego Wild Animal Park (SDWAP) are the only individuals in the Taif sample with any non-Thumamah ancestry). No significant differentiation was found between these two groups. All three Middle Eastern populations have a major component of ancestry from the SDWAP population, but significant input from other sources, and showed intermediate levels of differentiation from the SDWAP group. Finally, the Omani and two Saudi populations also have ancestry from the two major groups in Qatar (Al Sulaimi and Al Wukayr), but via independent sampling events. The highest divergence was shown by the two Saudi populations from the Omani population. To put these results in perspective, all R_{ST} values are relatively low and imply an effective migration rate of at least one individual per generation (data not shown).

Three samples of Arabian oryx horn collected on the Jiddat-al-Harasis, each believed to originate from a separate wild oryx from the pre-extinction population in Oman, were available. While the DNA yield from these desiccated samples which endured at least 20 years in the harsh conditions of the Arabian desert was low, it was

nevertheless possible to amplify microsatellites at five of the six loci for one of these samples (Table 8.15). At two of the five loci amplified, the individual was heterozygous for one allele found in contemporary oryx and one allele not found in contemporary oryx, and at another locus, the individual was homozygous for an allele not found in contemporary oryx. In other words 40% of the alleles in this sample represent genetic variants absent in contemporary Arabian oryx populations. This finding suggests the following. First, the observed genotype is consistent with the ancient sample being from an Arabian oryx, as it is improbable that a sample from any other species (e.g. sympatric mountain gazelles), would exhibit such a high level of band-sharing with contemporary Arabian oryx. Second, the genotype is not derived from contaminant DNA from one of the contemporary oryx samples, as alleles not found in contemporary oryx are found at some loci amplified from the ancient sample. Third, and most important, genetic variation existed in the pre-extinction population that is not represented in contemporary Arabian oryx derived from captive breeding programmes. The loss of microsatellite variation could be explored in more detail by obtaining samples from specimens of Arabian oryx preserved in museum collections.

Locus	Genotype	Comments
RBP3	136/136	136 allele not seen in contemporary oryx
OarFCB304	121/121	
MAF50	153/155	
MAF46	112/118	112 allele not seen in contemporary oryx
OarCP26	141/157	157 allele not seen in contemporary oryx
OarHH64	-	Locus could not be amplified

Table 8.15 *Genotype of sample from pre-extinction population in Oman. As only a very small amount of DNA could be extracted from the sample, autoradiograph bands tended to be faint relative to contemporary samples. This was especially true for RBP3, although for this locus the same genotype was derived from two independent PCR reactions.*

8.4.3 Inbreeding depression and outbreeding depression in wild Arabian oryx

Using data on Arabian oryx born at Yalooni, Mace (1989) compared average inbreeding coefficient of 12 calves that had died and 27 calves that had survived their first year of life. Those that died had a significantly higher mean inbreeding coefficient that those that survived (t = 2.52; d.f. = 37; $p < 0.02$). Spalton (1993) carried out a similar analysis of inbreeding in relation to juvenile survival based on all oryx born at Yalooni 1981-1992 and found a non-significant trend for lower survival among those with higher inbreeding coefficients (Mann-Whitney U = 1504; p = 0.096).

Both these analyses included oryx that were born in the pre-release enclosure where supplementary feed and water was available, and oryx that were born in the desert during the drought that ended in June 1986, when supplementary feed was also made available. Furthermore, during the early 1980s, the released Arabian oryx remained in an unnaturally rigid herd structure (Stanley Price 1989), and opportunities for individual oryx to mate outside the each herd were rare. My analysis of juvenile survival included only calves born 1987-1993. No supplementary feed or water were given to the oryx during this period, so the population was exposed to the rigours of natural selection in the desert environment. The social structure of the oryx was much more fluid from 1986 onwards (Stanley Price 1989; Spalton 1993), and probably represents a more natural social organisation of the species in this habitat.

The pedigree-based inbreeding coefficient data showed no significant effect of inbreeding on juvenile survival of calves born 1987-1993. In contrast the microsatellite-based measures individual heterozygosity and mean d^2 together yielded a surprising result. Reintroduced Arabian oryx in Oman exhibit both inbreeding depression and outbreeding depression for juvenile survival. Apart from male red deer calves (Coulson *et al.* submitted), this is the first time that outbreeding depression has been detected within any wild vertebrate species. In captive *Callimico* (Goeldi's monkeys), Lacy *et al.* (1993) analysed neonatal mortality and found outbreeding depression was occurring in addition to inbreeding depression, with the magnitude of the inbreeding depression a factor of 10 larger. In Arabian oryx, inbreeding depression is of similar magnitude to outbreeding depression: individual heterozygosity and mean d^2 each explain around 10% of the variation in juvenile survival.

Loss of fitness in wide crosses could arise from breakdown of co-adapted gene complexes by recombination in the offspring of distant crosses, leading to reduced fitness of their progeny (Templeton 1986), also called the epistasis model (Schierup and Christiansen 1996). Alternatively outbreeding depression might be driven by an ecological mechanism, local adaptation, so that wide crosses generate offspring carrying some alleles maladapted to each parental environment (Waser 1993a). The founders of the Arabian oryx in Oman originated from different parts of the Arabian oryx's former range and the finding of outbreeding depression suggests that prior to extinction in the wild Arabian oryx had diverged co-adapted gene complexes or were to some extent locally adapted.

Overall there is evidence for both inbreeding depression and outbreeding depression acting on juvenile survival in reintroduced Arabian oryx in Oman, but non-genetic effects, especially drought (accounted for in the statistical models as crude protein content of the vegetation), are also important determinants of juvenile survival. It is possible that inbreeding and/or outbreeding have an influence on other life history traits such as female fecundity and male reproductive success, but there are insufficient data available to assess this.

8.4.4 Genetic and environmental determinants of population viability

Nothing can be done to resurrect genetic variation lost as a result of the extirpation of Arabian oryx from the wild. However neutral variation such as microsatellite polymorphism is the type of variation that is most easily lost and provides a worst-case estimate of loss of selectively important variation (Chapter 9). In particular, variation in quantitative traits is much less easily lost (Lande and Barrowclough 1987). The key question is how well Arabian oryx that have lost variation as a result of a bottleneck can adapt and survive in the wild. Judging by the rapid increase of Arabian oryx reintroduced to the Jiddat-al-Harasis in Oman (Figure 7.4) despite episodes of prolonged drought (Stanley Price 1989; Spalton 1993), there do not appear to be intrinsic genetic factors holding back the species from successful rehabilitation to the wild. Both inbreeding depression and outbreeding depression act on juvenile survival in these animals but these effects do not appear to prevent the population in Oman from increasing at a rapid rate. Given the current population size (n = 399) it

does not seem likely that the impacts of inbreeding depression and outbreeding depression are likely to increase greatly in the future.

Three factors do limit future growth of this and other reintroduced populations of Arabian oryx. These are drought (Stanley Price 1989; Spalton 1993), poaching (Lawrence 1996; Rowe 1997) and encroachment of human development and domestic livestock on oryx habitat (Spalton 1993; Lawrence 1996). Of these, poaching may represent the greatest threat as this was the cause of the Arabian oryx's original extirpation. Furthermore poaching has the potential to afflict all reintroduction projects across the Arabian peninsula simultaneously if demand for live oryx increases beyond its current level.

In general, I suggest that a useful distinction may be drawn between intrinsic and extrinsic threats to population viability. Intrinsic threats include genetic and demographic uncertainty (Shaffer 1987; Lande 1988, 1993, 1995), while extrinsic threats include environmental uncertainty (Shaffer 1987; Lande 1993) and human influences: Diamond's 'evil quartet' of overkill, habitat destruction and fragmentation, introduced species and chains of extinction (Caughley 1994). There is no evidence that Arabian oryx are currently under threat from intrinsic factors (although demographic uncertainty has not been tested explicitly), while at least some extrinsic factors, principally drought and poaching, at times limit population growth (7.5.4) and have the potential to send the population into decline. A characteristic of extrinsic factors is their unpredictability. Intrinsic factors may be more analytically tractable, but extrinsic factors probably represent more common primary threats to population persistence, at least for populations such as the Arabian oryx with census sizes in the hundreds.

8.5 Summary

The Arabian oryx provides an opportunity to study the effects of loss of variation and inbreeding on a species reintroduced to the wild after surviving a documented population bottleneck. 343 Arabian oryx representing most of the major groups of the species world-wide were typed at six microsatellite loci. Significant but small population differentiation was found between most groups and there was no evidence

for hybridisation between Arabian oryx and other oryx species. 40% of alleles in one Arabian oryx sample from the pre-extinction population in Oman were not found in any contemporary Arabian oryx, suggesting that the species has lost neutral variation through the bottleneck. Inbreeding was common in reintroduced Arabian oryx in Oman, with parent-offspring and father-daughter matings accounting for 10% of the total. The microsatellite-based measures individual heterozygosity and mean d^2 revealed inbreeding depression and outbreeding depression to be occurring in this population simultaneously, with drought also being an important determinant of juvenile survival. However there is no evidence to suggest that lack of genetic variation, inbreeding depression or outbreeding depression are likely to slow population growth of reintroduced Arabian oryx in Oman.

9 Genetics and conservation - a critical perspective

9.1 Introduction

Genetics is relevant to conservation in two ways. First, genetic factors may influence the probability of decline or extinction of species or populations. Second, molecular techniques may be used to inform management decisions, and also to clarify the influence of genetic factors on a particular species or population. In this chapter, I attempt to draw together recent theoretical and experimental work on these topics with the empirical research discussed in Chapter 1 and in the light of the results from my work with red deer and Arabian oryx. Although the chapter extends beyond the immediate scope of my own research, I feel that it is important to set in context results on inbreeding and outbreeding in wild ruminants with concurrent research on other genetic processes and techniques of relevance to conservation of species in their natural habitats. Inevitably, some sections of this chapter are speculative, and are intended to be thought-provoking rather than definitive.

9.2 Genetic factors in species and population viability

Two genetic forces, inbreeding depression and loss of adaptive genetic variation, have long been recognised as possible threats to populations of animals and plants at low numbers. Outbreeding depression has also been discussed (Templeton 1986) but hitherto has had no empirical support from work on wild mammals or birds. Recently another genetic force has been suggested as a potential threat to small populations. Genetic drift is a stronger force in small populations than in large populations, and slightly deleterious mutations are therefore more likely to accumulate in small populations where selection is less effective in removing them relative to drift. If sufficient accumulation of mutations occurs, the mutational load may be sufficient to drive the population into negative population growth and rapidly to extinction, a process described as mutational meltdown (Lynch *et al.* 1995).

For conservation purposes, it is not viable individuals but rather viable populations that are important (Soulé 1987). The existence in a population of individuals with fitness reduced by genetic factors does not necessarily mean that the population is in imminent risk of extinction. In this section I examine the relationship between genetic factors and individual fitness, and how this relates to the conservation of natural populations.

9.2.1 Inbreeding and outbreeding

A major finding of this thesis and of Coulson *et al.* (submitted) is that outbreeding depression occurs as a consequence of mixing genetic stocks, both in red deer (Chapter 6) and in Arabian oryx (Chapter 8). These observations have important consequences for conservation. First, when initiating captive breeding programmes it is important to ensure a sufficiently broad founder base to avoid close inbreeding (Ralls *et al.* 1979) but equally it may be important to avoid mixing founders from distant geographical areas (Lacy *et al.* 1993). Second, when translocating wild animals from one area to supplement a dwindling population in another area, there is a risk that the offspring of individuals from a distant source may be maladapted to the local environment (Greig 1979).

Several authors have described inbreeding and outbreeding as being part of a continuum (Ralls *et al.* 1986; Shields 1993; Waser 1993b). There are alternative views on the form of the fitness function along this continuum (Knowlton and Jackson 1993), illustrated in Figure 9.1. The traditional view is that significant inbreeding depression occurs under close inbreeding, followed by a large plateau of high-fitness outcrossing and a step-like drop at the species boundary. On the other hand a habitually inbreeding species (e.g. a predominantly selfing plant) may suffer relatively little inbreeding depression (Husband and Schemske 1996), but on the other hand may have relatively restricted gene flow leading to genetic isolation by distance. In this context local adaptation can occur, leading to a steady decline in fitness with more distant outcrossing events (e.g. the ascidian *Botryllus schlosseri*, Grosberg 1987, 1991), the process called outbreeding depression. A third model incorporates elements of both the first two, with inbreeding depression accompanying close inbreeding but

outbreeding depression being evident in more distant crosses. This model has been called optimal inbreeding (Shields 1983) or optimal outbreeding (Bateson 1983).

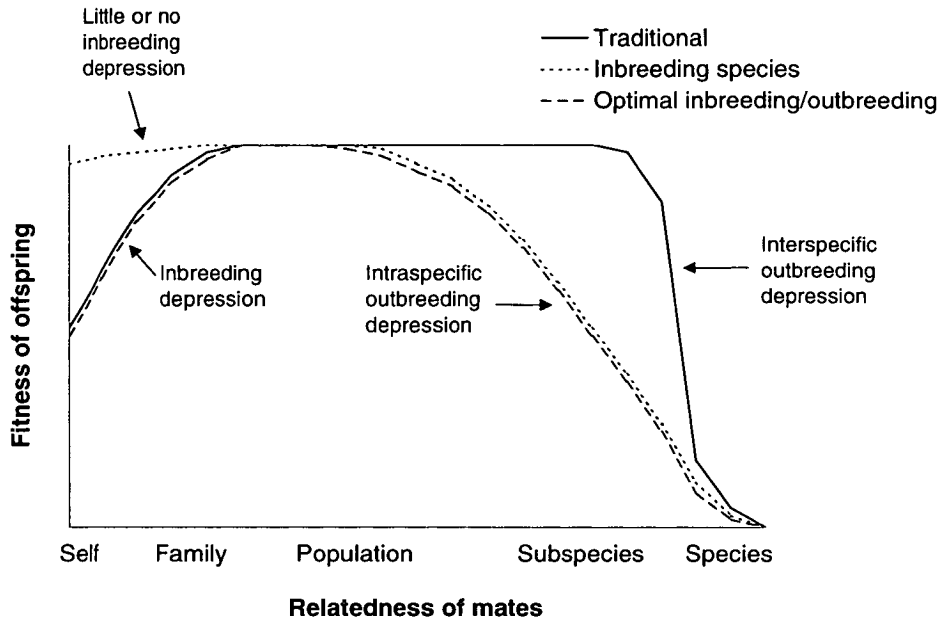

Figure 9.1 *The continuum of inbreeding and outbreeding, illustrated as the relationship between the degree of relatedness between mates and the fitness of their offspring. Adapted from Knowlton and Jackson (1993).*

Figure 9.1 makes it obvious that the mating patterns of natural populations of animals and plants should evolve towards a fitness optimum (Lande and Schemske 1985). However the form of the fitness function is malleable, and almost certainly varies considerably both between species and spatially and temporally within species. Even if the mating system is optimal, relatedness of individual pairs of mates is likely to vary around the optimum for stochastic reasons or because the optimal mating system involves some kind of mixed strategy (e.g. dispersive morphs in the naked mole rat: O'Riain *et al.* 1996). Consequently a certain level of inbreeding depression and outbreeding depression is likely even in natural populations with optimal mating systems. What the results of Coulson *et al.* (submitted) and this thesis seem to show is that the traditional view is wrong, in other words that there is intraspecific outbreeding depression in species of conservation interest.

While it is interesting to unravel the effects of inbreeding and outbreeding at the individual level, it is the consequences of these effects on population viability which are important for conservation. It is quite clear that both red deer and Arabian oryx can tolerate both inbreeding depression and outbreeding depression, being populations stable at carrying capacity (red deer) or increasing (Arabian oryx). There is only one convincing study examining the consequences of inbreeding depression on *population* viability (Saccheri *et al.* 1998). Saccheri *et al.* showed that small populations of Glanville fritillary butterfly (*Melitaea cinxia*) with lower allozyme heterozygosity were more likely to go extinct than populations with higher heterozygosity, and this effect was independent of population size. Many more studies of this type are needed, but practical difficulties are formidable since many replicate populations are required of a species in which local extinction can be unambiguously determined.

9.2.2 Maintenance and loss of genetic variation

In this section, I summarise the theory underpinning the maintenance and loss of quantitative genetic variation in finite populations, on the assumption that the majority of adaptive evolution is based on quantitative characters (Lande and Barrowclough 1987).

If an ideal population has N individuals reproducing by random union of gametes in discrete generations, the effective population size N_e may be defined as the number of individuals in an ideal population that would have the same rate of random genetic drift as in the actual population (Lande 1988). Assuming no mutation, heterozygosity is lost under genetic drift according to the equation:

$$\frac{H_t}{H_o} = \left(1 - \frac{1}{2N_e}\right)^t \qquad \textit{Equation 9.1}$$

where H_t is the heterozygosity after t generations (Falconer and Mackay 1996). Given the definition of effective population size given above, one can compute the effect of unequal sex ratios, variance in reproductive success, fluctuations in population size and overlapping generations on loss of neutral genetic variation in populations of different sizes (Chepko-Sade *et al.* 1987; Lande and Barrowclough 1987; Harris and Allendorf 1989; Nunney 1991; Nunney and Campbell 1993; Nunney and Elam 1994;

see also Vucetich *et al.* 1997). In nearly all species the effective population size is less than the census population size, and a recent review suggested that an average value for the ratio N_e/N is only around 10% (Frankham 1995). The equations upon which these calculations are based assume that the effects of different factors on N_e are independent, but recent data on Soay sheep suggests that subtle interactions between factors may mitigate the effects of genetic drift in natural populations (Pemberton *et al.* 1996).

Noting that additive genetic variation responds to genetic drift in the same way as heterozygosity (i.e. following Equation 9.1), Franklin (1980) suggested that an effective population size of 500 would be sufficient for mutation to mitigate loss of genetic variation for quantitative traits due to genetic drift. This "magic number" of 500 has been widely cited in the conservation literature and incorporated into guidelines for assessing population viability (Mace and Lande 1991). Lande and Barrowclough (1987) point out that an equilibrium between mutation and drift can occur at any population size, but show that an effective population size of approximately 500 is necessary to maintain a heritability as high as 0.5 for an additive genetic trait under mutation-drift balance.

These calculations are based on changes in additive genetic variation for bristle number in *Drosophila melanogaster,* a trait for which mutation and genetic drift might be expected to be prevailing forces. However it is not clear that a simple mutation-drift model is relevant to adaptive variation, since adaptive variation is more likely to be influenced by natural selection as well as genetic drift. Calculations of effective population size only have relevance to the maintenance of adaptive variation if genetic drift has greater influence on adaptive variation than natural selection.

Most adaptive variation is thought to be in the form of quantitative variation under stabilising selection (Lande and Barrowclough 1987). The ability of natural selection to maintain quantitative genetic variation in natural populations is unclear, but population substructure (Goldstein and Holsinger 1992) and a fluctuating environment (Kondrashov and Yampolsky 1996) are both capable of producing higher levels of quantitative variation under stabilising selection than would be expected in panmictic populations of the same size in a constant environment. Prescriptions for maintaining

adaptive genetic variation in small populations based only on the balance between mutation and genetic drift do not seem sufficiently rooted in reality.

It has been suggested that adaptability may be quantified via estimations of heritability of fitness traits (Storfer 1996). However estimating heritability in natural populations is notoriously difficult, and aside from the difficulty of collecting fitness data from known relatives while controlling for shared-environment effects, reliable heritability estimation also requires large sample sizes which might be difficult to achieve for most species of conservation interest, especially in the wild (Barton and Turelli 1989). Furthermore the technique will only assess variation for fitness in the environment in which heritability is measured. Adaptive variation may have no measurable fitness consequences in one environment but be of profound importance in another environment. Barton and Turelli (1989) stress that quantitative genetic variance exhibits strong environmental dependence and therefore cannot be expected to reveal long-term genetic potential. It therefore seems unlikely that adaptability can be quantified in a way that is meaningful in conservation.

If maintenance of adaptive genetic variation is to be a primary aim of conservation of species in the wild, it is essential to achieve a better understanding of how adaptively important quantitative genetic variation is maintained in finite populations existing in a fluid adaptive landscape, and to gather data on quantitative genetic variation for fitness in a wide range of organisms. In particular, we know very little about how selection operates during ecologically-driven population declines. A working hypothesis might be that selection is increased in periods of population decline. If this is the case, increased selection for outbred individuals may allow more variation to be maintained through the period of decline than would be expected under neutral models. On the other hand increased selection may be directional, and this would have the effect of removing variation more rapidly than would be anticipated by neutral models.

At the same time it is important to recognise that there is cost to overestimating the size of population required to maintain an appropriate level of adaptive variation. There is very little evidence that maintaining a species at effective population sizes significantly smaller than the traditional "magic number" of 500 for many generations will in fact compromise its present or future persistence and adaptability. By having a

high target (particularly Lande's (1995) recommendation of $N_e \geq 5{,}000$), we may decide not to attempt to conserve a population or species that may be perfectly viable, or we may abandon the idea of setting up a reserve because its size is deemed insufficient to maintain a genetically viable population of the species which the reserve is intended to protect.

9.2.3 Mutation accumulation

Mutation is a constant process occurring in all organisms, and is ultimately the source of all genetic variation upon which natural selection subsequently may act. Most mutations are thought to be either neutral or deleterious. Truly neutral mutations are of no consequence to the organism which bears them (but may be useful molecular markers for research scientists). On the other hand lethal or very deleterious mutations are rapidly selected out of any population in which they arise, even when the population is small (Keightley *et al.* 1998). Recent attention has been focused on the possible importance of mutations of intermediate effect, those which are slightly deleterious or quasi-neutral.

Genetic drift is caused by the random sampling of parental alleles to generate the genotypes of offspring. A larger sample more accurately captures the variation in the preceding generation, meaning that genetic drift will occur more rapidly in small populations than in large ones (Equation 9.1). If the force of natural selection acting on individuals is of equal strength in small and large populations, the force of genetic drift will be stronger relative to natural selection in small populations. The implication is that slightly deleterious mutations are more likely to be expelled from large populations under natural selection, while in small populations they may reach fixation under genetic drift despite the simultaneous action of natural selection.

Most work on mutational load and genetic drift in small populations has tended to assume that the small populations are founded from large populations, and considers only the effect of small population size in the context of a pre-existing mutational load derived from the large population. However it has been suggested that new mutations acquired while populations are at low numbers might have a significant impact on long term population viability. Lande (1994) and Lynch *et al.* (1995) investigated this problem analytically using data on frequency of mutations and their effect on viability

in *Drosophila* (Mukai *et al.* 1972). Lande and Lynch *et al.* found that for populations of census sizes of 100-1000, typical of the levels at which legislative protection is applied to populations or species, there is a substantial extinction risk arising from the fixation of novel mutations. Under a wide range of conditions, populations with effective population size of 100 (i.e. having a census size of approximately 1000, assuming Frankham's (1995) average value of around 0.1 for the ratio N_e/N) have a mean time to extinction of a few hundred generations. Lynch *et al.* describe mutation accumulation in terms of a two stage process. First there is a longer period of gradual accumulation leading to progressive slowing of population growth, followed by a relatively short period when negative population growth sets up a positive feedback between population decline and mutation accumulation leading to rapid extinction. The second stage Lynch *et al.* (1995) termed mutational meltdown, and potentially forms another vortex to add to Gilpin and Soulé's (1986) description of the extinction process. On the basis of their analyses, Lande (1995) and Lynch *et al.* (1995) suggest that many current conservation management plans are inadequate to ensure that the protected species are secure from the threat of mutation. If these authors' models are correct, the implications for long-term species conservation are devastating.

Recently new experimental investigations of mutation accumulation in *Drosophila melanogaster* have been published. Shabalina *et al.*'s (1997) results were similar to those of Mukai *et al.*'s (1972) data and hence support Lande (1994, 1995) and Lynch *et al.*'s (1995) models. However Gilligan *et al.* (1997) found no relationship between population size and rate of mutation accumulation. It is also possible that the mutation rate in *Drosophila melanogaster* is not representative of the mutation rate in other species. Keightley and Caballero (1997) found that the deleterious mutation rate in *Caenorhabditis elegans* is two orders of magnitude lower than that found in *Drosophila.* Furthermore Keightley and Caballero found that the total mutational load was not dominated by a large number of slightly deleterious mutations, as assumed in the models of Lande (1994) and Lynch *et al.* (1995).

The recent work on mutation accumulation neglects two important assumptions. First, selection is presumed to be a linear function of mutational load. If some form of truncation selection operates, in which all individuals with mutational load greater than a certain level fail to reproduce, the dynamics of mutation accumulation are

likely to be very different. Little data is available suggesting what form selection functions might take in natural populations, but in any population where many individuals fail to reproduce (e.g. red deer: Chapter 6), there is certainly potential for truncation selection. Second, selection is assumed to be independent of population size. If the force that is keeping a population small exerts a stronger selection pressure than would be found in another larger population of the same species, selection would be inversely related to population size, and the effects of drift in small populations would be mitigated by higher selection coefficients.

Lande (1994) and Lynch *et al.* (1995) have identified a possible genetic risk incurred by populations of wild animals and plants at low numbers. However current theoretical models are based on empirical data for which interpretation is in some doubt (Keightley *et al.* 1998), and experimental work testing the new models has produced contradictory results. The pessimistic conclusions of Lande (1994, 1995) and especially Lynch *et al.* (1995) do not currently seem well justified.

9.3 Use of molecular markers in conservation

Journals such as *Molecular Ecology* and *Conservation Biology* contain increasing numbers of papers describing the application of molecular techniques to endangered species. To the conservation biologists wishing to investigate a new study species, the application of molecular techniques may seem almost a necessity to give credibility to the conservation programme. However it may be case that a molecular approach is not required at all, and if it is, it may not be clear how to choose between the many techniques available.

There are just two major uses of molecular techniques in conservation. The first is to use variation to uncover relationships between individuals, groups, populations, races or species. The second is to relate variation to fitness or viability of individuals, groups, populations, races or species. In broad terms the study of relationships is easier since the samples themselves, providing they are representative, carry all necessary information, and, of equal importance, there is a solid theoretical base upon which results can be interpreted. To study variation in relation to fitness or viability, some measure of fitness or viability is required in addition to a measure of variation.

While it may be straightforward to demonstrate some correlation between fitness or viability and variation, it may be difficult to convincingly demonstrate a causative link.

In this section, I want to focus on the suitability of molecular approaches to answering questions in conservation management. I consider separately the use of markers to infer relationships between groups and to correlate variation with fitness. I also outline a checklist that may help evaluate whether or not a molecular approach is appropriate to any particular conservation problem.

9.3.1 Relating groups and individuals

The most popular use of molecular techniques in conservation is for phylogenetic analysis at the species level or between higher taxonomic groups (Avise 1989). In particular analysis of DNA sequence data is an immensely powerful method for probing the ancestral relationships of groups at many taxonomic scales (Moritz 1994). Only a small number of samples are required, and automatic sequencing makes the data collection routine. There are now many computer programs to aid analysis of sequence data, typically presenting results in the form of a phylogenetic tree (e.g. PHYLIP, PAUP). The major difficulty is to decide whether or not the sequence data has allowed identification of the correct tree from the very large number of possible trees. Bootstrapping (Felsenstein 1985) is the standard first step, and confidence may be further increased by obtaining the same topology from two or more independent sequences, or if molecular and morphological data give concordant trees. Moritz (1994) cautions against exclusive reliance on trees from mitochondrial DNA data.

Often sequences are used to assess the genetic distance separating pairs of taxonomic groups. Interpretation can be controversial if the objective is to turn distance estimates into absolute time, as this requires assumptions about the rate of molecular evolution (Avise 1994). Nevertheless sequence data can often be used to decisively resolve taxonomic uncertainties in a conservation context (Avise 1989).

Relationships within species are more commonly determined using marker polymorphisms, either at a series of individually defined loci (allozymes, single-locus minisatellites, microsatellites, RFLPs, AFLPs) or at multiple anonymous loci

simultaneously (multilocus minisatellite fingerprinting, RAPDs), although rapidly evolving DNA sequences such as mitochondrial DNA D-loop may also be used. Techniques vary in their expense, ease-of-use, reliability and informativeness, and detailed reviews comparing individual techniques are available elsewhere (Queller *et al.* 1993; Schlötterer and Pemberton 1994). Two general comments will be made here. First, PCR-based techniques allow analysis of a wider range of tissues, including faeces, hair, museum specimens and samples preserved by mud, ice or desiccation (Höss *et al.* 1992; Morin *et al.* 1994; Taylor *et al.* 1994; 8.4.2). Second, single-locus techniques give data more amenable to computer coding and statistical interpretation (Queller *et al.* 1993), but with the exception of allozymes, may be more expensive because several separate loci are normally required to allow meaningful analysis. Microsatellites are probably the most popular technique for within-species work at present.

At the level of populations and races, many computer programs are available to aid analysis of single locus data in terms of the traditional genetic differentiation measure F_{ST} (1.2.1) (e.g. GENEPOP: Raymond and Rousset 1995), and marker data can often be used to identify substructure within populations as well as relationships between populations. A program is also available to calculate microsatellite-specific differentiation measures R_{ST} and $\delta\mu^2$ and estimate migration rates between populations (Goodman 1997; 8.3.2).

At the level of groups and especially individuals, markers need to show high levels of polymorphism to be informative. Large-scale sampling also becomes necessary in order to probe population structure at this fine scale. Two types of analysis can be carried out: relatedness estimation and parentage analysis. Relatedness estimation relies on the principle that related individuals have increased probability of sharing alleles or haplotypes identical by descent. At its simplest level relatedness between pairs of groups or individuals involves only the calculation of band-sharing coefficients (e.g. multilocus minisatellite fingerprinting). Single-locus techniques allow allele frequency information to be incorporated into the calculations (Queller and Goodnight 1989), which at least in principle increases the power of the analysis (Blouin *et al.* 1996). Parentage analysis uses the principle of Mendelian segregation of alleles from parents to offspring in order to identify putative parents from other

individuals. Barring mutations, null alleles and typing errors, all offspring alleles must have originated with one or other parent. Again a simplistic approach such as multilocus minisatellite fingerprinting uses only band-sharing information (in some situations this can be highly effective), while single-locus approach allows incorporation of frequency information (Chapter 3) and is more amenable to large scale study. If the parentage of all individuals can be inferred, a pedigree can be constructed which allows direct calculation of all pairwise relatedness coefficients. Computer programs for analysis of both relatedness (RELATEDNESS and KINSHIP, Queller and Goodnight 1989) and parentage (CERVUS, Marshall *et al.* 1998) using single locus data are now available. It should be stressed that the power of molecular markers to estimate relatedness or resolve parentage is often surprisingly low (Pamilo 1989; Lynch and Milligan 1994; Figure 4.1). This tends to be a particularly acute problem in species of conservation interest which often have relatively low levels of neutral marker diversity (Mace *et al.* 1992; 8.3.1).

It is important to consider how the array of techniques just described can be applied to specific conservation problems. I mention just three applications here; there are many others. First, molecular techniques have great potential for inferring past and present demographic processes (Milligan *et al.* 1994), and demography has frequently been highlighted as being a crucial component in conservation (e.g. Lande 1988). Second molecular techniques can be used to identify both Evolutionarily Significant Units (i.e. distinct taxonomic groups) and Management Units (i.e. distinct populations of a single species) (Moritz 1994), clarifying management priorities. Third, molecular techniques have been used on a number of occasions to identify poached wildlife (Arabian oryx: Marshall 1997; marine mammals: Baker and Palumbi 1994, Baker *et al.* 1996; British raptors: J. Wetton pers. comm.), and as such may act as a deterrent to poaching which is the primary threat for many endangered species.

9.3.2 Correlating marker variation with individual fitness or population adaptability

Molecular marker variation has been correlated both with individual fitness and proposed as an indicator of population adaptability. First, individual marker heterozygosity or other molecular measures have been used in place of individual

inbreeding coefficients and correlated with individual fitness measures to uncover the presence of inbreeding depression within populations (Allendorf and Leary 1986; Mitton 1993; Coltman *et al.* in prep; note that many of the studies reviewed by Allendorf and Leary and Mitton infer a relationship between heterozygosity and fitness from changes in population genotype frequencies rather than by demonstrating a correlation between heterozygosity and fitness of individuals). Outbreeding depression can be studied similarly. Second, markers have been used to compare levels of variation between species or populations in order to identify species or populations which may carry less adaptive variation and therefore may be more vulnerable to extinction (e.g. O'Brien *et al.* 1985; Quattro and Vrijenhoek 1989).

The use of neutral marker heterozygosity as a surrogate for the inbreeding coefficient has a sound theoretical basis (Falconer and Mackay 1996), and is equally applicable to individuals as it is to populations. Protein polymorphisms have been widely used, but these often have poor statistical power (even outbred individuals tend to be homozygous at most loci), and furthermore protein polymorphisms may not behave in a selectively neutral way (Powell and Wistrand 1978; Pemberton *et al.* 1988, 1991; Gulland *et al.* 1993; Bancroft *et al.* 1995). DNA-based marker polymorphisms such as microsatellites have higher individual variability and are more nearly neutral (although linkage of microsatellites to loci under selection (Slatkin 1995a) may lead to departure from neutral expectations). It is therefore surprising that no published studies have correlated individual heterozygosity across microsatellite markers with measures of fitness.

In contrast to heterozygosity, the theoretical basis of the microsatellite-based measure mean d^2 (Coulson *et al.* 1998b) as an individual-based measure of inbreeding and/or outbreeding has not been thoroughly explored. However mean d^2 has been found to be correlated with individual fitness where individual heterozygosity is not (Coulson *et al.* 1998b, submitted; Coltman *et al.* 1998a; Chapter 6). Mean d^2 should be considered in addition to rather than as an alternative to individual heterozygosity (Chapter 8). It is clear that mean d^2 is capable of measuring some important property or properties of the variation in a diploid genome, and is a convenient and useful measure for the study of natural populations. Future theoretical and experimental effort could usefully be directed towards understanding exactly what it is that mean d^2 is measuring.

Although genetic factors may not be the most important problem facing small populations (Lande 1988), molecular markers can be helpful for identifying the fitness consequences of inbreeding and outbreeding in populations of conservation interest where pedigree information is either not available or not sufficiently informative (8.3.6). Gathering individual fitness data may represent a greater challenge than estimating inbreeding levels. In particular caution should be exercised in extrapolating the results from early fitness components such as juvenile survival to reproductive traits or overall fitness, given that the impact of inbreeding/outbreeding on different fitness components can vary not only in magnitude but also in direction (Chapter 6).

The use of population-level marker heterozygosity to infer adaptability of different species (and hence extinction risk) has a chequered history (Caughley 1994; Merola 1994). For example, O'Brien *et al.* (1985) argue that because the cheetah exhibits low protein polymorphism relative to other felids, the cheetah as a species is more vulnerable to extinction because it is less able to adapt to novel environmental challenges. However protein variation does not represent most of the adaptively important variation in a species (Hedrick and Miller 1992), and there are numerous examples of species with low protein polymorphism that appear to have viable, self-sustaining populations (e.g. Northern elephant seal: Bonnell and Selander 1974; fallow deer: Pemberton and Smith 1985). The lack of polymorphism at protein loci reflects past demography (Milligan *et al.* 1994) rather than current adaptability, or lack of it (see also Nei *et al.* 1975). Indeed O'Brien and colleagues (O'Brien *et al.* 1983, 1987; Menotti-Raymond and O'Brien 1993) suggest that the observed levels of variability in the cheetah across various types of marker loci resulted from one or more population bottlenecks in the late Pleistocene; Hedrick (1996) points out that the data are equally compatible with the cheetah having a low effective population size due to the species' metapopulation structure. Either way, marker variability should be interpreted in terms of its demographic signal rather than its adaptive significance. Comparison of heterozygosity among populations within a species (e.g. Vrijenhoek *et al.* 1985) may be more informative, especially if differences in mean fitness, or components of fitness, between populations are associated with differences in heterozygosity (Quattro and Vrijenhoek 1989). However demonstration of fitness

differences between populations is not the same as showing that inbreeding is a threat to the viability of those populations.

In summary, molecular markers may be used to assess the relationship between inbreeding or outbreeding and individual fitness, bearing in mind that inbreeding or outbreeding depression rarely represent the primary threat to the persistence of natural populations of any size (Lande 1988, but see Saccheri *et al.* 1998). On the other hand it is not possible to use molecular markers to assess population or species "fitness", as molecular markers indicate demography rather than adaptability. Indeed it is not clear that adaptability can be measured at all (9.2.2).

9.3.3 The role of the scientific method in molecular conservation genetics

Given that the application of molecular techniques is expensive and time-consuming, it is important that they are deployed in conservation with prudence (Mace *et al.* 1992). A number of questions can be posed to help arrive at a decision (Table 9.1).

Step	Question
1	What is the uncertainty which it is hoped molecular techniques can resolve?
2	Is it possible to formulate unambiguous hypotheses which frame the uncertainty?
3	Do appropriate molecular techniques exist with which to test these hypotheses?
4	Are there alternative non-molecular techniques that could be used to test the hypotheses?
5	Do alternative management decisions or plans depend on the uncertainty under consideration?

Table 9.1 *Steps that might help to decide whether or not molecular techniques are appropriate to a particular conservation problem. Steps 1-3 are equally relevant to both general scientific study and conservation management, whereas steps 4 and 5 are more specifically relevant to conservation management.*

Molecular techniques are only of value in conservation if the results obtained are informative. At the outset it is important to be sure that there is a question at all, and that molecular techniques may have some relevance to the question. The second step

is to be sure exactly how the molecular data will resolve the question. If it is not possible to identify alternative hypotheses that may be clearly distinguished using molecular data, it is likely that the initial question is not sufficiently well-defined. Third, it is important to be sure that one or more molecular techniques are available that are likely to deliver the relevant data. This involves evaluating different techniques for reliability, practicality and so on, and also some attempt to evaluate whether or not the technique(s) will yield data with sufficient statistical power to distinguish the hypotheses identified in step 2. To evaluate statistical power, reference can be made to the same techniques being applied in related taxonomic groups. It is important to consider both the number of samples required and the amount of genetic variation required of each sample. For phylogenetic work it is important to have samples from one or more appropriate outgroup, and for assignment of individuals to taxonomic groups it is essential to have reliable reference samples for all of the candidate taxa (Baker *et al.* 1996). Several analysis packages for molecular data offer a simulation function (e.g. CERVUS: Marshall *et al.* 1998; KINSHIP: Queller and Goodnight 1989), and these can be used to estimate statistical power given various assumptions.

If the question defined in step 1 concerns general scientific research, answers to steps 1-3 are sufficient in order to make a decision on whether or not to proceed with a molecular approach. If on the other hand the question defined in step 1 is a conservation management question relevant to one particular taxonomic group, species or population, two additional steps are required. Step 4 concerns value for money. It may be possible to identify some cheaper alternative to molecular analysis that is also likely to be able to resolve the hypotheses stated in step 2. For instance, taxonomic inference may be possible using morphometric analysis rather than DNA sequence data, dispersal could be monitored using radio-tracking rather than via indirect estimates of gene flow or pedigrees could be inferred from behavioural rather than molecular data. Particularly at the individual or population level, substantial ecological data should be collected in any case in order to identify possible threats. Step 5 takes a broader perspective on conservation management. There is little point in spending money on molecular techniques if, for whatever reason, the same

management decision is likely to be made irrespective of the outcome of the analysis, or alternatively if there is no management decision to be informed by the outcome.

An evaluation process such as the one outlined in Table 9.1 could help avoid the temptation to apply molecular techniques in conservation simply because they represent a high-tech solution. Molecular techniques can be highly informative, but only if they are used appropriately.

9.4 Summary

Three genetic processes have been suggested as potential threats to small populations: inbreeding depression, loss of adaptive genetic variation and accumulation of deleterious mutations. Although inbreeding depression may be severe in the artificial structure of a captive populations, there is little evidence that inbreeding depression threatens viability of small natural populations. Loss of adaptive variation is a threat that has been principally evaluated by theoretical study of mutation and genetic drift, but natural selection is likely to play a more significant role in natural populations, and there is little empirical evidence that small populations are compromised by a lack of adaptive variation. Mutation accumulation has recently been suggested as posing a major threat to small populations, but the experimental evidence is contradictory and there are no field data.

Molecular techniques have frequently been used in conservation. In general it is rarely the genetic variation itself which is of interest, but the information that variation conveys about past or present evolutionary or demographic processes. There may be a temptation to use molecular techniques without a clearly defined goal. I suggest a checklist which may help to restrict the use of molecular techniques to those conservation problems in which such techniques are the best approach.

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Appendix

A1 Additional statistics for paternity inference

A1.1 Derivation of likelihoods that take account of typing errors

Defining x_i to be the unknown, true genotype of individual i that has been mis-typed at the locus under consideration, Equation 3.2 becomes:

$$
\begin{aligned}
L(H_1|g_m,g_a,g_o) = (1-e)^3.T(g_o|g_m,g_a).P(g_m).P(g_a) + \\
e(1-e)^2\begin{bmatrix} T(g_o|g_m,x_a).P(g_m).P(x_a) + \\ T(g_o|x_m,g_a).P(x_m).P(g_a) + \\ T(x_o|g_m,g_a).P(g_m).P(g_a) \end{bmatrix} + \\
e^2(1-e)\begin{bmatrix} T(x_o|g_m,x_a).P(g_m).P(x_a) + \\ T(x_o|x_m,g_a).P(x_m).P(g_a) + \\ T(g_o|x_m,x_a).P(x_m).P(x_a) \end{bmatrix} + \\
e^3.T(x_o|x_m,x_a).P(x_m).P(x_a)
\end{aligned}
$$

where e is the error rate. Since x_i can be any genotype, $P(x_i) = 1$ and $T(g_j|x_i) = P(g_j)$ for any i, j, and so this simplifies to:

$$
\begin{aligned}
L(H_1|g_m,g_a,g_o) = (1-e)^3.T(g_o|g_m,g_a).P(g_m).P(g_a) + \\
e(1-e)^2\begin{bmatrix} T(g_o|g_m).P(g_m) + \\ T(g_o|g_a).P(g_a) + P(g_m).P(g_a) \end{bmatrix} + \\
e^2(1-e)\left[P(g_m)+P(g_a)+P(g_o)\right] + e^3
\end{aligned}
\qquad \textit{Equation A1}
$$

Equation 3.3 becomes:

$$L(H_2|g_m, g_a, g_o) = (1-e)^3 . T(g_o|g_m) . P(g_m) . P(g_a) +$$
$$e(1-e)^2 \begin{bmatrix} T(g_o|g_m) . P(g_m) . P(x_a) + \\ T(g_o|x_m) . P(x_m) . P(g_a) + \\ T(x_o|g_m) . P(g_m) . P(g_a) \end{bmatrix} +$$
$$e^2(1-e) \begin{bmatrix} T(x_o|g_m) . P(g_m) . P(x_a) + \\ T(x_o|x_m) . P(x_m) . P(g_a) + \\ T(g_o|x_m) . P(x_m) . P(x_a) \end{bmatrix} +$$
$$e^3 . T(x_o|x_m) . P(x_m) . P(x_a)$$

which simplifies to:

$$L(H_2|g_m, g_a, g_o) = (1-e)^3 . T(g_o|g_m) . P(g_m) . P(g_a) + \qquad \textit{Equation A2}$$
$$e(1-e)^2 \begin{bmatrix} T(g_o|g_m) . P(g_m) + \\ P(g_o) . P(g_a) + P(g_m) . P(g_a) \end{bmatrix} +$$
$$e^2(1-e)\left[P(g_m) + P(g_a) + P(g_o)\right] + e^3$$

The likelihood ratio is Equation A1 divided by Equation A2, as before, and reduces to Equation 3.4 when $e = 0$.

When mothers are unsampled, errors may occur in the offspring or the alleged father. The likelihood that the alleged father is the true father is:

$$L(H_1|g_a, g_o) = (1-e)^2 . T(g_o|g_a) . P(g_a) +$$
$$e(1-e)\left[T(g_o|x_a) . P(x_a) + T(x_o|g_a) . P(g_a)\right] +$$
$$e^2 . T(x_o|x_a) . P(x_a)$$

which reduces to:

$$L(H_1|g_a, g_o) = (1-e)^2 . T(g_o|g_a) . P(g_a) + e(1-e)\left[P(g_o) + P(g_a)\right] + e^2 \qquad \textit{Equation A3}$$

The likelihood that the alleged father is a male selected at random is:

$$L(H_2|g_a, g_o) = (1-e)^2 . P(g_o) . P(g_a) +$$
$$e(1-e)\left[P(g_o) . P(x_a) + P(x_o) . P(g_a)\right] +$$
$$e^2 . P(x_o) . P(x_a)$$

which is:

$$L(H_2|g_a, g_o) = (1-e)^2 . P(g_o). P(g_a) + e(1-e)\left[P(g_o) + P(g_a)\right] + e^2 \quad \textit{Equation A4}$$

The likelihood ratio is Equation A3 divided by Equation A4, and reduces to Equation 3.5 when $e = 0$.

A1.2 Average probability of exclusion

The average probability of excluding an unrelated individual from parentage, given the genotypes of the offspring and other parent and assuming Hardy-Weinberg equilibrium, was derived initially by Jamieson (1965), but is most easily calculated in the form given by Chakravarti and Li (1983) and Jamieson (1994). In Chakravarti and Li's notation, the average exclusion probability P_l at a locus l with k codominant alleles is given by:

$$P_l = a_1 - 2a_2 + a_3 + 3(a_2 a_3 - a_5) - 2({a_2}^2 - a_4)$$

where $a_n = \sum_{i=1}^{k} {p_i}^n$ and p_i is the frequency of allele i, and $a_1 = 1$. Note that this equation was incorrectly cited in Morin *et al.* (1994). The equivalent average probability of excluding an unrelated individual from parentage given only the genotype of the offspring is derived below.

For homozygous offspring *AA*, an exclusion occurs if the candidate parent is neither *AA* nor any of the *k*-1 heterozygotes *AX*. For heterozygous offspring *AB*, an exclusion occurs if the candidate parent is neither *AA*, *BB*, any of the *k*-1 heterozygotes *AX* nor any of the *k*-1 heterozygotes *BX*. The heterozygous candidate parent *AB* occurs both in the set of genotypes *AX* and the set of genotypes *BX*. Defining the probability of genotypes *AA*, *AB*, *AX* and *BX* as $p(ii)$, $p(ij)$, $p(ix)$ and $p(jx)$ respectively and summing across all pairwise genotypic combinations, the average probability of exclusion at locus l, P_l, can be written:

$$P_l = 1 - \left\{ \sum_{i=1}^{k} \sum_{x=1}^{k} p(ii)p(ix) + \frac{1}{2} \sum_{i \neq j}^{k} \sum_{x=1}^{k} p(ij)\left(p(ix) + p(jx) - p(ij)\right) \right\}$$

Substituting the expected frequencies of the relevant genotypes, assuming Hardy-Weinberg equilibrium, yields:

$$P_l = 1 - \left\{ \sum_{i=1}^{k} p_i^2 \left(\sum_{x=1}^{k} 2p_i p_x - p_i^2 \right) + \sum_{i \neq j}^{k} p_i p_j \left(\sum_{x=1}^{k} 2p_i p_x - p_i^2 + \sum_{x=1}^{k} 2p_j p_x - p_j^2 - 2p_i p_j \right) \right\}$$

where p_i is the frequency of allele i as above. Given that $\sum_{i=1}^{k} p_i = 1$, this simplifies to:

$$P_l = 1 - \left\{ \sum_{i=1}^{k} p_i^2 . p_i(2 - p_i) + \sum_{i \neq j}^{k} p_i p_j (p_i + p_j)(2 - p_i - p_j) \right\}$$

By writing $\sum_{i \neq j}^{k} p_i p_j$ as $\sum_{i=1}^{k} p_i \left(\left(\sum_{j=1}^{k} p_j \right) - p_i \right)$ and similarly for other powers, P_l becomes:

$$P_l = a_1 - 4a_2 + 4a_3 - 3a_4 + 2a_2^2 \qquad \textit{Equation A5}$$

where $a_n = \sum_{i=1}^{k} p_i^n$ as above.

The overall average probability of exclusion across n independently-inherited loci, P, may be calculated in the usual way:

$$P = 1 - \prod_{l=1}^{n} [1 - P_l]$$

A1.3 Estimating the rate of typing error

Equation A5 may be used in estimating the rate of typing error, if a large number of parent-offspring pairs are known without error. Defining an error as the replacement of the true genotype with a genotype selected at random under Hardy-Weinberg assumptions, the rate of typing error e_l for locus l is approximately:

$$e_l \approx \frac{1}{2P_l} . \frac{m_l}{M_l}$$

where m_l is the observed number of parent-offspring mismatches in M_l comparisons, assuming that the probability of both parent and offspring being mistyped is negligible. All parent-offspring pairs used in estimating e_l must be independent. One must avoid including multiple representatives of a maternal half sibship and also avoid including an individual both as an offspring and as a female parent, otherwise the error rate is liable to be overestimated.

If error is constant across loci, a better estimate of the underlying error rate, e, is the average across n loci:

$$e = \frac{1}{n}\sum_{l=1}^{n} e_l$$

A2 Protocols for microsatellite typing of Arabian oryx

A2.1 DNA extraction

Skin samples had been preserved only by freezing; the oryx samples were of variable condition, ranging from very fresh to highly desiccated. Small samples were cut (up to 2mm x 2mm) and transferred to 1.5ml Eppendorf tubes containing 0.55ml of digestion solution containing proteinase K. For horn samples, scrapings were obtained from the horn bone or if no bone was present, from the inside of the horn sheath, and the scrapings treated as for skin. For white blood cell fractions, a small volume (< 0.1ml) of the fraction was mixed with digestion solution as above. For heparinised whole blood, 0.275ml of whole blood was mixed with an equal volume of the digestion solution in a 1.5ml Eppendorf tube. In all cases, the tubes were placed in a 65°C water bath for 10-15 minutes, followed by a 37°C water bath overnight.

To remove proteins 0.55ml of phenol was added, the tube mixed vigorously and microfuged at 13,000rpm for 12 seconds. The aqueous layer (up to 0.5ml) containing the DNA was transferred to a fresh Eppendorf tube, to which 0.5ml of chloroform was added, the tube mixed vigorously once more and microfuged at 13,000rpm for 12 seconds.

The aqueous layer was again transferred to a fresh tube to which 150µl of 10M LiCl was added to precipitate RNA. After gentle mixing, the tube was place in a -20°C freezer for 20 minutes, and then microfuged at 13,000rpm for 5 minutes. 0.5ml from this tube was transferred to a fresh Eppendorf tube, to which 1ml of 100% ethanol was added. In most cases gentle mixing yielded visible strands of DNA immediately, but for a few samples it was necessary to place the tube in a -70°C freezer for 20 minutes to produce a visible product. In either case the tubes were spun at 13,000rpm for 5 minutes to pellet the DNA, the 100% ethanol was poured away and replaced with 1ml of 70% ethanol. After standing at room temperature for 20 minutes, the tube was microfuged for 5 minutes at 13,000rpm, as much as possible of the 70% ethanol was poured off, and the sample was air dried for at least 30 minutes. To the dried pellet 1ml of TE buffer was added. The tube was placed in a 65°C water bath for 10 minutes in order to dissolve the pellet, and then permanently labelled.

The extracted samples were run on a 0.7% agarose gel in TBE buffer at 100V for one hour, in order to assess quality and concentration of DNA. Bands were visualised using ethidium bromide staining (35µl in a litre of TBE) and UV illumination. Providing there was a visible DNA band on the gel, 0.5ml of the DNA solution was transferred to a 96-well Beckmann deep well plate, to permit the use of an 8-channel pipette when setting up PCRs.

A2.2 PCR amplification

For the purpose of finding polymorphic microsatellite loci in oryx, a test panel of unrelated oryx were used, varying in number between three and eight. 66 microsatellite loci isolated in cattle, sheep, red deer or gazelle were tested for polymorphism in Arabian oryx (Table 8.3). For each locus, a control sample from the source species was amplified along with the oryx samples in order to confirm that unfavourable planetary conjunctions were not interfering with the PCR process.

PCR reactions were performed in Hybaid 96-well OmniPlates. For each sample, the reaction mix consisted of 1µl dNTPs, 1µl DMSO, 1µl PARR buffer, 0.1µl $MgCl_2$, 6µl sterile distilled water, 0.05µl Taq polymerase, 2x 0.2µl primer pair solutions and 0.025µl ^{32}P-labelled dCTP. This mixture was combined with 1µl of DNA sample, giving a total reaction volume of approximately 11µl; the aqueous layer was then

covered with a 20µl drop of mineral oil. Details of these and other reagents appear in Table A1.

Short name	Full name	Concentration
dNTPs	Deoxynucleotide solution	1mM; 0.1mM dCTP
DMSO	Dimethyl sulphoxide	Neat
PARR	PARR-excellence buffer	10x working concentration
$MgCl_2$	Magnesium chloride solution	0.05M
Taq	Taq polymerase	5U/µl
Primer pair	Single stranded DNA primer pairs	0.01mM
dCTP	^{32}P-labelled deoxycytosine	10mM, in Tricine buffer
TE	Tris-EDTA buffer (pH 7.6)	10mM Tris.Cl (pH 7.6)
		1mM EDTA (pH 8.0)
TBE	Tris-borate/EDTA buffer	0.045M Tris-borate
		0.001M EDTA

Table A1 *Reagents and concentrations used. Short names are those used in the text.*

PCR amplification was carried out in Hybaid OmniGene machines programmed as outlined in Table A2. The machines were used in simulated tube mode.

Stage	Cycles	Temp (°C)	Length (sec)	Function
1	1	93	120	Denature
2	7	93	30	Denature
		46*	60	Anneal
		72	30	Extend
3	25	89	30	Denature
		48*	60	Anneal
		72	30	Extend

Table A2 *PCR programme used for amplifying microsatellites. Annealing temperatures (marked with a *) were adjusted for some loci in order to improve the quantity or quality of the PCR product.*

A2.3 Manual sequencing gels

For each sample in the OmniPlate, the aqueous layer containing the PCR products was mixed with 5µl of sequence loading buffer. The samples were denatured in the OmniPlate by placing in a PCR block and heating to 95°C for at least two minutes. 2.5µl of this mixture was loaded on to a 0.4mm 6% polyacrylamide sequencing gel in TBE buffer heated to 50°C. A ^{35}S-labelled M13 sequencing reaction was also loaded on the gel so that allele lengths could be assigned to any products found. Samples were run at 30-40mA for 1-2 hours (depending on expected size of PCR product), the gel was then covered with cling film and vacuum-dried on a paper backing and exposed to X-ray film overnight.

A2.4 Automated sequencing gels

Additional typing was carried out for some loci using an ABI377 automated DNA sequencing machine. PCR procedures were as above except all dNTPs were at 1mM concentration in unlabelled form, and one of the primers carried a fluorescent tag appropriate for detection by the ABI system. The standard ABI sequence loading buffer was used. Analysis of the gel image was carried out using GENESCAN and GENOTYPER software.

A2.5 Typing procedure

When testing new loci, they were scored for presence, quantity, quality and variability of product, and if polymorphism was found the allele lengths of test animals were determined (using the M13 standard sequence). For some loci a second test run was performed with an altered PCR program, typically using a different temperature at the annealing step in stages 2 and 3. When screening all animals for a particular locus, animals were scored for the alleles they carried. Alleles were sized in base pairs using the standard sequence tracks and with reference to any samples on the gel for which allele sizes had already been determined. Gel scoring was double-checked by a second person, and data were entered into a dBASE IV database for later analysis.